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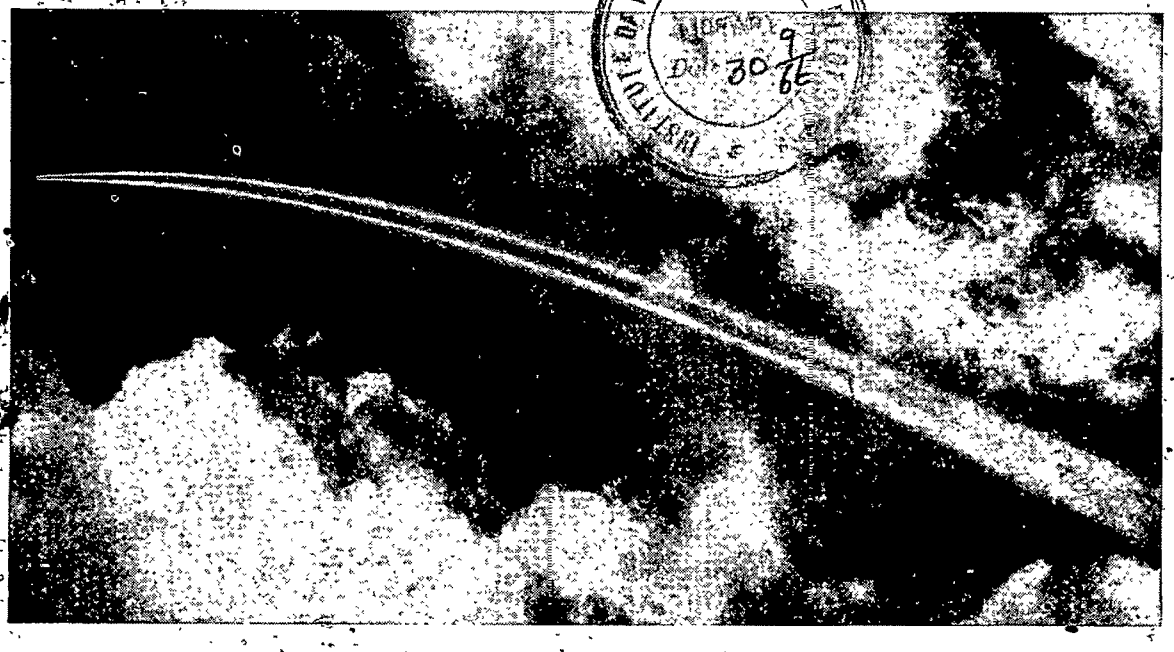
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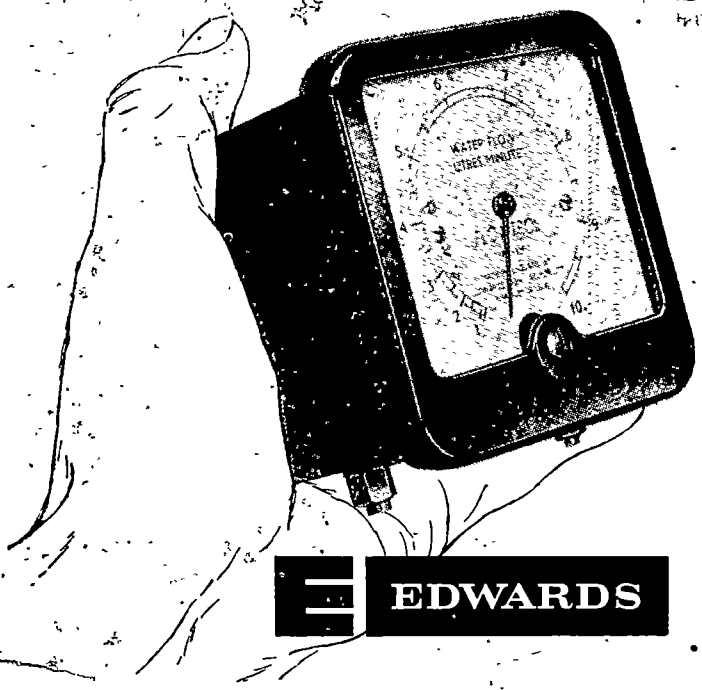
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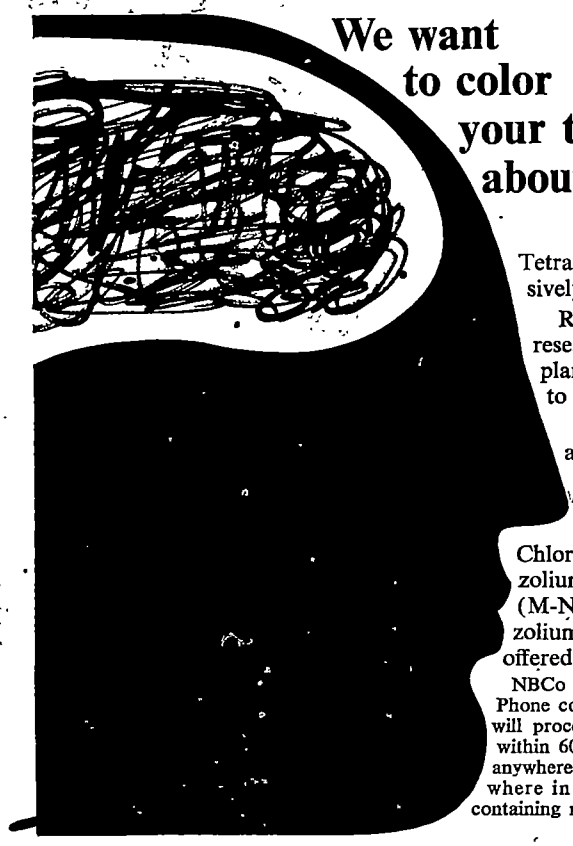
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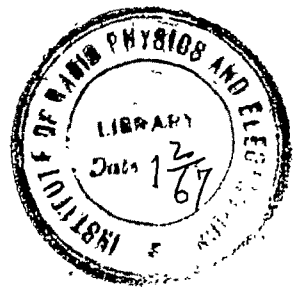
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INDEPENDENCE FOR UNIVERSITIES

NOT very long ago, the University Grants Committee was like Caesar's wife. To universities, it was a sure defence of independence. To British governments, it was a way of spending large sums of money without worrying too much about the details and the responsibility. To visitors to Britain it was, or at least was said to be, a triumph of public administration, a marvellous embodiment of the art of compromise, and the last word in common sense. And certainly there was something remarkable and even admirable about the concept of an institution which could intervene in the financing of universities from public funds in such a way as to prevent the donor from feeling cheated and the beneficiary compromised. But all that was three years ago.

The present mood in the universities is very different. The University Grants Committee no longer seems a comfortable bulwark against interference from outside. Instead, it has taken to asking awkward questions about cost-effectiveness. Sometimes it seems to the universities to be a branch of the Civil Service. Ironically, this transformation has happened since the Robbins Report sketched out what seemed to be a glorious future for the universities. Sir Ifor Evans was right, in his farewell address to the faculty at University College, to single out that document as a turning point (see page 2 of this issue), but the reasons are much more complicated than mere misinterpretation, mischievous or otherwise.

It is sometimes forgotten that the Robbins Report was not merely a charter for the universities but a proposal that there should be a massive expansion in higher education as a whole. Two governments in succession have accepted that as many young people as possible should receive some form of higher education. Increasingly, however, they have found it necessary to make decisions about the allocation of resources among different kinds of institutions—teacher training colleges, technical colleges, regional colleges and universities.

It is perhaps inevitable that the present Government should be tempted, first of all, to spend money where it will be most quickly effective in increasing the amount of higher education. This is one of the reasons why universities have found themselves, in the past two years, ill-equipped to carry out the expansion which the Robbins Report declared necessary. This is also why the University Grants Committee itself has been required to see that the funds available to the universities are spent as efficiently as possible. Even if the University Grants Committee were still a dependant of the Treasury rather than the Department of Education and Science, it could not hope to be immune to pressures in these uncomfortable directions. The scale of the current growth of higher education in Britain makes the isolation of one sector impossible.

The universities have probably increased their own difficulties by complacency since the Robbins Report, which was more concerned to demonstrate the need and feasibility of more higher education than to decide how best to provide it. It was probably sensible to assume, as a first approximation, that a substantially enlarged university system would not be qualitatively different from what there is at present. One consequence, however, was the foolish conclusion that an increase of the university population would require the creation of more small universities. The Government has done right to set its face against this tendency. A few really big universities would be a better investment, academically as well as financially. It would have been better if the universities and not the Government had first made this point.

Another consequence of the Robbins Report was the sanctification of much that is traditional in British academic life. The ratios of staff to students assumed by the Robbins Commission have, for example, become magic numbers, yet the universities would be better able to defend themselves against suspicions of extravagance if they had seriously considered what economies could be won from greater size, or by means of variations of the ratio from one academic discipline to another. And they would be able to put their arguments more effectively if more power resided with the vice-chancellors who must represent them in the outside world.

If there are defects in the position of the universities, however, it does not follow that the Government is blameless. The present shortage of funds, especially for buildings, and the uncertainty about the immediate future, may be a spur to economy but it is also damaging to the health of the universities. At the same time, the consequences of what is called the "second tier" policy have only been loosely thought out. It is right, of course, that the Government should strengthen the technical and regional colleges; grouping them into units called polytechnics should do a lot to help. But is it really wise to create a system in which there will be two sharply contrasting types of institutions for higher education? It is surprising that the Secretary of State for Education and Science has not recognized the beginnings of a problem which is the adult equivalent of the old gulf between the grammar and the secondary modern schools.

How, then, should the universities defend their interests? The first need is to recognize that the protective influence of the University Grants Committee has probably gone for good. A retreat from dependence on public money, which appeals to Sir Ifor Evans, is also impracticable. It follows that there has to be some coherent body which can speak for the universities as a whole. The Committee of Vice-Chancellors is the obvious instrument, but it will have to become much more active if it is to be effective. The ideal is that the universities should collectively determine how they can best satisfy the needs outlined in the Robbins Report and the frequently conflicting needs of the

British economy. The best guarantee of independence would be to take the initiative in solving the problems which exist.

DEAD DUCK INTO PHOENIX

STUDENTS of the Ministry of Technology, enemies as well as friends, must now acknowledge that a change for the better has overtaken that agency of the British Government originally created to foster change elsewhere. One sign of what is happening was the speech which the Minister of Technology, Mr. Frank Cousins, made to the Confederation of Shipbuilding and Engineering Unions on June 22. Almost for the first time there were traces of what could well become, with diligence and persistence, a coherent strategy to replace the rudderless philosophy of the past two years. What seems to have happened is that the Ministry has recognized how much of its work must be dull and unspectacular. In some fields, such as computers and electronics, it will have to lavish special care and attention until somebody finds a solution to the problem of how comparatively small societies can remain in the forefront of technological change without losing money. But the potential benefits of steadily increasing efficiency in less-spectacular industries are just as great, and this is the lesson that is sinking home. It is especially welcome that, in his speech at Torquay, Mr. Cousins should have gone out of his way to emphasize the promise of a link between increasing efficiency and increasing prosperity, for technology is not an end in itself but a means of living better.

Some of the tangible benefits of the new realism at the Ministry of Technology are the National Computer Centre soon to be started at Manchester, and the solid work on industrial standardization now under way. There have also been a number of recent development contracts which are at once imaginative and down-to-earth. The programme to develop methods of computer control for chemical plants is a good example, and of course it is entirely sensible that the Ministry should now try to make good use of the regional offices which the old Department of Scientific and Industrial Research established in the hope of helping industry to greater efficiency. Unfortunately, however, sweet reason is not everywhere apparent. The Ministry's chronic trouble has been the pretence that it could fly before it could walk. Traces of this old Adam are still there. Mr. Cousins, for example, was talking of the "Breakthrough Programme" that would be built around the laboratories of the old Ministry of Aviation as if he still thought office had put a magic wand into his hand. There is still a danger that the Industrial Reconstruction Corporation, which gives the Board of Trade powers to buy into private industries, will go to Technology's head. And the Ministry has not yet begun to tackle some of the great and outstanding problems such as industrial retraining. It is too soon for watchdogs to look elsewhere.

NEWS AND VIEWS

Plain Words

SIR IFOR EVANS, the retiring Provost of University College, London, put into words some of the current discontents of British universities in his address to the Assembly of Faculties on June 23. In particular, he lamented "the complete surrender of the universities to government finance and in so many insidious ways to government control. It has, of course, all grown worse since the Robbins Report . . ." But the trouble, Sir Ifor said, was not to be found in the report itself, but in the way in which it had been interpreted. By implication, the Robbins Report had become a licence for interference.

For much of his fifteen years as Provost, Sir Ifor has evidently been captivated by a vision of independence for University College, and this has plainly become more attractive as the forces of expansion and inflation have made independence steadily less attainable. At current prices, Sir Ifor estimated, it would cost £80 million to buy complete autonomy, and, though he is evidently hoping that a benefactor of such generosity may somewhere exist, most of those who heard him speak considered such a sum of money a sufficient proof of the necessity of the University Grants Committee.

Even so, Sir Ifor had a pointed tale of how in 1931 the College had to sell, for lack of £35,000, a piece of land which it is now having to acquire again for more than £1,000,000. He complained that the University Grants Committee in the fifties backed the founding of the University of Sussex because congestion on the sites of the London colleges meant that the "development in that area of a university institution is a particularly difficult and expensive operation". This restriction has now been eased, if not entirely dispelled, and Sir Ifor seems to have persuaded the present chairman of the University Grants Committee that the College should be allowed to expand outside its traditional rectangular plot, and that it should be allowed to pay market prices as well. He has had less luck on student housing, and confessed himself "astounded" that the University Grants Committee says that "not a pound of their money" should go on hostels. But he feels most strongly of all about interference from outside, and about "the imposition of regulations and memoranda that may ultimately turn the best creative and scientific minds in this country into mere university administrators, keeping their departments tidy for Government inspection, or the next round of reform". For his part, Sir Ifor said, he would have preferred to live in the "age of Rutherford than in the age of Franks". Because he is known to be a traditionalist, some of what Sir Ifor said may be discounted, but his break with the tradition of public gentility for the heads of universities could itself be an important move in the imminent tussle about the financing of the next university quinquennium.

No Pause in France

FRENCH science appears to be deliciously immune from the talk of deceleration of the growth of expenditure to be heard in the United States and the United Kingdom. The Fifth Plan makes it plain that expen-

diture on scientific research and development in France is intended to grow steadily, absolutely and as a proportion of the Gross National Product, until it amounts to 2·5 per cent of the wealth of France by 1970. In a comment on these plans prepared by the French Embassy in London, it is made quite plain that "France's financial outlay must be determined by reference to that of neighbouring countries". From this point of view, the argument goes, the target of 2·5 per cent is, if anything, a minimum. (The current British rate of expenditure works out at something between 2·5 per cent and 2·7 per cent according to the basis for calculating the GNP.) The pace of the proposed French development is measured by the fact that, in 1962, scientific research and development accounted for a mere 1·8 per cent of the GNP. Another yardstick is that the numbers of people employed on scientific work and in higher education in France are expected to double between 1963 and 1970, which in turn requires an increase of the annual output of trained scientists and engineers from French universities from 10,000 in 1963 to 21,000 in 1970.

So far as science is concerned, the Fifth Plan is uncommonly explicit about the way in which effort shall be distributed among the various fields of expenditure, largely on the strength of survey of possible developments carried out by the Research Commission. Most of the development in prospect is concentrated in the physical sciences, as shown by the following table of the allocations to capital expenditure from public funds :

	Expenditure (millions of francs)
Mathematics	315
Physics	1,254
Chemistry	400
Earth sciences	284
Oceanography	150
Biology and medicine	590
Agriculture	390
Building, planning	162
Human sciences	120
Patent rights	10
Inter-disciplinary operations	225
Space	2,000
Total	5,900

France will gain from this expenditure an impressive range of establishments and institutes. The way in which it has been decided that certain universities and university departments should be singled out for rapid growth in a particular field is also an echo of the "centres of excellence" doctrine of the United States, and possibly an example to other European countries in this respect. But other countries, of course, will envy most of all the buoyant growth planned for the next five years.

Next Leap Forward

THE proposal that a 300-GeV proton accelerator should be built somewhere in Europe and within the organization of CERN moved a little nearer reality in June with the thirty-second meeting of the Council of CERN at Geneva. It will be a long time yet before European governments are required finally to say whether they will contribute to this project, and how much. Nevertheless, by the end of this year it should be plain which are the favourites among the twelve sites from nine countries now being studied in considerable detail. Given the great cost of the 300-GeV machine and the fact that its siting will employ a nearby work force of between 10,000 and 15,000 men and their families, it is unlikely that the interested countries will be able to separate from each other decisions on where the accelerator should be built and how its cost should be apportioned.

Enthusiasm for the machine, however, remains as great as ever. The European Committee for Future Accelerators, meeting during May, reaffirmed its belief in the Amaldi Report (1963) which first seriously recommended the 300-GeV machine and which argued for a great expansion of comparatively smaller accelerators as well. In the three years since the proposal for the accelerator first became tangible enough to feature in diplomatic discussions, there has already been a considerable growth of interest in less ambitious machines. The Committee for Future Accelerators was pleased, in May, to see how plans are being advanced for a meson factory at Zurich, a 2·5-GeV electron accelerator at Bonn, and the electron-storage rings intended to work with the accelerator at Frascati, near Rome. But the "summit programme", as it is called, remains the Committee's chief objective. It has also declined to budge from the view that what a country spends on high-energy nuclear physics within the structure of CERN should be balanced by at least an equal amount of expenditure in national centres and universities. The passage of time may well ensure that this hope is fulfilled, for there are those who think that each month sees a strengthening of the centrifugal tendencies which tempt individual countries, from time to time, to think it would be better to have a machine of their own, smaller though it would have to be.

Migraine Research

A NATIONAL appeal to help the "millions of migraine sufferers" has been launched by Lord Brain for the Migraine Trust, which is trying to raise £750,000 to finance a research programme. In particular, it wishes to establish a hospital in-patient unit of 16 beds, at a cost of £370,000, so that full metabolic and radiological studies can be carried out on patients during and after attacks. Research is already being carried out at the Migraine Clinic in the National Hospital, London, but this needs £30,000 to speed and to expand its work on the variation and associated change in the size of blood vessels in certain parts of the brain during an attack, and on the connexion of migraines with abstinence from food.

Describing a survey into the incidence of migraine which has been started in the Rhondda Valley with support from the Medical Research Council, Lord Brain said, "As soon as possible we plan to develop the survey into a detailed population study, taking into account other branches of medical research, and covering a period of three years". To allow for regional variations, a survey in Scotland under the auspices of the University of Glasgow is being planned.

The most important work of the Trust is to co-ordinate and assess similar research in medical centres throughout the world. Lord Brain stated that in addition to co-ordinating work in Britain the Migraine

Trust plans to publish a quarterly journal which will carry abstracts from important world-wide literature as well as original papers on migraine. A symposium is planned for this autumn and the first international symposium will be held the following year.

Project for Engineers

THE Department of Education and Science launched a new magazine this week in its continuing attempt to interest senior school students in engineering as a career. *Project* will be published three times a year and 24,000 copies will be distributed to fifth and sixth forms throughout Britain. The engineering institutions are anxious to see the magazine succeed and are contributing articles and information but not money. The aim is to give a favourable impression of various types of engineering, the training required to become a qualified engineer, and the example of well-known engineering innovators—this first issue includes a profile of Dr. Barnes Wallis of the British Aircraft Corporation, whose ideas have not always been immediately accepted by the British Government but whose inventiveness and originality, according to the editor of *Project*, make him one of the outstanding engineers in Britain.

The magazine, a £10,000 glossy product, is produced by the Central Office of Information for what is known as the Publicity Working Party on Engineering Technology—the custodian of the British Government's hope that more students will be attracted into engineering and technology. Other projects have included a travelling exhibit for schools entitled "Technology Today", which has visited forty-five centres in the past 7 months, and an earlier publication, *Engineering: a Creative Career*, which attempts to present a broad spectrum of engineering problems, the routes to qualification beginning at both 'O' and 'A' levels, and typical university syllabuses. A national exhibition "Engineer's Day" will be held in London in the autumn. Although the new magazine is aimed for students, anyone interested can obtain copies from the Government bookshop or his newsagent at an annual cost of 15*s*.

Safety Cars

THE Road Research Laboratory of the Ministry of Transport has made a tentative bid to do for Britain what Mr. Ralph Nader seems to be doing for the United States. In its most recent report on *Safety Cars* (obtainable either from the Laboratory or the Ministry of Transport), Mr. G. Grime has applied Newton's Second Law to collisions between cars, and has been able to infer a great deal about the relative motion of cars and passengers in the fraction of a tenth of a second which seems usually to be required to crush the front of a car involved in a head-on collision by 60 cm or so. His general conclusion is that seat-belts are among the best means of protection, so that it is no surprise that Mrs. Barbara Castle, the Minister of Transport, should have announced a regulation that will in future require that cars should be equipped with anchorage points for seat-belts.

One of the simplest of Mr. Grime's conclusions is that passengers tend to come into contact with the vehicles which carry them only when the collision is almost at an end. In one calculation, for example, he found that by the time a human body had shot forward by 41 cm from the front seat to strike the instrument panel of a medium car in a head-on collision at 50 km per hour, the crushing of the front of the car would have only 0·5 cm to go. This means that the unlucky passenger would strike an essentially static object, and even the best padding on the instrument panel would entail a deceleration of 64 *g*. A suitably padded and flexible steering-wheel might reduce the deceleration in these circumstances to 35 *g*, but a seat-belt capable of a modicum of stretch could ensure that deceleration amounts to 17·1 *g* in similar circumstances, and that the attendant forces are "applied in a controlled manner over a large and predetermined area". In all this the principle is that a person should be partially anchored to the car in which he rides, so that he can expect to benefit from the energy expenditure involved in crushing the front of the car.

Mr. Grime is not optimistic about the prospects of greater safety by designing the front of a motor-car so as to minimize the consequences of collision. According to the calculations, seat-belts are always better. Mr. Grime does, however, conclude from American experience with would-be astronauts that it would be feasible to design seat-belts capable of decelerating people by something like 30 *g*, compared with the 20 *g* which is now common. His dynamical calculations also persuaded him that there would be advantages in a design for a seat-belt capable of applying a greater force at the limit of its extension, and that the ideal is a situation in which seat-belt and seat are built as an integral whole. Finally, for those who will not wear seat-belts, there are some benefits to be won from crushable fittings on the instrument panel of a motor-car, especially if they can be so arranged as to help decelerating a passenger soon after the beginning of a collision.

Put Out More Books

WHEN Blackwells first opened their doors and started selling books in Oxford, there was room in the shop for only three customers at a time. When their new extension was opened by Sir William Haley on June 16, three hundred guests browsed in traditional Blackwell fashion among the shelves, uncrowded and undisturbed. The new extension was christened the Norrington Room, after the president of Trinity College, under whose quadrangle Blackwells have found room to expand by digging a subterranean bookroom of enormous size.

Despite its size, and the need for artificial lighting, the room is prevented from seeming oppressive by the use of four different floor levels, and a variety of ceiling materials. There is shelving to carry 160,000 books, arranged to make the best use of space, and to break up the room into units of more human size. The expansion will enable Blackwells to carry even greater stocks on the premises, which is important in a service industry where orders must be dispatched as soon as possible. Although the personal shopper represents less than 10 per cent of the turnover, the increase in stock will also be a great convenience to him, particularly in science and technology, where the expansion is concentrated. A quick check in familiar fields confirmed that the increase in stock is quite genuine, which is good news for scientists both in Oxford and elsewhere.

Parliament in Britain

In a written answer to a question from Mr. Boston on June 20, the Parliamentary Secretary to the Ministry of Public Building and Works, Mr. J. Boyden, stated that on the basis of a special enquiry made in 1964 it was estimated that in that year the construction industry spent about £2·5 millions on research. This is the equivalent of 0·3 per cent of its gross output, compared with a national average for all industries of almost 2·7 per cent.

In opening an adjournment debate on building methods and bad-weather precautions on June 21, Mr. Boston returned to the question of the attitude of the building industry to research, and Mr. Boyden, replying for the Government, said that the Minister, Mr. R. Prentice, had that day held a conference with those concerned with setting up a new organization for research and information in the construction industry. The discussions had gone well, and many sections of the industry were sharing a keen interest in raising the necessary funds. The question of continuous working during bad weather and the winter would be an important part of both research and information work. He hoped that the new body would follow up the steps already taken by the Ministry and that in future it would be customary for the industry to work a normal week, except perhaps during cataclysmic weather The need of research was fairly well known and, while agreeing that the construction industry as a whole spent less on research than many manufacturing industries, Mr. Boyden hoped that the new organization would give just the impetus needed.

In reply to a question in the House of Lords on June 21, the Parliamentary Under-Secretary of State for Commonwealth Relations and for the Colonies, Lord Beswick, said that a National Reports Centre had been set up at the National Lending Library for Science and Technology as a British counterpart to the United States Clearing House which produced a fortnightly list entitled *United States Government Research Reports*. The British Centre published a monthly bulletin entitled *British Research and Development Reports* in which accessions to the stock of reports were announced. The service started only this year, and Lord Beswick agreed with the Earl of Bessborough about the importance of distributing the bulletin widely. Any organization could be put on the mailing list if it applied to the National Lending Library.

In a written answer in the House of Commons on June 20, the Minister for Land and Natural Resources, Mr. F. Willey, said that a hydrographic survey of the Wash area, with gravity coring of the sea-bed, current gauging and seismic investigations with tests of samples, would form an integral part of a feasibility study of a Wash barrage. It would be wrong, Mr. Willey said, to embark on such a scheme except as part of a comprehensive investigation. The report on the Water Resources of the Great Ouse Basin suggested a five-year programme of investigations which, with the preparations of designs and estimates, would cost about £1·5 million. The hydrographic survey and related soil investigations, including boreholes, would account for almost £1 million and would take two to three years.

University News:

Aberdeen

Mr. D. F. Kerridge has been appointed to the chair of statistics as from August 1.

Aston

Mr. A. J. Ede has been appointed professor of mechanical engineering as from August 1.

Leeds

Dr. D. Dowson has been appointed to the chair of fluid mechanics and tribology in the Department of Mechanical Engineering.

London

Dr. G. S. Nelson, reader in medical parasitology in the London School of Hygiene and Tropical Medicine, has been appointed to the William Julien Courtauld chair of helminthology tenable at that school.

Manchester

Mr. C. R. Illingworth has been appointed to a newly established additional chair of mathematics; Dr. F. D. Kahn has been appointed to a newly established second chair of astronomy; Dr. D. B. G. Edwards has been appointed to a newly established chair of computer engineering.

Announcements

Mr. Nigel Calder, editor of the *New Scientist* since 1963, will be leaving his present post on October 1 this year. He will be succeeded by Mr. Donald Gould, who has won a considerable reputation in recent years, chiefly as a free-lance writer on medical subjects. Mr. Calder intends to devote himself to free-lance writing.

Over 300 British scientists have signed an appeal to the French Government to cancel the new series of nuclear tests about to be made in the Pacific. The protest was published this week as a full-page advertisement in the Paris weekly *Le Nouvel Observateur*. Over 30 of the signatories are Fellows of the Royal Society or heads of departments at British universities.

Dr. Columbus O'Donnell Iselin has been awarded the fourth Henry Bryant Bigelow Medal for Oceanography by the Woods Hole Oceanographic Institution. Dr. Iselin is a former director of the Institution and has held the Henry Bryant Bigelow chair there since 1958; he is also professor of oceanography at Harvard University and the Massachusetts Institute of Technology. Dr. Bigelow was the first director of Woods Hole, holding the post from 1930 until 1940, and was also the first recipient of the Medal, which was awarded him in 1961.

Prof. T. Bergeron has been awarded the International Meteorological Organization Prize by the Executive Committee of the World Meteorological Organization. This Prize is awarded annually for outstanding work in meteorology and international collaboration.

Dr. Paul-A. Giguère, director of the Department of Chemistry in the Université Laval in Quebec, has been elected president of the Chemical Institute of Canada during 1966–67.

The following have been elected honorary fellows of the Institute of Biology: Sir Macfarlane Burnet, Prof. E. B. Chain, Dr. J. C. Kendrew, Sir Peter Medawar.

Dr. E. A. Walker, president of the Pennsylvania State University and chairman of the National Science Board, has been elected president of the U.S. National Academy of Engineering, in succession to Dr. A. B. Kinzel.

AT its annual general meeting, the Association of Applied Biologists elected the following honorary officers for 1966–67: *President*, Sir Vincent Wigglesworth; *Vice-President*, F. Raw; *President-Elect*, D. J. Watson; *General Secretary*, F. T. Last.

THE first volume of the newly launched *British Film Guide* lists some 1,700 films directly or indirectly related to medicine. The intention is that the completed *Guide* will list by subject all non-fiction films available in Great Britain. It will appear in twelve volumes, the first of which can be had from Film Centre (International), Ltd., 24 Conduit Street, London, W.1, price 39*s*. 6*d*.

ARRANGEMENTS have been made for the Russian journal *Genetika*, first published in Russia in 1965, to be translated into English on a continuing basis. Further information concerning this journal can be obtained from Clearinghouse for Federal Scientific and Technical Information, Joint Publications Research Service, Tempo E., 6th and Adams Drive, S.W. Washington, D.C. 20442.

THE next joint meeting of the Challenger Society and representatives from the Marine Laboratories will be held in the University of Southampton during July 21–22. Further information can be obtained from N. J. Holmes, Department of Oceanography, University of Southampton, Southampton.

THE third annual meeting of the Society for Cryobiology will be held in Boston during August 8–10. Further information can be obtained from Dr. C. Huggins, Department of Surgery, Massachusetts General Hospital, Boston.

THE second international congress of food science and technology will be held in Warsaw during August 22–27. Further information can be obtained from Dr. A. Borys, Instytut Przemyslu Miosnego, Warsaw 12, Rakowiecka 36.

A SYMPOSIUM on "Solar-Terrestrial Physics" will be held in Belgrade during August 29–September 2. Further information can be obtained from Dr. D. Bajic, URSI Belgrade Symposium Committee, P.O. Box 356, Belgrade.

THE eleventh international symposium on "Combustion" will be held in the University of California, Berkeley, during August 14–20. Further information can be obtained from the Combustion Institute, 986 Union Trust Building, Pittsburgh, Pennsylvania.

THE sixth international congress for electron microscopy will be held in Kyoto during August 28–September 4. Further information can be obtained from the Chairman of the Organizing Committee of the Congress, Institute for Virus Research, Kyoto University, Kyoto.

THE tenth international conference on "Low Temperature Physics" will be held in Moscow during August 31–September 6. Further information can be obtained from the Organizing Committee of the Conference, Institute for Physical Problems of the Academy of Sciences of the U.S.S.R., Vorobyevskoye Shosse 2, Moscow B-334.

A SYMPOSIUM on "Neutron Monitoring for Radiological Protection", under the auspices of the International Atomic Energy Agency, will be held in Vienna during August 29–September 2. Further information can be obtained from S. Somasundaram, Division of Health, Safety and Waste Disposal, International Atomic Energy Agency, Vienna.

A SYMPOSIUM on "Collection and Processing of Field Data", under the auspices of the Commonwealth Scientific and Industrial Research Organization, will be held in Canberra during August 30–September 2. Further information can be obtained from Dr. E. F. Bradley, Division of Plant Industry, P.O. Box 109, Canberra City, A.C.T.

THE fourth international Biometeorological Congress will be held at Rutgers University during August 26–September 2. There will be two plenary sessions on "Biometeorological Properties of the Atmospheric Environment" and "Impact of the Atmospheric Environment on the Integument of the Organism". Further information can be obtained from F. Sargent, 524 Burrill Hall, University of Illinois, Urbana.

A MEDICAL congress on the theme "Clinical Physiology and Internal Medicine", organized by the Czechoslovak Society of Internal Medicine, will be held in Prague during August 29–September 2. The congress will include a symposium on "Conception and Methods of Clinical Physiology". Further information can be obtained from the Congress Office, Fourth Medical Clinic Faculty of General Medicine, Charles University, U nemocnice 2, Prague 2.

A SYMPOSIUM on "Origin and Distribution of the Elements", organized by the International Association of Geochemistry and Cosmochemistry and co-sponsored by the United Nations Educational, Scientific and Cultural Organization and the International Union of Geological Sciences, will be held at the headquarters of the Organization in Paris during September 21–23. The symposium will be divided into five sections: theories of origin; solar, stellar and interstellar abundances; meteorites; planets, asteroids, comets and tektites; terrestrial abundances. Further information can be obtained from Prof. E. Ingerson, Department of Geology, University of Texas, Austin.

Appointments

THE Minister of Health, Mr. Kenneth Robinson, has appointed Dr. D. Dooley to be H.M. Inspector of Anatomy in England and Wales in succession to Dr. C. G. Nicol, who has retired. The post was established by an Act of Parliament in 1832; the duties involve issuing licences to teachers of anatomy and operative surgery and supervising the curriculum and teaching of these subjects in the medical schools and schools of anatomy in England and Wales.

WHEN Dr. L. Harrison Matthews retires from the Zoological Society of London this week, his work as scientific director will be divided into two new posts. Mr. L. G. Goodwin, director of the Nuffield Institute of Comparative Medicine, will be in charge of research as the director of science. Mr. C. G. C. Rawlins will be director of zoos with responsibility for managing both Regent's Park and Whipsnade zoos. Mr. Rawlins retired this year from the Overseas Civil Service after serving as a district commissioner and gaining considerable experience in administering fauna conservation and game areas. Dr. H. G. Vevers, now curator of the aquarium and invertebrates and assistant to Dr. Matthews, will be the assistant director of science.

MR. I. L. BAUMGART has been appointed assistant director-general of the New Zealand Department of Scientific and Industrial Research on the retirement of Mr. L. W. Tiller. Dr. A. T. Johns has been appointed assistant director-general of the Department of Agriculture, and Dr. G. Butler will replace Dr. Johns as director of the Plant Chemistry Division of the D.S.I.R.

DR. M. KENT WILSON, professor and chairman of the Department of Chemistry in Tufts University, has been appointed head of the Chemistry Section, Division of Mathematical and Physical Sciences, in the National Science Foundation as from July 1.

DR. J. F. CLARK has been appointed director of the Goddard Space Flight Center, Greenbelt, Maryland, where he has been acting director for a year.

NEW CURRICULA FOR SCHOOLS

WHAT should students be taught at school? For a long time this was a question that needed no answer. The practice of the schools was a sufficient guide to what their practice should be. The construction of the curriculum and its evaluation have for a long time been thought of as slowly changing entities the momentum of which carries them along. This is why it is remarkable that in the past decade or so there has been a rapid growth of interest and experiment designed deliberately to change the curriculum of the schools. Though experiments have been carried out in studies of all kinds—in modern languages and English, for example—the developments there have been in the deliberate design of science curricula stand out for their vigour, their adventurousness and their wide applicability in the schools.

The beginning of this movement is marked by the work of the Physical Sciences Study Committee (PSSC) which grew up at Massachusetts Institute of Technology in the years from 1957 on, and after a number of authorities in the United States had been alarmed by what seemed to be the neglect of science teaching in American high schools. In the United States the initiative in physics was quickly followed by similar developments in other fields—in chemistry and biology, for example. Funds were provided in rapidly growing amounts by the National Science Foundation and by other bodies concerned with the development of the curriculum—school systems, universities and even publishers.

In parallel with these developments in the United States have been similar experiments elsewhere. In Britain, the groups of teachers working within what used to be the Science Masters Association (now known as the Association for Science Education) embarked on a number of developments in the 'fifties. Working largely through voluntary committees, the schoolmasters were able to suggest ways in which more modern topics might be introduced in the school curriculum, and ways in which the style of teaching in science could be designed more adequately to excite the interest and the curiosity of children. At the end of 1961 this voluntary work was in part superseded by the creation of the Nuffield Foundation Science Teaching Project, which brought together a number of different interests in the evolution of the curriculum—the interests of the professional institutions such as the Institute of Physics, for example, and the interests of Scottish teachers and education authorities. The first part of the work of the Science Teaching Project has been published this week, and during the rest of the summer the books which embody O-level courses in physics, chemistry and biology will appear. It is hoped that some hundreds of schools will embark on these new courses in the autumn.

Elsewhere there have been similar tendencies. The book review which appears on page 13 of this issue refers to the work of a curriculum development project in Australia which appears already to have had an important influence on the pattern of science teaching in secondary schools. In Europe a great many countries other than the United Kingdom have undertaken curriculum development for their secondary schools. It is true, of course, that in several countries the pace at which change can occur is limited by the way in which curriculum may often have statutory validity as it does, for example, in Spain and Italy. The movement towards the reform of the science curriculum is not confined to industrial countries, and indeed the under-developed countries of Africa and south-east Asia are as keenly interested in what the new curricula may provide as are the nations with older-established educational systems. For this reason organizations such as PSSC have inspired curriculum developments overseas, particularly in Africa, with support from the Ford Foundation and under the label of Educational Services Incorporated. British interests in this field are likely to be channelled into help for less-developed countries through the newly formed organization called Centre for Curriculum Renewal and Educational Development Overseas which has been sponsored jointly by the Ministry of Overseas Development and the Nuffield Foundation, as well as through a number of older-established institutions, among whom the commercial publishers cannot be neglected.

The fact that such a diversity of curriculum developments has coincided in time is a measure of the way in which the educational systems of industrialized nations have been forced to tackle a number of basic problems of organization. In Britain, for example, the most sustained pressure on the schools in the past fifty years has been the steady growth of the secondary school population. This has meant an increasing diversity of ability and interest at all levels of the school system. It has also meant that the teachers could not count on the willingness of their pupils to grapple with the learning process without prompting. Instead they have had to become increasingly concerned with how best to help children learn and how best to give them the will to do so. In modern languages this has led to the growth of systems of teaching in which children are practically involved in speaking a foreign language from the beginning. In science the tendency has been similar. All the experiments in curriculum renewal now going on have as their common component a wish to give pupils first-hand experience of the process of science. In other words, didactic teaching is being replaced by what is intended to be a regimen of experimental discovery by the pupils themselves. In mathematics, the curriculum development projects, some modest and some ambitious in their intellectual content, also have in common an attempt to give children a feeling for the conceptual structure of mathematics without exercising them in the often dull routines of the subject.

The opportunities and the difficulties which attend the whole process of curriculum development are admirably exemplified by the experience and the ambitions of the Nuffield Science Teaching Project. Here, as elsewhere, the objective has been to devise programmes for teaching science in which experiments by children play an integral part. The argument is that only by such means can children be expected to acquire a deep understanding of physics, chemistry or biology. For one thing, of course, real science is largely an empirical study. For another, doing something with the hands is in practice a vivid way of learning that something. It is also an enjoyable activity, and the devisers of the Nuffield courses have sought deliberately to exploit enthusiasm first captured in a laboratory experiment.

The new science course must also seem like science to the children who will follow it. This means that it is hard to omit all mention of parts of a subject which children know about from their reading of newspapers and books—electrons, for example, or nucleic acids. At the same time, the problems of teaching children about such things without being trapped into didactic talk at the blackboard are substantial. This is why a great deal of effort in the design of new curricula has been put into the design of equipment that can somehow bring large parts of modern science into the school classroom.

But devising a new curriculum is more than just writing a text-book and a set of instructions for teachers.

In reality, it is not possible to predict with anything like certainty the chances that a curriculum devised at the desk will suit the classroom. For this reason the Nuffield courses, like the new mathematics courses of the School Mathematics Project, and like the courses which have been evolved by the American curriculum development projects, are the result of close interaction between the classroom and the developers. The Nuffield courses now being published have been tested in 170 secondary schools over a period of three years. The thoroughness of such feasibility testing is certain in future to be the measure of the quality of a new curriculum.

With a new curriculum must go new styles of examination. If the object is somehow to deepen children's understanding of science, such examinations as there may be must also test understanding if they are to be meaningful. Given the importance of examinations in school systems such as that of Britain, teachers inevitably look to curriculum developers for an initiative in the design of examinations. It may not be the coincidence it seems that the examinations authorities in Britain were prepared to welcome the new style of examination at the time when curriculum development itself became fashionable, for the same pressures are at work throughout the school system. The Nuffield courses have been accompanied by specially designed examinations provided by the examinations boards during the trial period. The examination questions frequently provide the information that would constitute the answer to a more conventional question. Their style is almost certain to influence examinations in school science which are quite unconnected with the Nuffield development.

The design of the curriculum is, as it happens, less difficult than its dissemination in the schools. The pioneering work tends to be done by enthusiasts who are, almost to a man or woman, among the more able teachers. Although well-run feasibility trials must involve teachers of various kinds and schools with varying facilities, it is nevertheless a curious problem in techniques of propaganda to know how best to communicate the intentions of the curriculum developments to those who must in the end make use of the work that has been done. This is why most of the schemes for curriculum development have concentrated on providing materials for use by teachers. By comparison, books for pupils, while not unimportant, take second place. Even so, there are evident and serious limits to what can be done by the sale of books. Teachers the world over are too busy to be able to change their style simply by thinking. It follows that teachers must somehow be given opportunities to inform themselves about new methods of teaching and about new advances in the subject they aim to teach. In the United States a vigorous programme of in-service training, financed largely by the National Science Foundation, has made it possible to introduce some tens of thousands of teachers to the new curricula which have been developed in the past decade. Summer institutes are held at universities throughout the United States and teachers attend them for periods of six weeks or so, often with the benefit of a modest payment for foregoing their normal holiday. In the United Kingdom, circumstances are different; for example, summer holidays are not as long. Even so, the provision of opportunities for teachers to keep up to date in method and content is still far from satisfactory, although in recent months there have been welcome signs of improvement. In the summer this year, for example, British local authorities have arranged upwards of one hundred courses for as many as one hundred teachers at a time to introduce some of the Nuffield materials to some of those who may later make use of them. This will have to be a continuing activity, whether or not the Nuffield courses themselves win general acclaim.

The ideal is a state of affairs in which the involvement of the teachers in the evolution of the curriculum is taken as a matter of course. Just as the involvement of children in practical laboratory work is the best way of helping them to learn science, so the involvement of teachers in the evolution of the curriculum is the best way to give them a feeling for its practicability and educational virtue. The difficulty, of course, is that most teachers are too busy to be concerned with anything but tomorrow's classes. The remedy is that they should be given the time to work on curriculum development either in their own schools, or in groups of teachers recruited locally, or by relying on institutes. So far, permanent arrangements along these lines have not been made in the United Kingdom, although there are now a number of interesting developments involving the creation of science centres for teachers in schools. In some places, local authorities have taken the initiative. Elsewhere the arrival of a new curriculum has first created the need to convey information to teachers and has then suggested the profit there can be in a regular interchange of ideas and suggestions among groups of teachers. It would seem that professional and learned societies have a valuable part to play in furthering arrangements of this kind. In Britain the Institute of Physics is already engaged on a scheme intended to create a close link between a university (Manchester University Institute of Science and Technology) and a large group of physics teachers in the region. This, however, is only a beginning, and it is claimed that there is an urgent need for further experiments in the design of machinery whereby teachers may become caught up in the process of curriculum development, and in which university and professional scientists can more easily and convincingly help in this same process.

The other obvious and continuing need is that of money. So far, in Britain the lion's share of money for curriculum development has been provided by the Nuffield Foundation. Altogether £1 million has been committed to the schemes so far begun. By comparison with investment in the United States, for example, this is a tiny sum, especially because of the range of the projects now being carried through. There are, for example, a scheme for dealing with primary school science, schemes to carry the science courses for children between eleven and sixteen through into the sixth form, and schemes intended to cater for children of varied ability, and for schools wishing to teach science as a single subject and not several. In the long run the obvious source of finance for continuing work of this kind is the Schools Council, recently formed and jointly financed by the local authorities in Britain. Individual authorities themselves, and the publishers as well, have a further part to play. But in a sense the greatest danger may be that the current wave of curriculum development may be regarded by those who hold the purse-strings as a once-and-for-all endeavour, complete in itself.

In reality the last thing that any school system needs is a static curriculum. The ideal is a dynamic process of change in which the content of what is taught as science, and the way in which it is taught, are sensitively matched to the needs of pupils in schools, the ability of their teachers, and the demands of the society in which they live. This implies that the success of a new curriculum should not be judged by the degree to which it is accepted in its entirety by teachers, but rather by the degree to which it fires the enthusiasm for change of all concerned with education. In practice, this means that those who launch new curricula must particularly value those teachers who look critically at a new course and decide that some parts of it can be successfully taught, and that some parts of it should—in the circumstances of a particular school—be left aside. For these are the teachers whose criticism may eventually mature into individual contributions to a still better course of study. In other words, each new curriculum is only a stepping-stone to a better one.

BIOGENESIS OF MITOCHONDRIA*

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"MITOCHONDRIA can be seen to swell, coil, branch, fragment, coalesce, put out pseudopods, even to get tied in knots. They can change their form until they resemble bubbles, strings of beads, dumb-bells, lemons or snow-shoes" (Ritchie and Hazeltine, 1953). Despite this variability in form, mitochondria from yeasts, fungi, plants and animals resemble one another in both enzyme content and function. They contain traces of deoxy-ribonucleic acid (DNA)[1], significant quantities of ribo-nucleic acid (RNA)[2] and large quantities of phospholipid and protein. Much of the protein is, moreover, unique to the mitochondrion and does not occur elsewhere in the cell. We now accept that the DNA of the nuclear genes codes for formation of several different types of RNA and that one of these, messenger RNA, in turn codes for the amino-acid sequence of proteins formed on the cytoplasmic ribosomes. Where then are the mito-chondrial proteins synthesized; are they formed on the ribosomes and transferred to the mitochondria to be self-assembled therein, or are they formed by some in-dependent system within the mitochondria? Isolated mitochondria, suspended in buffered succinate, will un-doubtedly incorporate radioactive amino-acids into proteins over long periods[3]; nevertheless, malic dehydro-genase and cytochrome *c* (typical mitochondrial proteins) when extracted from these labelled mitochondria are themselves completely devoid of any radioactivity[4]. This paradox presents something of a challenge and has occupied much of our attention.

Although much of the progress made in the understand-ing of mechanisms of protein synthesis has been achieved by the use of cell-free systems, the very existence of this paradox indicated that it might be advisable to return for its resolution to the study of whole cells. When "Krebs" ascites tumour cells§ are incubated in a suitable nutrient medium there is rapid protein synthesis. At 37° C the rate of synthesis is too rapid to permit successful pulse labelling experiments, but if the temperature is lowered to 22° C protein synthesis is slowed down sufficiently so that a 'chase' of cold amino-acid added to the medium stops (almost instantly) the incorporation of a radioactive amino-acid; the effect is shown graphically in Fig. 1.

In a series of experiments the chase was added at times varying between 4 min and 20 min. While longer periods of labelling give a less selective labelling of microsomes, it was obvious in every experiment that, after the chase, there was a rapid release of labelled protein from the microsome fraction and that the bulk of this radioactive protein appeared in the cell sap. It will be noted that there is a significant but small increase in the radioactivity of the mitochondrial protein after addition of chaser. While this type of experiment is only indicative, the ab-sence of any close resemblance between the kinetics of labelling of mitochondrial protein and cell sap protein certainly suggests that a limited amount of mitochondrial protein is synthesized independently of the microsome-cell sap complex. As cytochrome *c* was not labelled when mitochondria were incubated *in vitro* with a labelled amino-acid[4] this protein was the obvious choice for further

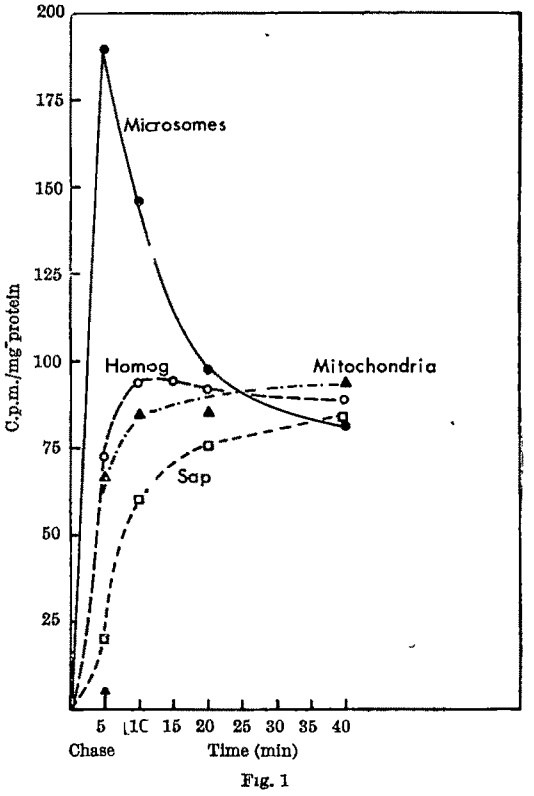


Fig. 1

investigation. As can be seen from Table 1, the labelling of cytochrome *c* in the absence of a chase resembles that of whole mitochondrial protein. If, however, a chase is added to the cells at 30 min the subsequent increase of specific activity of cytochrome *c* is substantial (60 per cent), but the change in specific activity of whole mito-chondrial protein is negligible, there being, if anything, a decrease in specific activity. This result suggests that the peptide chain of cytochrome *c* is synthesized on non-mitochondrial cytoplasmic ribosomes but that some other mitochondrial proteins are synthesized by a separate intramitochondrial protein synthesizing system.

These experiments encouraged us to take as our working hypothesis the assumption that mitochondrial proteins are synthesized by two independent routes. We suppose that one group of proteins is synthesized inside mitochon-dria and the other group is synthesized on non-mito-chondrial cytoplasmic ribosomes and later transferred to

Table 1. LABELLING OF CYTOCHROME *c* WITH ^{14}C-VALINE BEFORE AND AFTER A CHASE OF ^{12}C-VALINE
(Results expressed as mμc /mg protein)

Fraction	Time of incubation and treatment: 20 min	60 min, no chase	60 min, chase at 30 min	Remarks
Whole homogenate	233	411	217	Chase stops labelling
Whole mitochondria	296	540	247	" "
Cytochrome *c* (1)	50·7	126	81·5	60 per cent increase after chase
Cytochrome *c* (2)	52·5	—	89·5	" "
Cell sap*	35	—	72	101 per cent increase after chase

* Separate experiment, chase added at 20 min.

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§ The Krebs II ascites tumour is a cell line which originated as a mammary carcinoma and was adapted to growth as a free-living form of cell which can be propagated in the peritoneum of the mouse (Klein, G., and Klein, E., *Cancer Res*, 11, 466; 1951).

the mitochondria, possibly as porphyrin-free unfolded peptide chains (mitochondria can certainly synthesize porphyrins[6]). In seeking confirmation of this hypothesis, we measured the effect of a whole series of inhibitors on the synthesis of microsomal and mitochondrial proteins in Krebs ascites tumour cells. With the concentrations used, none of the classic inhibitors of protein synthesis showed any selective inhibition of one or other of these two postulated independent systems. We had found in earlier incubations of mitochondria *in vitro*, however, that ribonuclease had no inhibitory effect on mitochondrial protein synthesis, presumably because the enzyme failed to penetrate into the organelle[6]; whereas it is well known that microsomal protein synthesis is inhibited by ribonuclease. Conditions were sought, therefore, for the introduction of ribonuclease into intact Krebs cells. Ribonuclease added to a suspension of normal washed Krebs cells has no effect on protein synthesis, but if cells suspended in buffered saline + ribonuclease are osmotically shocked by adding seven volumes of water, briefly incubated, then restored to an isotonic medium and incubated with ^{14}C-valine, it is found that the presence of ribonuclease has induced substantial inhibition of protein synthesis. The results of one such experiment are shown in Table 2.

Table 2. INHIBITION OF PROTEIN SYNTHESIS IN KREBS CELLS EXPOSED TO HYPOTONIC SHOCK AND RIBONUCLEASE

Period of osmotic shock	Concentration of ribonuclease (μg/ml.)	Counts incorporated (per 1×10^8 cells)	Percentage of inhibition by ribonuclease
0*	0	3,300	—
0*	100	2,660	20
5	0	1,053	—
5	100	800	24
15	0	399	—
15	50	328	18
15	250	209	47
15	500	152	62

Washed Krebs cells (6×10^8 in 4 ml. of phosphate buffered saline) were poured into 28 ml. water containing ribonuclease. At various intervals thereafter osmotic pressure was restored to normal and a sample of cells was incubated at 37° C with ^{14}C-valine for 15 min.

* It is not possible to carry out all the necessary operations in zero time and in fact these cells were exposed to osmotic shock for perhaps 30 sec before tonicity was restored to normal.

The strong inhibition of protein synthesis induced by osmotic shock even in the absence of ribonuclease seemed to indicate at first that we had damaged the cells irreversibly; nevertheless, shocked cells could be propagated in the mouse peritoneum and in rate of growth they could not be distinguished from fresh cells. In accordance with expectation, synthesis of mitochondrial protein was less inhibited by ribonuclease than was synthesis of microsomal and cell sap proteins. The results of two experiments are given in Fig. 2. In each experiment cytochrome *c* was isolated after addition of mouse liver cytochrome *c* as carrier. It will be noted that the inhibition of incorporation of isotope into cytochrome *c* was of the same order as the inhibition of microsomal protein synthesis whereas the synthesis of total mitochondrial protein was much less inhibited.

It is difficult to explain these results except by the assumption that the peptide chain of cytochrome *c* is synthesized on the non-mitochondrial cytoplasmic ribosomes whereas the peptide chains of some other mitochondrial proteins are synthesized by a separate system resistant to ribonuclease and presumably therefore inside pre-existing mitochondria. It must be emphasized that we do not propose the existence of two mechanisms for protein synthesis but rather the existence of several independent sites separated by barriers impermeable to ribonuclease but with selective permeability for cytochrome *c* peptide.

Despite their plasticity (indicated by our opening quotation) mitochondria are well-defined complex multimembrane structures. It is impossible to say precisely how many proteins are present in the typical mitochondrion, but a survey of the literature indicates that something of the order of at least twenty soluble and twenty insoluble proteins are likely to be involved in the formation of this one organelle. There is good evidence both from electron microscopy[7] and from comminution and reconstruction experiments that most and perhaps all of these proteins are fitted together in an orderly repeating pattern[8].

In earlier investigations of the synthesis of mitochondrial proteins *in vitro* we found that, while isolated mitochondria could readily incorporate labelled amino-acids into protein, very little of this radioactivity was found in the soluble readily extractable proteins[9]. These proteins constitute perhaps one-quarter of the total protein of liver or Krebs cell mitochondria, and as a result of our present investigation we now suggest that these proteins are synthesized on non-mitochondrial cytoplasmic ribosomes under the control of an RNA code supplied by the nucleus.

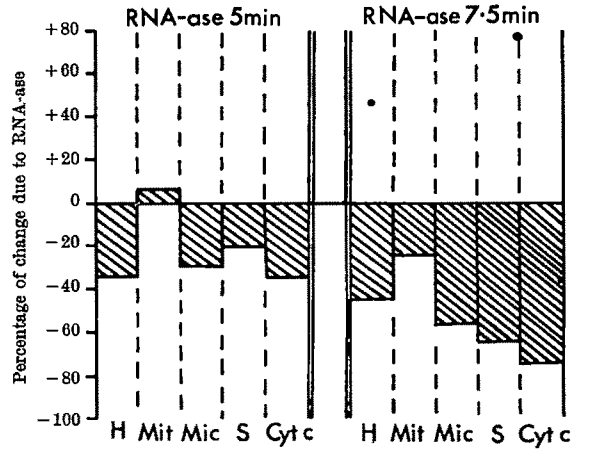


Fig. 2. The inhibition of protein synthesis in Krebs ascites tumour cells after osmotic shock in the presence of ribonuclease. In the first experiment the duration of osmotic shock was 5 min, in the second 7·5 min at 37° C. After shock, cells were incubated for 15 min and 20 min, respectively, in isotonic buffered glucose containing ^{14}C-amino-acid. At the end of this time cells were disrupted and individual fractions isolated by the appropriate methods

While the classic work of Ephrussi on petite mutants of yeast and of Sonneborn on extrachromosomal determinants of phenotypic character in *Paramecium* strongly suggested the existence of extrachromosomal genes (plasmagenes), it is only in the past 18 months that conclusive evidence has become available[1] that several types of mitochondria, including Krebs cell mitochondria[10], contain DNA. There is no direct evidence as yet that this DNA controls protein synthesis, but it is certainly pertinent to ask whether such evidence can be found. As a first approach to this problem, we re-examined the pattern of incorporation of radioactive amino-acids into the insoluble proteins of liver mitochondria during incubation of intact washed mitochondria in buffered sucrose with succinate as energy source. The insoluble mitochondrial proteins are not solubilized by the orthodox techniques of protein chemistry, but they can be obtained in solution by extracting mitochondria with a mixture of sodium dodecylsulphate, sodium-deoxycholate and sodium-cholate[11]. Addition of ammonium sulphate under suitable conditions leads to the precipitation of a fraction which Green and his colleagues have described as mitochondrial structural protein. This material constitutes nearly half the entire protein of ox heart mitochondria, but it possesses no known enzyme activity; nevertheless it seems to be an essential component which must be present before any part of the electron transport chain can be reconstructed by addition of dehydrogenases and cofactors. Some time back[12], Roodyn, in this laboratory, applied the Green technique to liver mitochondria previously labelled by incubation

in vitro with ^{14}C-valine. He found that the Green structural protein fraction was radioactive. We have recently found that this fraction prepared from liver mitochondria is a mixture of several proteins and that it can be successfully resolved into a number of discrete protein bands by electrophoresis in acrylamide gel using as solvent either a mixture of sodium dodecylsulphate and urea in phosphate buffer[13], or alternatively a mixture of phenol, acetic acid and water[14]. The number of components present was discouragingly large, so we tried instead the more recent improved method for preparation of structural protein[15]. This method, applied to liver mitochondria, yielded a multi-component product, but four major bands were readily resolved on acrylamide and it seemed worthwhile to analyse the distribution of radioactivity on an identical gel. This was done using a gel homogenizer[16], and the results are shown in Fig. 3.

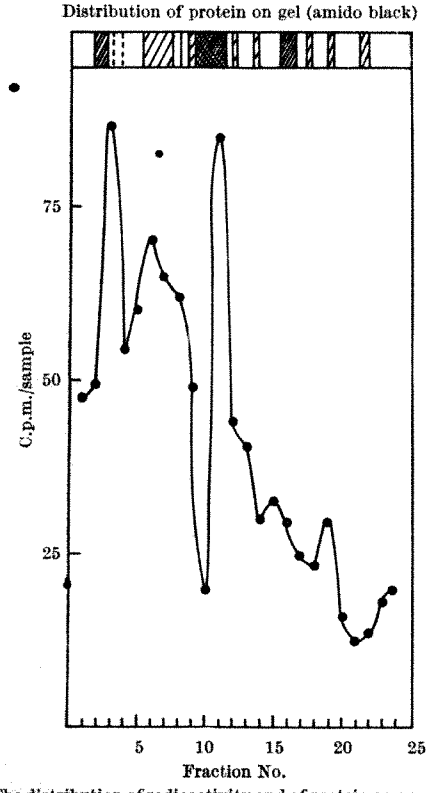


Fig. 3. The distribution of radioactivity and of protein on an acrylamide gel after electrophoresis of 400 μg of structural protein prepared from liver mitochondria. The mitochondria were incubated with ^{14}C-protein hydrolysate in buffered succinate for 1 h (ref. 9)

It is obvious from the diagram that several distinct proteins become labelled when mitochondria are incubated *in vitro* with labelled amino-acids. It occurred to us that we might be able to identify one of these bands by comparing it with Racker's coupling factor-4* (one of the major insoluble structural proteins of the mitochondria) which has been obtained in a state approaching purity[17]. Dr. Racker very kindly supplied us with a sample of his coupling factor-4 and this was fractionated on acrylamide at the same loading and under exactly the same conditions as our radioactive structural protein. The resulting gels were stained and photographed side by side (Fig. 4). It appears from Fig. 4 and from Fig. 3 that one of our major radioactive bands is similar to Racker's coupling factor-4. It must be emphasized, however, that we have not unequivocally identified one of our radioactive bands with Racker's coupling factor-4, and full characterization awaits isolation in quantity and fingerprinting of the radioactive bands. It seems reasonable, however, to extend our working hypothesis and to propose that mitochondrial DNA may code for a limited spectrum of insoluble proteins which form a part of the mitochondrial matrix. (Since this article was prepared, it has been suggested that mitochondrial structural protein of respiration deficient mutants of *Neurospora* differs from the structural protein of wild type in its amino-acid composition—Woodward, D., and Munkres, K., *Proc. U.S. Nat. Acad. Sci.*, **55**, 872; 1966). This extension is supported by the studies of Kalf and Gréce[18], who have obtained evidence that when lamb heart mitochondria are incubated with ^{14}C-amino-acids *in vitro*, the adenosine triphosphatase (ATPase) of the mitochondria becomes radioactive and that the radioactivity does not result from terminal addition of radioactive amino-acids to pre-existing protein.

* Racker's coupling factor-4 is one of four proteins required for the full coupling of phosphorylation to oxidation of substrate by partially disintegrated mitochondria.

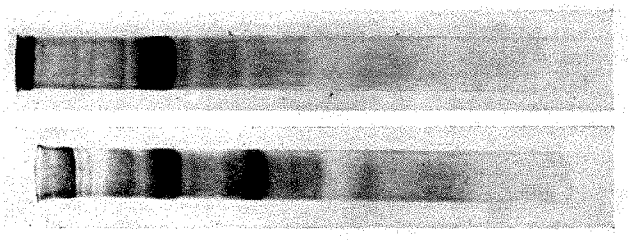


Fig. 4. Comparison between Racker's coupling factor-4 and structural protein from rat liver mitochondria. Both gels were heavily loaded with protein (400 μg) so as to show up minor bands and were afterwards stained with amido black

We can, so far, provide no direct evidence for the function of mitochondrial DNA, but we already know that mitochondria can incorporate thymidine into mitochondrial DNA *in vitro* (Fig. 5) as well as *in vivo*, that mitochondria contain a DNA-dependent RNA polymerase[19], that amino-acid activating enzymes are present in the soluble protein extracted from mitochondria[3] and that isolated mitochondria can incorporate radioactive amino-acid into certain insoluble proteins. The mere fact that when mitochondria are thus incubated some proteins are strongly labelled, whereas other equally typical mitochondrial proteins are entirely unlabelled, is a powerful argument for assuming that the labelling which occurs *in vitro* is a genuine and selective protein synthesis. The fact that this synthesis *in vitro* is inhibited by both puromycin and *cycl*oheximide strongly suggests that it is proceeding by essentially the same mechanism as that already characterized for the cytoplasmic ribosomes. Indeed, it may be advisable to introduce a slight modification of present nomenclature and to suggest two new terms—"cytoribosomes" and "mitoribosomes"—to describe the ribosomes of the extramitochondrial and of the intramitochondrial protein synthesizing systems.

In the light of these results it is informative to review various observations recorded for biological systems of widely differing complexity. In such a review there is an obvious element of risk because we cannot be equally expert in all disciplines, but if we rush in like fools perhaps we may be forgiven if we trip over facts which by their very familiarity may sometimes be invisible.

So far as we are aware, bacteria contain no centriole, no basal body or kinetoplast, no mitochondria and no plastids. Nevertheless, aerobic bacteria do contain cytochromes, flavoproteins and quinones and use a series of coupled enzyme reactions for the aerobic oxidation of acetate which closely resembles that found in higher forms; some even show glucose repression and oxygen induction of the cytochrome system closely resembling that found for yeast mitochondria[20].

Many forms of bacteria do develop before division a complex membranous structure which appears to be

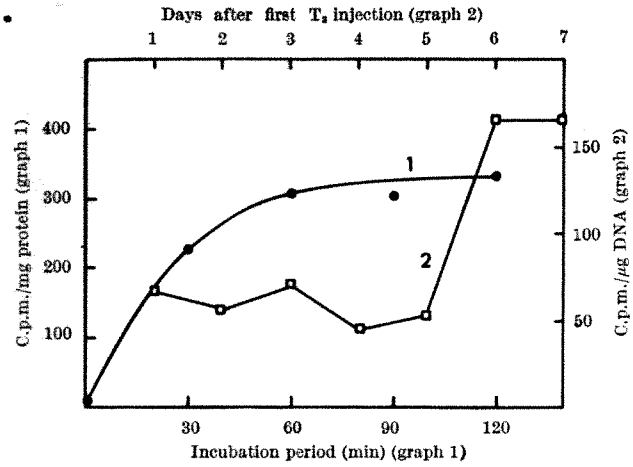


Fig. 5. The effect of repeated injection of triiodothyronine into thyroidectomized rats on the rate of incorporation of radioactive thymidine into mitochondrial DNA. Graph 1 shows the kinetics of incorporation during incubation of a single sample of washed liver mitochondria incubated under the usual conditions. In Graph 2 each point represents the mean value for the mitochondria obtained from the livers of three rats treated as indicated

formed by invagination of the plasma membrane. This structure, now usually referred to as the mesosome region, shows some of the character of a primitive mitochondrion in that it reduces tetrazolium; it also exhibits some of the character of a centriole as it seems to initiate the separation of the bacterial chromosomes[21]. Add to this the observation that in the primitive ciliates the mitochondrion and the kinetoplast form a single body[22], that in various higher forms the kinetoplasts, the mitochondria, the basal bodies and the plastids all seem to contain traces of DNA which can be lost (provided loss is not lethal), that moreover the photosynthetic apparatus of the plastid has many biochemical similarities to the mitochondria[23], and one seems almost forced to suggest that the mesosome which had the capacity to attach to the bacterial chromosome may well have, during evolution, acquired a few of the chromosomal genes and then to have differentiated further into the various related structures of higher forms, namely, the centrioles, the mitochondria, the kinetoplasts, the basal bodies and the plastids. If this were indeed so, we might well expect that the same type of DNA-dependent DNA polymerase would be responsible for the replication of the DNA of all these bodies. There are, it seems, a few hints that this is so; for example, one such polymerase seems to be present in both nucleus and cytoplasm of fibroblast cells, and this polymerase is concentrated in the nucleus during periods of chromosome replication but released into the cytoplasm during interphase[24]. Moreover, in the sea urchin embryo it has been reported that mitochondrial formation and mitotic activity are antagonistic[25]. We thought, therefore, that because thyroxine is known to induce mitochondrial hyperplasia in animals[26] it would be interesting to measure the effect of thyroid hormone on the rate of DNA synthesis and on the DNA level in rats injected with triiodothyronine. We found that when thyroidectomized rats were given repeated injections of triiodothyronine, there was little effect on DNA level or on the rate of DNA synthesis for 5 days. On the sixth day, however, as shown in Fig. 5, isolated rat liver mitochondria showed an increased ability to incorporate tritiated thymidine, and by the seventh or eighth day the DNA level in rat liver mitochondria was increased by as much as three-fold (per mg protein) compared with controls.

We scarcely need to direct attention to the interesting problems in control which are presented by the existence of a number of extra-chromosomal genes, the reading of which must be fully integrated with the reading of chromosomal genes so that the right proteins are fitted together in the appropriate order to produce functional mitochondria. It is noteworthy that few mitochondrial proteins failed to penetrate acrylamide gel (10 per cent monomer) and this would imply an average peptide molecular weight below 40,000. Nevertheless, many mitochondrial enzymes, especially the more firmly bound enzymes, are of extremely high apparent molecular weight[8]. It would, therefore, not be unreasonable to suppose that the majority of these enzymes are aggregate structures compounded of a number of distinct peptide chains. It is obvious from the investigations of Slonimski *et al.*[27] that a whole spectrum of nuclear genes controls mitochondrial maturation and the synthesis of cytochrome enzymes in yeast. It could well be, therefore, that some of the multi-chain enzymes of the mitochondrion depend on nuclear genes for one chain of a multi-chain structure but on mitochondrial genes for some other parts of the aggregate three-dimensional active enzymes. Even in those acridine mutants which lack some mitochondrial DNA (ref. 28) there is a primitive form of mitochondrion[29]. This primitive mitochondrion has some structural resemblance to a mesosome and perhaps contains a lipoprotein similar to that of the primitive mesosome. Moreover, the recent investigations of Jakob[30] on complementation between respiratory mutants of *Saccharomyces* strongly suggest that there is a coupled interaction between the chromosomal genes and the cytoplasmic genes of respiration-deficient mutants. Such coupling might be expected if the reading of two or more entirely independent polycistronic messengers had to be integrated before a functional mitochondrion could be produced.

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BOOK REVIEWS

SCHOOL SCIENCE REFASHIONED

Science for High School Students
Pp. 1040. Single-volume Library Edition. 5.50 A.dollars. Two-volume Students' Edition. 3 A.dollars each volume. Teachers' Manual. Pp. 480. 3 A.dollars.

Abridged Science for High School Students
Vol. 1: Pp. 288. 2 A.dollars. Vol. 2: Pp. 334. 2 A.dollars.

Senior Science for High School Students
Part 1: Physics. Pp. 480. 3 A.dollars. Part 2: Chemistry. Pp. 448. 3 A.dollars. Part 3: Biology. Pp. 320. 2.75 A. dollars. Teachers' Manual. Pp. 320. 6 A.dollars. By the Nuclear Research Foundation School Certificate Integrated Science Textbook Group of Authors and Editors, under the Chairmanship of H. Messel. (Sydney: Nuclear Research Foundation within the University of Sydney; London: Pergamon Press, Ltd., 1964, 1966.)

THESE science text-books were designed to provide a complete science course for secondary pupils in the schools of New South Wales, but the authors hope that they will be found useful elsewhere. The first 4 years of the course are covered in *Science for High School Students* (SHS), while the final 2 years are distributed between the three separate volumes of *Senior Science for High School Students* (SS). *Science for High School Students* is available in two formats: a library edition complete in one volume and a classroom and laboratory edition in two separate volumes. An abridged edition has also been designed for use by pupils following the Ordinary Level course. (The unabridged edition is intended for those taking School Certificate at the Credit or Advanced Level.) A manual for teachers has been prepared for both *Science for High Schools* and *Senior Science for High Schools*. Appearing as they do some years after the various American courses and just before the publication of the Nuffield texts in Britain, these books are of more than usual interest.

The general SHS volume is very full in content and covers elementary physics, chemistry, biology and geology with encyclopaedic thoroughness. It suffers, however, from the main disadvantage of other texts of this kind: it gives the pupil all the facts without any reference to the way in which the content is actually to be taught in the classroom. Many experiments are described, but there is little to indicate that they are part of a consistent course in which the pupil, his experiments, the teacher and the text all have a part to play in the learning process. An extreme example of this comes early in the first chapter where the pupil in almost his first lesson meets the Bohr atom, nucleons, elements and compounds. How can there be any hope that the pupil will think critically about his science when he is introduced, at the very outset of his course, to a series of facts about the more crucial concepts of physical science designed for ready acceptance?

SHS is written for use over a wide range of age. When a pupil starts on the course he is twelve and he passes to the succeeding volumes when he is sixteen. It is therefore surprising to find little variation in reading difficulty between the prose of the earlier and later chapters. For example, the following sentence occurs very early in the abridged version, and is intended for 12-year-old pupils about to follow the ordinary course, and thus unlikely to be among the most able: "Nevertheless Democrites would have reasoned: suppose that I imagine that I have a perfect tool and that I can keep on dividing the material for as long as it is divisible—can I keep going for ever, or will I eventually have the smallest piece of matter which is indivisible?"

The *Teachers' Manual* is helpful; it contains hints on the experiments in addition to sources of material and questions. The balance of the course would have been improved if more emphasis had been placed on this manual and thus on the teacher. In the pupils' book, the result of an experiment is given almost in the same breath as the experiment itself. How much better if the result had only appeared in the manual together with the development following from the experiment. (These comments also hold good for the *Teachers' Manual* to SS.)

These criticisms are directed at SHS as part of the school learning situation, and should not be allowed to detract from the obvious thoroughness and care which have gone into the writing. Many of the sections are models of clarity and compression. Among examples of these is the discussion of fields on pages 42–46 and 42–47. The geology content, which is very full, is not usual in British courses of a similar kind, but it fits naturally into these text-books. Here, at least in Britain, we are given cause to examine our own neglect of the subject, and also of astronomy which receives similar extended treatment. Many teachers will find SHS useful, especially those sections covering other than their own speciality.

The senior course has been conceived as a whole, and because of this there is no wasteful overlap between the three parts into which it has been divided. There is, however, a deliberate overlap between the content of the more advanced *Senior Science* and that of the more elementary *Science for High School Students*. Part 1 is solely concerned with physics, and is an unusual book. There is no reason to doubt the authors' claim that certain concepts and the mode of their presentation have never before appeared in a school text. In Britain, books at this level tend to be written by schoolmasters and, although usually reliable and sometimes distinguished, are usually derived works and seldom contain anything so fundamentally different as to give one a mental start. The physics section of SS does just this. It continuously excites and makes the reader eager to press on and follow a theme to its conclusion. Those of us who work in schools have reason to thank members of university faculties who give part of their time and energy to write texts of this nature.

To some degree the content follows general modern trends. There is little calorimetry; Joly's differential steam calorimeter is not the ghost at this feast. Geometrical optics, transfer of heat-energy and electrical circuit theory are only covered to a standard somewhere below Ordinary Level in Britain, and the space and time so gained are used adventurously. First comes a most careful treatment of dynamics. Points that are all too often passed over briefly are given more extended attention; examples are the discussions of action and reaction, and of inertial forces in circular motion. No use, however, is made of the calculus, here or elsewhere, and this leads to some difficulties.

A treatment of electricity and magnetism follows, in which the latter is treated as a second-order effect. It is made clear at the outset that the student is asked to regard magnetism as inevitable given Coulomb's law and the transformations of special relativity. The derivation of the law of force between current-carrying conductors is approximate and depends on low charge-velocity. This is the section which makes the book unique as a school text. There has been a growing body of opinion that special relativity ought to be formally taught in schools, and this

book makes it possible for us to estimate the feasibility of such a step. A discussion of wave theory leads to a very clear section on the wave nature of matter. This is very fine and will serve as a source of inspiration to all who teach these topics.

A text-book cannot be judged apart from its intended users. It is to be hoped that a strong feeling that the majority of pupils at the late secondary stage will find this book very hard going indeed will prove ill-founded. For this reason, reports on the reactions of such students in Australia to the book are eagerly awaited.

A few points give rise to queries. The coulomb is first defined as a certain number of electronic charges. Later Millikan's experiment is described and the outcome given in terms of coulombs. It would seem in fact that it is the permittivity of free space which is arbitrarily assigned, but the point is clouded—possibly deliberately. The mathematical analysis is regrettably printed in a very cramped manner.

The other two SS volumes are more orthodox in character. Part 2 contains a coherent and didactic account of modern chemistry. The theme begins with a traditional description of the behaviour of gases, reactions between gases, and formulae and equations. At this stage, a less conventional but reasonably clear and simple account of energy in chemical reactions and bonding is introduced. This is followed by a consideration of the elements of the Periodic Table and their halides and oxides. The history of atomic structure is dealt with in a section which precedes a modern introduction to structure which includes reference to the Born-Haber cycle. The final part of the text is directed at an up-to-date look at chemical change from the point of view of equilibria, acids and bases, electron transfer mechanisms and rates, and oxidation states. Organic chemistry is crammed for the most part into two chapters—one under the guise of the hydrides of carbon and the other on substitution and addition compounds.

Some criticisms of the details of the text should perhaps be given. The most important of these concern the introduction to formulae and equations. This is very conventional and makes no use of the mole concept. A small but important point which should be corrected concerns the energy diagrams, such as they are. The higher energy species are shown at the bottom and lower energy at the top: this is most confusing!

On the face of it, the text is clear, straightforward and modern. But this book represents the complete standard work for chemistry at senior high school level in New South Wales. What do the students actually do? Do they listen to expositions by the teacher and then read the book? What experiments do they perform? The experimental work in the book is very weak, and few experiments are inserted at the end of each chapter. Although some of these experiments are "open ended", their position at the end of the chapter implies that they are confirmation for, or illustrations of, the theories which have been expounded in the preceding text. In no case does the need for this theory derive from the evidence of an experiment. It may be, of course, that there is a great shortage of laboratory space in New South Wales. In Britain the tradition of chemistry as an experimental subject dies hard, and new movements in the teaching of chemistry are aimed at making Advanced Level courses even more laboratory-based. The New South Wales course is clearly text-book based. To our eyes, therefore, despite its modern content. Part 2, on chemistry, has an old-fashioned look.

Part 3, on biology, is an authoritative and informative book arranged in three main sections. The first deals with Darwin and evolution, Mendel and genetics, variation and the modern view of natural selection. This is followed by an excellent discussion on the diversity and abundance of species and an investigation of the effects of environment on populations. Ideas new to this level are included, for example underpopulation and crowding. In the section on population, examples from Australian ecology are used to illustrate features of ecology, which makes a pleasant change from time-worn European situations. The second section is a treatment of three particular aspects of an individual organism: reproduction, control and co-ordination and behaviour. Some modern material is included but the treatment is very ordinary. The final section on cell structure and function uses much biochemistry and electron microscopy and is an up-to-date and authoritative account. The ground covered in these topics is dealt with in a logical and concise manner: the general level is about that of the present Advanced Level in Britain.

The main difficulty with Part 3 is that its approach to biology is not one of enquiry and investigation depending on practical observation and experiment. In fact, the practical section of the book is relegated to twenty pages at the back and the whole emphasis of the text is one of encyclopaedic coverage of a series of complex topics. Each chapter is provided with a group of questions, often well designed, but they are based on the text rather than on experiment.

In general, this is an interesting volume that could be of considerable value to teachers as a book of reference and to pupils as a background reader. However, the general approach runs counter to present trends in the teaching of biology in Britain, which seek to make the subject an activity of experiment and discovery.

A select bibliography follows many of the chapters in the SS volumes and especially in Part 3; this will help the pupil considerably in his selection of "follow up" reading. Throughout the books, pages are identified by chapter: thus a page labelled 36–15 is the fifteenth page of Chapter 36—in places the index is somewhat confusing because of this system.

The criticisms which we have made stem, in the main, from a divergence between our conception of the function of a science text and that of the authors. However, the drive and industry which have gone into the production of these volumes are obvious to all. Prof. Messel and his colleagues are to be congratulated on the completion of this huge task—done in record time. Their efforts can only encourage and inspire all engaged on the refashioning of science courses.

M. J. W. ROGERS
R. SCHOFIELD
D. STANBURY

THE MOON IN PICTURES

Photographic Atlas of the Moon

By Zdenek Kopal. Pp. 277. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 128*s*.

THIS book represents part of the latest in a now considerable series of systematic photographic surveys of the Moon. Sponsored by the U.S. Air Force Aeronautical Chart and Information Center, more than forty thousand exposures of all or part of the Moon were obtained at the Pic du Midi by a team from Manchester led by Kopal, to produce the largest homogeneous set of lunar photographs ever made. 300-m contour charts have been produced from these.

The present work consists of three introductory chapters, a selection of 217 prints taken from the total amassed so far and nine photographs from the *Ranger VII* series. The first chapter is essentially a presentation of the physical properties of the Moon, the main topics being the characteristics of the orbit, the librations, mass and density, internal structure and conditions, and a lengthy consideration of the possibility of a gaseous atmosphere.

Following a brief description of the familiar surface features, the second chapter is concerned with the origin

of the larger ones. Attention is given to both external and internal processes, particularly for the formation of ring structures, but the origin of the surfaces of the maria, and also of domes, rilles and wrinkle ridges, are considered. An outline of the chronology of lunar structures is given using the criteria of overlap, ruggedness and albedo for dating purposes.

The third chapter considers the detailed structure of the lunar ground. The evidence from photometric, polarimetric, thermal and radio investigations is presented well, with only one obvious error—the phase at which the plane of polarization of moonlight rotates by 90° is given as the quadrature, when in fact it has the value of about 23·5°. In the interpretation of the data, however, several points of controversy arise. The undoubtedly vesicular nature of the surface is attributed to small crater-pits produced by micro-meteorites in the overlying coating of interplanetary dust; the difference in albedo between maria and continents is explained as due to inmixing of native material by more energetic impacts. This argument ignores two points: it has been shown by several workers that no combination of regular crater pits can fully reproduce the lunar photometric curves, some degree of interconnexion between adjacent pores being almost certainly necessary; and also that falling interplanetary dust will probably build up a dendritic "fairy-castle" structure in any case. Both these points deserve mention, as does the often neglected possibility that the differences between maria and continents may not only be those of albedo: as any sufficiently vesicular dust or rock may have most of the lunar properties, the two types of area could be quite different in origin, as would be the case if the maria were relatively recent flows of vesicular lava.

In terms of absolute resolution the photographs given do not represent any improvement over, for example, the *Photographic Lunar Atlas* edited by Kuiper, but do have the advantage of homogeneity, making intercomparison of different areas more meaningful. In general this work is a useful addition to the literature on lunar surface problems by way of a review. Perhaps no single work can cover every facet of the subject, but this book presents an outline of the appearance and nature of the Moon adequate for many purposes.

L. WILSON

BACKWATER OF SCIENCE

Melting and Crystal Structure

By A. R. Ubbelohde. Pp. xi + 325. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 63*s*. net.

Melting and Crystal Structure is mainly concerned with the principal thermodynamic parameters of melting, particularly melting points and the changes of entropy and volume at melting, and the attempts which have been made to understand what regularities can be perceived in these parameters. There is little detail with regard to crystal structure, and "Melting and Molecular Motions" would have been a more accurate title. Save for one chapter on "Rate Processes in the Solid–Liquid Transition" (chiefly nucleation), and another on glasses, the book concentrates on equilibrium properties. If a good monograph is one of which one can say, "Read this; accept it, and use it to light your path in neighbouring fields of science", this does not qualify as such, but if the test of merit is whether it is likely to promote the advance of natural knowledge, it can. For no one can read this book without the conviction that there is much more yet to be discovered about even so simple a phenomenon as melting, at every level from the most to the least sophisticated. The good book in the former sense could scarcely be written, because the basic data are often missing or unreliable. One would like to be able to congratulate the author for bringing the data, such as they are, together. Unfortunately, at any one place he usually quotes only a single value, without declaring a basis of selection, and repeated data seldom agree with precision.

The author himself directs attention to the substantial discrepancies between dT_f/dp and the ratio of directly measured ΔV_f and S_f (12 per cent discrepancy for sodium chloride: page 142). It should be sobering for scientists, taking pride in their achievements over the really difficult measurements, to appreciate the paucity of reliable data on such elementary matters as these. Ought we to tolerate this situation? How can we complete the surveys in areas which science has passed by on the way to the frontiers? Why should only graduates get that incentive to do their best in a measurement which comes from knowing that it produces new knowledge, which someone needs? Schoolboy athletes try to do better than has been done before: why should not schoolboy scientists? In Britain, about 50,000 people each year take Advanced Level examinations in physics and chemistry: with a little organization they could contribute measurements of latent heats and dilatations at fusion for a host of substances in the easy temperature range, say 0°–200° C, with such multiplicity of independent checks that the reliability of the results should be easy to ascertain. Some enterprising schools, and some undergraduate laboratories, could extend into more difficult temperature ranges: and a few of those involved would come to appreciate the worth of more thorough research, say into the thermodynamics of melting at constant volume.

Against a background of imperfect data, the theory of the subject remains, apart from the basic thermodynamics, a mixture of near misses (such as the Lindemann melting point formula, percipient when new, but something which surely ought to have been overtaken by deeper understanding by now), crude though instructive models (such as theories of melting which still leave the liquid molecules on a lattice) and wild ideas often stemming from dubious experimental observations. The author cites them systematically, with perhaps too uniform a sceptical deprecation: for he tends to be tentative in introducing the obvious or even the necessary.

A chapter on liquid crystals contains an interesting miscellany of citations, but its very brief section on cholesteric phases, when contrasted with Friedel's article (*Annales de Physique*, 18, 273; 1922), serves remarkably to illustrate the way in which scientific understanding can lapse in a field which is not continuously cultivated.

F. C. FRANK

POSITIVIST MEASUREMENT

Basic Concepts of Measurement

By Brian Ellis. Pp. ix + 220. (London: Cambridge University Press, 1966.) 45*s*. net; 8.50 dollars.

THE aim of this book is "to give a consistent positivist account of the nature of measurement", which is regarded as "a central concern of the philosophy of science". It is clearly written; the author faces the problems honestly and does not hesitate to say when he has failed to solve them, and the book is a lively stimulus to thought.

It has, however, one fundamental defect, which appears to arise from the desire to regard the commonsense and scientific views of the world as expressible in the same terms. This is impossible, and if possible would be undesirable. There is no reason why the astronomer should be forced to express terrestrial rotation in terms of sunrise, or why, in daily life, we should speak of dawn in terms of the position angle of the Earth. In shopping, measurement is always measurement of a quantity of something already there—a pound of bacon, a yard of

cloth. In science, on the other hand, the measurement is primary, and there may or may not be a "thing" which is measured. What thing lies behind the altitude of the First Point of Aries? The spectroscopist measures λ, and afterwards chooses whether to call it the length of a wave or the reciprocal of the momentum of a particle: he does not first observe the wave or particle and then measure one of its properties. It follows that a true fundamental account of measurement must not presuppose a metaphysical something of which the measurement ascertains the magnitude.

Mr. Ellis's account is based on the presupposition that every measurement is the measurement of a "quantity" possessed by a "thing". He has then to define "quantity", and his "final position" is that it depends on "the existence of a set of linear ordering relationships"; he leaves it to others to decide which quantities are entitled to be given names. On this definition probability, for example, is a quantity, and it is discussed at length. It is scarcely too much to say that all the problems dealt with are created by the unnecessary introduction of quantities, and the acuteness of the analysis does not compensate for its superfluity.

Nor are quantities merely superfluous; their introduction as entities prior to their measurement (there is, of course, no reason to forgo the convenience of using such terms as wave-length for descriptive purposes) is a fundamental error, for it misrepresents empirical discoveries as logical necessities. Thus, the agreement between measurements of distance by laying rods end to end and by triangulation follows inevitably on the "quantity" assumption, for they are measurements of the same thing. When there is reason to think that it breaks down over long distances, all we can do is to try to discover which method is "right". But if we regard both as valid but different measurements the results of which are approximately equal, their disagreement over long distances reveals an important fact about the universe. Whether it is best described as the curvature of space or the non-rectilinear propagation of light or what not can then be discussed, but in any case it is a discovery about the universe which the presupposition of quantities would have made impossible.

HERBERT DINGLE

HIGH-ENERGY NUCLEAR PHYSICS

High Energy Nuclear Reactions

By A. B. Clegg. (Oxford Library of the Physical Sciences.) Pp. vi+130. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 18s. net.

NUCLEAR reactions induced by low-energy nucleons are usually complicated processes, but at higher energies in the region above 100 MeV and for light target nuclei a great simplification is possible in certain types of reactions. This book is especially concerned with reactions induced by protons and neutrons in the energy region between 100 and 400 MeV, in which a simple change is made in the target nucleus. In this case it is possible to understand the reaction mechanisms in some detail and in some instances also to obtain information about the structure of the nuclei involved.

In the first chapter it is pointed out that at these energies the incident nucleon frequently interacts with only one nucleon at a time, and in subsequent chapters the reactions considered are those in which only one collision with a nucleon changes the state of the nucleus. In the next chapter the reasons why this type of reaction is likely to occur are discussed and the matrix elements involved are related to the nucleon–nucleon scattering amplitudes. Distortion effects on the incident and outgoing waves are next considered and the quasi-classical approximation introduced. The third chapter is concerned with the experimental features of the investigation of high-energy nuclear reactions and contains a brief discussion of the measurement of particle energies, the measurement of γ-ray energies from the decay of excited states and polarization phenomena in nuclear reactions.

Chapter 4 discusses the elastic scattering of nucleons by nuclei. Here the treatment in some places is brief and not complete because several other books have recently been published on the theoretical analysis of elastic scattering data using the optical model. Dr. Clegg instead concentrates on the relationship to the nucleon–nucleon scattering amplitudes, and an extensive comparison is made between the theoretical calculations and the available experimental data. The contributions from spin-flip and isotopic spin-flip processes are considered in some detail.

Chapter 5, which is by far the longest in the book, is concerned with inelastic scattering. Using the plane-wave approximation the contribution from spin flip effects are considered together with the information which can be obtained from the angular distribution of γ rays from the excited nucleus. Distorted wave calculations are then introduced and the effects due to a spin-dependent distorting potential are found to be small. These calculations are then compared with the experimental measurements of the polarization and angular distributions and where possible related to the nucleon–nucleon scattering amplitudes. Other excitation mechanisms which might contribute to inelastic scattering reactions and how they can be related to single nucleon excitation are then considered.

Nucleon knockout reactions in which the incident nucleon collides with a nucleon in the nucleus with the result that both nucleons leave the otherwise undisturbed nucleus are dealt with in the next chapter. After a brief discussion of (p,d) reactions, $(p,2p)$ reactions are considered in great detail, especially the interpretation of the results in terms of the shell model. In the final chapter reactions in which a cluster of nucleons are ejected from the target together with a nucleon are discussed. Although a comparison with experimental results is difficult because of the lack of data there is an extensive discussion of the relationship between the cluster model and the shell model and it is shown that in many cases the predictions of these two models are very similar.

This book can be strongly recommended. The reactions are considered from a theoretical point of view, but the results are extensively compared with the experimental data. The underlying physics of the nuclear model or nuclear reaction is kept continually in mind and the calculations made using simple models so that the physical principles are not obscured by mathematical or computational complexities. The book is likely to be of considerable interest to the nuclear physicist studying nuclear reactions at lower energies and to the physicist working with nucleons in the 100 to 400 MeV energy region. It is particularly valuable at a time when there is considerable interest in the use of high-energy particles to study nuclear structure.

C. J. BATTY

ERDTMAN'S PALYNOLOGY

Pollen and Spore Morphology/Plant Taxonomy—Gymnospermae, Bryophyta (Text)

By G. Erdtman. (An Introduction to Palynology, III.) Pp. 191+24 plates. (Stockholm: Almqvist and Wiksell, 1965.) 88 Sw. kr.

Pollen and Spore Morphology/Plant Taxonomy is the most recent volume of Prof. Erdtman's impressive series of contributions to the studies of spores and pollen of living plants. The rather disconcerting companionship of Bryophytes and Gymnosperms in the one volume is a reminder that palynology is still suffering the pains of over-rapid growth. The series (*An Introduction to Palynology*)

started bravely with Part 1, the Angiosperms, in a single volume of text and illustrations in 1952. Gymnosperms, Pteridophytes and Bryophytes (Part 2)—the illustrations only—followed in 1957. The present Part 3 is the text to the Gymnosperms and Bryophytes—the text for the Pteridophytes is still to come. This seemingly rather confused situation simply reflects the dilemma of the integrator in a field where research is outpacing the integration: Prof. Erdtman has elected "for various reasons" to postpone a description of Pteridophyte spores to a later volume.

But the present work is much more than merely a text to a volume of illustrations. It carries an additional twenty-four plates, and numerous half-tone illustrations in the text. These are of the usual excellent quality which has become the hallmark of Erdtman and his students at Solna. They include ordinary-light micrographs of acetolysed spores in distilled water; electron micrographs of shadowed replicas, to show exine topography; and thin sections of exine taken with phase contrast and ultra-violet. The refinement of technique in illustration since Volume 1 is a tribute to the painstaking application to the problems of spore illustration made by Prof. Erdtman and his students.

The body of the work is a description in catalogue form of spore and pollen morphology with the original figures of Volume 2 greatly augmented by new photographs. In every genus there are detailed references to the specimens examined and to the relevant literature. Occasionally, one regrets that Prof. Erdtman's enthusiasm for a logical and consistent terminology has taken us far from terms already well entrenched in 1957. Under his 'N.P.C.' system the monolete proximal aperture of a moss spore now falls into the same 'character' category as the (distal) colpus of, say, a cycad. As a result, some may be sorry to see the proximal aperture of *Splachnum* (katalept) referred to as a 'colpus' (with or without inverted commas!)—the same term being applied to the distal 'furrow' of cycad pollen.

There are two areas which show up in the book as being particularly in need of further work. First, our knowledge of the megaspore membrane within the seed of the gymnosperms and its structure and homologies with the microspore exine is sparse. (The megaspores occupy four pages, the pollen seventy.) Secondly, there is still lack of knowledge of orientation with respect to the tetrad in some pollen and spore types; even in such a large and well-studied genus as *Ephedra*, for example, there is controversy as to whether the elongated axis is polar or equatorial. Since any comparative pollen morphology must be based on the basic orientation of the spore, this is a feature of fundamental significance.

This work is a valuable contribution to the various fields of botany through which palynology ramifies—Quaternary palaeobotany, gymnosperm and bryophyte systematics and the more refined study of cell wall formation in general. There can be no greater praise of the book than to say that Prof. Erdtman has maintained the high standard of illustration, meticulous observation and description that he set in his own earlier work.

W. G. CHALONER

THE SIPHONOPHORA

A Synopsis of the Siphonophora

By A. K. Totton, assisted by H. E. Bargmann. Pp. vii + 230 + 40 plates. (London: British Museum (Natural History), 1965.) 220*s*.

SOME animal groups are easier to describe and classify than others, especially when creatures have hard skeletal characters. The softer-bodied animals have, however, given great trouble and, among these, the planktonic siphonophores are extreme examples. Not only are they liable to crumple and distort on preservation, but they consist of polymorphic colonies carrying floats, swimming organs, stomachs, stinging tentacles, sexual and asexual bells. Any one of these parts is liable to break off and a preserved collection may consist of fragments belonging to a number of different species. It is no wonder that the early systematists got into a muddle and different parts were assigned to wrong parent species. Even to-day many species are known only from fragments and the complete animals still remain to be described. But their beauty and strangeness have attracted the most famous zoologists, among the first to write large works on them being T. H. Huxley and Ernst Haeckel. In more modern times, however, the leader in the field was undoubtedly Henry B. Bigelow, who produced a classical work in 1911 based on the fine collections of the *Albatross*, to be followed by a number of subsequent reports.

Now we have before us *A Synopsis of the Siphonophora*. The author is A. K. Totton, whose first published work on siphonophores was in 1932 when he reported on the collections of the Great Barrier Reef Expedition. Totton, who was on the staff of the British Museum (Natural History), has had the unrivalled collections of the *Discovery* investigations at his disposal, but he has never been content to examine preserved material and, as a true naturalist, has spent much time in the field studying the living animal and its development, more especially at Villefranche, where upwelling at times brings rare species to the surface.

A quick perusal of his synopsis shows at once the leading position that Totton himself has earned in the study of this difficult group. He states that about one hundred and thirty certain species are now known. A check on the identity of these species shows that twenty-five can be attributed to Totton, who has thus named more than twice as many species as any other author, Bigelow coming second with eleven. Out of one hundred and fifty-three text-figures, some one hundred and twenty are Totton's own drawings, and of the forty plates twelve include original photographs by him.

The synopsis is not a comprehensive monograph. It lacks much information on general biology, on histology and geographical distribution, as Totton himself admits. Some of the species descriptions are indeed also somewhat cursory, and full synonymy lists are not given under the species. Synonyms should also have been included in the index. The possibilities of writing full-scale monographs such as A. G. Mayer's *The Medusae of the World* are growing less. It is all a matter of time. It is only those who have given many years to a study of a group who are competent to undertake the task and the coverage of the literature grows ever more time-consuming.

It is, however, becoming very essential that those with the knowledge should gather it together and make it available for those who follow. It is certain that in some groups, in which there is confusion in synonymy and of which the specific descriptions are scattered through a wide range of publications, young workers are deterred from entering the field. The publication of a comprehensive work always seems to produce a quick response of new workers.

The phylogeny of the siphonophores has always intrigued zoologists, and among these Walter Garstang was pre-eminent. In his introductory pages, Totton has given an informative review of the work and ideas of the most prominent investigators of the past. Included on the title page is the name of Dr. Helene E. Bargmann, the value of whose assistance can well be appreciated in putting together a work of this nature. Among the many attractions of the *Synopsis* are the reproduction in the plates of so many of the illustrations of the earlier naturalists and the amusing frontispiece.

The whole volume is splendidly produced and should long remain a standard work and an encouragement for the recruitment of future investigators. F. S. RUSSELL

Elementary Analysis

By Richard F. McCoart, Malcolm W. Oliphant and Anne E. Scheerer. (Holden-Day Series in Mathematics.) Pp. xv+251. (San Francisco and London: Holden-Day, Inc., 1964.) 7.95 dollars.

THIS book consists of a careful introductory course to differential and integral calculus. It is intended for a first-year course at a U.S. university and would be useful to sixth forms in Britain and to some extent to first-year students at a university doing a rather pure mathematical ancillary course. Subjects treated include the concept of functions, the ε–δ definition of limit, continuity, and introduction to the derivative and the Riemann integral by way of the concept of bounds. None of these subjects is developed very far, but so far as it goes the treatment is clear, careful and unhurried. There are plenty of exercises with answers supplied and some worked examples. R. L. PERRY

Slow Neutrons

By V. F. Turchin. Translated from the Russian. Pp. vi+304. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 107*s*.

THIS book is to be welcomed, for the field of neutron scattering is as yet not easily accessible except through original papers. At the time of its publication in Russian (1963) the book was the only one to treat thoroughly dynamic, inelastic scattering processes; this translation of it is likely to remain a valuable text for many years even though some other books are now available.

Turchin covers the basic neutron-scattering processes from individual nuclei and from condensed systems. He presents the theory necessary for the interpretation of elastic (Bragg) diffraction from crystals, and inelastic scattering both from crystals and from disordered (liquid-like) systems. This latter topic is thoroughly treated by the space–time correlation method.

The sections devoted to the various approximations useful for realistic situations are likely to prove particularly valuable to workers in the field. The section on magnetic scattering is short, but adequate. The second part of the book is entitled "Thermalisation and Diffusion of Neutrons" and discusses briefly the nature of these processes and their significance in reactor calculations. There are many existing better sources for this work, and in general a reader would do well to treat these chapters as introductory.

The standard of translation is high, but the formulae are badly set and difficult to read; the only error that I found, however, was the retention of Russian subscripts in one formula. The direct reproduction from typescript necessarily gives a slightly untidy appearance to the page, but a little more attention to the quality of the mathematical formulae and diagrams would have made the book far more pleasant to read. W. M. LOMER

General Genetics

By Adrian M. Srb, Ray D. Owen and Robert S. Edgar. Second edition. (A Series of Books in Biology.) Pp. xi+557. (San Francisco and London: W. H. Freeman and Company, 1965.) 50*s*.

THIS substantially revised, second edition of a most valuable text-book appears thirteen years after the first edition of 1952. The two original authors have been fortunate in securing the collaboration of a third, Dr. R. S. Edgar, a distinguished representative of the new generation of geneticists who, in his work with bacteriophage, has opened a new approach to physiological genetics at the molecular level.

Many things have happened since 1952; they are incorporated in this new edition. Just about that time fine genetic analysis was forcing a replacement of the concept of genes as beads on a string by that of words in a sentence, with each word coding for a protein. Soon afterwards the Watson–Crick model of DNA gave precise chemical meaning to this picture and suggested the way in which genes are replicated and the colinearity of the fine structure of a gene with the amino-acid sequence of a polypeptide. It took only a few years to confirm the former, but more than ten years to confirm the latter.

Thanks to these early developments and the many others, too well known to need mentioning, on the genetic code and protein synthesis, the biochemical and the genetical approaches to heredity, differentiation, development and evolution have become completely interwoven. Demarcation disputes, unfortunately still rampant, are becoming boring. This book may well help to bury them for good.

There are a few minor errors, such as the legend to Fig. 3·1, on page 67. The treatment of human genetics (Chapter 15) is inadequate and not up to date; better to omit it or treat the subject more significantly. Apart from these minor faults, there is little doubt that this new edition will, as its predecessor, secure many years of deserved success as an introductory text-book of genetics.

G. PONTECORVO

Research in Pesticides

Edited by C. O. Chichester. (Proceedings of the Conference on Research Needs and Approaches to the Use of Agricultural Chemicals from a Public Health Viewpoint, held at the University of California, Davis, October 1–3, 1964.) Pp. xx+380. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 128*s*.

SINCE the publication of Miss Rachel Carson's book *Silent Spring* in 1962, more words must have been spoken and written about pesticides, and the hazards of their residues in foods or of environmental contamination, than about any other aspect of applied biology. *Research in Pesticides* offers an up-to-date review of the present position on pesticides, not agricultural chemicals as stated in the sub-title, with the exception of legislative control.

At the conference reported in this volume, twenty-six specialists read papers covering a great diversity of aspects of pesticide usage in relation to public health. These aspects, according to the titles of the seven parts into which the conference was divided, cover criteria for public policy in the use of pesticides; approaches in the investigation of pesticidal action (on the pest); whole animal responses to pesticides; ecological systems (fresh water, land drainage, sea water, wildlife, domestic animals, plants); removal or persistence of pesticides (public water, air, soils, foods); alternate methods of pest control; and interpretation of data with respect to human health. Each aspect was covered by two to six papers, followed by one to three formal contributions to a discussion, but on only three occasions was there open discussion from the floor. There is a short, but helpful, topical index.

The great majority of the papers reach a high standard of summary and review, but most authors seem to have been unable to see further ahead than the clear need to amass more data. However, I particularly liked the contributions by O'Brien on target enzymes, Frawley on synergism, Freed on control of plants, Crosby on the international removal of residues, McNew on regulating the interaction of pest and host, and that portion of the open discussion to Part 7 commenting on the interpretation of animal in relation to human toxicology.

The standard of editing is high, but on pp. 52–55 appear three mentions of a contribution on pitfalls in studies of metabolism by Dr. Casida, who was in the audience. No explanation is offered for the omission from print of what seems to have been a very interesting paper. The book will clearly be an excellent reference volume for libraries, but those workers who would find a personal copy valuable, because of their need to take a broad view of pesticides in committee work or scientific administration, may be deterred by the price. E. A. PARKIN

MEETINGS

COSPAR — VIENNA, 1966

THE ninth Plenary Session of Cospar and the eighth International Space Science Symposium was held at the Congress Hall of the New Hofberg, Vienna, Austria, during May 10–19, 1966. Some 600 delegates and scientists from 33 countries attended. Three special topical symposia were held on life sciences and space research, the Moon and planets, and interactions between the neutral part and the ionized part of the atmosphere

Maurice Roy, representative of the International Union of Theoretical and Applied Mechanics, was elected to a second three-year term as president. The Bureau of Cospar is now composed of A. A. Blagonravov (Academy of Sciences, U.S.S.R.) and R. W. Porter (National Academy of Sciences, United States) as vice-presidents, with L. Egyed (Hungarian Academy of Sciences), K. Lauter (German Academy of Sciences at Berlin), Sir Harrie Massey (Royal Society of London), and M. Nicolet (Royal Academy of Sciences of Belgium). The tenth meeting of Cospar will take place during July 17–28, 1967, in London; the first week will be a joint session with the fourth General Assembly of the IQSY, and the special symposium at the meeting will be about the scientific results of that programme.

Working Group I—Tracking, Telemetry and Dynamics. Concern was expressed over the need to protect certain radio frequencies used for tracking satellites and for receiving beacon and telemetry signals. It is difficult to assign a protected frequency to satellite transmitters, for the regulations of the International Telecommunications Union require that in such a case an identifying call name must be assigned This is usually impractical, because satellite transmissions often contain only the telemetry channels for command and data relay. Some satellites continuously broadcast data through telemetry channels, often so as to relate satellite data directly with data obtained from the ground simultaneously. These telemetry emissions also require protection. These matters are to be discussed further with representatives of the International Radio Consultative Committee, and formal requests for frequency allocations will be filed through channels to the International Telecommunications Union.

Optical means of tracking satellites range from accurate astronomical telescopes of the Baker–Nunn type and others to observations made with an ordinary press camera with a 4 in. × 5 in. negative. The latter has proved effective in filling in orbit information, especially during the early days of a satellite before accurate prediction ephemerides are available to guide the narrow field astronomical instruments. Visual observations by skilled teams are also of considerable importance in the early and late phases of an orbit. Recent work by King-Hele indicates the usefulness of skilful camera and visual observation in determining atmospheric drag—and hence deducing atmospheric density—especially for low-perigee satellites. The Working Group urged continued attention by amateur groups to good observation, especially in the Southern Hemisphere, and urged that coverage should be increased.

The Working Group also agreed to study the possibility of distributing listed observations in a form directly accessible to machines. The need was pointed out for the determination of position and proper motion of stars in the southern half of the celestial sphere, so that better optical tracking can be achieved in the Southern Hemisphere. Support for the efforts of the International Astronomical Union to compile a southern hemisphere star atlas was also recommended.

Mrs. Alla Massevitch (U.S.S.R.), who has chaired this Working Group since the inception of Cospar, resigned. J. Kovalevsky (France) is the new chairman and D. J. King-Hele (U.K.) vice-chairman.

Working Group II—Design and Co-ordination of Experiments. One of the important programmes discussed by the panel on the dynamics and structure of the neutral atmosphere was the sounding of the atmosphere above balloon ceilings by small meteorological rockets. Some four thousand soundings are now available, chiefly from the United States network of some fifteen stations, but regular firings are also conducted by the U.K. Meteorological Office at South Uist in the Hebrides, and rockets have been fired from India, Pakistan, Australia, France, Italy, Iceland, Norway, Sweden, Spain, Poland, Egypt, Israel, U.S.S.R., Argentina and Brazil. German groups have fired rockets from various locations outside the Continent; the Japanese are testing rockets in preparation for regular firings in a year or so, and interest has been expressed by Canada, Greece and Columbia. Rockets have been fired at McMurdo Sound, Antarctica, and at Thule, Greenland, and from ships. In a few years, a world-wide network of meteorological rockets may be a distinct possibility. Regional groups have been organized for the Indian Ocean and the Americas. The panel strongly endorsed these efforts, and hoped that such groups could become the mainspring for an effort to obtain good latitude coverage along particular meridians, and urged that additional groups should evolve, particularly in Europe and in the Western Pacific and East Asian Regions. A latitude profile based on observations from two ships in the western Pacific undertaken by the U.S.S.R. attracted interest, and it was suggested that such efforts be coordinated into land-based latitude chains.

Because of the wide interest in data from the rapidly evolving network of rocket stations, among ionospheric physicists as well as meteorologists, an effort has been made in the past year to centralize the collection and publication of rocket profiles. World Data Center *A* for Meteorology (United States) has agreed to publish data on a month-by-month basis, and the first volume (for July 1965) was distributed. Countries obtaining rocket meteorological data are now urged to submit data to the World Data Center for meteorology within 4 weeks of a firing for inclusion in the appropriate volume due to appear after a delay of 6 months.

The importance of dust in the solar system and the upper atmosphere of the Earth was discussed by an *ad hoc* group, which suggested that a formal Cospar panel should be organized on this subject. This suggestion will be considered at the London meeting following a scientific symposium on dust to be arranged by the *ad hoc* group, whose goals would then be further clarified.

The Working Group also turned its attention to experiments or programmes that would benefit from coordination on a multilateral or global basis, with particular attention to the period of solar maximum a few years hence.

Working Group III—Data and Publications. The desirability was again emphasized that launching groups should supply descriptive summaries of rocket flights, giving brief information on the experiments, and similar summaries for satellites and space probes. In order to simplify the mechanics of distributing data on satellite launchings, it was decided to use telecommunications only for those satellites with which speed is essential, as, for example, when real-time telemetry is employed, or where the spacecraft is of optical or radio interest (flashing lights, radio beacons, etc.).

Working Group IV—International Reference Atmosphere. Support was added to the request of other working groups for more data in the region between balloon ceilings (30–40 km) and satellite level (several hundred km) and particularly for data from meteorological rockets on the upper stratosphere and mesosphere, and data on density and temperature in the region of 150–200 km by the tracking of low-altitude satellites. Interest was also expressed in attempts to take account of essentially regular dynamical effects, such as the solar heating bulge in the upper atmosphere and the solar tidal motion in the middle atmosphere which is now regarded as a consequence of solar heating in the ozonosphere and not a resonant response to the gravitational tide. Interest was also expressed in an attempt to develop a reference ionosphere. These two topics, and particularly the latter, are complex and it is probably premature to make decisions on these elaborations of the reference atmosphere.

Luigi Jacchia (United States) was elected chairman of the Working Group to succeed Dr. W. Priester (West Germany), and V. I. Krassovsky (U.S.S.R.) vice-chairman.

Working Group V—Space Biology. The question of space probe sterilization received considered attention, with an exchange of information on techniques now in use in the U.S.S.R. and the United States and some plans for future probes. A conference on space-probe sterilization was planned as a prelude to the London meeting. There will also be a two-day symposium at this meeting, covering areas such as the biology of the inner planets, strategy and tactics of life-detection schemes, orbiting biological experiments, and long-term effects of weightlessness on the cardiovascular system.

Working Group VI—Scientific Space Experiments concerned with the Properties and Dynamics of the Troposphere and Stratosphere. The terms of reference of this Working Group, established at the meeting last year in Argentina, imply support for the interest of the International Council of Scientific Unions and the World Meteorological Organization in developing a world-wide system of meteorological observations. It is therefore mainly concerned with determining atmospheric parameters over the globe through indirect techniques of electromagnetic sounding and atmospheric probes (constant-level balloons) and at the surface (remote astromatic station, ocean buoys) located by satellite ranging systems.

The Working Group agreed to prepare a document on (i) the specification of a global meteorological experiment; (ii) the design of experiments to test atmospheric predictability; (iii) the design of experiments to study the spectral distribution of atmospheric energy μ, a function of the scale size of disturbances; (iv) the status of the art of indirect probing and a look to the future for such parameters as temperature, density, pressure, wind, surface state, cloud, radiation flux, water vapour and ozone; and (v) a status report on the constant-level balloon systems now under test (GHOST balloons, United States) and development (by the EOLE group in France), and on the satellite ranging and interrogation schemes which are under development

Popular Education and Training. A panel with this title was organized in response to the United Nations request to the scientific unions for advice on ways in which space science could be used to accelerate technical training and education in developing countries. The panel suggested that the use of certain geodetic satellites for accurate surveying in Africa shows how this kind of work may be a good possibility for many developing countries. Another possibility is the improvement of local weather services by means of automatic equipment taking pictures from the TIROS satellites. Many such units have been built in universities, and it was considered that such developments are not in principle beyond the reach of universities in developing countries.

STANLEY RUTTENBERG

OBITUARIES

G. R. Crowe

A COLLEAGUE writes:

G. R. Crowe, who died in June aged 73, spent most of his working life in the Cavendish Laboratory. Starting as a boy in 1907, and leaving the Laboratory only during the First World War when he worked at the Cambridge Instrument Company, he retired as principal assistant in 1959. To a few he was "Crowe", but to most of us he was "George".

Crowe became Rutherford's personal assistant in 1919, a post which he held until Rutherford's death in 1937. Throughout this period many of Rutherford's best experiments owed much to Crowe's skill and perseverance. Essentially he was a man with good hands who was skilled in most of the contemporary laboratory techniques. He could do most things that required to be done whether it was with metal or glass or photography. With such old-fashioned things as wax or mica he was an artist. Perhaps his greatest single contribution to Rutherford's work was the preparation of the thin glass tubes in which radioactive emanation was sealed. It would still be an exercise for an apprentice to make the tubes. When one adds the difficulty and the hazards of the radioactive filling it was a task for a craftsman.

One of the greatest delights in the 1930's for Rutherford and Crowe were the Royal Institution lectures. For both of them the lectures were at once a challenge and a holiday. It is doubtful whether Rutherford ever spent so much time preparing a set of lectures, and much of the work of the Laboratory came to a halt until the preparations were complete. The master's ideas were shaped and made practicable by Oliphant and Wynn Williams, but the detailed execution fell largely to Crowe. It was not trivial in 1934 to build a portable Cockcroft and Walton accelerator with subsidiary counting equipment and to erect it in London on a "one-night stand" and therewith to give the first public demonstration of nuclear transmutation. This, of course, was a laboratory effort, but Crowe did his part, a very large part, supremely well.

The "If's" of history are many. Not the least interesting to a physicist is the speculation as to how much, or how little, some of the eminent would have achieved without skilled assistants. Whittaker, in his *History of the Aether*, records of Oersted that he was "a man of genius but a very unhappy experimenter: he could not manipulate instruments". On the other hand, we can be quite certain that the president of the Royal Society in 1966 would still have been president if laboratory assistants had never existed. Crowe's opinions on this subject were interesting and emphatic. When he entered the Cavendish as a boy, J. J. Thomson was the Cavendish professor. From his own observation supported by gossip with Everett, Thomson's assistant, Crowe concluded that J. J. was never allowed to touch any apparatus whatsoever—otherwise disaster inevitably followed. Of his own master, Rutherford, Crowe had many colourful stories, many of them bearing on this subject. The tenor of them can be summarized in the Rutherford exclamation, "Don't shake the bloody bench, Crowe", when the great man was patently failing with something which required manipulative dexterity. Although, in this case, Crowe was the only witness—as well as judge and jury—there is little reason to doubt the substantial correctness of his comments.

In his prime, a man of considerable physical strength and an enthusiastic player in the Cavendish cricket match, Crowe was endowed with a calm temperament and was not easily ruffled. He allowed the sometimes violent storms, of which Rutherford was the sole and only origin, to pass over him. In this he was assisted by his partial deafness. It was always very difficult for Rutherford in

one of his tempers to know whether Crowe could not or would not hear him.

It was natural that Bragg should turn to Crowe for help in the preparation of his own Royal Institution lectures when he succeeded Rutherford in the Cavendish chair. These lectures were distinguished by a quite exceptional clarity and thoroughness of preparation and owed a great deal to Crowe's patience and skill in mastering extremely ingenious and delicate techniques. It was not only in this work, however, that Bragg found Crowe's practical skill a match for his own inventiveness. They worked together during the Second World War, and almost immediately afterwards Crowe ventured into a new field, assembling and operating the first electron microscope in the Laboratory, one of the first in the country. Even Crowe's temperament was stretched to the limit by this wayward instrument and he always maintained it was during this period he learnt to swear "aloud". Nevertheless, Bragg's wisdom in setting Crowe to the task bore rich dividends in Crowe's contributions to the technique of preparing specimen replicas.

In the Cavendish, Crowe was respected for what he could do and well liked for what he was. In experimental physics his death marks the end of an era. No one now in pursuit of the highest prizes plays with wax or bothers to split mica. Yet these trivialities played a not negligible part in an intellectual revolution. It is right that the man who could do them and did them should be remembered.

Prof. J. M. Mackintosh

PROF. J. M. MACKINTOSH died at his home in Bristol on April 20, at the age of 75. In his lifetime he spanned almost the whole of our experience of public health, first as County Medical Officer of Northamptonshire, then as Chief Medical Officer of the Department of Health for Scotland, professor of public health at Glasgow, and as dean and professor of public health at the London School of Hygiene and Tropical Medicine. A highlander by origin and temperament, he was born in Kilmarnock where his father practised as a solicitor, and educated at Glasgow High School and University. He was a graduate in arts and medicine in the older Scottish tradition of the educated doctor: at the University of Glasgow he was one of a vintage generation whose studies were interrupted by active service in the First World War. Severely wounded as a subaltern in the Cameron Highlanders, he was sent home and finished his medical studies. He returned to the war as a medical officer in the R.A.M.C. It was war-time service which determined his choice of public health as a career.

After service as an assistant medical officer in Dorset, Burton-on-Trent, and Leicestershire he was appointed County Medical Officer of Northamptonshire in 1930. There he made a celebrated series of surveys of rural housing, and his reports, illustrated with his own pen-and-ink sketches, are a unique piece of English social history. He loved his period of public health service.

From 1937 until 1941 he served as Chief Medical Officer of Scotland This was a period when attention had turned in Scotland to the need for major reform of the health services, and a period of hectic planning in anticipation of war. This experience was formative of his thinking about hospital planning and strategy At this time, too, James Mackintosh discovered with a special delight and appreciation the Highlands and Islands Medical Service which had pioneered the provision of health services for remote and scattered populations; in retirement it was one of his unrealized hopes that he might be able to write a history of the service.

In 1941 he resigned from central government work, which he had felt restricting and lacking in those individual contacts whose stimulus he found rewarding. He moved to the chair of public health of the University of Glasgow and began the academic career by means of which his name has become widely known. To begin with, however, he was still concerned with war and was moved to the United States to advise on the medical aspects of civil defence. This was the beginning of an association with American colleagues which continued for the rest of his life. In 1944 James Mackintosh was appointed dean and professor of public health at the London School of Hygiene and Tropical Medicine and continued there until his retirement in 1956. The end of the war was a time of ferment. Young men out of the forces were eager to make their mark in their country's health services. Increasingly, postgraduate students came to learn from British experience and teaching. The times welcomed the gifts of James Mackintosh as a thinker and teacher. His lectures on the history and development of health services were a delight, tantalizing to those who wanted a text-book exposition, but profoundly educative in giving a sense of historical perspective and the development of health services as a function of contemporary culture and thought. Mackintosh was beyond all else a social philosopher of the health services, weaving his text from wide reading and experience, and illuminating generalizations from the particular example with a special skill of his own.

When he retired from his chair in 1956 he was already frail and in poor health. He nevertheless had two further successful careers before he died. He worked in Geneva as a consultant to the World Health Organization, and was largely responsible for the production of its *First Report on the World Health Situation*, a classic of its kind. He later worked in the United States for the American Hospital Association and the Milbank Foundation, and his volume of essays on *Topics in Public Health* published last year was partly produced at that time.

J. H. F BROTHERSTON

Dr. A. C. Stephen

DR. ALEXANDER (ALASTAIR) CHARLES STEPHEN, who was keeper of the Department of Natural History in the Royal Scottish Museum from 1935 until 1958, died suddenly at his home in Edinburgh on June 3 at the age of seventy-two.

Born at Garvock, Kincardineshire, he was educated at Robert Gordon's College and Marischal College, Aberdeen. His academic course was interrupted by service in France and Belgium, but he graduated at Aberdeen in 1919. His first appointment was junior naturalist on the scientific staff of the Fisheries Board for Scotland in 1920, where he was responsible for an investigation into the distribution of animal life on the bottom of the North Sea and adjoining areas.

He joined the Royal Scottish Museum in June 1925 as assistant in the Department of Natural History, and his period of service was notable for the opening of new halls and galleries which, in their presentation of material, were well in advance of other museums in Britain. He continued his research in marine zoology and was the first to apply techniques for quantitative study to the intertidal fauna of sandy and silty shores—work which yielded valuable information on the ecology of these zones and formed his thesis for the D.Sc. in 1934. He wrote on the distribution of cephalopods in British waters and was an authority on the taxonomy of echiurids, sipunculids and priapulids, publishing several reports on material from Africa collected during the *Discovery* and John Murray Expeditions, and from the B.A.N.Z.A.R. Expeditions. He completed a synopsis of the British species and, at the time of his death, was engaged in the preparation of a monograph of the world species.

For many years he was editor of the *Scottish Naturalist*, and he served also as president of the Royal Physical Society of Edinburgh and the Astronomical Society of Edinburgh. He was also a Fellow of the Royal Society of Edinburgh.

A. RODGER WATERSTON

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INTERSTELLAR VEHICLE PROPELLED BY TERRESTRIAL LASER BEAM

By PROF. G. MARX
Institute of Theoretical Physics, Roland Eötvös University, Budapest

THE extreme difficulties of interstellar space travel are well known[1]. It is a commonly accepted view that, apart from the technical difficulties involved, the laws of conservation of energy and momentum forbid the visiting of other planetary systems in the human life-span[1]. This article sets out to show that this is not necessarily the case. To arrive at the nearest stars in the life-span of the astronaut a relativistic velocity is needed. The kinetic energy, K, of vehicle of rest mass, M, moving at the velocity $c\beta$ is:

$$K = Mc^2\left(\frac{1}{\sqrt{1-\beta^2}} - 1\right)$$

Here c is the velocity of light. To achieve a relativistic velocity the vehicle would need to have a propellent energy, W, that greatly exceeds the most optimistic estimates of the technical possibilities in the foreseeable future. The reason for this is the very low mechanical efficiency of rocket propulsion in the relativistic domain[2]. A rocket can be accelerated only by the reaction of its exhaust gases. The greater part of the energy liberated from the propellant is lost in the form of the unavoidable recoil motion of the small masses of the exhausted gas particles. This is a direct consequence of the conservation of energy and momentum. To prevent wastage of energy, the only possibility seems to be to transfer the recoil momentum to the Earth. Because of the large mass of the Earth, its associated recoil energy is negligible, as in the case of a car, running on a solid road. As a possible realization of this principle, consider the following propulsion system: a departing vehicle has an effective cross-sectional area, f, as seen from the Earth, and acts as a perfect reflector of light in the backward direction. A light beam of constant intensity, I, coming from a terrestrial light source, is reflected by it. If the vehicle moves with the speed $c\beta$, the intensity of the reflected light, I', turns out to be smaller than I. The momentum gained by the vehicle is $c^{-1}f(I+I')\mathrm{d}t$, and the energy gained is $f(I-I')\mathrm{d}t$ during the time interval $\mathrm{d}t$, and the vehicle is accelerated from the velocity $c\beta$ to $c(\beta+\mathrm{d}\beta)$.

The equations expressing momentum and energy conservation are:

$$\frac{If}{c^2}c\,\mathrm{d}t + \frac{Mv}{\sqrt{1-\beta^2}} = \frac{M(v+\mathrm{d}v)}{\sqrt{1-(\beta+\mathrm{d}\beta)^2}} - \frac{I'f}{c^2}c\,\mathrm{d}t$$

$$If\,\mathrm{d}t + \frac{Mc^2}{\sqrt{1-\beta^2}} = \frac{Mc^2}{\sqrt{1-(\beta+\mathrm{d}\beta)^2}} + I'f\,\mathrm{d}t$$

These can also be written as differential equations of the motion:

$$I + I' = \frac{Mc^2}{f}\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{\beta}{\sqrt{1-\beta^2}}\right)$$

$$I - I' = \frac{Mc^2}{f}\frac{\mathrm{d}}{\mathrm{d}t}\left(\frac{1}{\sqrt{1-\beta^2}}\right)$$

Adding and subtracting these equations give:

$$I = \frac{Mc^2}{2f}\frac{\mathrm{d}}{\mathrm{d}t}\left(\sqrt{\frac{1+\beta}{1-\beta}}\right) \qquad (1)$$

$$I' = -\frac{Mc^2}{2f}\frac{\mathrm{d}}{\mathrm{d}t}\left(\sqrt{\frac{1-\beta}{1+\beta}}\right) \qquad (2)$$

If the incoming intensity, I, is constant in time, then, by integration, the terminal velocity:

$$\frac{v}{c} = \beta = \frac{(1+2\tau)^2-1}{(1+2\tau)^2+1} \qquad (\beta = 0, \text{ if } \tau = 0) \qquad (3)$$

Here τ is the ratio of the radiant energy, W, used up during the time t, to the rest energy of the vehicle:

$$\tau = \frac{Ift}{Mc^2} \equiv \frac{W}{Mc^2}$$

Substituting equation (3) back into equation (2), the following expression for the "instantaneous mechanical efficiency" is obtained:

$$\eta_m = \frac{\mathrm{d}K}{\mathrm{d}W} = \frac{I-I'}{I} = 1 - (1+2\tau)^{-2}$$

If $\tau \to \infty$, that is, if $v \to c$, the instantaneous efficiency rises up to 100 per cent; almost the whole energy of the light falling on the mirror is converted into the kinetic energy of the vehicle in the extreme relativistic limit.

The "total mechanical efficiency" can be defined as a ratio of the kinetic energy of the vehicle (starting from rest) to the radiant energy of the beam incident on it:

$$\eta_t = \frac{K}{W} = \frac{1}{1+(2\tau)^{-1}}$$

The total efficiency starts from a very low value (valid for non-relativistic motion) and rises to 100 per cent when the vehicle has reached relativistic velocity. It can thus be seen that, with "a solid ground below the feet", all the principal difficulties of interstellar space flight disappear. A vehicle can be accelerated almost to the speed of light if an emitter on the Earth can accurately project light on to its mirror.

To make this technically possible, an ideal focusing is needed. All light sources emitting natural light must be excluded because of the divergence of the small wave pockets. The only possibility is offered by the coherent radiation of the laser. If the divergence angle of the laser beam is taken as:

$$\alpha = \mathrm{tg}^{-1}(\lambda/\rho)$$

where λ is the wave-length and ρ is the radius of the coherently radiating surface of the laser, then the maximum range of operation, r, may be estimated (in units of light years) as:

$$r = 0{\cdot}1\,a\,\rho\,\varepsilon$$

where a and ρ are the radii of the mirror and laser surface, respectively, both in the kilometres, and $\varepsilon = hc/\lambda$ is the energy of the light quanta in kiloelectron volts; h is Planck's constant. It can be seen that the technical conditions are extremely exacting. A range of operation of 0·1 light year would require a coherently radiating surface of the order of 1 km² which emits hard X-rays, and the vehicle would need an X-ray mirror with an effective cross-sectional area of several km².

As a matter of fact, this propulsion system would also operate without an ideal mirror. If the mirror were replaced by a black surface, and if the absorbed light

energy were radiated out isotropically into the space in the reference system of the vehicle, the instantaneous mechanical efficiency turns out to be:

$$\eta_m = \frac{dK}{dW} = \beta \frac{1+\beta}{1+\beta^2}$$

and thus:

$$\lim_{\beta \to 1} \eta_m = 1$$

In spite of this, the technical realization of these conditions would seem to be practically impossible in the next few decades. (But the construction of a photon rocket with 90 per cent mechanical efficiency is perhaps an even more difficult task.)

Laser propulsion from the Earth may be possible only in the very distant future. It would solve the problem of acceleration, but not the problem of deceleration on arrival at the destination. But if radio contact had been reached with intelligent beings on another planet, a man-carrying space-ship could start from the Earth, accelerated by our laser stations It could be accelerated up to 0·99c just after leaving the Earth and could then run with this speed through interstellar space. The receiving planet could then decelerate the vehicle to zero velocity. Travelling home could take place in the same manner.

No planet could be invaded in this fashion by unexpected visitors from outer space, which would perhaps explain why no real evidence of such invasions has been found on Earth up to now.

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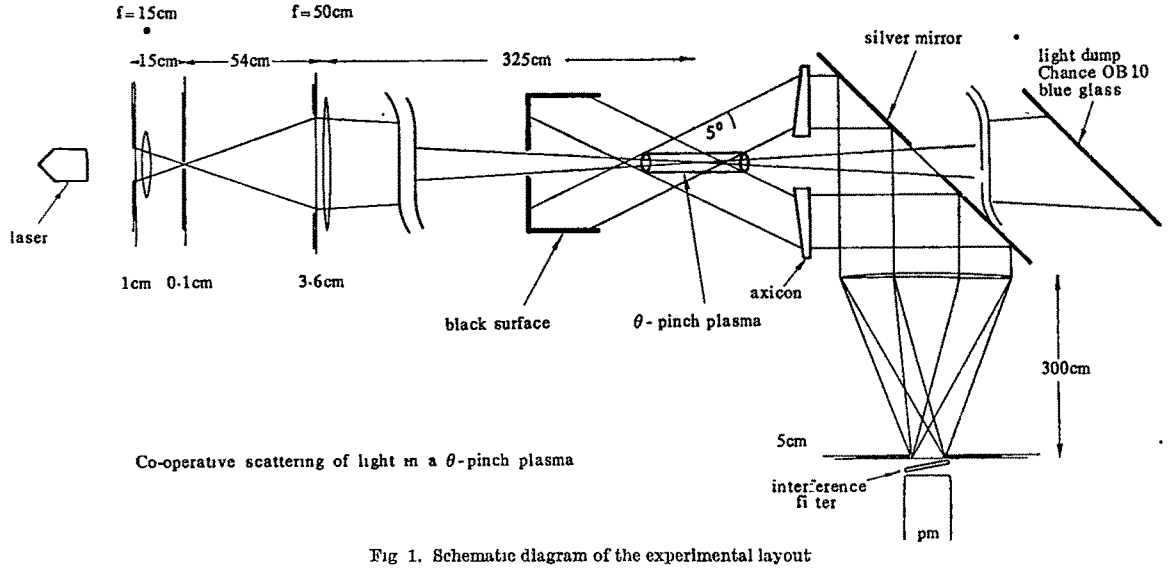
CO-OPERATIVE SCATTERING OF LASER LIGHT BY A THETATRON PLASMA

By D. E. EVANS, M. J. FORREST and J. KATZENSTEIN

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It is now well known that the illumination of a plasma by a monochromatic uni-directional light beam results in a spectral distribution of scattered light the shape of which depends on the ratio, α, of the scale length for scattering to the plasma Debye length. It has been shown theoretically[1] that the spectrum can be regarded as the sum of two parts, first the electron feature which predominates when $\alpha \ll 1$ and has a Gaussian shape, and second, the ion feature which accounts for most of the scattered light when $\alpha \gg 1$. In the neighbourhood of $\alpha = 1$ the electron term is no longer Gaussian but begins to be depressed in the centre and to show some signs of resonances displaced from the centre frequency by $\pm \sqrt{2}\omega_p$, the plasma frequency. By the time α is 3 or 4 these resonances are well developed and the Gaussian character of the distribution has entirely vanished. Evidence for the deformation of the Gaussian electron feature near $\alpha = 1$ has been reported by Kunze *et al.*[2] and Ramsden and Davies[3], and more recently Chan and Nodwell[4] and Ramsden and Davies[5] have described the appearance of the plasma frequency resonance for higher α.

This article reports measurements of the distribution of light scattered into the electron feature in circumstances when α varied between 1·2 and 1·5. A Kerr-cell *Q*-switched ruby laser and amplifier provided 70-MW pulses within a divergence angle of 6·7 milliradians and these were used to illuminate the plasma produced by a 45 kJ (30 kV) theta pinch machine, *MAGGI* II. The experimental arrangement is shown in Fig. 1. Laser light is focused into a 6-mm diameter cylinder along the axis of the coil within which the plasma is formed, by the usual arrangement of lenses and stops, and is conveyed away through a 3-m long tube to a system of absorbers of blue glass (Chance *OB*10) chosen for its very low coefficient of surface scatter. Light scattered at 5° to the forward direction by a 10-cm length of plasma at the centre of the coil passes through an annular window concentric about the coil axis, then through an axicon from which it emerges parallel to the axis. This is followed by a silvered mirror which reflects the light through 90°, a lens of focal length 3 m, and in its focal plane a 5-cm aperture which defines the range of angles around 5°, namely ± 0·5°, into which light is accepted by the detector. The latter consists of a dielectric multilayer filter supplied by Thin Films Inc., and an RCA 7265 photomultiplier having a photocathode with an *S*20



Co-operative scattering of light in a θ-pinch plasma

Fig 1. Schematic diagram of the experimental layout

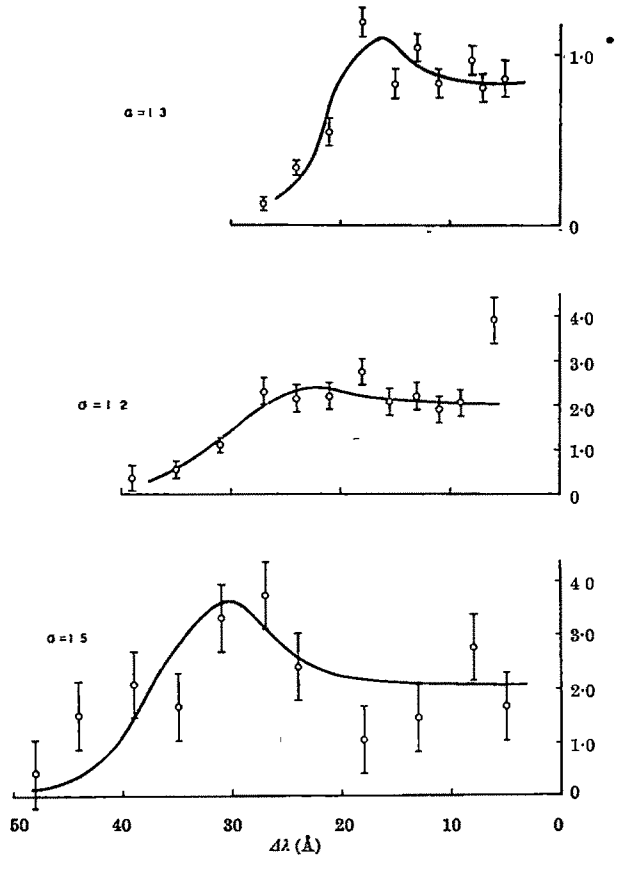


Fig. 2. Spectra of light scattered at 5°. Abscissa is the shift from the laser wave-length in Ångstrom units. Ordinates are arbitrary intensity units. Curves are best-fit theoretical scattered light functions

response. The filter is centred at 6943 Å and has a half-width of 5 Å when illuminated normally by monochromatic light reaching it through the system already described. Its pass-band shifts to shorter wave-lengths as it is tilted away from normal incidence, thus permitting its use as a spectral resolving instrument, though confining investigation of the spectrum to the blue side of the laser wave-length.

Spectral distributions of scattered light obtained under various machine operating conditions are presented in Fig. 2. They were built up from a series of individual shots—about a hundred shots being required to produce each spectrum. Each point represents the difference between the average of a set of shots with plasma, and that of a set without plasma at the same setting of the filter. The errors on the points are experimental in that they are derived from the root mean square deviation from the mean of the sets of experimental readings. Probable sources of the observed error are plasma light noise and fluctuations in the plasma parameters from shot to shot. Variations in the intensity of the laser output were detected by a photodiode monitor and were normalized out of scattered light signals. A residual fluctuation in the normalized stray light signals suggests an additional source of noise which has not been identified as yet. Stray light at the laser frequency was equivalent in intensity to that which would be produced by Rayleigh scattering on cold nitrogen gas at a pressure of about 160 torr, so that although scattered light could be detected at the centre of the spectra, it gave an enhancement over the stray light of only about 20 per cent. Nevertheless, the contrast of the filter was so high that beyond about 4 Å from the centre, scattered light was readily distinguished from stray.

To each set of experimental points a theoretical spectrum function of the form:

$$\Gamma_\alpha(x) = e^{-x^2}\{[1 + \alpha^2 - \alpha^2 f(x)]^2 + \pi\alpha^4 x^2 e^{-2x^2}\}^{-1}$$

where $f(x) = 2xe^{-x^2}\int_0^x e^{t^2}\,dt$ was fitted.

The abscissa was scaled according to the relation:

$$\frac{x}{x_s} = \frac{\Delta\lambda}{(\Delta\lambda)_s}$$

in which the subscript 's' refers to the value of x or $\Delta\lambda$, the wave-length shift, at the peak or shoulder of the function. It is of interest that where α lies between 1 and 2, $x_s \cong \alpha$ to better than 5 per cent, from which it follows that the position of the peak or shoulder depends only on plasma density. By a process of trial and error the value of α was determined to the nearest tenth, for example in the first of the spectra it became plain that $\alpha = 1{\cdot}3$ was a better fit than either 1·2 or 1·4. Similarly the position of the peak value, $(\Delta\lambda)_s$, was estimated to be correct to the nearest Ångstrom unit, since a change of 2 Å either way produced a readily detectable deterioration in the apparent goodness of fit.

The first two spectra were observed when the discharge tube was filled with hydrogen at a pressure of 20 mtorr, and 1 kG of reverse bias field was applied. A pre-ionizing pulse (110 J, 34 kV) ensured breakdown of the gas in the first half-cycle of the discharge of the main condenser bank. The first spectrum was measured half-way in time to the peak of the first half-cycle current pulse; the second was measured at the peak. The difference between the two is attributed to the change in the plasma properties as compression occurs. The best fit for the first spectrum was characterized by the parameters $\alpha = 1{\cdot}3 \pm 0{\cdot}05$ and $(\Delta\lambda)_s = 16$ Å $\pm$ 1 Å, from which electron density $n_e = \left(6{\cdot}0^{+1{\cdot}0}_{-0{\cdot}6}\right) \times 10^{15}$ cm^{-3} and electron temperature $T_e = 103^{+29}_{-17}$ eV were calculated. The second spectrum yielded $\alpha = 1{\cdot}2 \pm 0{\cdot}05$ and $(\Delta\lambda)_s = 22$ Å $\pm$ 1 Å, giving an electron density $n_e = \left(1{\cdot}16^{+0{\cdot}14}_{-0{\cdot}06}\right) \times 10^{16}$ cm^{-3} and electron temperature $T_e = 233^{+51}_{-28}$ eV.

The third distribution in the figure was measured at the peak of the first half-cycle of the current at a filling pressure of 50 mtorr of hydrogen and in the presence of 500 G of reverse bias field. The best fit in this case gave $\alpha = 1{\cdot}5 \pm 0{\cdot}05$ and $(\Delta\lambda)_s = 30$ Å $\pm$ 1 Å, corresponding to density $n_e = (2{\cdot}15 \pm 0{\cdot}15) \times 10^{16}$ cm^{-3}, and electron temperature $T_e = 278^{+40}_{-36}$ eV.

An independent measurement of the electron temperature in this plasma has been made under the same plasma conditions as those applying to the second of the three distributions in the figure by D. Beach *et al.*[6]. Their experiment, which also dealt with laser light scattering, was performed at 90° and so had an $\alpha \ll 1$. Their estimate of temperature is $T_e = 240 \pm 36$ eV, which agrees favourably with that measured by us.

We consider that the importance of these measurements lies in their application as a plasma diagnostic technique, for it is only when α is in the neighbourhood of 1 or 2 that measurements of T_e and n_e can both be derived from the spectrum of the scattered light without any knowledge of the absolute intensity.

We thank the Laser Group of the Atomic Weapons Research Establishment, who provided the laser, and Dr. J. A. Reynolds and Mr. J. A. Daniel for advice on and operating the thetatron.

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ROLE OF AMMONIA IN THE ATMOSPHERIC CORROSION OF MILD STEEL

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THE atmospheric corrosion of mild steel in Great Britain is associated with a high level of pollution, and sulphur dioxide in particular has received considerable attention as a predominant factor in atmospheric corrosion. Although some corrosion rates have been correlated with the sulphur dioxide level, anomalies do exist and this is considered to indicate that other pollutants may also be important.

As long ago as 1788 it was known that ammonia is one of the products of corrosion[1], and in recent years Ambler[2], Harrison and Tickle[3], and Young[4] have all investigated its presence in natural corrosion products.

The source of the ammonium ion found in such material has now been established and light thrown on its role in the corrosion process[5].

Examination of the rust formed on steel exposed to air in Manchester, as well as that formed in controlled laboratory atmospheres, has shown that the nitrogen content of the steel contributes mainly to the adherent inner rust, whereas atmospheric pollution contributes to the loose outer rust.

The seasonal variation in the total ammonia retained on a specimen exposed in Manchester is presented in Fig. 1; irrespective of when the specimen was first exposed, the ammonia retention rose in the winter months, falling again in summer. When the percentage of ammonia in the rust is plotted as in Fig. 2, it can be seen that the specimens of short exposure length exhibit a higher percentage of ammonia in the rust, as for example for specimens first exposed in September 1964 and February 1965. Furthermore, the ammonia is higher in the adherent rust in these early stages, but with prolonged exposure the ammonium content of the loose outer rust becomes predominant. Thus the seasonal variation in the amount of ammonia retained on a specimen follows a similar relationship to that noted for sulphates formed as a result of sulphur dioxide pollution. More significant, however, is the high ammonia content of the rust in the early stages of the corrosion process, particularly as it is associated with the adherent rust close to the metal surface.

To establish the role of the ammonium ion in the initial stages of corrosion, clean specimens of mild steel were subjected to a fine mist of atomized salt solutions for about 20 sec, and the effects of the salt solutions observed. The following solutions were atomized:

Salt solution (10 per cent w/v)	pH	Salt solution (10 per cent w/v)	pH
Sodium chloride	6·1	Ammonium chloride	5·2
Sodium sulphate	8·3	Ammonium sulphate	5·1
Sodium carbonate	11·0	Ammonium carbonate	9·2

Ammonium chloride and sulphate reacted more rapidly than the equivalent sodium salts, and also appeared to involve the entire surface of the specimen in the corrosion process. The attack in the presence of sodium ions was usually at discrete sites, and on drying salt nuclei were still discernible. Neither specimen contaminated with carbonates exhibited corrosion. The severe attack occasioned by ammonium salts may be due to the acidity of their solutions, but the observation that the attack in general affected the entire surface, giving it a 'wetted' appearance, was considered to be important.

The surface tensions of the solutions used were all greater than that of water[6], and would not therefore encourage wetting. However, once electro-chemical corrosion commences, hydroxyl ions are liberated at cathodic sites by the oxygen reduction reaction, and these ions together with the cation of the contaminant form the appropriate hydroxide. It would seem that it is the cathodic hydroxide that characterizes the corrosion process in the presence of the ammonium ion, for whereas sodium hydroxide solutions have a surface tension greater than that of water, that of ammonium hydroxide is lower. These properties are illustrated in Fig. 3, where the surface tension of water at 20° C is taken as an arbitrary zero, and surface tension-concentration profiles drawn. Thus, solutions possessing a negative surface tension with respect to water would preferentially wet a solid surface, while those with positive surface tensions would be non-wetting. The surface tensions of solutions of sodium chloride, sodium sulphate, sodium hydroxide, ammonium chloride and ammonium sulphate are all positive, and

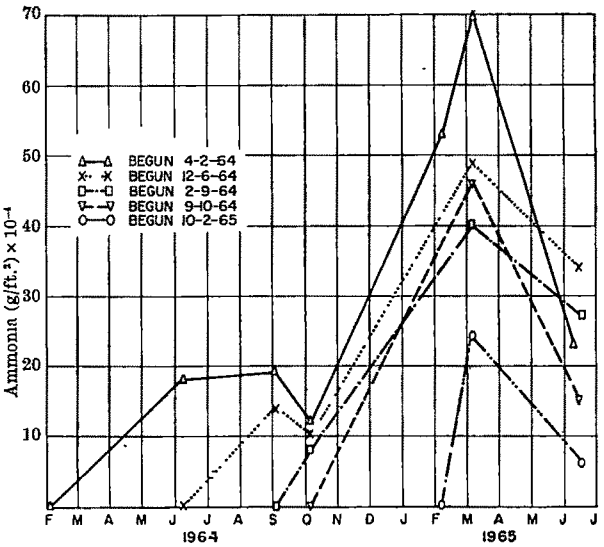


Fig. 1. Seasonal variation of the total ammonia retained on a specimen of mild steel

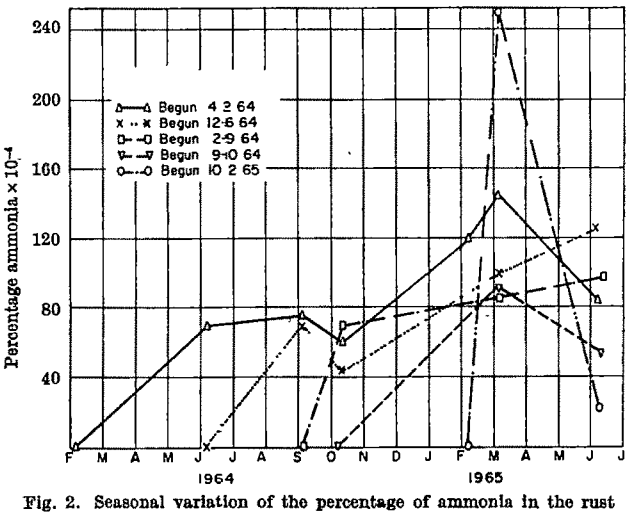


Fig. 2. Seasonal variation of the percentage of ammonia in the rust

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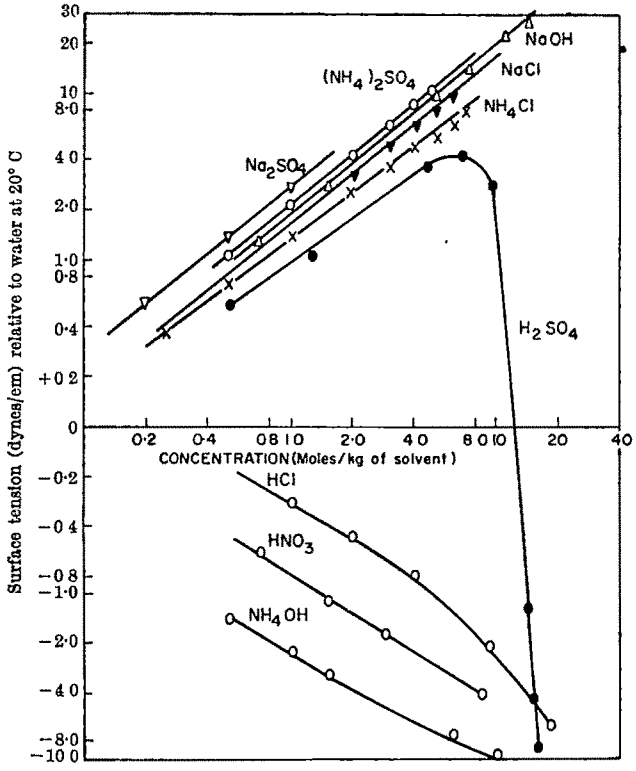


Fig. 3. Surface tensions of salt solutions at various concentrations

increase with concentration. Although not plotted in Fig. 3, the surface tensions for solutions of comparable salts of potassium, lithium, magnesium, and calcium are also greater than that of water, so that the phenomenon is apparently peculiar to ammonium compounds. Also included in Fig. 3 are the surface tensions of solutions of hydrochloric, nitric, and sulphuric acid. Solutions of the former acids also exhibit surface tensions lower than that of water, while sulphuric acid is distinctive in that the surface tension rises at first, then falls below that of water. Droplets of moisture on a steel surface are known to develop on outer annular cathodic zone where, in the presence of ammonium ions, the surface tension would fall, enabling the drop to spread. The presence of almost any other cation in solution would appear to cause such a drop to shrink. Thus the principal effect of ammonium in atmospheric corrosion may be that it promotes wetting and the subsequent involvement of greater areas of metal.

The severity of the attack experienced in the presence of ammonium salts may to some extent be due to the acidic nature of solutions of these salts, while the solubility of ammonia is an added factor. Brasher[7] has pointed out the error in designating certain electrolytes as corrosive and others as inhibitive, and stipulates that all electrolytes are corrosive in solutions of sufficiently great dilution. This would probably account for most of the discrepancies noted in the literature regarding the corrosiveness of the ammonium ion, and also indicated the importance of the ammonium ion in the initial stages of the corrosion process. It is in these stages that the hydroxide causes the corrosion to spread. At higher concentrations, such that ferrous ions are precipitated, the reaction will become blocked.

Considerations of the surface tension of the catholyte resulting from electrochemical corrosion in the presence of the ammonium salts, and observations on the high ammonia content of the adherent rust in the initial stages of corrosion are considered to suggest that the ammonium ion may play an important part in the mechanism of atmospheric corrosion.

One of us (B. G. C.) thanks the South African C.S.I.R. for financial assistance.

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OXIDATION OF TITANOMAGNETITES AND SELF-REVERSAL

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THE recent statistical correlation of petrology and the polarity of natural remanent magnetization of rocks[1] has led to a renewed interest in the possibility of self-reversal. It has been found that if rocks are categorized according to the degree of oxidation of the iron titanium oxide minerals[2], then the fraction of reversed rocks in each category increases with increasing degree of oxidation. The lowest category contains no reversed rocks and the highest category 80–90 per cent reversed rocks. Although this cannot be taken as definite proof of self-reversal, no attempt has yet been made to provide physical models by which oxidized titanomagnetites can be made self-reversing. The purpose of this article is to consider the oxidation processes and products and the consequent magnetic effects of the oxidation of titanomagnetites.

Oxidation of Titanomagnetites

(*A*) *To a mineral of spinel structure.* Cation deficient iron–titanium oxide spinels (analogous to maghemite, γ-Fe_2O_3) are found in Nature and are considered to be oxidation products of titanomagnetites. The cation distribution in single-phase spinel oxidized titanomagnetites has not been determined, but it is possible to apply the principles which govern cation distributions in titanomagnetites and other spinel oxides and make conjectures about how the cation distribution will change with oxidation.

The preference of Fe^{2+} ion for the tetrahedral sites of the spinel structure was first found by Verwey and Heilmann[3], who showed that if the chemical bonds in the spinel lattice are assumed to be purely ionic, the electrostatic energy dictates that tetrahedral sites should be occupied by the large divalent ions (for example, Fe^{2+}). However, chemical bonding in tetrahedral sites is not ionic but covalent[4] and the electrostatic energy lost by placing Fe^{3+} in tetrahedral sites is compensated by the energy of the covalent bond. This is the case in titanomagnetites[5] where tetrahedral sites are shared by Fe^{2+} and Fe^{3+} throughout most of the series. The absence of tetrahedrally sited Ti^{4+} is indicated by the neutron diffraction data of Ishikawa *et al.*[6].

The cation distribution in single-phase spinel oxidation products of titanomagnetites can be discussed on the basis of the foregoing data if the oxidation mechanism of Colombo *et al.*[7] for magnetite is extended to the titano-

magnetites. Cations diffusing through the crystalline lattice combine with adsorbed oxygen atoms at the surface which are ionized by the extra electrons of the Fe^{2+} ions. The speed with which the reaction occurs depends on the rate of diffusion of cations, and because of covalent bonding, tetrahedrally sited ions in spinels have a very low diffusion rate[8]. For this reason oxidation will occur largely at the expense of octahedral Fe^{2+} and the cation population of tetrahedral sites will remain constant. The cation distribution in the oxidation product will then be governed by the initial cation distribution, and the vacancies will occur largely in octahedral sites.

The degree of oxidation of the titanomagnetite

$$Fe^{2+}_{1+x}\ Fe^{3+}_{2-2x}\ Ti^{4+}_{x}\ O^{2-}_{4}$$

can be defined by an oxidation parameter z given by the reaction:

$$Fe^{2+} + \frac{z}{2}O \rightarrow z\,Fe^{3+} + (1-z)\,Fe^{2+} + \frac{z}{2}O^{2-}$$

and the number of vacancies produced per "molecule" is:

$$\frac{3 + \frac{z}{2}(1+x)}{4 + \frac{z}{2}(1+x)}$$

The number of Fe^{2+} ions per "molecule" is

$$\frac{(1+x)(1-z)\,4}{4 + \frac{z}{2}(1+x)}$$

The cation distribution may then be written:

$$Fe^{3+}_{1-\delta}\ Fe^{2+}_{\delta}\left[Fe^{2+}_{\frac{(1+x)(1-z)4-\delta}{4+\frac{z}{2}(1+x)}}\ Fe^{3+}_{\frac{2-2x+z(1+x)4}{4+\frac{z}{2}(1+x)}+\delta-1}\ Ti^{4+}_{\frac{4x}{4+\frac{z}{2}(1+x)}}\ \square_{\frac{\frac{3}{2}z(1+x)}{4+\frac{z}{2}(1+x)}}\right]O_4$$

where δ is the number of Fe^{2+} on tetrahedral sites and $\square$ denotes vacancies. From the arguments already given, δ will remain constant as oxidation proceeds and be equal to the value for the unoxidized titanomagnetite, δ_0. The range of oxidation parameter over which this is possible is shown on the $FeO - Fe_2O_3 - TiO_2$ ternary diagram (Fig. 1) and also for a number of values of x in Table 1. When all the octahedral Fe^{2+} ions have been oxidized the reaction can only proceed by the oxidation of tetrahedral Fe^{2+}, and for this range of composition δ is simply given by the total number of Fe^{2+} ions,

$$\frac{(1+x)(1-z)\,4}{4 + \frac{z}{2}(1+x)}$$

The oxidation of octahedral Fe^{2+} probably corresponds to the so-called first stage of oxidation of magnetite[7] which occurs quickly at a low temperature (1–2 h at 250° C). Further oxidation retaining the spinel structure may be impossible as the higher temperatures required to increase tetrahedral Fe^{2+} mobility could result in the decomposition of the cation-deficient spinel.

(*B*) *To a spinel-rhombohedral intergrowth.* The very common occurrence of intergrowths of ilmenite and magnetite led many workers in mineralogy to believe in the existence of a solid solution between the two, but Buddington and Lindsley[9] have shown that they are the result of oxidation of titanomagnetites either directly or by the formation of an intermediate cation-deficient spinel. The ilmenite (or ilmenite rich haematite-ilmenite solid solution, haemo-ilmenite) exsolves in the (111) planes of the spinel host.

Effect of Oxidation on Magnetic Moment

(*A*) *Spinel oxidation product.* Verhoogen[10] suggested an order–disorder mechanism for self-reversal which could apply to the case of spinel oxidation products. The disordered phase had a random distribution of the three types of ions and vacancies and the ordered one had a maximum amount of Fe^{3+} ions in tetrahedral sites, vacancies and Ti^{4+} ions in octahedral sites, with Fe^{2+} ions filling the remaining lattice sites. However, a random distribution of Ti^{4+} is not found in quenched samples of titanomagnetites. Thus it may not be probable in quenched oxidized titanomagnetites.

Another possibility is the oxidation of a ferrite with a *B* sub-lattice predominant moment (for example, a titanomagnetite) to a composition with an *A* sub-lattice moment. Using the cation distribution of the previous section, the variation of saturation magnetization, n_B, at 0° K varies with oxidation parameter, z, in the following manner:

$$n_B = \frac{56 - 24x - 4z + 4xz}{4 + \frac{z}{2}(1+x)} + 2\delta - 10\ \mu_B/\text{molecule}$$

For the first stage of oxidation, that is, $\delta = \delta_0$, it can be seen that for all compositions the spontaneous magnetization does not reverse. Further oxidation results in the reversal of magnetization for initial compositions corresponding to $x > 0{\cdot}6$ at the degree of oxidation given in Table 1 and shown in Fig. 1.

Table 1

Initial composition, x	Degree of oxidation, z First stage	Reversal
0	1	—
0·1	1	—
0·2	1	—
0·3	0 911	—
0 4	0·837	—
0·5	0·772	—
0 6	0 713	1·0
0·7	0 664	0·837
0 8	0·620	0·691
0·9	0 526	0 561
1·0	0·444	0·444

(*B*) *Spinel-rhombohedral intergrowth.* The intergrowth of ferrimagnetic and antiferromagnetic materials provides a system in which exchange anisotropy can operate[11]. The antiferromagnetic haemo-ilmenite exsolves in the (111) planes of the spinel host. It was first pointed out by Gorter[12] that as the (111) plane of the spinel has an oxygen arrangement which, like the (111) plane in haematite is

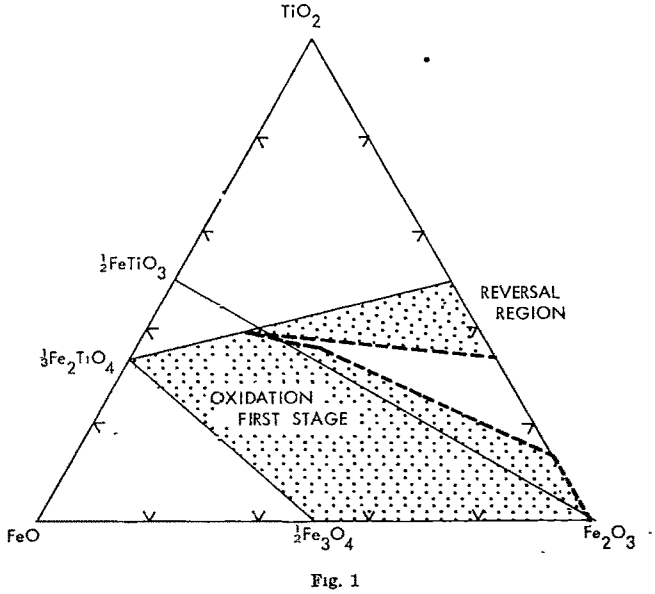


Fig. 1

close packed, and that as the unit cell dimensions are not too different it is possible for a super-exchange interaction to be active across the shared boundary layer. If, in such a system, the antiferromagnet is ordered in an external field above the Néel point of the ferrimagnet, and on cooling, the B sub-lattice spins of the ferrimagnet are coupled antiparallel to the cooling field, then the thermoremanent magnetization will be opposed to the external field.

Another mechanism for self-reversal lies in the decomposition of a highly oxidized cation-deficient spinel which has an A sub-lattice predominant moment. The remanence of the resulting magnetite–ilmenite intergrowth will be due to the B sub-lattice and so the decomposition will have the result of reversing the spontaneous magnetization of the system.

Discussion

Exchange anisotropy has been found in naturally occurring samples of maghemite partially inverted to haematite[13], but has not yet been reported for rocks containing oxidized titanomagnetites. A preliminary investigation of oxidized synthetic titanomagnetites[14] has not revealed exchange anisotropy.

Self-reversal by the oxidation of a titanomagnetite with a B sub-lattice predominant moment to one with an A sub-lattice predominant moment may not be possible because of the high degree of oxidation necessary for reversal. A further point is that the progressive increase in the number of vacancies may produce anomalous thermomagnetic curves of the L and N types It is more than possible that the Néel points will be below room temperature, or in the case of N-type curves, compensation points will be below room temperature and a B sub-lattice spontaneous magnetization will predominate at room temperature.

Because the cation distribution in quenched samples of titanomagnetites is now known[5], it seems probable that quenched samples of oxidized titanomagnetites will not have a random distribution of cations and that reversal by the order–disorder mechanism[10] is unlikely.

The production of a highly oxidized cation-deficient spinel at high temperatures is possible under the stabilizing effect of suitable magmatic gases. If this has the same cation distribution as the hypothetical oxidized titanomagnetite with the same composition, then an A sub-lattice moment will result. Subsequent decomposition to a magnetite–ilmenite intergrowth will then produce a reversal of magnetic moment. This would provide a physical model for explaining the correlation between reversed lavas[1,2] and the signs of a high degree of oxidation. To check this for natural samples, the bulk chemical compositions of titanomagnetites, with or without intergrowths, in rocks must be determined, and correlation between reversed polarity and chemical composition sought. If our hypothesis is correct, the bulk composition of reversed lavas should correspond to highly oxidized titanomagnetites of large x value. The most suitable technique for such an experiment would probably be electron probe X-ray microanalysis and chemical analysis.

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DISTRIBUTION OF THE NUMBER OF CELLS IN A CLONE—A MONTE CARLO CALCULATION

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WHEN mammalian cells from a monolayer culture are plated by the Puck technique[1] and each cell allowed to grow into a clone, then it is immediately obvious that there is a considerable range of the sizes of the clones. A detailed investigation of the distribution of the clone sizes for HeLa cells has been made by Nias, Gilbert, Lajtha and Lange[2] and a typical distribution shown in Fig. 1 has a mean clone size of twenty-four cells at 7 days; 10 per cent of the clones have less than four cells, 10 per cent more than forty-five cells and some clones have up to eighty cells. In such experiments cells are plated from an asynchronous exponentially growing population, and since the cells are at different phases of their cell cycle a range of clone sizes must be expected. However, if all cells had a constant generation time (time between consecutive cell divisions) then a total range of clone sizes of only twofold would be expected. The mean number of cells in the clones is observed to increase exponentially from the time of plating and so there is no net division delay which might disturb the early development of the clones.

The factors which might cause the wide distribution of clone sizes are: (*a*) a variation of the cell generation times; (*b*) the production of a proportion of cells at each division which undergo no further division; (*c*) a correlation between the generation times of the mother and daughter and between sister cells. A theoretical study has therefore been made of the influence of these factors

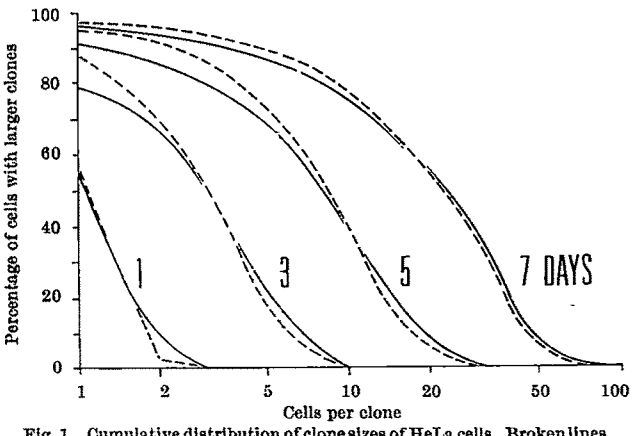


Fig. 1. Cumulative distribution of clone sizes of HeLa cells. Broken lines, experimental distribution. Fulllines calculated from the generation time distribution (*a*) of Fig. 2

on the clone-size distribution. As it was not possible to calculate an explicit probability distribution function for the clone sizes, an experimental numerical method was used, the so-called Monte Carlo method (for a general account of the methods see Hammersley and Handscomb[3]). Paper calculations are made for a sample number of clones (say, 100) and each clone is built up in detail by assigning to each cell a generation time determined in a suitable random way.

For the theoretical calculations it is necessary to have a clear set of basic assumptions. It is assumed that all cells are potentially the same (except for any cells which may have been designated sterile) and that essentially the same population could be grown from any individual cell. Cells are assumed to have generation times which vary in a random way but which conform to some postulated probability distribution function. The generation times may be quite independent of those of the ancestors of the cell or alternatively some degree of correlation between generation times of mother and daughter and between sister cells may be postulated. It is assumed that the cells which are plated are selected at random from a population which is in exponential growth and which has been growing long enough to be asynchronous.

The calculations were programmed for the *Atlas* computer at the University of Manchester and contain two essential parts: (*a*) the growth of a clone over an arbitrary time interval and (*b*) the selection of a cell to begin a clone. The basic steps in growing the clone from a time T_1 to a time T_2 are as follows: the clone at T_1 has n cells and the times, after T_1, at which they will next divide are stored in the first n locations of a store array. Each cell is scanned in turn until a cell is found the time for division of which is earlier than T_2. Division of this cell to create two new cells is then simulated. The next two random generation times are added to the time of this division to give the times at which the divisions of the two daughter cells will occur. The cell number is increased by one, and one daughter cell is stored at the end of the array of cells and the other in the place of the old cell. This cell is now re-scanned and, according to the time of its division, either another division made or the scan passed on to the next cell in the array. When the scan has passed all cells in the array (including the recently created ones), all cells will have their next time of division after T_2 and the total number of cells in the array is the size of the clone at T_2. In this way, starting from one cell at time zero, the size of the clone at a sequence of times $T_1, T_2, \ldots$ can be calculated.

The selection of the cells at zero time to start the clones must be made with care, because the times to their next division must conform to the statistics appropriate to an asynchronous exponentially growing population. The clones being grown by these calculations form just such a population and so previous clones provide material from which to sample for the next clone. The first cell of the first clone has, however, to be chosen in an arbitrary fashion, and, in effect, the whole population is grown from this first cell. A sampling population for initiating the clones is built up by storing the final cells of each clone and when the store is full by overwriting the earlier ones, so that an ever-changing population of finite size is maintained which has the proper statistical properties.

The random generation times required at each cell division are obtained as follows: first a random number, uniformly distributed in the interval 0–1, is generated by a standard computer routine, and then a transformation applied to it so that, in effect, a random selection from the postulated distribution of generation times is obtained. To produce correlations between mother–daughter and sister–sister generation times, two independent random times T_1 and T_2 are first obtained from a postulated distribution and then two generation times T_3 and T_4 are calculated from:

$$T_3 = \alpha T' + \beta T_1 + \gamma T_2$$
$$T_4 = \alpha T' + \gamma T_1 + \beta T_2$$

where T' was the generation time of the mother cell and:

$$\alpha + \beta + \gamma = 1$$

The degrees of correlations can be adjusted by the choice of α and β. A proportion of sterile cells can be obtained by assigning a very long generation time to some cells as a result of a draw of a random number. The distribution function and the correlations of the generation times actually used in the calculation are computed.

Before presenting the results of the calculations of clone-size distributions mention should be made of an interesting facet of the distribution of generation times in an exponentially growing population which was noticed during these calculations. If a random sample of cell divisions is taken from the growing population, then there is associated with each division one mother cell and two daughter cells. The average generation time of the mother cells is always less than the average of the daughter cells, except when all the generation times are equal and then the averages are also equal. The reason for the difference is because the selection of cell divisions gives an unbiased sample of daughter cells but a biased sample of mother cells; the mother cells with short generation times come from divisions later in the population growth and so will be overweighted. The relation between the mean generation time of the mother cells, T_m, and daughter cells, T_s, is given by the following equations:

$$T_s = \int_0^\infty f(t)t\,dt$$
$$T_m = 2\int_0^\infty f(t)e^{-bt}t\,dt$$
$$1 = 2\int_0^\infty f(t)e^{-bt}\,dt$$

where $f(t)$ is the differential distribution of the generation times of the daughter cells and b is the exponential growth-rate of the population. It should therefore be noted that when a distribution of generation times is observed by time-lapse cinematography it will be the distribution $2f(t)e^{-bt}$ which is most economically measured since the mother cell of all cell divisions except the first can be used.

On the basis of the foregoing methods, Monte Carlo calculations of clone-size distributions were first made on the assumption that there is no inter-cell correlation of the generation times and good agreement with experimental values could be obtained using a suitable distribution of generation times. This is shown in Fig. 2 (*a*) and

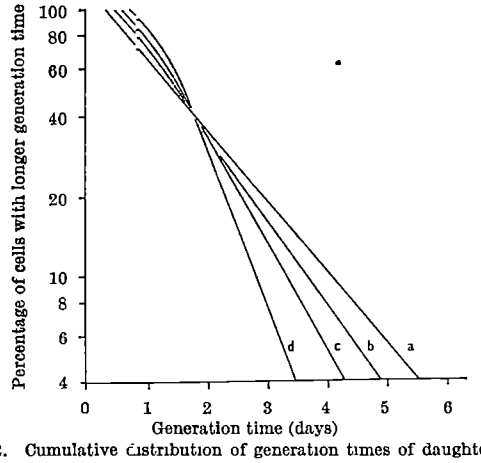


Fig. 2. Cumulative distribution of generation times of daughter cells

	Mean cell generation time, days	Coefficient of variation (per cent)	Correlation Mother–daughter	Correlation Sister–sister
a	1·96	82	None	None
b	1·90	73	0·07	0·12
c	1·86	61	0·14	0·30
d	1·77	45	0·31	0·53

the corresponding clone-size distributions in Fig. 1. No cell is assumed to divide before 0·35 day and thereafter the distribution of generation times has an exponential form, giving a total mean time of 1·96 days. Some other types of distributions were investigated, in particular sigmoid distributions based on the Gaussian curve, but these do not give the broad clone-size distribution observed. A similar type of calculation has been made by Hoffman, Metropolis and Gardiner[4].

Next the effect on the clone-size distribution of the production of a random proportion of sterile cells at each cell division was investigated. The clone-size distribution is only broadened significantly if the proportion of sterile cells is large; the Monte Carlo calculations of Till, McCulloch and Siminovitch[5] confirm this though they were made in a somewhat different context. Experiments show the HeLa cells to have a high plating efficiency and so the production of sterile cells is not an important factor in their clone-size distribution.

Calculations were made using various degrees of correlation between mother–daughter and sister–sister generation times and these gave a marked broadening of the clone-size distribution. The distribution of generation times shown in curves (*b*), (*c*) and (*d*) of Fig. 2 all predict clone-size distributions almost identical with those shown in Fig. 1, which had been calculated from (*a*) of Fig. 2. Correlation coefficients of the magnitude used here are not improbable since time-lapse ciné experiments of Dawson, Madoc-Jones and Field[6] have shown mother–daughter and sister–sister correlation coefficients of 0·19 and 0·56 for rat sarcoma cells in culture. All the distributions of generation times with the inter-cell correlations shown in Fig. 2 were obtained from the uncorrelated distribution of curve (*a*) by the method already described.

From the measurements of clone-size distributions alone it is not possible uniquely to determine the statistics of the cell generation times. Additional experimental evidence is needed and this can be got either from time-lapse cinematography of sample clones or perhaps from labelling experiments. Engelberg[7], and Anderson and Peterson[8] have shown that some information about the width of the generation time distribution can be obtained from measurements of the decay of an induced partial synchrony in a cell culture.

The results of these calculations show that the experimentally observed distribution of clone sizes can be adequately accounted for by a distribution of cell generation times with some degree of correlation between the generation times of the mother and daughter and the sister cells. It is emphasized that the wide distribution is largely developed during the early growth of the clone, for when the clone has more than about ten cells the variations average out and departures from the mean growth then become rare. The basic assumption of this model of clone development is that all cells are potentially alike. Cells will have generation times different from the mean because of stochastical variation of processes governing the development of the cell or because of local variations in its environment. It is not unlikely that some of these effects also influence the next generation of cells, but as the potentiality of all the cells is the same the effects do not persist through an indefinite number of generations.

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IDENTIFICATION OF TWO FOWL β MACROGLOBULINS WITH PRECIPITATING ANTIBODY ACTIVITY

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ALTHOUGH there are well-documented similarities between avian and mammalian serum proteins, some significant differences have already been recorded. These include the faster electrophoretic mobility of chicken γ globulin[1,2], the high salt requirement for optimal precipitation of fowl antigen–antibody complexes, and the greater sensitivity[3] to 2-mercapto-ethanol of fowl 7*S** γ globulin[4].

A further difference between mammalian and avian species is evident from the results of two related investigations which are reported here. The first concerns the isolation and characterization of three fowl macroglobulins and, in the second study, two serum proteins associated with the early precipitating antibody response in fowls are identified.

Recently, attention has been focused on the antibody activity of fowl macroglobulins, but conflicting results have been obtained. Franklin[5], in an investigation of the physico-chemical properties of chicken macroglobulins by ultracentrifugation in sodium chloride, isolated two high-molecular-weight components from the serum. Benedict *et al.*[6], using chromatography of immune fowl serum on DEAE-cellulose, obtained three distinct macroglobulin fractions, each of which possessed antibody activity. In contrast, antibody could not be detected in a macroglobulin fraction obtained by elution of immune fowl serum from a 'Sephadex *G*-200' column[7]. Likewise, Orlans *et al.*[8] were unable to obtain positive evidence of macroglobulin in specific precipitates of fowl antiserum and bovine serum albumin.

Fractionation procedures investigated in the present work included column chromatography on DEAE-cellulose and on 'Sephadex *G*-200', and preparative ultracentrifugation. Immunoelectrophoretic analysis and analytical ultracentrifugation were used to determine the individual components of particular fractions. By stepwise elution with increasing concentrations of sodium chloride on DEAE-cellulose, only very low yields of macroglobulins were obtained. Column chromatography on 'Sephadex *G*-200' produced one peak of macroglobulin, but this was shown by immunoelectrophoresis to contain both α and β globulins. These methods were therefore discarded in favour of ultracentrifugation and, of several

* In this article the old-style electrophoretic nomenclature is used, and not the proposed World Health Organization nomenclature, as the structure of avian antibodies is not yet known.

modifications tested, sucrose gradient centrifugation was found to give the most satisfactory separation of high-molecular-weight components.

Fresh chicken sera were initially concentrated by spinning in a No. 40 rotor at 60,000*g* in a 'Spinco' model *L* preparative ultracentrifuge for 16 h at 4° C. The resultant supernatant was discarded and the pellet was taken up in a minimum quantity of *tris* buffer containing either 1·0 M urea or 0·5 M glycine at *p*H 7·6. Of the total protein in this preparation, 30–40 per cent was macroglobulin. A volume of 0·5 ml. of the dissolved pellet was layered on top of 20 ml. of 10–30 per cent sucrose gradient prepared in the same *tris* buffer.

After centrifuging in an '*S.W.*' swinging bucket rotor at 50,000*g* for 16 h, fractions of 0·5 ml. were collected by downward displacement. Alternate fractions were diluted with 3·0 ml. of the same *tris* buffer solution. Their optical densities, measured at 275 mμ in a Beckman spectrophotometer, were plotted against the fraction number. The remaining fractions were analysed by immunoelectrophoresis.

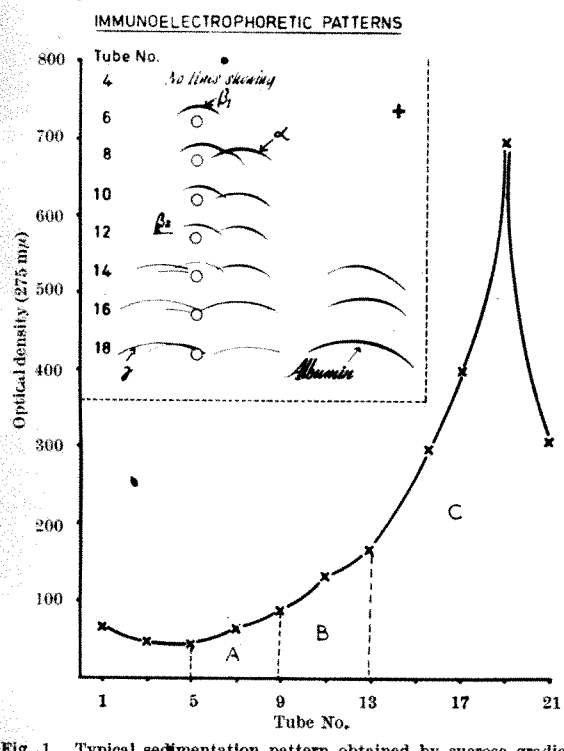


Fig. 1. Typical sedimentation pattern obtained by sucrose gradient ultracentrifugation of fowl serum, with inset showing immunoelectrophoretic patterns of alternate fractions developed against rabbit anti-fowl serum. Region *A* (fractions 5–9) contains 19*S* components, region *B* (fractions 10–13) contains 19*S* and >19*S* material, and region *C* (fractions 14–21) contains both 7*S* and 4*S* components

Fig. 1 shows a typical sedimentation pattern of the dissolved pellet after sucrose gradient centrifugation, together with immunoelectrophoretic patterns of alternate fractions.

In order to locate the macroglobulin components, analytical ultracentrifugation was carried out on several samples of the eluate using a 'Model *E*' analytical ultracentrifuge at 59,780 r.p.m. and 20° C. Region *A* (fractions 5–9) was shown to contain components with sedimentation constants of greater than 19*S*, while region *B* (fractions 10–13) contained 19*S* material and some material greater than 19*S*. Region *C* (fractions 14–21) contained both 7*S* and 4*S* components. As no trace of 7*S* material was evident in Region *B*, the line which first appears in the β_2 region in fraction 12 represents a β_2 macroglobulin and cannot be attributed to the 7*S* γ globulin component. This latter protein appeared in subsequent tubes and gave a characteristic immunoelectrophoretic line clearly distinguishable from that of the 19*S* macroglobulins (Fig. 1, fractions 14–18).

The macroglobulins, therefore, were eluted in fractions 4–13, and by immunoelectrophoresis were shown to contain three components: two β globulins and one α globulin.

Of the two β macroglobulin components, the β_2 macroglobulin has been recorded previously[9], and has immunoelectrophoretic characteristics similar to human β_2 macroglobulin. The other is a macroglobulin which does not migrate from the origin and therefore we suggest that it be referred to provisionally as a β_1 macroglobulin. Antibody studies to be described below discount the possibility that this is denatured material at the origin. Dreesman *et al.*[10], in a recent investigation of a component in chicken serum of similar electrophoretic mobility, reported on its antibody activity and called it a β_2A globulin. However, its position relative to the transferrin and γ globulin lines is different from that of human β_2A globulin, which, moreover, has a lower sedimentation range.

Fowl serum, therefore, possesses two β macroglobulins compared with one in human serum.

Having established the immunoelectrophoretic characteristics of these 19*S* components, we then sought to identify the type of proteins involved in the antibody response of fowls to bovine serum albumin (BSA). Fowls were given immunizing doses of BSA in Freund's incomplete adjuvant. Each bird received two injections of antigen (40 mg/kg) given 16 days apart. Serum samples were collected daily. A previously described modification[11] of immunoelectrophoresis was used to localize in the immunoelectrophoretic spectrum those globulins which possessed antibody activity. The immune serum was subjected to electrophoresis and a solution of the immunizing antigen was placed in the outer troughs. However, since the optimal precipitation of fowl antigen–antibody complexes occurs in the presence of high salt concentrations, a further modification was introduced. Following electrophoresis of the immune serum, the agar strips were removed from the glass supports and placed on agar slabs prepared in 10 per cent saline.

No antibody was detected in the sera for 5 days after the first injection or for 3 days after the second injection. On the sixth day after the second injection, two lines of specific antigen–antibody precipitate were evident (Fig. 2*a*), one remaining at the origin and one extending from the origin into the β_2 region. Thereafter the β_2 line diminished and the β_1 line became more prominent

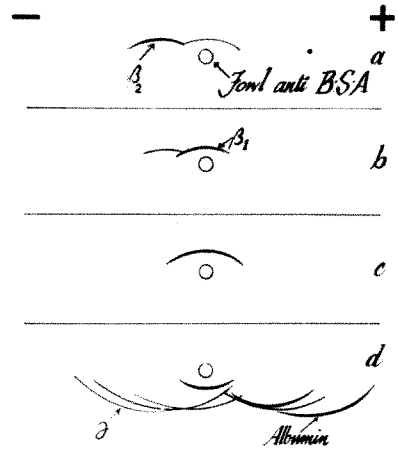


Fig. 2. Localization of antibody activity in immunoelectrophoretic spectrum of fowl antibody to BSA. Diagrams 2*a*–2*c* represent lines of precipitation of electrophoresed fowl anti-BSA developed by linear diffusion against BSA, and represent progression from a preponderance of β_2 to β_1 type antibody: (*a*) at 6 days; (*b*) at 10 days; (*c*) at 15 days. For reference, diagram 2 (*d*) is a normal immunoelectrophoretic pattern of fowl anti-BSA serum developed against rabbit anti-fowl serum

(Fig. 2*b* and *c*). There was a temporary disappearance of these lines for 3 days immediately following the second injection of antigen, after which only the β_1 line was observed.

The β_2 line of anti-BSA precipitating antibody was distinguished from 7*S* γ globulin by direct comparison with the conventional immunoelectrophoretic pattern of fowl anti-BSA serum developed against rabbit anti-fowl serum (Fig. 2*d*). No 7*S* γ globulin line was seen using the "inverse" immunoelectrophoretic technique at high salt concentration.

Our failure to observe any 7*S* γ globulin precipitating antibody in the primary response of fowls to BSA is at variance with the findings of Dreesman *et al.*[10]. They have already noted considerable variation in the relative preponderance of 19*S* or 7*S* anti-BSA antibodies made by individual fowls, and suggest that this may be due to variations in either the exact nature of the antigen or the particular technique used to test antibody response. It may well be that the "inverse" immunoelectrophoretic technique in high salt concentration used in this investigation is more sensitive in detecting macroglobulin than 7*S* γ antibodies, but it has nevertheless enabled us to distinguish two separate β globulin components within the class of anti-BSA precipitating antibodies.

The sequence of appearance of antibody types reported here provides evidence that the β_1 line is not due to denatured material such as is sometimes seen at the origin, or to non-specific heterophil antibody activity[10].

Thus early fowl antibody may be associated with both β_1 and β_2 globulins. Since these two globulins share the immunoelectrophoretic characteristics of the two β macroglobulins isolated by sucrose gradient centrifugation, the results of the two investigations considered together suggest an immunological role for the two fowl β macroglobulins. Although no precipitating antibody activity was observed in the α globulin region, a reaction of antigenic identity was demonstrated between the α and β_1 macroglobulin fractions (Fig. 3). These two fractions were pooled and subjected to immunoelectrophoresis against antiserum from rabbits injected with these two fractions. The reaction of identity between these two macroglobulins is evidence that they share at least some common protein sub-unit in their structure and synthetic mechanisms, even if they do not share the antibody combining configurations.

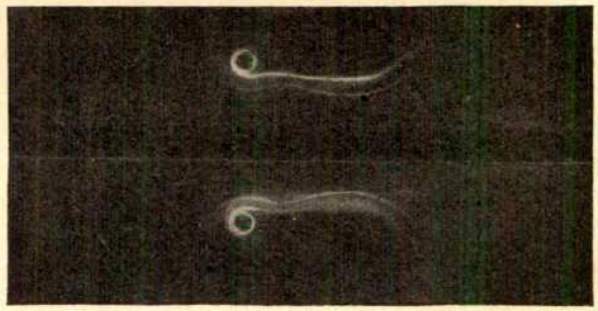


Fig. 3. Precipitation patterns obtained by electrophoresis of pooled α and β_1 fowl macroglobulins and linear diffusion against rabbit antiserum potent against these two fractions. This antiserum was obtained by booster injections of pooled α and β_1 macroglobulins into rabbits already producing polyvalent anti-fowl whole serum antibodies. This explains the faint precipitin lines seen here against trace contaminants of β_2 and γ globulin fractions

This finding prompts speculation concerning a possible link between mammalian immunoglobulin synthesis and the non-specific inflammatory response of α_2 globulin levels. A search for shared polypeptide chain structures between mammalian α_2 and γ globulins might well be profitable.

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ELIMINATION OF NON-SPECIFIC ANTIBODIES FROM RABBIT ANTISERA USING ACETONE-PRECIPITATED SOLUBLE PROTEINS

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AFTER repeated injections of influenza virus or its subunits into animals, non-specific antibodies were often found to be present in the antisera. These non-specific antibodies are directed against components of the host cells and may cause reactions, for example, in the complement fixation test and the fluorescent antibody technique.

If the same antigen is used for the induction of antibodies and the respective antigen–antibody reactions, it is necessary to eliminate the non-specific component from either the antigen or the antiserum or from both.

A number of methods are available for the elimination of non-specific immunoglobulins or free labelling agents: serum dilution, dialysis, serum adsorption with preparations of tissue or cells[1–4], tissue powders[5,6], tissue homogenates[7], charcoal[8], gel-filtration on 'Sephadex'[9], electrophoresis[1,10], chromatography on cellulose ion-exchangers[11–15], adsorption with antigen fixed to slides[16], and dissociation of specific antigen–antibody complexes[17,18].

For the complete removal of the non-specific antibodies we have used the homologous antigen in form of a water-insoluble protein precipitate of chorioallantoic fluid, prepared by a modified method of Coons *et al.*[6]. Using this powder for adsorption of the non-specific antibodies, no significant drop of virus specific complement fixation titre (CFT) and haemagglutination inhibition titre (HIT) of the antiserum could be observed. For preparation of protein powder see Fig. 1.

Influenza virus A_2/Singapore 1/57 was propagated in 10–12-day-old chicken embryos and purified by differential centrifugation. Subunits were prepared by treatment with ether-'Emasol-1130'[19], adsorption, and elution with fowl red-cells. Haemagglutinin was purified by chromatography on DEAE-cellulose[20], *s*-antigen by centrifugation[21]. Rabbits were immunized with either virus particles, haemagglutinin or *s*-antigen by one intramuscular and three intravenous injections. The immunosera were obtained two days after the last intravenous injection to produce virus or haemagglutinin antibodies. Ten days after the last intravenous injection, blood was withdrawn to obtain *s*-antibodies. The complement fixation technique was performed in accordance with the method of

Non-infected chorioallantoic fluid cleared by low speed centrifugation
↓
add 4 volumes of acetone slowly with stirring, allow the mixture to stand for 5 min, centrifuge for about 10 min at 500*g*, discard the supernatant.
↓
sediment
wash three times with 80 per cent acetone by centrifugation.
↓
sediment
dry over-night at 37° C, powder in a mortar.
↓
powder
shake with sodium chloride solution for 24 h at 4° C, wash three times with 80 per cent acetone by centrifugation, dry over-night at 37° C, powder in a mortar.
↓
water-insoluble protein powder.

Fig. 1. Preparation of water-insoluble protein powder from non-infected chorioallantoic fluid of 12-day-old chick embryos

Lief *et al.*[3]. For the haemagglutination inhibition technique on 'Plexiglass' plates we used serial two-fold dilutions of 0·25 ml. antiserum (RDE-incubated), 16 haemagglutinin units per 0·25 ml. and 0·5 ml. of 0·5 per cent fowl red cell suspension. The antisera from rabbits containing non-specific antibodies were shaken over-night at 4° C with protein powder followed by centrifugation at 2,000*g* for 15 min. Only 0·04 per cent of the protein powder was resolved after shaking for 24 h in isotonic sodium chloride solution at room temperature. The results of the serum treatment are shown in Table 1.

Table 1. ELIMINATION OF NON-SPECIFIC ANTIBODIES FROM RABBIT SERA

Experiment	Amount of powder (mg/ml. serum)	Antigens for CF-reaction infected CAF CFT	non-infected CAF CFT
	50	200 + + + +	< 5
	25	200 + + + +	< 5
1	10	200 + + + +	50 +
	5	200 + + + +	100 +
	0	400 +	100 + + +
	15	320 +	20 + + + +
	10	320 +	40 + + + +
2	5	320 +	40 + + + +
	0	320 +	80 + + + +
	0 without shaking	320 +	80 + + + +
	50	640 + + + +	< 5
	25	640 + + + +	< 5
3	10	640 + + + +	20 + +
	5	640 + + + +	20 + + +
	0	640 + + + +	20 + + + +

(Experiments 1 and 2: anti-*s*-sera; experiment 3: anti-haemagglutinin-serum.) Elimination by shaking the sera with protein powder over-night at 4° C. CFT, reciprocal serum dilution giving complete (+ + + +) or decreasing degrees (+ + +, + +, +) of fixation of complement.

The effect of adsorption corresponded to the amount of powder used. In the aforementioned experiments, 20 mg of protein powder caused complete adsorption of the non-specific antibodies. This indicates that elimination of non-specific antibody is due to an antigen–antibody reaction.

If a protein powder is prepared by only one short treatment of soluble protein, with acetone, the same amount of powder is required for the elimination of the non-specific antibody, but a higher resolution of powder takes place in this case.

In Fig. 2 the amount of protein powder is plotted against the level of resulting CFT of antibodies after treatment.

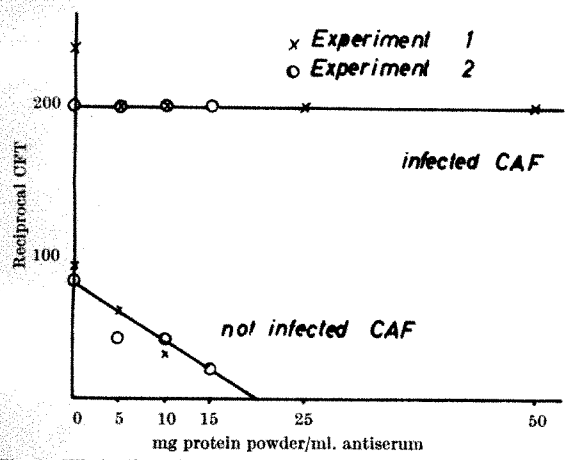


Fig. 2. Elimination of non-specific antibodies by protein powder from rabbit antisera immunized with *s*-antigen. Infected and non-infected chorioallantoic fluids in a constant dilution of 1 : 4 were used as antigens against serial dilutions of anti-*s*-serum for the CF-test. CFT see Table 1. CFT 1 : 320 + is plotted as CFT 1 : 200

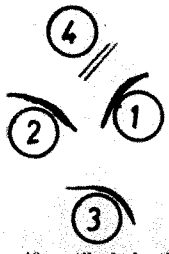


Fig. 3. Detection of non-specific antibody by the agar gel diffusion technique of Ouchterlony. (1) Anti-*s*-antigen serum before adsorption. (2) The same serum after adsorption. (3) Non-infected CAF. (4) RNA-containing fraction extracted from infected chorioallantoic membranes after removal of haemagglutinin by fowl and sheep red cells and after centrifugation of the *s*-antigen (40,000 r.p.m., 120 min, Spinco *L*, rotor 50)

It shows that the level of specific antibody remains constant in the serum, while the non-specific antibody is adsorbed.

Furthermore the adsorption of the non-specific antibody from anti-*s*-serum has been examined by agar gel diffusion. The corresponding data are shown in Fig. 3.

There was no precipitation of the adsorbed antiserum with the non-specific protein of allantoic fluid. By agar-gel diffusion no difference of the antigenicity was observed between *s*-antigen from the membrane and another from virus particles disintegrated with ether.

The effects of protein powder treatment on HIT and CFT are shown in Table 2. Adsorption was carried out with anti-virus, anti-haemagglutinin and anti-*s*-antigen sera. Non-specific antibodies could be adsorbed without visible decrease of virus-specific ones.

Table 2. EFFECTS OF PROTEIN POWDER TREATMENT ON HIT AND CFTP, USING ANTI-VIRUS-, ANTI-HAEMAGGLUTININ-, AND ANTI-*s*-ANTIGEN SERA

Antisera	Antigen CAF	Powder mg/ml. serum	Before treatment CFT	HIT	After treatment CFT	HIT
Anti-	infected	10	800 + +	2000	800 + +	2000
virus	not infected		50 +	0	< 5	0
Anti-	infected	10	880 + +	1200	800 + +	1200
hemaggl.	not infected		50 +	0	< 5	0
Anti-	infected	20	400 + + + +	0	400 + + + +	0
s-antigen	not infected		100 + + +	0	< 5	0

The use of powder from a soluble protein seems preferable for the following reasons: (1) The existence of corresponding antigens for adsorption of non-specific antibodies. (2) The absence of solid cell particles. (3) The low or non-existent adsorbing activity for specific antibodies. (4) The lack of dilution of the immune serum. (5) The insolubility of the powder in the sera. (6) The constancy of the antigenicity after storage for 1 week at 37° C. (7) The convenience of producing large amounts. (8) The possibility of sterile conditions.

The use of soluble proteins only, instead of cells, tissues, or tissue homogenates, may be more effective in the adsorption of non-specific antibodies.

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ANTIBODY FORMATION AND EXPERIMENTALLY INDUCED CHIMERISM IN VERY YOUNG RABBITS

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THE injection of antigens into animals may result in one of three possible events: immunological tolerance may be induced, antibody may be formed, or there may be failure to react to the antigen in any detectable way. Which of these three reactions is observed depends on the nature and quantity of the administered antigen and on the age and immunological experience of the injected animal[1]. We shall show that, given identical quantities of human albumin, any of these three responses may be observed in rabbits, depending on the form in which the antigen was administered to the new-born animal.

If new-born rabbits were given 2·8 μg N of human albumin, within 36 h of birth, antibody could not be detected in the circulation in any of these animals during the subsequent 4 weeks. Tests for the elimination of radioactively labelled (iodine-131) human albumin[2] showed that 28 per cent (2/7) of 30-day old animals and 11 per cent (3/27) of 70-day old animals were tolerant (compare with ref. 3). If a similar quantity of protein (1–4 μg N) was associated with nucleated adult rabbit cells, and was then injected into new-born animals, a considerable proportion of the animals contained antibody to human albumin in their circulation 2–4 weeks later. The questions arose whether, (*a*) antibody formed in these circumstances was the product of donor cells or whether it was synthesized by the recipient animals; and if it is the latter, whether (*b*) a product of the donor cell can be detected. The experiments described here were designed to answer these questions. They showed that the antibody induced by antigen, associated with nucleated donor cells, was produced by the cells of the recipient animals, and that in some recipient animals, soluble products from donor cells were formed and could be detected in the circulation for long periods of time.

Cells of normal donors were obtained from thymus, lymph node or as peritoneal exudates, rich in mononuclear cells. The separated cells (from six unrelated donors) were incubated for 30 min at 37° C with radioactively labelled (iodine-131) human albumin and were then washed ten times with Eagle's basal medium. The amount of human albumin associated with the washed cells was determined by radioactive counting. Twelve hours and 36 h after birth, rabbits were injected with 1–4 μg N human albumin, associated with about 80×10^6 lymph-node cells, thymus cells or with peritoneal exudate cells, rich in mononuclear cells. The injected animals were then bled at weekly intervals.

Within 2–3 weeks after the neonatal injection with albumin-treated cells, the majority of the animals had circulating antibody to human albumin which could be demonstrated by its capacity to agglutinate tanned erythrocytes, sensitized with human albumin[4]. Antibody was induced by albumin-treated peritoneal exudate cells (rich in mononuclear cells) in 43/56, by albumin-treated lymph-node cells in 42/72 and by albumin-treated thymus cells in 18/33 of the injected animals.

We had thus found that a quantity of human albumin, which had induced tolerance in 28 per cent of injected animals, when given in soluble form, induced antibody in 55–80 per cent of injected animals, when given in association with nucleated cells. It seemed quite possible that this difference in responsiveness was due to the production of antibody by the transferred cells, as had been found by Harris *et al.*[5] (see also refs. 6–10) under different experimental conditions. To investigate this possibility, we used allotypic markers for the discrimination between cell products of donor and recipient animals.

Animals, used as recipients and donors, differed in the allotypic specificity of their immunoglobulins and possessed either allotypic specificity *A*4 or *A*5, which are known to be located on the light chains and to be determined by two allelic genes A_b^5 and A_b^5 (ref. 11).

Antibody produced by animals injected with cell associated human albumin was removed from the sera by incubation with human albumin, covalently linked to cellulose particles[12,13]. After incubation, the cellulose particles were separated from the serum by centrifugation, were washed, and antibody was eluted at *p*H 3·0. The eluate, adjusted to *p*H 7·4, was then tested for antibody activity and for the presence of allotypic specificities of donor and recipient types.

The quantity of allotypically defined immunoglobulins in isolated antibody or in whole serum was estimated by two techniques: by haemagglutination inhibition[14] and by single diffusion[15]. The first of these techniques is capable of detecting very small relative quantities of allotypically marked immunoglobulin, but with a low level of precision. The second method gives relative immunoglobulin quantities with an error of 5–10 per cent, but is much less sensitive than the first. Where both methods could be used, the agreement between relative values obtained by these two methods was good.

The quantity of antibody recovered from the 'immuno-adsorbent' was generally between 25 per cent and 100 per cent of the total antibody, but for serum from 2–3-week-old animals was usually lower: between 5 per cent and 20 per cent.

The isolated human albumin antibody from animals injected at birth always contained allotypic specificity of the recipients and never the allotype of the donor of the transferred cells.

It seems reasonable to assume that the recovery of antibody of donor and recipient origin would be comparable and that low recoveries of early antibody will therefore not affect the validity of our conclusions. We conclude, therefore, that transferred cells do not synthesize an important fraction of the antibody; and that the remarkable difference between the response of new-born animals to the same antigen in soluble form and in association with nucleated cells must be attributed to the potentiation of the immunogenicity of the antigen and presumably to some kind of mediation between antigen and the synthesis component of the immune apparatus of the new-born animal.

While we had thus shown that the transplanted cells did not produce antibody to human albumin, it remained to be seen whether or not they or their descendants produced any product, detectable in the circulation. To test for this, we examined and assayed the immunoglobulins in the whole serum of animals, which had received human albumin associated with nucleated cells, using the same techniques as were previously used for the examination of the specificity of isolated antibody to human albumin. Although all the antibody to human albumin was synthesized by the recipient, substantial quantities of immunoglobulin of donor type, not directed against human albumin, were found in the serum.

The allotypic specificity of the donor cells could be observed, in most recipient animals, for a period of 4–10 weeks with a continuous decline in concentration until it was no longer detectable (Fig. 1). The half-life

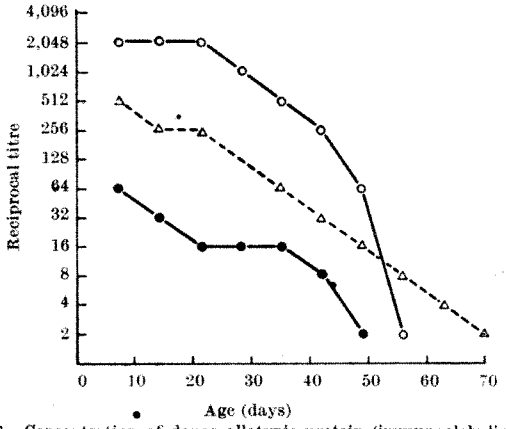


Fig. 1. Concentration of donor allotypic protein (immunoglobulin) in the serum of rabbits injected at birth with nucleated cells. Allotype of recipient animals was A5 and allotype of donor animals was A4. Concentration of allotypic immunoglobulin was measured by agglutination-inhibition. ———●——— and ———○———, Concentrations of allotypic protein of donor type in a rabbit injected at birth with lymph-node cells; – – – △ – – –, concentration of allotypic protein of donor type in a rabbit injected at birth with cells from peritoneal exudates, rich in mononuclear cells

of disappearance, measured by agglutination-inhibition, was about 7 days (not corrected for increasing body-weight) (Fig. 1). So as to be able to interpret the significance of half-life measurements, we determined the half-life of allotypically labelled immunoglobulins under conditions where synthesis could not affect its concentration, that is, after passive transfer of immunoglobulin of one allotype to new-born animals, homozygous for another allotype. The half-life determined under these conditions was 7 (± 1) days.

These results indicate that there was no synthesis of immunoglobulins by the transferred cells during the period of observation. However, in other individuals of the injected group, synthesis by the transferred cells did occur (Fig. 2). In 8 per cent (2/24) of the animals injected with albumin attached to lymph node cells and in 28 per cent (7/25) of the animals injected with thymus cells, the foreign allotypic specificity could be detected and measured for 5 months.

In the circulation of 8 per cent of animals injected with lymph-node cell suspensions we observed an immediate increase in the concentration of the foreign allotypic specificity, followed by a decline and then a second increase which lasted for about 2 months; later, the concentration of allotypic specificity remained fairly constant. In 28 per cent of the animals injected with thymus cell suspensions, there was a continued increase in concentration for the first 2 months of life. As in the case of animals injected with lymph node cells, the concentration of "foreign" allotypic specificity reached a fairly constant level when the animals were 8 weeks old. This stabilized concentration was maintained throughout the period of observation. In the case of the animals which had received thymus cells, the concentration was close to that of untreated heterozygous (A^4/A^5) animals, and was 10–20 per cent of this value in animals which had been injected with lymph node cells (Fig. 2).

Our experiments do not allow us to decide whether information transfer to recipient cells or colonization by donor cells is responsible for the formation of the allogeneic immunoglobulin. It seems reasonable, however, to assume that chimerism[16–31] has been produced within a non-inbred animal population by the transfer of cells to new-born animals and that some of these animals produced the allogeneic allotype for the whole period of observation, and at concentrations identical with those of heterozygous animals.

The question arose whether the allotypic specificity of the donor animal could be demonstrated only in the homologous organ of chimeric rabbits, or whether it could be found in other parts of the body as well. To test this, lymph node, spleen, and bone marrow cells from an animal, in which the specificity of the donor had persisted for 6 months, were transferred to a new-born animal, and we could show the appearance and rapid decline of the foreign allotype possessed by the donor, in recipients of lymph-node cells (10/13), thymus (3/11) and spleen (2/2) but not of bone marrow cells (0/6).

It should be emphasized that the weight increase of the chimeric animals, produced in our experiments, was quite similar to that of normal animals and that there was no indication of runting.

We have thus observed that cell transfer within an allogeneic population has resulted in a remarkably high incidence of long-lasting synthesis of a serum protein which carries the marker of the gene-product of transferred allogeneic cells.

The diphasic appearance of the foreign immunoglobulin in the rabbits, injected with lymph-node cells, in which a prolonged increase in the concentration of allogeneic protein was observed, is of considerable interest (Fig. 2). It seems quite possible that, during the first phase, immunoglobulin was being produced by cells which were already fairly far advanced in their differentiation at the time of transfer and that the decline of immunoglobulin concentration reflected the life span of these differentiated cells. The second, long-lasting rise might be attributable to the synthetic activity of cells, much less differentiated at the time of transfer. Products from these cells would constitute an appreciable fraction of the immunoglobulins only after cell differentiation and cell division had progressed sufficiently. The difference between allotype production in animals injected with lymph-node cells and with thymus cells can, in all probability, be attributed to higher concentration of relatively undifferentiated cells in the thymus cell population, so that the initial phase of production by mature donor cells would be obscured, even if it should occur.

In summary, we have shown that the injection into new-born rabbits of a small quantity of albumin, associated with nucleated cells, induces an antibody response in the majority of animals, whereas the same quantity of antigen in solution fails to stimulate antibody formation or induces tolerance. The antibody produced after the injection of albumin, associated with nucleated cells, is of recipient origin. However, immunoglobulin carrying the marker of donor cells can be demonstrated in the

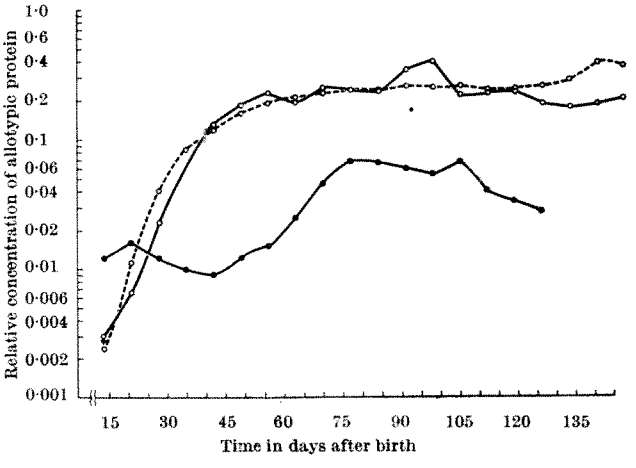


Fig. 2. Concentration of donor allotypic protein (immunoglobulin) in the serum of rabbits injected at birth with nucleated cells. Comparison between concentration of allotypic immunoglobulin in chimeric animals and in normal heterozygotes. Allotype of recipient animals was A5 and allotype of donor animals was A4. Allotype concentration was measured by a single diffusion method. ———○———, Concentration of allotypic protein of donor type in a rabbit injected at birth with thymus cells; ———●———, concentration of allotypic protein of donor type in a rabbit injected at birth with lymph-node cells; – – – ○ – – –, concentration of allotypic immunoglobulin in normal heterozygous animal

recipient animals, and may reach serum concentrations comparable with those normally present in animals which are heterozygous with respect to the marker.

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INTRANUCLEOLAR DEOXYRIBONUCLEIC ACID COMPONENTS IN INSECT CELLS AS REVEALED BY ELECTRON MICROSCOPY

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IN most plant and animal cells the nucleolus is associated with a particular chromosome of the complement at a specific region called the nucleolar organizer. As seen in usual cytological preparations, the chromatin of the organizer or the "associated chromatin" may be perinucleolar in many cells, including mammalian cells, or it may be at the central portion of the nucleolus as in chironomids. The deoxyribonucleic acid (DNA) of the organizer is sometimes referred to as "nucleolar DNA", but this part of the nucleolar-chromosomal complex is a specialized locus of the chromosome. Apart from it, no DNA is demonstrable in nucleoli in general by light microscope cytochemical methods and thus the widely held view that this organelle is devoid of DNA. In agreement with this view, DNA appeared to be absent in the nucleolus of the salivary gland cells of the chironomid *Smittia*[1,20]. This article presents evidence from electron microscope investigations to show that chromatin-like material which appears to contain DNA occurs inside the nucleolus proper and this will be referred to as "intranucleolar DNA".

The salivary glands were fixed for 20 min in cold (4° C) 10 per cent formalin, 6·5 per cent glutaraldehyde or a mixture of acrolein–formalin, 10 per cent each, in phosphate buffer at *p*H 7·4–7·6. Dehydration as well as embedding were in glycol methacrylate as described by Leduc, Marinozzi and Bernhard[2,3]. Sections were cut on a Porter–Blum 'Servall' microtome and subjected to various treatments including enzymatic extractions. The enzymes used were: 0·1 and 0·2 per cent RNase (Lights, five times crystallized) in distilled water adjusted to *p*H 6·8; 0·1 per cent DNase (Lights, once crystallized) in distilled water, *p*H 6·2; 0·5 per cent pepsin (Lights, twice crystallized) in 0·1 N hydrochloric acid; 0·33 per cent trypsin (Difco, 1 : 250) in distilled water, *p*H 8, and 0·25 per cent pronase (Calbiochem, grade *B*) in phosphate buffer at *p*H 7·4. Sections were stained either in 2 per cent aqueous uranyl acetate for 1 h or this followed by lead citrate[4] for 40 min and a 20-min stay in water.

A high-power electron micrograph of a section not treated with any enzyme, but double stained with uranium and lead, is shown in Fig. 1. The interesting feature here is the presence of extremely dense fibrillar masses dispersed in the nucleolus. Both in fine structure and electron density, they bear a close resemblance to the contents of the chromosomes. The fibrillar masses were not present in all sections of the nucleolus; when present, their numbers varied from one to four except in the few sections of the central portions of the nucleolus where a good number of them occurred. The larger masses (up to about 450 mμ in average diameter in this section) were situated close to the site of the organizer and the smaller ones (65–160 mμ in diameter) were dispersed in the inner regions of the nucleolus.

Many experiments were carried out with enzymes either singly or one followed by another and on material fixed in different aldehydes. The results which have a bearing on the chemical organization of the nucleolus at the ultrastructural level will be reported later (together

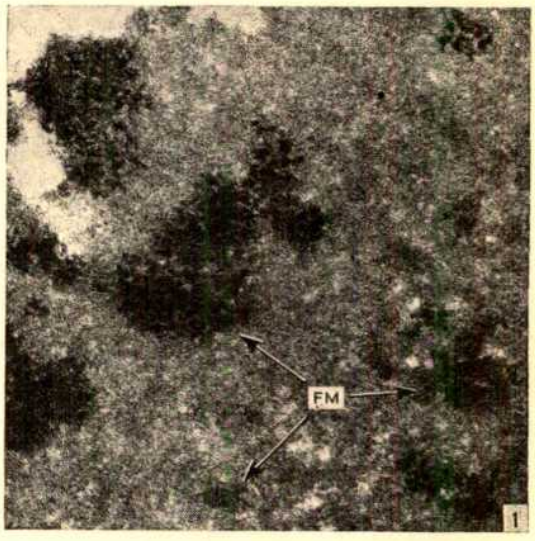


Fig. 1. Electron micrograph of nucleolus with dense fibrillar masses (*FM*) scattered within; the larger of these occur close to the site of the organizer. (Glutaraldehyde fixed and double stained with uranyl acetate and lead citrate. ×37,300)

with the methods in detail) and only those relevant for the present discussion are presented here.

If sections of glutaraldehyde-fixed glands are treated first with pepsin for 1 h and then with RNase also for an hour (all at 37° C), the nucleolar constituents are markedly extracted compared with normal. In such preparations the chromosomes are retained (Fig. 2) and small dense patches of chromatin-like material are seen distributed all over the nucleolus. In Fig. 2, the large patch close to the nucleolar organizer measures about 600 mμ in average diameter and is probably continuous with the chromatin of the organizer. The smaller ones within the nucleolus vary in size with average diameters of 50–170 mμ. If the order of enzymatic extractions is reversed and RNase is used first, most of the nucleolus is still removed, but the appearance of the chromosome is different. In these preparations, too, material as in chromosomes has been

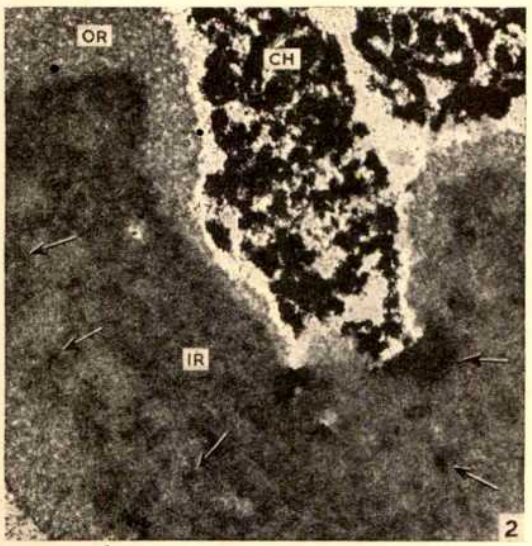


Fig. 2. Nucleolus and chromosomes (*CH*) after extraction, first with pepsin, and then with RNase. Note the small patches of chromatin-like material (arrows) dispersed in the nucleolus. The inner region of the nucleolus (*IR*) is denser than the outer (*OR*) in such preparations and the chromatin patches occur predominantly in the former region. One or two large patches are probably continuous with the chromatin of the organizer. (Glutaraldehyde fixed and double stained. × 9,300)

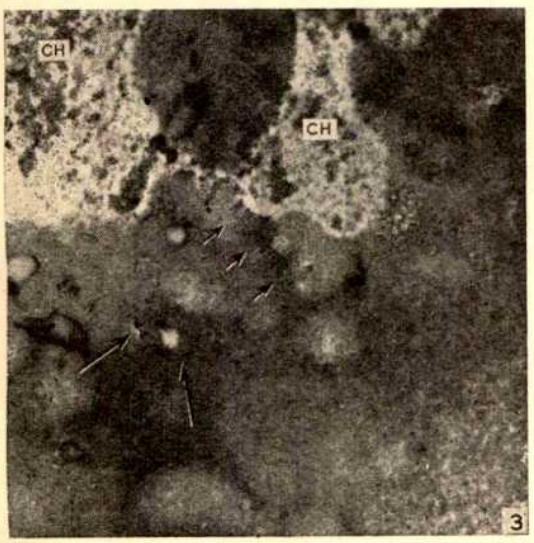


Fig. 3. Nucleolus and chromosome after extraction with RNase followed by pepsin. Material of similar appearance as chromatin (*CH*) is observable inside the nucleolus (arrows). Some of these appear to be continuous (small arrows) with organizer chromatin and others (large arrows) isolated. (Glutaraldehyde fixed and stained in uranyl acetate. × 10,000)

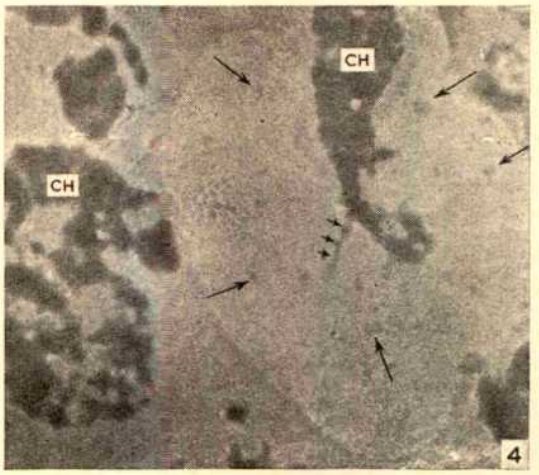


Fig. 4. Nucleolus and chromosomes (*CH*) as they appear after extraction with pronase and RNase. Note small patches of dense chromatin-like material (arrows) dispersed inside the nucleolus which appears almost as negative image of control. An extension of the organizer chromatin is indicated (small arrows). (Acrolein-formalin fixed and stained in uranyl acetate. × 6,600)

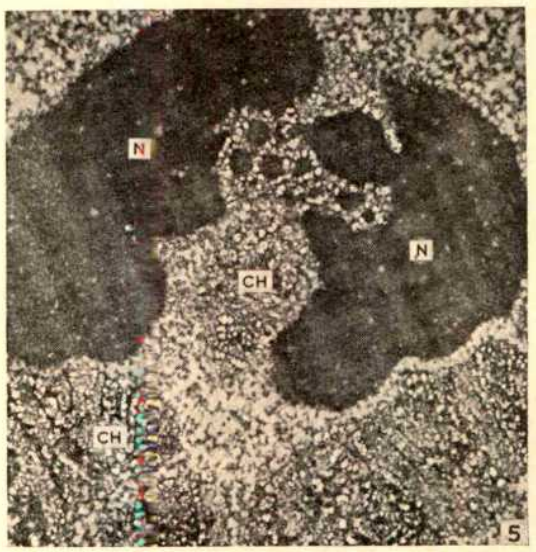


Fig. 5. A section extracted with DNase. See the chromosomes (*CH*) riddled with holes or empty spaces. Similar holes are also present in nucleolus (*N*) which otherwise appears unaffected by DNase. No holes noticeable in the interchromosomal (sap) regions of the nucleus. (Formalin fixed and stained in uranyl acetate. × 8,000)

found within the nucleolus (Fig. 3). Some of this material appears as extensions of the organizer chromatin and some is not so. Fig. 4 is a micrograph showing nucleolus and chromosomes after extraction first with pronase for 20 min and then with RNase for 2 h. The nucleolus is almost a negative image of the controls whereas the chromosomes are largely retained and dense, though with no discernible ultrastructure. In certain sections, such as that in Fig. 4 for example, small patches of chromatin-like material are seen dispersed in the nucleolus.

DNase extraction of glutaraldehyde-fixed cells causes variable decrease in the binding of uranium by chromosomes. After digestion of formalin-fixed cells for 90 min, however, the chromosomes contain numerous holes or empty spots as shown in Fig. 5. (DNase used with mg^{++} gives similar results in a shorter period.) It may also be noted that such holes occur within the nucleolus, which is otherwise unaffected by DNase. The size and distribution of the holes in the nucleolus correspond fairly closely to those of the intranucleolar chromatin patches observed after other procedures.

Extraction with trypsin for 30 min results in marked loss of chromosomal constituents (no figure presented).

A similar striking loss of constituents occurs confined to small spots up to 500 mμ within the inner region of the nucleolus; there is also a general extraction of the matrix material in the outer region of the nucleolus.

In a previous electron microscope investigation[5] in which osmium fixation was used, the electron density of all nuclear structures was uniformly high and no small patches of chromatin were detected within the nucleolus. With aldehydes as fixatives, a certain amount of distinctive staining of cellular components seemed possible and they have the added advantage of enabling specific extraction by enzymes. On these accounts, the present investigation was carried out on aldehyde-fixed glands.

In sections stained with uranyl acetate alone, the chromosomes and nucleoli were more or less uniformly stained. After the double-staining procedure described earlier, it was seen that the chromosomes were distinctively denser and this enabled small patches of chromatin-like material to be easily detected within the nucleolus. It has been pointed out before[6] that, after formalin and acrolein fixation, uranyl acetate at *p*H 5 stains the deoxyribonucleoproteins more intensely than the ribonucleoproteins, although the chemical basis of this distinctive contrast is not known. Earlier, Huxley and Zubay[7] had noted that pretreatment of lightly fixed (5 min in buffered osmium tetroxide) tissue with uranyl acetate seemed to render DNA-containing regions accessible to lead hydroxide which increased their density preferentially. On this basis they observed that some differentiation between DNA and RNA may be possible by comparison of "uranium–lead" and "lead only" stained tissues.

The investigations with enzymes have further served to elucidate the nature of the intranucleolar chromatin-like material. By successive digestions on same sections with pepsin and RNase in any order, it was shown how patches of chromatin occur within the extracted nucleolus. This procedure was observed by Bernhard and Granboulan[8] to be an effective method to demonstrate intranucleolar DNA. The intranucleolar chromatin is retained even when the nucleolar extraction is virtually complete, as when pronase is used instead of pepsin in the above procedure. In the present investigation the most indicative evidence of intranucleolar DNA comes from the digestion experiments with DNase. Extraction with trypsin also shows that, at the probable sites of intranucleolar DNA, proteins also are present as in chromosomes. All these experiments point to one conclusion, that is, that chromatin-like material occurs inside the nucleolus and this in all probability contains DNA.

Intranucleolar chromatin or DNA has recently been demonstrated in a few mammalian cells by electron microscope cytochemical procedures[2,6,8–13] and the present instance is the first from an insect cell. However, this approach to the problem has had such limited application so far that no generalization concerning intranucleolar DNA can be made yet. Based on light microscope cytochemical tests, there have been many reports (see review by Busch *et al.*[14]) of DNA inside the nucleolus, but some of these require confirmation and on the whole the tendency has been to view them with scepticism. Chemical methods of analysis have yielded a wide range of values for DNA in nucleolar fractions[14,15] and although a part of this in all cases may be due to contaminating chromatin, it would seem that not all the DNA can be dismissed as such. A definite solution to the problem of the intranucleolar DNA will probably come from concerted attempts by chemical methods and ultrastructural approaches.

Concerning the distribution of intranucleolar DNA it may be noted that most patches occur in that part described previously[5] as the inner homogeneous region. It was mentioned that some of the patches situated close to the organizer are apparently extensions of the organizer (Figs. 2–4), but the question of whether the smaller patches dispersed within the nucleolus are linked to each other or the organizer cannot be answered at present. This will be a laborious task as a considerable number of serial sections will have to be examined. In a mammalian cell, it has been shown[13] that the intranucleolar chromatin and the associated chromatin form a continuous system.

In future, if intranucleolar DNA proves to be of widespread occurrence, the mode of origin of this DNA will invite enquiry, but, for the present, this must remain in the realm of speculation. Apart from the obvious possibility of its extension from the organizer locus—the situation with regard to the DNA may be analogous to that in Balbiani rings—it may be asked if minute quantities of DNA (so far undetected) might find their way into the large organized nucleolus by way of prenucleolar bodies reported in the literature[16,17] (other references in Kopac and Mateyko[18]). From the manner of origin of similar bodies from within chromosomes, the possibility that certain of their constituent elements might contain DNA was raised before[19] and this hypothesis is now open to investigation in suitable material by methods of ultrastructural cytochemistry.

The search for intranucleolar DNA was made in the hope that the results would contribute to a better understanding of the functional aspects of nucleolus in *Smittia* investigated[1,20,21] by various experimental approaches. With regard to the nature of nucleolar RNA, it is generally held that the nucleolus is the site of synthesis of ribosomal RNA, and recent biochemical investigations show[22,23] that the DNA complements of ribosomal RNA are located in the organizer chromatin. The nucleolus of the salivary glands of late larval stage of *Smittia*, however, appears to be a site of synthesis of transfer RNA as well as high molecular weight (> 28*S*) RNA[24–28], and several indications point to the non-involvement of the nucleolar organizer in this process[1,20,21,29]. An implication of the present finding, therefore, is that in *Smittia* the intranucleolar DNA may be the primer for the RNAs synthesized in the nucleolus.

I thank Mr. M. D. McKean for technical assistance. I also thank Dr. W. Bernhard and colleagues for help with the methods of ultrastructural cytochemistry during my visit to their laboratory at Villejuif early in the course of this work. This work was supported by the British Empire Cancer Campaign for Research and the Damon Runyon Memorial Fund for Cancer Research.

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REQUIREMENT FOR RIBONUCLEIC ACID SYNTHESIS FOR DEOXYRIBONUCLEIC ACID REPLICATION IN BACTERIA

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A REQUIREMENT for protein formation for continued replication of deoxyribonucleic acid (DNA) in bacteria has been observed[1–8]. It has been proposed that the formation of protein at some point in the cycle of replication of the bacterial chromosome is necessary for continued DNA formation[6,7]. This proposal was based on the observation that blockage of protein synthesis in a bacterial culture leads to a cessation of DNA synthesis after that relative increase in quantity of DNA which would account for all chromosomes being replicated. The present article describes experiments which show that incubation in the absence of required amino-acids of an auxotroph of *Escherichia coli* which requires amino-acids and thymine, thus preventing protein synthesis, and at the same time preventing DNA synthesis during the incubation through the absence of thymine from the medium, leads to a requirement for ribonucleic acid (RNA) formation (but not for protein formation) if DNA synthesis is to continue to the end of the replication cycle. The results support the premise of a recent suggestion[9] that RNA is an essential component of the DNA synthetic system.

The techniques for measurement of nucleic acid and protein and the bacterial growth procedures have been described[10]. The organism used was *E. coli* strain *TAU-BAR*, an auxotroph requiring thymine, uracil, arginine, methionine, tryptophan and proline, and furnished to this laboratory by Dr. Phillip Hanawalt, of Stanford University. The supplementation of minimal growth medium was 8 μg per ml. thymine, 20 μg per ml. uracil, and 40 μg per ml. of the required L-amino-acids, except for L-tryptophan, which was 20 μg per ml.

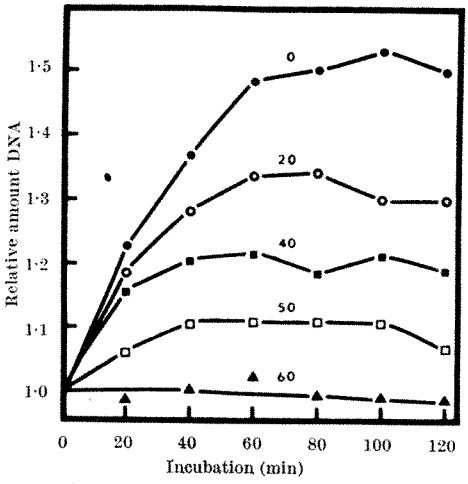


Fig. 1. Formation of DNA by *E. coli* strain *TAU-BAR* in medium lacking required amino-acids after different periods of incubation in medium lacking thymine and the required amino-acids. Log phase cultures of this strain were filtered rapidly on membrane filters, washed and re-suspended in minimal medium with all supplements except thymine, methionine and tryptophan. After incubation at 37° C for the period of time (in min) indicated by the number labelling each curve, thymine was added (zero incubation time) and incubation continued. A comparable effect was found with incubation in the individual absence of arginine, tryptophan or methionine (but not proline) but the combined absence of methionine and tryptophan proved most effective and was used in these investigations. The vertical axis is in units of 3·6 μg of DNA per ml. of culture. All incubations were at 37° C with vigorous aeration through rotary shaking

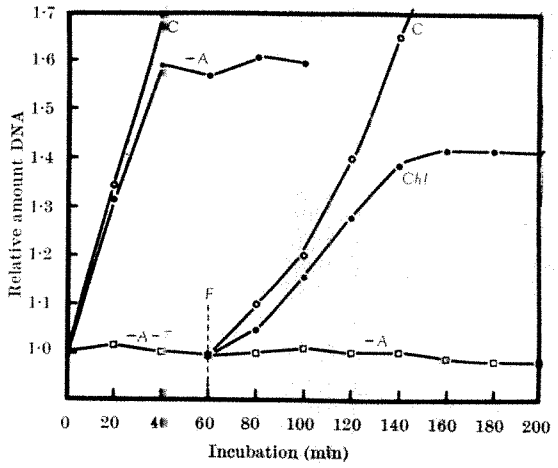


Fig. 2. Recovery of DNA synthesis by *E. coli TAU-BAR* after incubation for 1 h in the absence of thymine and required amino-acids. Log phase cultures of this strain were rapidly filtered, washed and re-suspended in warm medium lacking thymine, methionine and tryptophan (−*A*, −*T*) and incubated for 1 h (0–60 min). Control cultures were re-suspended in warm complete medium (*C*) and medium containing thymine but lacking methionine and tryptophan (−*A*). After 60 min incubation, the −*A*, −*T* culture is filtered, washed and rapidly re-suspended (in less than 2 min) in warm complete medium (*C*), complete medium to which chloramphenicol (40 μg/ml.) has been added (*Chl*), and medium lacking methionine and tryptophan (−*A*). In addition, the effect of incubation in medium lacking methionine and tryptophan, but containing thymine and chloramphenicol was determined (not shown). In this case relative DNA concentrations with incubation were: 60 min, 1·0; 80 min, 1·03; 100 min, 1·11; 120 min, 1·25; 140 min, 1·36; 180 min, 1·38. Units, 3·6 μg of DNA per ml. of culture

Incubation of strain *TAU-BAR* for increasing periods in medium which prevents DNA synthesis (through the absence of thymine) and RNA and protein synthesis (through the absence of amino-acids) leads to a progressive loss of capacity for subsequent DNA synthesis after addition of thymine (Fig. 1). Approximately 60 min of prior incubation under these conditions is necessary for complete loss of capacity to form DNA later in amino-acid-deficient medium containing thymine. If thymine and the required amino-acids are restored to the culture, DNA synthesis resumes (Fig. 2). The possibility exists that lack of cellular capacity for RNA synthesis under these conditions is involved in failure of DNA synthesis, since it has been shown that a deficiency in a required amino-acid leads to cessation of RNA synthesis[11]. The antibiotic, chloramphenicol, was utilized to determine whether RNA formation in the absence of protein synthesis would restitute the capacity for DNA replication with these cultures. It has been established that nucleic acid synthesis will proceed in the presence of this antibiotic, though protein synthesis is blocked[12,13]. While chloramphenicol blocks the formation of ribosomes, it allows formation of RNA to occur[14,15]. The expected increase in DNA in the absence of protein formation, assuming synthesis to the end of the replication cycle (or

an increase of approximately 40 per cent)[7], occurs in the presence of chloramphenicol (Fig. 2) and a relative increase in RNA approximately equal to the increase in DNA is seen (Table 1). No increase in DNA is observed in the presence of thymine when the absence of the required amino-acids is used to block RNA and protein synthesis (Fig. 2) and no formation of RNA or protein is observed (Table 1). That blockage of RNA synthesis and not some other effect of incubation without amino-acids is involved in the limitation of DNA synthesis observed is supported by the finding that addition of chloramphenicol to the culture in the absence of the required amino-acids leads to the recovery of DNA synthesis, as well as RNA synthesis. It has been shown that addition of chloramphenicol to a culture lacking an essential amino-acid endows that culture with the capacity for RNA synthesis[1,2].

Incubation without thymine and the required amino-acids does not lead to any observable breakdown of RNA during the first 60 min, but breakdown does occur after this time (Table 1). This RNA breakdown takes place whether or not thymine is re-added at 60 min of incubation. If thymine is present during the first 60 min of incubation without amino-acids, allowing the completion of the DNA replication cycle, little breakdown of RNA is observed, even with prolonged incubation without amino-acids with or without thymine. Apparently, completion of the DNA replication cycle endows the cellular RNA with an immunity to the RNA breakdown induced by incubation without amino-acids and without thymine. This compares with the finding of Maaløe and Hanawalt[6] that completion of the DNA replication cycle prevents the death of cells due to lack of thymine.

Table 1. RNA AND PROTEIN FORMATION BY *E. coli* STRAIN *TAU-BAR* AFTER INCUBATION WITHOUT THYMINE AND REQUIRED AMINO-ACIDS

Relative amount of RNA (*R*) or protein (*P*) with incubation

	Min:	60	80	100	120	140	160	180	200
C	*R*	1·00	1·14	1·21	1·30	1·42	1·65	2·21	2·43
	P	1·00	1·11	1·14	1·27	1·41	1·71	2·24	2·51
Chl	*R*	1·00	1·17	1·20	1·26	1·36	1·36	1·34	1·33
	P	1·00	0·95	0·95	0·90	0·90	0·93	0·88	0·88
−A	*R*	1·00	1·00	0·91	0·84	0·81	0·69	0·66	0·51
	P	1·00	1·03	1·00	1·00	0·94	0·97	0·97	0·94

For the first 60 min, the culture was incubated without thymine, methionine and tryptophan. It was then filtered, washed, and re-suspended in complete medium (*C*), complete medium containing chloramphenicol (*Chl*), or medium lacking methionine and tryptophan (*−A*). See the legend to Fig. 2 for details of this experiment. 1 unit is 22·0 μg of RNA and 65·3 μg of protein per ml. of culture.

These results suggest the possibility that incubation in the absence of required amino-acids and thymine results in some damage to the DNA (ref. 16) or to an RNA component of the DNA synthetic system[9]. This damage ultimately leads to a breakdown of cellular RNA in the absence of RNA formation. Incubation without RNA formation (that is, in the absence of required amino-acids) does not result in DNA replication because of the damage. If, however, incubation is under conditions where RNA formation takes place, the capacity to make DNA is recovered and DNA synthesis proceeds to the end of the cycle, where it is limited in chloramphenicol by the requirement for protein synthesis.

Kellenberger *et al.*[17] have shown in a "DNA stringent" strain of *E. coli* an increased short-term incorporation of radioactive thymidine, when chloramphenicol is added to cultures incubated in the absence of required amino-acids. They proposed that DNA is under the same control observed by Stent and Brenner[11] for limitation of RNA synthesis in the absence of required amino-acids. This appears unlikely in view of the fact that in most strains (including the one utilized here) DNA synthesis continues to the end of the cycle in the absence of required amino-acids and suggests that the results of Kellenberger *et al.*[17] may be explained by the requirement for RNA formation for subsequent DNA synthesis described here. Okazaki and Okazaki[18] observed with *Lactobacillus acidophilus* that DNA synthesis ceases in a manner comparable to *E. coli*[7] if amino-acids are deleted from the medium but the DNA content of the culture increases several-fold in the presence of chloramphenicol. These results may be understood if incubation without amino-acids leads to loss of the RNA required for continued DNA synthesis, while incubation with chloramphenicol allows retention of the required RNA and its participation in several rounds of DNA formation in this *Lactobacillus* strain. This interpretation would be compatible with these investigations on *E. coli* into the effect of incubation without amino-acids and incubation with chloramphenicol on DNA replication.

Doudney[8] has related the relative increase in RNA in cultures of an amino-acid-requiring strain (previously brought to the end of its replication cycle by starvation of an essential amino-acid) to subsequent relative increase in DNA and suggested the possibility that RNA formation on the DNA template is required "at the molecular or genetic level" for subsequent DNA replication. Hanawalt[19] has proposed that synthesis of complementary RNA normally precedes DNA replication, in explanation of the involvement of RNA synthesis in death due to lack of thymine. On the basis of investigations of the formation of RNA during the recovery of the DNA-synthetic system of bacteria damaged by ultra-violet light, we[9] have suggested that RNA is an essential component of the DNA-synthetic system in the unirradiated bacterial cell and that the requirement for RNA synthesis in DNA replication after exposure to ultra-violet light is in replacement of this ultra-violet-damaged RNA. Our investigation[9] suggested that the involvement of protein formation in initiation of the cycle of DNA synthesis may be indirect and dependent on the necessity of protein formation for continuation of RNA synthesis past the chromosomal origin (or some other site). The present investigations are in agreement with this suggestion and support the hypothesis that in *E. coli* each cycle of DNA replication is preceded by a cycle of RNA formation which may be effectively limited by a requirement for protein synthesis. This would explain the accumulation of capacity for more than one cycle of DNA replication under conditions of blocked DNA synthesis (that is, thymineless incubation[20,22], or incubation after ultra-violet exposure[21,22]) since it appears reasonable according to the aforementioned hypothesis that prior formation of RNA would effectively by-pass the limitation on DNA synthesis imposed by blockage of protein synthesis, initiating a new round of DNA synthesis[20].

We have observed that residual synthesis of DNA in the absence of required amino-acids exceeds the expected 40 per cent increase, which accounts for the chromosomes coming to the end of their cycle in a log phase culture[7] (Fig. 2). However, increase of DNA in chloramphenicol after incubation without amino-acids or thymine is only 40 per cent (Fig. 2). This could be explained if it is assumed that the "node" of RNA synthesis is normally somewhat in advance of the "node" of DNA synthesis. Thus, with withdrawal of the required amino-acids, DNA synthesis would proceed to the point which RNA synthesis has reached. In general, the distance along the chromosome between the site of RNA synthesis on the DNA template and the site of DNA replication would determine the residual capacity for DNA replication after limitation of RNA formation by any means, and this might be expected to vary in various strains and under various conditions. With a culture lacking in the RNA required for DNA replication because of incubation without thymine and the required amino-acids, synthesis of RNA and DNA in chloramphenicol would proceed only to the end of the cycle (the point where the requirement for protein formation limits RNA synthesis[9]) accounting for the 40 per cent increase.

This investigation does not determine whether the requirement demonstrated for RNA formation in DNA replication is indirect and supporting or whether the RNA

is actually involved in "information transfer" by way of complementary hydrogen bonding of purine and pyrimidine bases. The control of DNA replication by prior RNA formation appears to constitute an effective cellular mechanism for relating gene replication to previous cytoplasmic increase.

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ELECTROPHORETIC SEPARATION OF SERUM ACID PHOSPHATASE ISOENZYMES IN GAUCHER'S DISEASE, PROSTATIC CARCINOMA AND MULTIPLE MYELOMA

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PATIENTS with Gaucher's disease[1], prostatic carcinoma[2] and multiple myeloma[3,4] may have elevated serum acid phosphatase activity. In a search for characteristic isoenzymes, sera from normal individuals and patients with these diseases were investigated by disc electrophoresis on polyacrylamide-gel columns[5]. These columns were later stained for acid phosphatase activity by the method of Barka[6].

Electrophoresis was performed (1 m.amp, 100 V) for 2 h at 4° C. The test serum (25 μl.) was directly layered on the spacer gel while it was beneath the buffer. (Anti-convection gel was not added, as is sometimes the case, to increase the amount of serum subjected to electrophoresis.) After separation, columns were fixed in 10 per cent neutral formalin-acetate (NFA) for 30 min at 4° C and then briefly rinsed in cold distilled water. Columns were later stained for 12–24 h (4° C) using Barka's azo-dye method with naphthol *AS-BI* phosphate as substrate (*p*H 5)[7]. After incubation, the columns were rinsed and stored in test-tubes containing distilled water. Zones containing acid phosphatase activity stained bright red while albumin stained non-specifically a light yellow (Fig. 1).

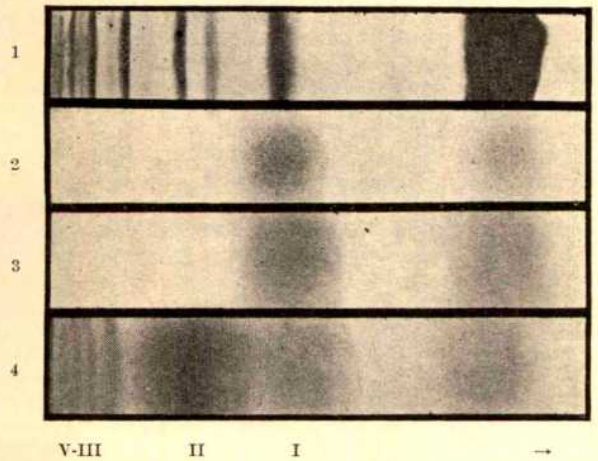


Fig. 1. Normal serum protein electrophoretic pattern in polyacrylamide-gel column (1) compared with electrophoretic pattern of acid phosphatase activity in normal (2), prostatic carcinoma (3), and Gaucher's disease patients (4). Isoenzyme bands are labelled I–V. Acid phosphatase activity stains red while albumin is non-specifically coloured a light yellow

Fixing the acrylamide gels with NFA before the histochemical procedure minimized background staining and retarded diffusion of the enzyme during incubation. No major decrease in the intensity of the stained isoenzyme patterns occurred after fixation in normal individuals or diseased patients. Electrophoresis of haemolysates of normal red blood cells was performed and stained for acid phosphatase activity. If the columns were fixed in NFA (30 min) after electrophoresis and before the histochemical treatment, no enzyme activity of erythrocytic origin was observed, thus confirming the observation that red blood cell acid phosphatase is inhibited by formalin[8].

Gel columns which contained electrophoretically separated serum were boiled in distilled water (100° C, 10 min) to destroy enzyme activity and then stained for acid phosphatase activity. No enzyme activity was demonstrated. The Gomori method[9] was tried as a histochemical procedure and found unsuitable. Specific zones of enzyme activity could not be differentiated from a similarly stained background.

Acid phosphatase activity in controls (six males and eight females) with normal serum acid phosphatase activity had one broad red staining band on electrophoresis which migrated about midway between the origin and the albumin fraction (Fig. 1, No. 2). No sex difference was observed.

Five of six patients with prostatic carcinoma investigated had increased total serum acid phosphatase activity (1·5–4 Bodansky units—B.U.) (normal 0–1). The sixth patient had a serum acid phosphatase of 0·9 B.U. All patients exhibited only one staining band which migrated similarly to normal acid phosphatase (Fig. 1, No. 3). All carcinoma patients had considerably increased staining compared with normals. The intensity of this reaction product was proportionate to the total serum acid phosphatase level.

The columns of prostatic carcinoma patients which were treated with tartrate solution (0·1 M, 30 min) after electrophoresis and before enzyme staining had the acid phosphatase activity in Band I markedly, but not completely, inhibited compared with its untreated control. Negligible inhibition occurred in similarly treated normal serum.

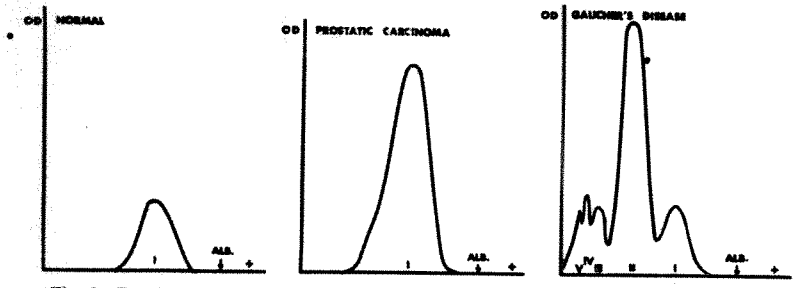


Fig. 2. Densitometric tracing of electrophoretic serum acid phosphatase pattern in polyacrylamide-gel column in normal, prostatic carcinoma, and Gaucher's disease

Nine patients with Gaucher's disease (six males and three females) had elevated serum acid phosphatase values (8–25·8 King-Armstrong units) (normal 1–4). All had at least two enzyme bands (Fig. 1, No. 4). Band I migrated similarly to that of normal patients. Band II was the broadest and most intensely stained. Other minor bands (III, IV, V) were considerably narrower and found in five of the nine patients. Of these five patients, three had three bands while two patients had two minor bands. Compared with the serum protein electrophoretic pattern[5,11] (Fig. 1, No. 1), Band I migrated in a position similar to transferrin. Band II was in the fast moving haptoglobulin region while Bands III, IV, and V had mobilities of gamma globulins and the slower haptoglobins. No differences were observed between male and female. In one family, a male with Gaucher's disease had five isoenzyme bands. His apparently normal parents, sister and her two daughters had normal electrophoretic patterns and serum acid phosphatase activity.

A semi-quantitative estimate of the amounts of acid phosphatase was made by passing the stained column through a densitometer ('Chromoscan *J*288', Joyce Loebl and Co.). Variations in the quantity and difference in the isoenzyme pattern of a normal individual compared with a patient with prostatic carcinoma and another with Gaucher's disease are illustrated (Fig. 2).

Sera from six patients with multiple myeloma which exhibited normal total serum acid phosphatase activity (B.U.) exhibited one electrophoretic band which migrated similarly to the normal controls. This band stained with normal or slightly less than normal intensity. Four patients had hypogammaglobulinaemia and two had a monoclonal spike migrating in the beta region on protein electrophoresis.

Tissue from a human plasmocytoma and from an experimentally induced mouse plasmocytoma[10] were examined for acid phosphatase activity. Each tumour was homogenized with two volumes of 0·25 M sucrose (4° C). A portion of the centrifuged supernatant (4° C, 105,000*g*, 90 min) diluted to ten times the original tumour weight with 0·25 M sucrose was subjected to disc electrophoresis and stained for acid phosphatase activity. Two isozyme bands were observed in the human plasmocytoma homogenate. The faster one migrated just behind Band I while the slower component was in the haptoglobin region. One band was demonstrated in the mouse plasmocytoma homogenates which migrated in a similar fashion to the first band of human myeloma tissue.

Only a very small number of normal individuals and those with prostatic carcinoma have had their sera electrophoretically examined for isoenzymes of acid phosphatase. Earlier electrophoretic investigations on paper[12,13], in starch gel[14–16] and starch block[17] demonstrated one enzyme zone in normal and prostatic carcinoma patients. In contrast, Dubbs and Hilburn, also using a starch-gel medium, found three isoenzyme fractions in normal individuals[18]. Naiman and Oski described a distinctive pattern on starch-gel electrophoresis in prostatic carcinoma patients, but were unsuccessful in demonstrating serum acid phosphatase isozymes in normal individuals[18]. While our investigations were in progress, Czitober *et al.*[20], using starch gel electrophoresis, also reported three isoenzyme zones in normal subjects and found four in prostatic carcinoma. In all these reports[12–20] different enzyme staining techniques were used. The reported differences observed in the number, width and migration of acid phosphatase isoenzymes in the normal and pathologic sera may be related to the specificity of the various enzyme-staining methods and substrates used as well as to the different electrophoretic techniques[21].

Normal serum acid phosphatase is reported to be composed mainly of platelet acid phosphatase[22], and this isoenzyme is not significantly inhibited by tartrate[22,23]. Prostatic acid phosphatase, however, is sensitive to tartrate[2,8]. The marked reduction in the staining of the enzyme band in all our prostatic carcinoma cases which were treated with tartrate confirms the prostatic origin of this enzyme component. This phenomenon was also evident in the patient with a normal total serum acid phosphatase. This inhibition procedure was of diagnostic value in a fashion similar to differential serum enzyme investigations of prostatic acid phosphatase[2].

The three patients with Gaucher's disease investigated by Czitober *et al.* were reported to have five acid phosphatase fractions; however, this is not quite clear from their drawing[20]. The fact that some of our patients did not have any or all of the minor acid phosphatase bands (III, IV, V) suggested that these isoenzymes might arise from some organ, for example, spleen, liver or bone, or perhaps represent intrinsic molecular differences in the disease itself. It was found that the four patients who had no minor bands had all undergone splenectomy. These findings suggest that the spleen may directly or indirectly be the source of certain serum isozymes. The two individuals with only two extra bands were our youngest patients (17- and 23-year-old males). The ages of the three persons with three minor bands (two males and one female) ranged from 30 to 54. All had enlarged spleens palpated 3–9 cm below the right costal margin. Although less splenic enlargement was found in those with two minor bands, one patient with three bands also had a smaller-sized spleen.

The role of acid phosphatase activity in Gaucher's disease is unknown. The increased amounts and differences in isoenzyme pattern in this disease compared with normal suggest that the basic abnormality in this illness is not only restricted to disturbed cerebroside metabolism. In a search for some inherited abnormality in the absence of apparent Gaucher's disease, the relatives of one patient were investigated and no extra isoenzymes were observed.

Although Band I migrated at essentially the same position in normals, prostatic carcinoma, myeloma and Gaucher's disease patients, slight differences in migration were nevertheless present (Fig. 1). This further suggests intrinsic differences among these isoenzymes.

Although the cells of multiple myeloma contain considerable acid phosphatase activity[3,4,7,24] like the Gaucher cell[25], elevated serum enzyme-levels are most unusual[3,26]. They were not increased in the cases investigated. The elevated serum enzyme values in one case[4] may be secondary to obstructive phenomena due to infiltrating myeloma cells in the prostate itself or to the associated renal insufficiency[27] or both, rather than represent a primary sign of multiple myeloma. The possibility of specific serum isozymes originating from myeloma tissue or associated with this disease cannot, of course, be excluded from our data.

We thank Mrs. Myrna Lewis for her technical assistance and the staff of the Department of Urology, New York

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IN VIVO FIBRINOLYSIS WITH A DIALYSABLE CONSTITUENT OF RECALCIFIED HUMAN PLASMA CLOTS AND GLUCONATE

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Two kinds of fibrinolysin are known to occur in the body: one is a proteolytic enzyme, plasmin, which attacks fibrin and fibrinogen as well as other proteins and amino-acid esters; the other acts solely on fibrin and seems to arise from the vascular endothelium in response to great stress such as severe exercise, electroshock, and injection of pyrogens, and in sudden death. The role of this second substance has been suggested to be merely activation of the enzymatic precursor plasminogen to plasmin[1], but the evidence is not conclusive. Despite a high level of this activator, plasminogen and plasmin concentrations in plasma have generally been found unchanged. This has been attributed to a potent plasmin inhibitor in plasma, antiplasmin. It has been claimed that only the plasminogen of the fibrin clot itself could be converted into functional plasmin by adsorbed activator. However, definite proof for this contention, such as demonstration of differences between plasminogen and plasmin contents of plasma before clotting and of lysed fibrin, is still lacking.

In 1962, I reported[2] the ability of a strain of *Streptococcus faecalis* grown in human serum or on beef brain heart infusion agar to form a fibrinolytic metabolite in the presence of sodium gluconate. Fibrin heated to 85° C, to destroy plasminogen, was actually more readily decomposed than unheated fibrin, leaving no doubt that the agent under study was a true fibrinolysin rather than an activator. Later, an apparently identical basic substance was shown to occur[3] in the extracts of recalcified human plasma clots, obtained by their decomposition in distilled water at 5° C over a period of several weeks. The dialysate of such extracts could again be converted into a fibrinolysin by the bacteria in the presence of gluconate.

To speed up the preparation of extracts, plasma clots, retracted and washed repeatedly, were digested with crystalline trypsin at 37° C overnight. The centrifuged digest was dialysed three times for 24-h periods against distilled water. The dialysates were dried at 37° C and then extracted with a mixture of methyl alcohol and distilled water in the proportion of 2 to 1. Following centrifugation, the supernatant was dried again, leaving a brown amorphous material. It was weighed and dissolved in water to make a 10 per cent solution. The residue in the bag was precipitated with two volumes of methyl alcohol, and the dried supernatant treated like the dialysate. More of apparently identical material was recovered, but some turbidity remained in aqueous solution. In later preparations dialysis was omitted, and the final solution passed through a Seitz filter to clear it. Its ultra-violet spectrum showed a distinct pattern with a maximum at 275 mμ and a minimum at 247 mμ. The yield per 100 ml. of clotted human plasma was 100–150 mg; with bovine plasma it was 200–300 mg. Human serum turned out to be an even richer source of the material. Precipitated serum proteins, after digestion with trypsin, drying and extraction with 66·7 per cent methyl alcohol, yielded about 2–3 g per 100 ml. of serum.

In vitro, none of the preparations displayed fibrinolytic activity with or without sodium gluconate.

Cats and dogs were used in *in vivo* experiments. They were anaesthetized with sodium pentobarbital (25 mg/kg). To produce blood clots in the jugular veins of animals, a modified Wessler technique[4] was first tried. A vein segment is clamped off, and a small amount of serum is injected into it with a 30-gauge needle. However, the clots formed by this method were usually too small to obstruct the blood vessel. A different technique proved more satisfactory in our hands. Blood was expressed from a portion of the external jugular and the external maxillary veins above and below their juncture in the upper part of the neck, and 45-mm seraffine clamps (A. H. Thomas and Co., Philadelphia) were applied. From a superficial leg vein 1 ml. of blood was withdrawn and kept in a glass syringe for half the clotting time, previously determined. A sufficient amount to produce bulging of the jugular vein was injected into it above the point of confluence of the two veins; this varied from 0·4 to 0·7 ml. A clamp was immediately placed below this point, and the two clamps compressing the veins above it were removed, whereon they were flushed with saline to prevent blockage through clotted blood. Clots of a given size could thus be formed. They were generally made 100 mm long. A ligature narrowing the lumen of the vessel to about half its normal size was placed below the proximal clamp, and the two clamps confining the clot were released. In some experiments the vein, instead of being ligated, was pinched off with a clamp just enough to prevent escape of the clot. The clot acted as a haemostat; the vein below it was free of blood. A catheter was inserted into the jugular vein above the clot. When fluid was administered through it, blood flow was reversed in the medial external maxillary vein for the short distance between the jugular and the hyoid venous arch, leading to the veins of the opposite side. A clamp was placed above the point of insertion in experiments in which the clot was to be exposed to the

infused material alone; without the clamp, infusion mixed with blood would pass the clot. In systemic experiments the catheter was inserted in the opposite external jugular vein in the lower part of the neck.

Solutions infused were physiological saline and sodium gluconate in concentrations of 1, 5 and 10 per cent with or without 0·5 per cent of fibrin extract. They were administered at the rate of about 1 ml. per min locally and about 2 ml. per min systemically.

Clot dissolution was determined by angiography with 50 per cent sodium diatrizoate ('Hypaque'). The radio-opaque dye was injected before the start of the infusion and as soon as changes in the size and consistency of the clot became noticeable. This was usually accompanied by an observable restoration of blood flow below the ligature. Occasionally the vessel lumen was made too narrow, and even a shrunken clot would continue to obstruct it; however, such a clot was readily dislodged by the pressure of injected dye.

Experimental results were consistent. No clot dissolution was observed 30 min after infusion above the clot of 40 ml. of saline with or without fibrin extract, mixed with blood, or of 40 ml. of 1 per cent sodium gluconate without blood. Blood clots were broken down partially by direct infusion of 40 ml. of 1 per cent or 20 ml. of 5 per cent sodium gluconate with blood or of 40 ml. of 1 per cent sodium gluconate and 0·5 per cent fibrin extract without blood.

In systemic experiments, carried out in dogs weighing 8–11 kg, 95–125 ml. of 5 per cent or 50–60 ml. of 10 per cent sodium gluconate were required for partial clot decomposition. Clot breakdown seems to be limited to a small local area in direct contact with the vascular endothelium. This is illustrated in Fig. 1.

Even 24 h after an infusion parts of a clot too large to pass the narrowed lumen were still clearly visible in X-ray pictures. Likewise, small free-floating clots, produced in some experiments, remained unchanged regardless of the amount and nature of the infused solution. In practical application this should cause no difficulty since remnants of clots could be expected to pass to ever narrower blood vessels, finally to disappear completely.

An opportunity presented itself to test the effect of gluconate in an animal suffering from a severe thrombotic condition. In a mare, weighing 450 kg, a large blood clot was palpated at the bifurcation of the aorta. At the time of treatment the thrombus was at least 2 weeks old; the animal experienced great difficulty in standing and walking on its hindlegs, and some muscular necrosis, as evidenced by flaccidity, had set in. Three hundred g of sodium gluconate in 1·5 l. of distilled water were administered through the jugular vein over a period of 2 h. Even before termination of the infusion marked changes were observed in the behaviour of the animal. First there were muscular tremors, starting in the flaccid muscles, spreading to the muscles of the hindquarters and eventually of the entire body. As they subsided, profuse perspiration began. Within a few hours the horse appeared normal. It has now had full use of its hindlegs for about 4 months.

The systemic administration of considerable amounts of sodium gluconate was found to produce a temporary increase in the bleeding time of infused animals but left their clotting time unaffected. The appearance of cadaver fibrinolysin is likewise associated with enhanced blood vessel wall permeability[5], both being manifestations of the shocked state. Locally, such a change in blood vessel walls is a common feature of inflammation. The limited fibrinolytic reaction in the obstructed vein would seem to be comparable with an inflammatory focus in the skin. Intradermal injection of 5 mg of fibrin extract with or without 5 mg of gluconate, but not of gluconate alone, into albino rabbits invariably produced some reaction, which became more distinct in the presence of a suitable adjuvant. Without gluconate the predominant feature was oedema with induration, with gluconate pronounced erythema, occasionally with incipient haemorrhage. When fibrin extract mixed with 50 μg of epinephrine was injected into the skin, haemorrhagic necrosis resulted. Thus far, only bacterial endotoxins have been reported to have this specific effect with epinephrine[6]. However, it has already been suggested[7] that these metabolites might in fact elicit the formation of other substances by the body.

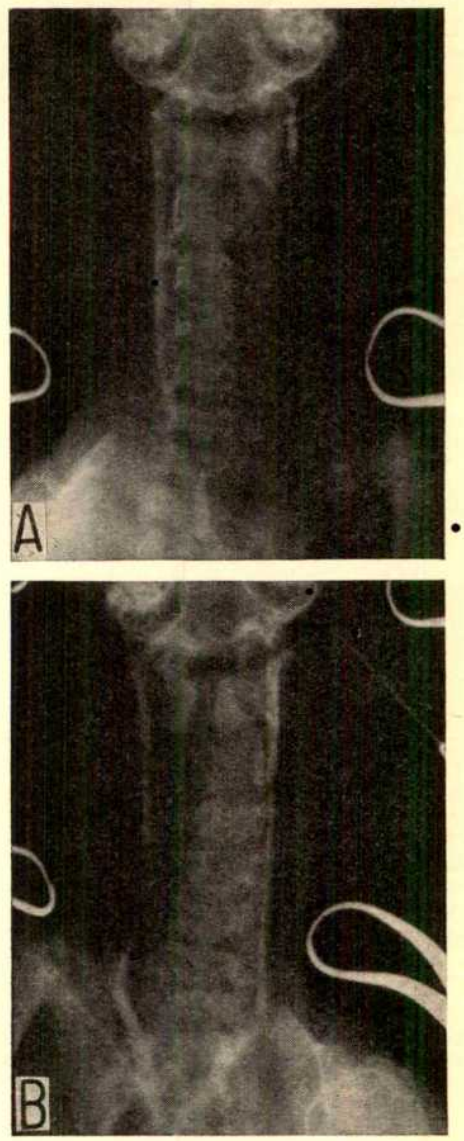


Fig. 1. Partial lysis of a blood clot in the jugular vein of a cat. *A*, Left jugular vein completely obstructed, with clear space above the clot. *B*, Circulation re-established. Central core of the clot is left behind and has been swept back by pressure of the injected dye

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NUCLEATION BY ELASTIN

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A FUNDAMENTAL step in the process of mineralization is the formation of nuclei of crystal growth. In this connexion, the isolation of substances that can induce nucleation is basic to the understanding of calcification in living organisms. As a result of the interest directed toward the calcification of bone, collagen has been identified as a substance capable of inducing the formation of nuclei of hydroxyapatite crystal growth from a metastable solution[1-8]. Mineralization occurs, however, in many areas other than bone. Among those investigated by our group, in addition to collagen, are: rat tail tendon, rat and rabbit aorta, epiphyseal cartilage, inorganic salts, saliva extract, bovine submaxillary mucin and elastin[9-14]. It is of interest to investigate these systems, as new calcifying catalysts are being discovered as a result[13,14].

This communication presents evidence indicating that purified elastin is capable of inducing the formation of nuclei of hydroxyapatite crystal growth.

Evidence that elastin can be the site of calcification was shown by Lansing[15], using the optical microscope and histochemical investigations. Electron microscope investigations on the calcification of the aorta conducted by Eisenstein and Zeruolis[16] confirmed this. Yu *et al.* supplied additional information that calcification of human aorta is in the elastic fibre within the limiting membrane of the fibre which forms a continuous phase with attached collagen fibres[17]. Ayer[18] has prepared electron micrographs of calcium in elastic lamellae in addition to deposits in ground substances and in association with collagen in human aged arterio-sclerotic aorta.

Calcification *in vitro* investigations of the aorta by Eichel *et al.*[19,20] suggested that elastin might be involved in the induction of calcification *in vitro* of aorta. Later, Martin *et al.*[21] showed by electron microscopy the site of calcification *in vitro* to be elastin.

In February 1963, Dr. F. Lamy, of the University of Pittsburgh, provided Dr. A. E. Sobel with samples of purified elastin. It was possible to demonstrate that this material induced calcification *in vitro*. Confirmation of this was supplied, at the request of Dr. Sobel, independently by Dr. B. Sherman of the State University of New York, Downstate Medical Center, and Dr. R. Eilberg of the Colgate Palmolive Research Laboratories, New Brunswick, New Jersey.

In the same year, Taves and Neuman[22] suggested that elastin rather than the predominant collagen is responsible for the nucleation by acid-extracted tail tendon supplied by 'Sigma'. They supported their contention by demonstrating that a tissue rich in elastin (ligamentum nuchae) induced nucleation. Schiffmann *et al.*[23] also reported that bovine ligamentum nuchae extracted with phenol at 25° C induced calcification *in vitro*.

Two objections may be raised to the investigations of Taves *et al.*[22] and Schiffmann *et al.*[23]: (1) that the material used was a heterogeneous mixture, and (2) that tests to exclude the existence of inorganic seed crystals were not carried out. From our investigations we have evidence to indicate that acid-extracted rat tail tendon, collagen (Sigma), apparently similar to that used by Taves and Neuman is already pre-nucleated[24]. One billion (10^9) nuclei of hydroxyapatite (seed crystals) $25 \times 50 \times 100$ Å weigh only 4×10^{-4} μg and thus will escape detection by the usual chemical methods, but can be detected by other means recently developed[24]. In the presence of pre-existing nuclei (seed crystals) calcification represents crystal growth and does not provide rigorous evidence for the existence of an organic compound(s) capable of inducing nucleation.

In the present investigations several different batches of purified elastin prepared from ligamentum nuchae were used. The elastin was prepared by the modified method of Lamy *et al.*[25]. The amino-acid composition of this elastin is given in Table 1. The last three entries in Table 1 represent ninhydrin-positive peaks which do not correspond to any of the thirty standard amino-acids. They appear between ammonia and lysine and have been observed by others. Desmosine and isodesmosine have been described by Partridge *et al.*[26,27]. Because of its position in the chromatogram the compound listed as ornithine probably corresponds to another amino-acid described in a preliminary fashion by Franzblau *et al.*[28] as X^4.

Table 1. AMINO-ACID COMPOSITION OF ELASTIN

Amino-acid residues	Amino-acid residues (g/100 g elastin)	Amino-acid residues	Amino-acid residues (g/100 g elastin)
Cysteic acid	0·162	Leucine	9·249
Aspartic	0·606	Tyrosine	1·697
Methionine sulphone	0·040	Phenylalanine	5·574
Threonine	0·743	Ammonia	0·449
Serine	0·797	Lysine	0·400
Glutamic	2·263	Histidine	0·043
Proline	12·306	Arginine	0·820
Glycine	27·409	H-Proline	0·849
Alanine	17·974	'Desmosine' (refs. 26 and 27)	2·660
Valine	12·994	'Isodesmosine' (refs. 26 and 27)	3·840
Isoleucine	2·409	'Ornithine' (ref. 28)	0·211
		Total	98·495

4 mg of elastin were placed for 24 h in 30 ml. of calcifying solution similar to those which were used in our earlier investigations for calcification *in vitro* of hypertrophic epiphyseal cartilage of saliva extract and collagen[1,5,6,8,13]. Thymol was added at a level of 10 ml. per l. The calcifying solution contained (per l.): 70 mmoles sodium chloride, 22 mmoles sodium bicarbonate and 5 mmoles potassium chloride. Calcium was added as calcium chloride. Phosphate phosphorus was added as a mixture of sodium dihydrogen phosphate and sodium monohydrogen phosphate (4 : 1).

The calcifying solution containing the basal salts (sodium chloride, sodium bicarbonate, potassium chloride) and phosphate were acidified (and made oxygen free) by passing in carbon dioxide until a *p*H of 6·0–6·1 was reached. Calcium was then added and made up to volume. In the presence of the electrodes of a *p*H meter, nitrogen was passed through the solution until a *p*H of 7·28–7·30 was reached. The solutions containing the samples and controls were then incubated at 37° C for 24 h or 48 h, depending on the investigations, after which time equal portions of the supernatant were taken for analyses.

The amount of calcium and phosphate incorporated was measured by the change in supernatant liquid using the methods previously used in similar investigations[2,5,6].

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Some of the control solutions were kept at least 24 h longer than the sample investigation, and if any precipitate developed the experiment was considered invalid. If the experimental solutions were in any way turbid, the experiment was discarded.

The first investigations indicated that the elastin prepared by Dr. Lamy induced calcification within the elastin body with the formation of hydroxyapatite (courtesy of Dr. A. Hirschman). However, nitrous acid treatment which inactivates nucleation by saliva extract, treatment with boiling water which inactivates rachitic cartilage and bovine submaxillary mucin, and treatment for 24 h with 20 mmoles cupric chloride in methanol or water which inactivates various collagens and saliva extract had no effect on the ability to calcify untreated elastin. These treatments do not inhibit crystal growth of pre-existing hydroxyapatite nuclei[8,12–14,24].

Thus we were confronted with the problem that the treatments which inactivate the nucleating ability of various nucleating catalysts do not inactivate the nucleating ability of elastin or the elastin that is already nucleated (that is, contains inorganic seed crystals). In this connexion, it must be borne in mind that pre-existing nuclei (seed crystals) of hydroxyapatite crystal growth may be due to inorganic substances other than calcium phosphates[12,29,30].

To remove inorganic seed crystals, we extracted 4 mg elastin with 25 ml. of 0·1 N hydrochloric acid in methanol for 3 days with vigorous shaking. This was followed by exhaustive washings with 50 per cent methanol and then triple-distilled water. The washing was continued until the extract was free of chloride ions. The elastin was then covered with solutions used for calcification *in vitro* and stoppered—care was taken to exclude all visible gas space. The supernatant was removed and the samples washed with triple-distilled water and fresh calcifying solutions were added for an additional 24 h when necessary.

Cupric chloride had no inhibiting effect on purified elastin unless it was specially treated in advance (Table 2). This finding can be compared with the results given in Table 3 in which there is no uptake of calcium or phosphorus after 24 h when acid extracted elastin is treated with 20 mmoles/l. of cupric chloride in methanol. However, it would seem that this situation is reversible after calcification for an additional 24 h.

The cupric chloride has no effect on the calcification *in vitro* of 'nucleated' elastin as can be seen from Table 4.

Table 2. INFLUENCE OF CUPRIC CHLORIDE ON THE CALCIFICATION *in vitro* OF UNTREATED ELASTIN, CALCIUM × PHOSPHORUS = 15 × 5 MG PER CENT²

Specimen	Centrifuged supernatant solution Calcium (mg per cent)	Phosphorus (mg per cent)
Control solution	14·9	4·64
Control solution	14·8	4·92
Control solution	14·9	4·76
Untreated elastin	8·1	2·2
Untreated elastin	7·4	2·1
Elastin* + 24 h, cupric chloride 20 moles/l. in methanol	8·1	2·1
Elastin* + 24 h, cupric chloride 20 moles/l. in methanol	8·4	2·3

* Untreated elastin.

Table 3. INFLUENCE OF CUPRIC CHLORIDE ON CALCIFICATION *in vitro* OF ELASTIN EXTRACTED WITH 0·1 N HYDROCHLORIC ACID IN METHANOL FOR 5 DAYS

Sample	Centrifuged supernatant solution Calcium (mg per cent)		Phosphorus (mg per cent)	
	24 h	48 h*	24 h	48 h*
Control solution	15·1	14·7	5·2	4·8
Control solution	14·9	14·9	4·9	5·0
Ext. elastin	8·1	7·4	1·86	1·61
Ext. elastin	8·3	7·9	1·84	1·70
Ext. elastin + 24 h, cupric chloride 20 moles/l. in methanol	14·3	8·26	4·68	1·89
Ext. elastin + 24 h, cupric chloride 20 moles/l. in methanol	15·2	10·6	4·8	3·6
Ext. elastin + 24 h, cupric chloride 20 moles/l. in methanol	15·4	12·1	5·0	4·0
Ext. elastin + 24 h, cupric chloride 10 moles/l. in methanol	7·6	7·4	2·2	1·8
Ext. elastin + 24 h, cupric chloride 10 moles/l. in methanol	8·3	7·6	2·0	1·9

* New calcifying solution after end of 24 h.

Table 4. INFLUENCE OF CUPRIC CHLORIDE ON CALCIFICATION *in vitro* FOR 24 H OF 'NUCLEATED' ELASTIN AND HYDROXYAPATITE ($Ca_3(PO_4)_2$)

Sample	Centrifuged supernatant solution Calcium (mg per cent)	Phosphorus (mg per cent)
Control solution	14·8	4·9
(calcium × phosphorus = 15 × 5 mg%²)	14·9	4·8
	14·7	4·8
	15·1	4·9
Elastin	10·5	2·4
	9·9	2·2
'Nucleated' elastin	10·1	2·8
+ 20 moles/l. cupric chloride in methanol, 24 h	10·6	2·9
$Ca_3(PO_4)_2$ + 20 moles/l. cupric chloride in methanol, 24 h	9·0	2·2
	9·1	2·2
$Ca_3(PO_4)_2$ + 20 moles/l. cupric chloride in water, 24 h	9·3	2·3
	9·0	2·1
$Ca_3(PO_4)_2$ + methanol, 24 h	8·5	1·6
	7·8	1·8
$Ca_3(PO_4)_2$ + water, 24 h	8·0	1·6
	8·1	1·8

Elastin was extracted for 3 days in 0·1 N hydrochloric acid in methanol. 'Nucleated' elastin was extracted elastin calcified *in vitro* for 24 h.

This table also indicates that when seed crystals of ($Ca_3(PO_4)_2$) are treated with 20 mmoles/l. of cupric chloride they are not prevented from inducing precipitation of the calcifying solutions.

The minimum calcium × phosphorus product at which nucleation occurs in acid-extracted elastin is 60 mg per cent² after 24 h. When 48 h have elapsed, the calcium × phosphorus product necessary for nucleation is lowered to 50 mg per cent². Unextracted and nucleated elastin calcify at an even lower product (40 mg per cent²; Tables 5 and 6), which again shows that once a substance becomes nucleated, it continues to calcify at a lower calcium × phosphorus product than is required for nucleation[8,12,13,24].

Table 5. MINIMUM CALCIUM × PHOSPHORUS PRODUCT FOR CALCIFICATION *in vitro* OF EXTRACTED AND UNEXTRACTED ELASTIN

Sample	Initial calcium + phosphorus (mg per cent)	Centrifuged supernatant solution calcium (mg per cent) 24 h	48 h*	phosphorus (mg per cent) 24 h	48 h*
Control solution	8 + 5	8·1	8·6	5·1	4·8
Ext. elastin		8·0	7·3	5·0	4·6
Unext. elastin		7·0	5·3	4·0	4·1
Control solution	10 + 5	9·8	9·9	5·2	5·1
Ext. elastin		9·8	5·5	5·1	3·1
Unext. elastin		6·1	5·4	3·8	3·2
Control solution	12 + 5	11·5	11·6	5·2	5·2
Ext. elastin		10·9	6·8	5·0	3·9
Unext. elastin		8·5	6·0	3·1	2·9
Control solution	13 + 5	13·0	12·9	5·0	5·0
Ext. elastin		9·9	6·2	4·0	2·4
Control solution	15 + 5	15·0	14·8	4·9	5·0
Ext. elastin		10·4	8·5	3·1	—

* New calcifying solution after end of 24 h.
Extracted elastin was extracted for 72 h with 0·1 N hydrochloric acid in methanol.

Table 6. CALCIUM × PHOSPHORUS FOR CALCIFICATION *in vitro* OF 'NUCLEATED' AND 'NON-NUCLEATED' ELASTIN*

Sample	Initial calcium + phosphorus (mg per cent)	Centrifuged supernatant solution Calcium (mg per cent) 24 h	48 h†	Phosphorus (mg per cent) 24 h	48 h†
Control solution	10 + 5	9·8	10·4	5·2	5·1
'Nucleated' elastin‡		4·4	3·8	2·4	2·3
Ext. elastin		9·8	5·5	5·1	3·1
Control solution	8 + 5	7·8	7·9	5·0	5·1
'Nucleated' elastin‡		4·7	4·7	3·7	3·7
Ext. elastin		8·0	7·3	5·0	4·6
Control solution	4 + 5	3·9	4·3	5·1	5·1
'Nucleated' elastin‡		2·9	2·8	4·5	4·5
Ext. elastin		3·9	4·1	5·0	5·0

* Elastin was first extracted for 3 days with 0·1 N hydrochloric acid in methanol.
† New calcifying solution after end of 24 h.
‡ 'Nucleated' elastin was obtained by preliminary calcification *in vitro* of extracted elastin for 24 h.

The results indicate the importance of testing for pre-existing nuclei before proposing that a substance is capable of inducing the formation of nuclei, and show that purified elastin is a nucleating substance. It can therefore be proposed that certain configurations of elastin may play some part in pathological calcification in aorta, skin and other sites in the body. The role of elastin in the

nucleation of bone (and dentine) needs to be further explored, as some elastin is present in bone[31].

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CARBONIC ANHYDRASE DEFICIENCY WITH PERSISTENCE OF FOETAL HAEMOGLOBIN: A NEW SYNDROME

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BETA-THALASSAEMIA major as well as hereditary persistence of foetal haemoglobin are characterized by marked elevation of foetal haemoglobin. Beta-thalassaemia major is associated with severe anaemia, while hereditary persistence of Hb F is not. In both conditions there is no carbonic anhydrase deficiency[1,2].

We now report on a syndrome in a 47-year-old Greek male, whose pattern of synthesis of haemoglobin and erythrocytic carbonic anhydrase resembled that of a new-born infant, namely, pronounced increase of Hb F, an extremely low level of Hb A_2 and a substantial carbonic anhydrase deficiency. The patient was non-anaemic but had red cell anisocytosis and poikilocytosis, target cells, circulating normoblasts, and abnormal and immature white blood cells in the bone marrow. He had hepatosplenomegaly and suffered from weakness, tendency to muscle cramps, evanescent dermal lesions, night sweats and episodes of partial loss of vision.

Analysis of his haemolysate by starch-gel electrophoresis[3] using various alkaline buffers revealed the presence of a large slow-moving haemoglobin, with the mobility of Hb F, and a smaller Hb A component (Fig. 1). The identity of the major component as Hb F was confirmed by its resistance to alkali denaturation and its mobility on agar-gel electrophoresis, *p*H 6·2. The alkali-resistant haemoglobin, examined on many occasions by the method of Singer *et al.*[4], ranged between 58 and 69 per cent, while the distribution of Hb F in the erythrocytes[5] resembles that of a new-born infant. Hb A_2 was detected on starch-gel only with a highly sensitive benzidine stain (Fig. 1). Quantitative estimation on diethylaminoethyl (DEAE) cellulose column chromatography[6] gave very low Hb A_2 values, 0·3–0·5 per cent. On starch-gel[7], the patient's non-haemoglobin protein pattern resembled that of the new-born infant (Fig. 2). Specific staining for carbonic anhydrase by Haüsler's modification[8] of Kurata's method[9] disclosed a marked deficiency of carbonic anhydrase activity (Fig. 3). Also with the method of Tashian and Shaw[10] it was demonstrated that both the CA-I type as well as the CA-II type of carbonic anhydrase, identical with respectively *B* and *C* type[11], were deficient (Fig. 4), and the deficiency was also indirectly demonstrated by the nearly complete absence of esterase activity on those spots (Fig. 5). Additional unusual carbonic anhydrase activity was not demonstrated. Quantitative estimation of erythrocytic carbonic anhydrase by the

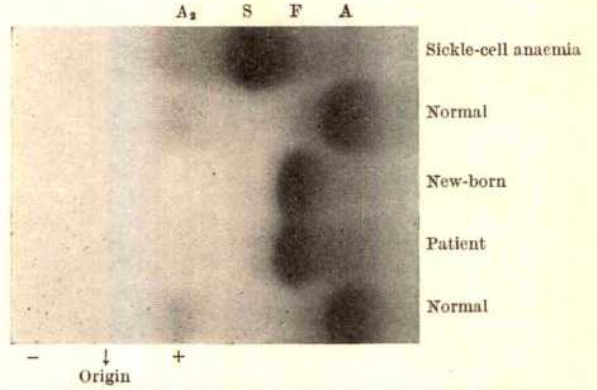


Fig. 1. Starch-gel electrophoresis in *tris*-EDTA-borate buffer *p*H 8·6 and specific benzidine stain, showing patient's haemolysate compared with controls. Note almost absence of Hb A_2 in the patient and the new-born infant

method of Wilbur and Anderson[12] showed pronounced deficiency in activity. Catalase examined by Tudhope's method[13] showed normal activity and mobility.

The patient's parents were dead. His only son and only daughter, two brothers and two maternal uncles had normal amounts of Hb A_2, no increase of Hb F and no clear evidence of red blood cell abnormalities except for one brother who, however, had alcoholic cirrhosis of the liver. No deficiency of erythrocytic carbonic anhydrase was detected in these relatives.

From the foregoing data, it can be concluded that the patient could not be homozygous for beta-thalassaemia or hereditary persistence of Hb F, while the increase of Hb F was too high for the heterozygous state. Hereditary persistence of Hb F associated with beta-thalassaemia is improbable since the children did not have increase of Hb F or Hb A_2. In both hereditary persistence of Hb F associated with alpha-thalassaemia as well as with delta-thalassaemia[14], one would not expect the increase of Hb F to be higher than 50 per cent as in both conditions only one gene leads to depression of the production of beta-chains. In the only reported case of hereditary persistence of Hb F with alpha-thalassaemia[15] the Hb A_2 level was normal, and the Hb F was increased to only 17 per cent. In the recently described cases of hereditary persistence

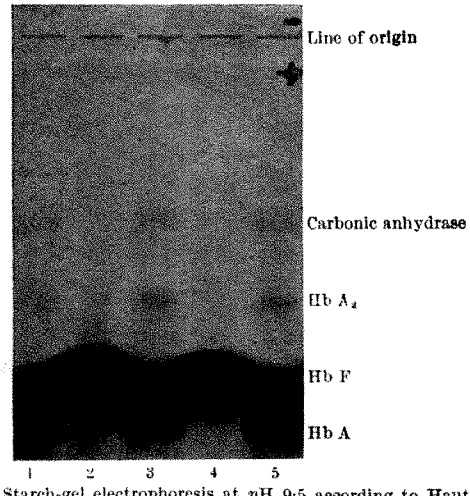


Fig. 2. Starch-gel electrophoresis at *p*H 9·5 according to Haut *et al.* (ref. 7) of patient's haemolysate compared with those of a new-born infant and of normals. 1, 3 and 5, Normals; 2, patient; 4, new-born infant. Note the almost absence of Hb A_2 in patient and the resemblance of the non-haemoglobin protein pattern with that of the new-born infant

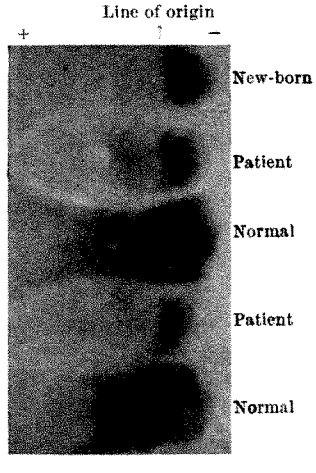


Fig. 3. Paper electrophoresis in veronal buffer *p*H 8·6, followed by specific staining with Haüsler's modification (ref. 8) of Kurata's method (ref. 9). Patient's haemolysate compared with those of a new-born infant and two normal adults. Note marked deficiency of carbonic anhydrase in the patient and new-born infant. Haemoglobin spots had migrated to the left, not seen in the picture

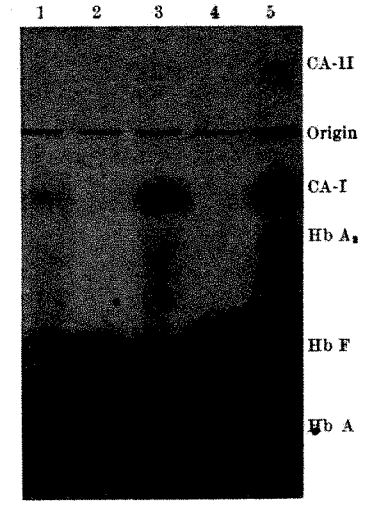


Fig. 4. Starch-gel electrophoresis in borate buffer *p*H 8·77, 0·02 M, (ref. 10). Protein stain with amido black. 1, 3 and 5, Normals; 2, new-born infant; 4, patient. Note marked deficiency of CA-I as well as CA-II type carbonic anhydrase, almost absence of Hb A_2 and increase of Hb F in the haemolysate of the patient and the new-born infant

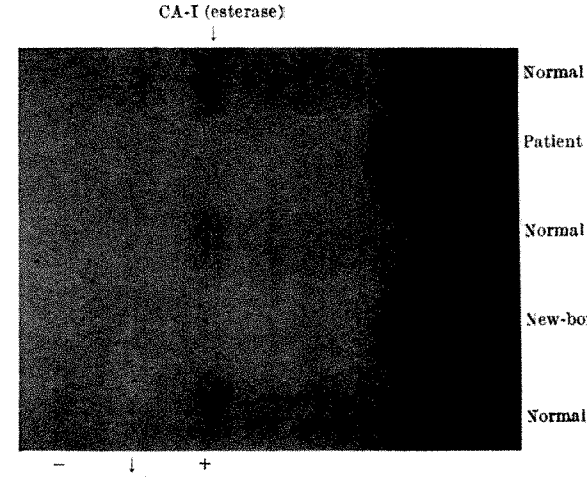


Fig. 5. Starch-gel electrophoresis in borate buffer *p*H 8·7 for esterase activity pattern. Enzyme activity visualized with Blue *RR* salt as dye-coupler and α-naphthyl acetate as substrate

of Hb F in association with delta-thalassaemia[16] the increase of foetal haemoglobin was less than 50 per cent, while the trait delta-thalassaemia was associated with a low Hb A_2 level. Therefore, it seems that our patient's condition does not fit in any of the hitherto known disorders.

In addition, the patient had pronounced deficiency of carbonic anhydrase, an important enzyme in the erythrocytes that catalyses the reaction

$$CO_2 + H_2O \underset{\leftarrow}{\overset{\text{carbonic anhydrase}}{\rightleftharpoons}} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

both ways. It is to be expected that a severe deficiency of carbonic anhydrase would lead to slowing of the reaction and possibly to accumulation of carbon dioxide and to difficulties in taking up oxygen. It appears fortunate that most of the patient's haemoglobin was of the foetal type, just as in a new-born infant, since foetal haemoglobin is a more suitable type under hypoxic conditions because of its high oxygen affinity and its ability to bind oxygen at a higher carbon dioxide pressure. This raises the question of the relationship between the carbonic anhydrase deficiency and the persistence of foetal haemoglobin in our patient. It has been shown that the synthesis of carbonic anhydrase and the hereditary persistence of Hb F are under separate genetic control[1]. In our patient,

the possibility that deficiency of carbonic anhydrase is the primary defect calling for compensatory adjustments in haemoglobin synthesis has to be considered and is suggested by the failure to find an increase of Hb F in the relatives, although at least four of those examined could have inherited the high *F* gene. The substantial carbonic anhydrase deficiency in our patient may represent the homozygous state or the heterozygous dominant trait of a hitherto undescribed abnormality. In the first assumption the trait condition may not be well expressed in this abnormality, thus the lack of convincing evidence in the relatives.

Abnormal young white blood cells in the bone marrow, and occasionally in the peripheral blood, and the hepatosplenomegaly in the patient raised the question of whether or not the patient had leukaemia. However, this would not explain his red blood cell changes and the biochemical findings. On the other hand, these changes might also have been brought about by the functional disturbances implied in the biochemical abnormalities.

A recently described case of deficiency of the *B* (CA-I) type of carbonic anhydrase[17] was not accompanied by deficiency of the *C* (CA-II) type and quantitative analysis of the total activity of carbonic anhydrase was normal. In both parents of that case no abnormality of carbonic anhydrase was demonstrated. A deficiency of carbonic anhydrase with variable increase of Hb F up to 30 per cent and decrease of Hb A_2 was described in Down's syndrome in neonates and young infants[18]. As the triad of high Hb F, low Hb A_2 and carbonic anhydrase deficiency is physiologically found in new-borns, it is not surprising to find it also in young infants having various disturbances. In our patient, however, the change-over from neonatal to an adult type of synthesis should have been well established. He also did not show the Down's syndrome and an investigation carried out by Dr. David Linder from the Childrens Hospital, San Francisco, showed the chromosomes to be normal.

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RESISTANCE OF *Plasmodium chabaudi*-INFECTED WHITE MICE TO A FULMINATING AND FATAL STRAIN OF *Plasmodium vinckei*

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IT is well known from studies of therapeutic malaria in general paresis that plasmodial infections of man induced by sporozoites or infected blood, and allowed to run a natural and protracted course, will confer on their hosts a state of protection against re-infection. This resistance, acquired as a result of mobilized and enhanced cellular and humoral defences, is strain specific and is less pronounced against other geographically remote strains. No true protective cross-immunity between different plasmodial species has been observed. The natural resistance encountered among certain human races against malaria infections, such as the resistance of the American Negro against *Plasmodium vivax*, has also been found to be only strain specific in its nature.

It has, therefore, generally been assumed that the state of resistance of populations in highly endemic malaria areas is a result of the total exposure of all individuals to the plasmodial species and strains of the region, and the degree of such resistance, a measure of the intensity and frequency to sporozoite exposure.

The *in vivo* protection afforded by a naturally suppressed malaria infection to its host has been used in the past as one of many criteria for the definition of a true plasmodial species. Thus, the late Prof. Rodhain, when working with two rodent malaria parasites, *P. berghei*[1] and *P. vinckei*[2] isolated from the region of Katanga in the Republic of the Congo (and carried by a common vector—*Anopheles dureni*), was able to show the absence of cross-immunity and protection among the two species[3]. This demonstration provided further clinico-immunological evidence to earlier observed morphological differences and host specificity of the two plasmodia. More recently, Zuckerman[4], when studying the antigenic components of these rodent malaria parasites, was able to demonstrate by methods of immunoelectrophoresis the differences in the mosaic antigenic structure of these two species, though antigens common to both plasmodia were also found.

The recent discovery in the Central African Republic (R.C.A.) of a new rodent plasmodium, *P. chabaudi*[5], has afforded an opportunity to investigate immunological relations and protective mechanisms among the different rodent malaria parasites. The observations which are the basis of the present article describe such a protective action of one plasmodium (*P. chabaudi*) against massive inocula of a fulminant and fatal related species (*P. vinckei*).

P. chabaudi (Landau, 1965) shares most of the morphological characters and patterns of behaviour of the earlier discovered *P. vinckei*. Both species show a very distinct preference for invasion and development in mature, haemoglobin-rich erythrocytes. Their schizonts occupy only part of the infected cell and contain from six to twelve merozoites. The white mouse, the young albino rat and the tree rat (*Thamnomys surdaster*) are susceptible hosts; the adult golden hamster (*Mesocricetus auratus*) is resistant to both. The claim of *P. chabaudi* to represent a true species rests at present in its distinct ecological environment, both in the rodent host (*Thamnomys rutilans*) and in its mosquito vectors, in the different growth and temperature requirements, and morphology of its sporogonic stages in experimental mosquito vectors (*A. stephensi*)[6] and differences in the pre-erythrocytic

schizogony (Garnham *et al.*[7]). A striking difference between *P. chabaudi* and *P. vinckei* may also be observed in the course of blood-induced infections of the two plasmodia in the white mouse. The course of *P. chabaudi* is prolonged and chronic. Parasitaemias progressively rise to reach a first peak at about the eighth day, descending to low levels and passing into latency, with an occasional exacerbation in subsequent months. The inapparent infection may be revealed by subinoculation of blood into a susceptible host. *P. vinckei*, on the other hand, causes in the white mouse a fulminating and invariably fatal infection. Death of the infected animals occurs within 5–7 days after intraperitoneal inoculation of a million parasitized red blood cells (RBC). Parasitaemias are always very high, ranging between 51 and 96 per cent at their peak.

A number of experiments were carried out in order to detect if cross-immunity between *P. chabaudi* and *P. vinckei* existed.

In a preliminary trial, thirty-two white mice (*C.F.I.* strain) were each inoculated with 1 million *P. chabaudi* parasitized erythrocytes (from the eighth blood transfer) and the course of their plasmodial infection followed. On the 43rd day following their inoculation, sixteen mice of this group were challenged, each with ten million *P. vinckei* parasitized red cells. The remaining animals were kept for observation of their chronic latent *P. chabaudi* course. Sixteen normal white mice of comparative age were also given identical inocula of *P. vinckei* and served as controls. Daily blood smears were obtained from all the animals, and the degree of parasitaemia determined by the percentage of infected erythrocytes per ten thousand cells counted. The results of this experiment have clearly shown that whereas all control *P. vinckei* mice succumbed to their fulminant plasmodial infections within 5 or 6 days, no deaths occurred among the latent *P. chabaudi* challenged mice. Parasitaemias in this group were generally low, and animals which showed elevated parasite counts were able afterwards to control and suppress their infection. Fig. 1 summarizes parasitaemias in each of the control and challenged groups of mice, as seen from daily parasite counts.

In a second experiment, twenty-four white mice were challenged with *P. vinckei* after shorter periods of exposure to *P. chabaudi* (3–5 weeks). The results were similar to those obtained in the first group. No deaths were observed among the challenged animals. Experiments since carried out have demonstrated that protection against fatal *P. vinckei* infections in mice previously exposed to *P. chabaudi* appears early. Mice infected with *P. chabaudi* and re-inoculated 7 days later with a million *P. vinckei* parasitized cells survived their challenge infection and were able to lower and even suppress parasitaemia. On the other hand, *P. chabaudi* and *P. vinckei*, inoculated in mice simultaneously, failed to protect their hosts, and the animals succumbed to their concomitant infections within 5–6 days with overwhelming parasitaemias (74·5–86 per cent).

It would seem, therefore, that resistance to the usually fatal inocula of *P. vinckei* develops in white mice within the first week of their active *P. chabaudi* infection. A more

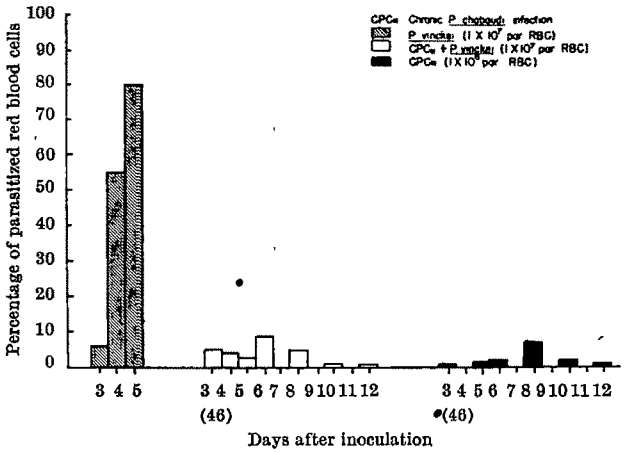


Fig. 1. *P. vinckei* and *P. chabaudi* parasitaemias in white mice, as compared with *P. vinckei* in chronic *P. chabaudi* mice

detailed investigation on this problem—the exact time of appearance of this protection, its duration and evanescence, as well as its manifestations *in vivo* and possibly *in vitro*—is at present being carried out.

Our experiments have further shown that the shielding action of *P. chabaudi* against fulminant and fatal *P. vinckei* infections is powerful enough to protect their hosts even against very massive inocula of 100 million and one billion (10^9) parasitized erythrocytes. Table 1 summarizes the results of a comparative study in which inocula of varying strength of *P. vinckei*, *P. chabaudi* and *P. berghei* were administered to groups of white mice, 43 days earlier exposed to *P. chabaudi*. It should be noted that at the time of their challenge inocula most mice had very light or suppressed *P. chabaudi* infections.

It will be seen from the results of these experiments (Table 1) that when a million *P. chabaudi* infected red cells are given to normal white mice (*C.F.I.* strain), a chronic and protracted infection ensues. Parasitaemias after early elevations fluctuate and the infection in the majority of the animals is suppressed to submicroscopic levels. However, in some animals maximum parasite densities may be encountered as late as the 70th day in their infection. A mortality of about 20 per cent among *P. chabaudi* mice has been observed. Death appears late in the course of the infection. Enhanced virulence has not at present been found to occur after eighteen successive blood transfers.

Higher inocula in normal white mice alter the course of the infection. Maximal parasitaemias are comparatively higher and more pronounced and the parasite peaks appear earlier in the course of the infection. Death in these animals is not the end result of a prolonged disease, but appears early in the 6th or 7th day in the course of their plasmodial infection.

In the groups of mice initially exposed to a million *P. chabaudi* and challenged 43 days later with 10 million or 100 million parasitized cells from stabilates of the homologous strain, all animals recovered. Parasitaemias were

Table 1. THE COURSE OF *P. chabaudi*, *P. vinckei* AND *P. berghei* IN WHITE MICE, 38 OR 43 DAYS EARLIER EXPOSED TO *P. chabaudi* INFECTIONS

Initial *P. chabaudi* inoculation	Challenge inoculation day 43 or 38	No. of fatal infect./ total No. mice inoc.	Per cent of fatal infections	Maximal parasit. per cent infected RBC's Mean	Range	Days of death Mean	Range
1 million inf. RBC's		2/10	20	18·9	0–60 0	—	64
None (controls)	10 million *P. vinckei* inf. RBC's	5/5	100	82·4	75·0–87·8	5 8	5–6
1 million inf. RBC's	10 million *P. vinckei* inf. RBC's	1/9	11	21 0	0·3–57·0	—	17
None (controls)	100 million *P. vinckei* inf. RBC's	5/5	100	82·0	72·0–88·0	5·4	5–7
1 million inf. RBC's	100 million *P. vinckei* inf. RBC's	3/10	30	19·4	1·6–65 0	12·7	6–26*
None (controls)	10 million *P. chabaudi* inf. RBC's	4/10	40	43·1	7·6–63·9	7·0	5–9
1 million inf. RBC's	10 million *P. chabaudi* inf. RBC's	0/10	—	15·7	< 0·1–31·4	—	—
None (controls)	100 million *P. chabaudi* inf. RBC's	2/7	28 5	44 6	38·1–49·4	—	6
1 million inf. RBC's	100 million *P. chabaudi* inf. RBC's	0/7	—	11·8	0·3–35 0	—	—
None (controls)	1 million *P. berghei* inf. RBC's	10/10	100	58·1	29·0–81·0	13 0	7–19
1 million inf. RBC's	1 million *P. berghei* inf. RBC's	10/10	100	51·6	18·2–81·0	10 5	7–17
10 million inf. RBC's	1 billion *P. vinckei* inf. RBC's	0/4	—	8·0	2·0–11·8	—	—
100 million inf. RBC's	1 billion *P. vinckei* inf RBC's	1/4	25	27·9	1·5–93·0	—	4

* Death occurring so late in the infection was probably due to *P. chaubaudi*.

lower than in the controls and were readily suppressed. 82 per cent of the mice challenged with massive (100 million or 1 billion (10^9) *P. vinckei* infected cells) survived and recovered.

Even in the fatally terminating infections of this group, the course of the disease was twice as long as in the *P. vinckei* controls (12·7 days against 5·4 days).

The course of *P. berghei* in the *P. chabaudi* latent mice was greatly different. The infection was invariably fatal, and though parasitaemias did not greatly differ in this group from that of the *P. berghei* controls, death, as the outcome of a combined plasmodial infection, appeared earlier in the *P. chabaudi* latent animals challenged with *P. berghei*.

The protective phenomenon described herewith from rodent malaria is of considerable interest. Though immune-reactions and *in vivo* protection have long been known to occur in some protozoal infections, and a state of pre-immunity to homologous plasmodial strains observed, it was generally assumed that such protective phenomena necessitated a prolonged period of immuno-alertness of the host and prolonged antigenic stimulation. The early appearance of a strong resistance in mice inoculated with *P. chabaudi* to fatal inocula of *P. vinckei*, a related yet geographically distinct species, points to the existence of other immuno-mechanisms, early awakened and powerfully triggered. The exact nature of this mechanism is at present unknown. Its general implications in malaria immunity and its exact significance in the relationship of *P. vinckei* to *P. chabaudi* are at present being investigated.

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SPONTANEOUS MOVEMENTS IN THE EMBRYO OF *Eleutherodactylus martinicensis*

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Larval development requires that a functional system of locomotion should be present from the beginning of free life, a necessity from which the embryo, provided with nourishment and sheltered from the outside world, is relieved. This consideration profoundly affects the development of neuromuscular co-ordination of bodily movement in these two sets of circumstances. Among those vertebrates in which part of the developmental period is spent in a larval form, the nervous and muscular systems are then made up of comparatively few elements arranged in relatively simple relationships. In the neural tube of larval Amphibia the primary sensory and motor systems are largely separate from the centres which will innervate the limbs at a later period of development.

The ontogeny of movement was first systematically investigated by G. E. Coghill in the embryo and larva of *Amblystoma punctatum*. On the basis of a long series of behavioural and neuroembryological experiments on this species, he stated a general thesis that from an early background of a total pattern of movement, individual elements gradually emerged and became distinct, and were ultimately independent of general activity[1]. At the present time this conception is considered of dubious relevance to the development of vertebrates higher in the series, the ontogeny of which is either embryonic or foetal[2]. The alternative view has been formulated that the behaviour pattern of the foetal mammal is built up by the summation of separate individual reflexes[3].

Recently, a series of investigations by Hamburger *et al.*[4–6] has shown that the chick embryo spends much of its time in spontaneous activity of its members, each movement of which generally lacks any co-ordination with that of any other. At the same time, locally co-ordinated reflex actions can result from external stimuli. The developing chick has thus two quite separate patterns of behaviour—a collection of reflexes which in normal development are never exhibited, and on the other

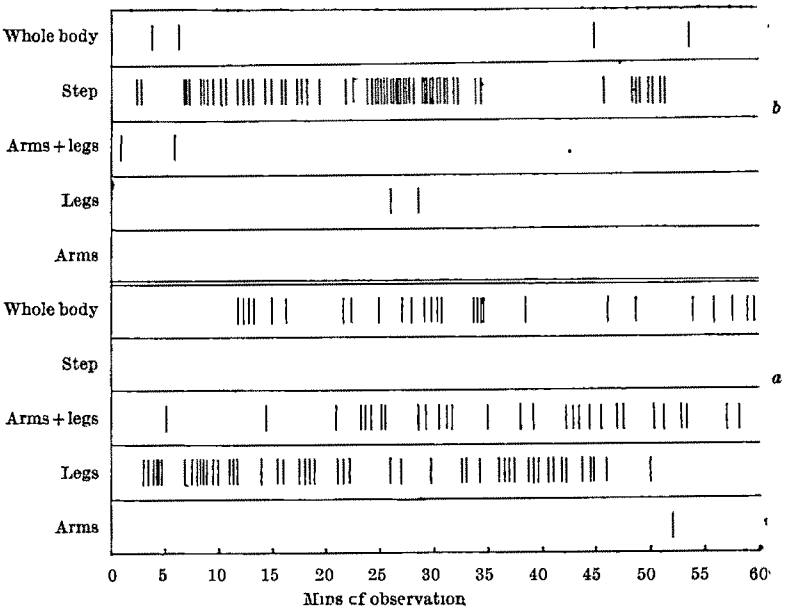


Fig. 1 Analysis of spontaneous movements occurring during 1 h in an embryo of *Eleutherodactylus*. (*a*) At 9 days, at the stage of reflex leg withdrawal, (*b*) 6 days later at juvenile stage, with no tail

hand its repertoire of spontaneous random movements of head, trunk and limbs.

How widely and on what scale does spontaneous behaviour exist in other embryos? Very little is known of such movements in mammals except that they exist[3]. Special interest attaches to the question whether two separate modes of developmental behaviour occur in those Amphibia which have an embryonic development, in view of the close relationships which members of the group with this type of ontogeny bear to the great majority the development of which is mainly larval.

In the bufonid genus *Eleutherodactylus*, which is widely distributed in the Caribbean and on the adjacent mainland, development is wholly embryonic[7]. Larval features in pharynx and aortic arches of other amphibia are suppressed, and the tail has acquired a new respiratory function within the egg envelope. In those species which are found in tropical habitats, development is very rapid. The eggs are laid in damp places but will continue to develop when freed from their envelopes and are immersed in water or saline. They are then completely accessible to observation and to experiment. So far, they have been used in endocrinological[8] and neuro-embryological researches[9–11].

The normal reflex pattern of behaviour in *E. martinicensis* has recently been described[12]. In this species, development at 20° to 30° C occupies 13–14 days from the laying of the eggs until the hatching of the fully formed juvenile. The first movements of the limbs occur at 7·5 days, during period of flexion of the trunk. At 8·5 days a withdrawal reflex of the hind limb independent of trunk movement can be seen on light stimulation. At 10 days, flexion of the hind limb is followed by extension, which gradually becomes more vigorous, and is then known as a "kick". In the following days, the leg becomes capable an increasing series of repeated kicks, which build up into bilateral swimming movements, as the arms become co-ordinated therewith. At first, incomplete swimming strokes are seen, which gradually extend in scope until, at the hatching stage, the animal can swim continuously for several seconds. Thus the reflex behaviour of *Eleutherodactylus* builds up into bilateral swimming.

Recently, the spontaneous behaviour of the embryo has been investigated in the absence of external stimuli. Observations were made on embryos in groups of two continuously over periods of 1 h, and the time of each movement of the embryo was noted under various headings. The data presented here refer mainly to two embryos which were followed over a period of 8 days from the 8·5-day stage when the legs retract on touching, to the second day of juvenile life with no vestige of the tail remaining. Movements of the limbs which were recorded were of the arms alone, of the legs alone, and of arms and legs together, diagonal movements of which were separately scored. In addition, occasions when the animal jumped forwards, or turned round, were noted. Two sets of records are given in Fig. 1*a* and *b*, while in Table 1 the numerical data are summarized, and are also set out graphically in Fig. 2.

The number of individual movements recorded during 1 h ranges from 15 to 107. They are less frequent during the early phase of atrophy of the tail. They are mostly of brief duration; no attempt was made to measure the time occupied by each movement, but it was always less than 1 sec. If we assume an average duration of 0·5 sec.

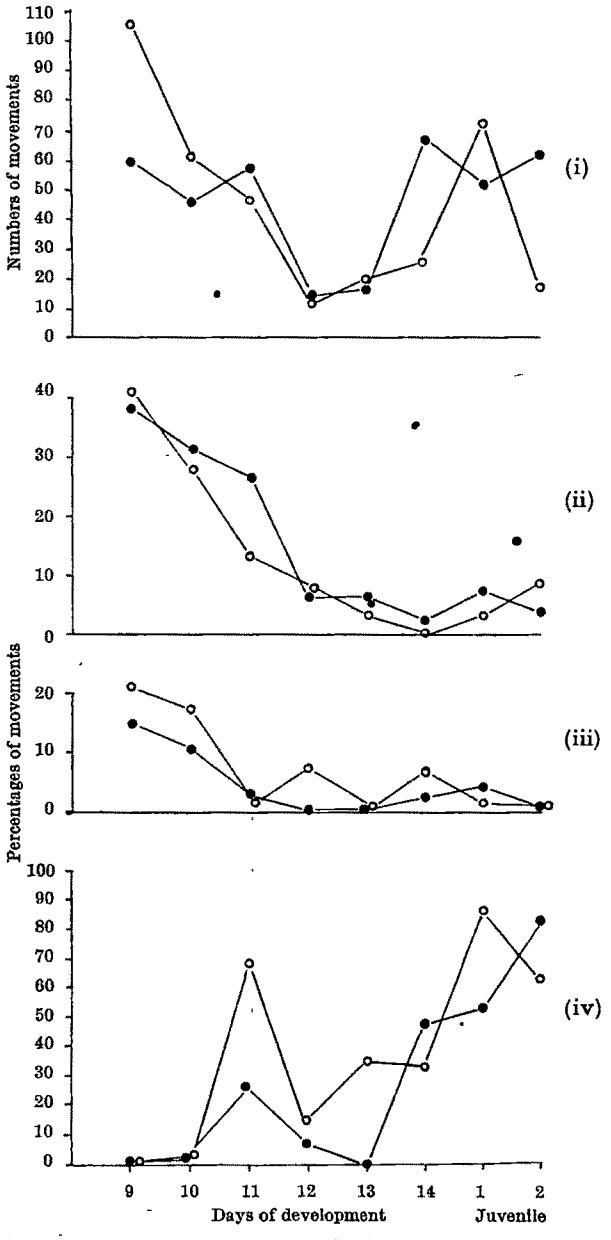


Fig. 2. Spontaneous movements occurring during periods of 1 h in two embryos of *Eleutherodactylus* at the stage indicated. Observations on 8 successive days. (i) Total number of movements (as in Table 1). (ii) Percentages of leg movements alone. (iii) Percentages of trunk movements. (iv) Percentages of steps, that is, diagonal arm and leg movements

then the proportion of time that the animal spent in movement during the periods of observation is generally less than 1 per cent, a very small proportion compared with the chick embryo, which may be active for 80 per cent of the time. In *Eleutherodactylus* embryos the number of spontaneous movements is at a minimum a day before the resorption of the tail begins.

A second point of difference from the chick is that although single limbs may show spontaneous movement,

Table 1. ANALYSIS OF SPONTANEOUS MOVEMENTS IN TWO EMBRYOS OF *Eleutherodactylus martinicensis*

Days of development	9	10	11	12	13	14	Juvenile +1	Juvenile +2
Stage	Egg tooth	Early xanthophores		Tail dwindles 1·5–2·0 mm	1·2–1·5 mm	0·6–1·0 mm	Tail gone Air breathing	
Reflex behaviour	Leg retraction	Leg extension	Repeated kick	Swims				
Total movements	61, 107	47, 61	49, 59	13, 15	17, 20	27, 68	51, 73	18, 83
Leg movements alone	24, 44	15, 17	7, 16	1, 1	1, 1	0, 2	2, 3	1, 2
Movements involving trunk	9, 24	5, 11	1, 2	0, 1	0, 0	2, 2	2, 1	0, 0
Stepping movements	0, 0	2, 3	15, 34	1, 2	0, 7	9, 33	27, 64	12, 52

the proportion of total movements of this kind falls to a very low level by the end of development. Most movements involve both arms and legs, and must therefore originate in impulses within neurones elsewhere than in the motor cells of the ventral horns. The extent to which trunk movements are involved decreases with time in both spontaneous and in reflex behaviour.

Simultaneous arm and leg movement towards the end of development is largely diagonal in pattern, and clearly foreshadows ambulation. In the late embryo, these conjoint movements gradually build up into complete walking steps. There is then a strong tendency for repetition of step movements, to the gradual exclusion of other types of spontaneous behaviour. Thus, whereas reflex behaviour in *Eleutherodactylus* builds up into bilateral swimming, spontaneous behaviour builds up equally gradually into diagonal walking. Accordingly, Hamburger's dictum that the two modes of activity are entirely separate[13] is further exemplified.

At the "repeated kick" stage of reflex behaviour, the embryo is spontaneously attempting ambulatory movement. There is no control over balance in these first faltering steps; the embryo often falls forwards, or on its side. Each movement is incomplete and the embryo may hold bizarre poses with an outstretched arm or leg until the action is resumed many seconds later. It thus seems that proprioception does not enter into these early ambulatory movements, although its presence has been postulated to explain why leg extension follows flexion in the contemporary pattern of reflex behaviour[11].

The separate nature of the two types of embryonic behaviour in *Eleutherodactylus* was further illustrated in some experiments in which embryos had been subjected to some 12 h of anaesthesia induced by a 1/10,000 w/v solution of *MS* 222 (ref. 14) and were recovering on re-immersion in saline. Control embryos of the same age by this time were showing the kick reflex, but this did not yet appear in the anaesthetized embryos, even after full recovery. Before this point, however, spontaneous movements appeared after 5–10 min in saline, and were then maintained at a level greater than normal for embryos of this age. No reflex responses were obtained by touching the legs until 35–50 min from the beginning of the recovery period. Thus, for about 30 min while the reflex pathways were still anaesthetized, spontaneous behaviour was the only mode of activity of the embryo.

The existence of two modes of behaviour may thus be regarded as a feature of embryonic life in which *Eleutherodactylus* resembles the chick, despite the differences in their respective patterns of spontaneous movement. Thus we can already say that this type of movement is not found exclusively among amniote vertebrates, the development of which *Eleutherodactylus* again resembles in that the limb buds appear relatively early in development[9], and independently of any influence of thyroxin[8]. The ontogeny of *Eleutherodactylus* is in this respect unlike that of larval amphibia. It may well be that larval development among tetrapods is as much a specialization as a primitive feature.

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CLASSIFICATION, PROBABILITY AND UTILITY

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In recent publications, Williams *et al.*[1,2] discussed the logical basis of classification, with special reference to numerical methods and to the role of probability in the process. Such a discussion was long overdue, and many of the points they make will win general assent.

Williams and Lance[2] point out that the individuals classified on one hand, and the attributes used in their classification on the other, may each be a finite set, completely known, or may be samples from an infinite population. The same, they say, may apply to the uses to which the classification is to be put. Where the uses constitute the only infinite set, they argue, probability has no meaning and the classification must be based on profitability. Williams and Lance do not attempt to define "profitability", but some discussion of this will be attempted; the term "utility" seems, however, preferable.

Utility in single-purpose classification. A particular use for a classification may often be expressed in terms of another classification. A classification of microfossils might, for example, be directed to identification of the geological strata from which they were derived. Their stratigraphical origins, when known, then provide a use-classification. In such cases, the utility of a proposed classification may be assessed by comparing it with this use-classification which, *ex hypothesi*, is optimal. A loss function is defined for each type of misclassification, and the classes of the proposed classification are mapped on to those of the use-classification in such a way as to minimize this loss function. The different minimal values for the loss function then provide a basis for comparing the utility of different proposed classifications.

In other cases, the use of the classification may be expressed in terms of prediction of a continuous variable. The utility of different classifications can then be compared in terms of the proportion of the total variability of the predicted variable contained in the inter-class comparisons.

Utility in multi-purpose classifications. Where the classification is to serve more than one use, the different loss functions for mis-classifications must be combined in order to compare the utility of two alternative systems. This is possible only where the loss functions can be expressed in the same terms, and even so they require arbitrary weighting. Where the loss functions are not even commensurable, comparison of different classifications on the strict basis of utility becomes impossible.

In all cases where the classification is to serve a finite set of uses, Williams and Lance term it "extrinsic", and expressly exclude it from consideration. Their remarks on profitability (= utility) therefore are strictly related to the case where the uses are infinite. But here the considerations of the preceding paragraph apply *a fortiori*, so that any treatment in terms of utility *sensu stricto* is out of the question. An artificial substitute may, however, be proposed. Any particular use will presumably be best served by separating the individuals into classes according to some function of their observed attributes. For different

uses, different functions may be required, and different weights given to the various attributes. It is accordingly suggested that the utility of a general-purpose classification be defined as its ability to predict the differing values of an attribute selected at random (compare with Williams and Lance's "informativeness").

With this arbitrary definition of utility for a general-purpose classification, probability and utility clearly come together. The estimate of utility will depend on the chance selection of an attribute for prediction. Estimates in respect of different attributes will form a frequency distribution, and permit a significance test of differences in utility between classifications.

It should be noted that this approach does not require either individuals or attributes to be a sample from an infinite population. It is valid even if the attributes are limited in number and completely known, and not only where those observed are regarded as a sub-set of an infinite set of observable attributes—the case envisaged by Williams and Lance when they write of the behaviour of further attributes as a test of profitability.

Measurement of the utility of a classification. To apply the general principle above requires that "ability to predict" the values of an attribute should be defined exactly. A solution to this problem will be outlined here.

Considering first the prediction of values of a quantitative (metric) attribute, if two classification procedures each divide the same set of individuals into the same number of classes, that procedure will have the greater predictive ability which gives the larger inter-class and the smaller intra-class variance—that is, which has the larger intra-class correlation. Where two classification procedures distinguish different numbers of classes, the appropriate basis for comparison seems to be the probability of the calculated intra-class correlation on the null hypothesis that the inter- and intra-class variances are equal.

For a qualitative attribute the information content of the inter-class comparisons may take the place of inter-class variance. Where the numbers of classes differ, an approach based on probability may again be suggested. For each number of classes, all possible partitions of the observed values of the attribute into that number of sets may be considered, and the proportion of such partitions with inter-class information content equal to, or greater than, that of the proposed classification may be computed. These estimates of probability, on the null hypothesis of random partition, may then be compared and the classification giving the less probable value of inter-class information selected.

We may now revert to the foregoing proposal that the utility of a general-purpose classification should be defined as its ability to predict the values of a random attribute. For any particular attribute we have suggested that prediction ability be regarded as a strictly decreasing function of the probability of the inter-class variance (information) on the hypothesis of random partition. The negative logarithm of the probability seems an appropriate function to choose. Accordingly, one may propose as a measure of utility:

$$U = E\ \{ - \ln P_i \}$$

where E stands for expectation, and P_i is the probability that a random partition of the values of the ith attribute into the same number of classes will concentrate as much or more information (variance) into the inter-class comparisons as in the classification considered. This expectation will be:

$$-\frac{1}{n} \sum_{i=1}^{n} \ln P_i$$

where the n attributes observed are considered as a complete set, and will be estimated by the same expression where they are a sample of a larger set.

It may be noted that this proposal can be immediately linked with classification for a specific purpose, for here:

$$U = -\ln P$$

where P is the probability, corresponding with P_i above, in respect of the particular attribute the prediction of which is desired.

The classification of samples. The foregoing remarks, and the concept of utility developed, apply irrespective of whether the set classified is to be regarded as complete, or as a sample from a larger population. It is to the latter case only that Williams and Lance regard probabilistic considerations as applying.

Sampling theory has never been satisfactorily applied to classification procedures. That the classifications derived by applying a specified procedure to replicate samples of individuals will themselves be subject to the vagaries of sampling is clear enough. But a classification is not itself a member of that class of objects to which probability concepts apply. Probability statements are made about events—real or hypothetical—and express a relationship between a defined sub-set of events and a defined set including it. A classification is an arrangement of individuals into classes, and as such has no probability whether or not the individuals form a sample from a larger set. One may ascribe a probability to a particular feature of the classification resulting when a specific procedure is applied to a sample of individuals from a specified population, and this may be what Williams and Lance had in mind. One may, for example, speak of the probability that this procedure will distinguish classes at all, or that individuals in a particular sub-set will all be allotted to the same class. But to ascribe a probability to the whole results of a classification procedure seems likely to be meaningless.

In order to apply probability concepts to classifications of repeated samples, it is accordingly necessary for attention to be fixed on particular features of the classification, rather than to consider it as a whole. It would be possible, for example, to determine a frequency distribution for the number of classes to be distinguished. The most suitable single expression of the value of a classification seems, however, to be its utility, as defined above. In repeated samples, this too will have a frequency distribution, and the frequency distributions of utility for classifications by two differing procedures could appropriately be used to compare their value.

It should be noted, however, that though this interpretation of the sampling properties of a classification has been described in connexion with an actual sampling of individuals, it is also meaningful where the observed set of individuals constitute the whole population. In this event, the observations can be treated, under a null hypothesis, as a sample from a hypothetical population in which the observed values of each attribute are distributed at random and independently between different classes. The single observed value of utility, not itself subject to sampling error, can then be compared with the expected distribution of utility in samples from the hypothetical population.

Willams and Lance suggest, as a possible strategy where probabilistic considerations apply, the independent classification of two separate samples followed by a comparison of the results. But even comparisons between classifications must usually be confined to extracted statistics (such as their utility) rather than covering all features. The first requirement for a general comparison between classifications of two samples would probably be that the classes of one should be mapped on to those of the other; in the general case this may be fraught with great difficulties. Consequently, the comparisons suggested by Williams and Lance would be unlikely to go beyond the rather subjective level they envisage.

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LETTERS TO THE EDITOR

ASTROPHYSICS

Lithium in the Peculiar Star *HD* 101065

In a recent article[1] Przybylski presented the results of a preliminary coarse analysis of element abundances in the atmosphere of the peculiar star *HD* 101065. This star, the spectrum of which consists almost entirely of rare earth lines, is of considerable interest as it apparently represents a very advanced stage of nuclear evolution. In order to decide where *HD* 101065 fits into the scheme of stellar evolution at present accepted, a quantitative knowledge of the composition of its atmosphere is necessary. Przybylski's work provides a valuable first step in this direction.

I will report here some observations made at the Radcliffe Observatory, Pretoria, in 1965, that provide additional information on the composition of *HD* 101065. A number of coude spectra were obtained with the 74-in. reflector giving complete coverage of the spectrum from 3370 Å to 6867 Å. In the photographic region the dispersion is 6 8 Å/mm and in the visual 13·6 Å/mm. The latter plate will be discussed here. The exposure was 6 h, and the spectrum was widened to 0·30 mm. The density is suitable for microphotometric measurements over most of the range 5500–6880 Å, and is particularly good in the region of Hα. Wave-length measurements on this plate confirm that the majority of the observed spectrum lines are due to the once ionized rare earth elements. In the region 5500–6880 Å nearly every rare earth ion line tabulated by Meggers *et al.*[2] appears in *HD* 101065 (accounting for nearly all the strong stellar lines) and with relative intensities roughly similar to the laboratory observations.

The main point to be made in this communication is confirmation of Przybylski's suggestion of the occurrence of the 6707·8 LiI line in the spectrum of *HD* 101065. Przybylski pointed out that the SmII line at 6707·45 Å should occur in *HD* 101065 and was unable to estimate whether the line at 6708 Å in the star was due to LiI or this SmII line.

Fig. 1 is a microphotometer tracing of the region 6685–6720 Å in *HD* 101065. A moderately wide slit was used in order to smooth out grain noise; the measurements to be described later were carried out with higher resolution. Line identifications originate from the tables provided by Meggers *et al.*[2] and their observed (laboratory) intensities appear in brackets. Fig. 2 shows the spectral region near Hα and is on the same scale as Fig. 1.

From the identified lines of SmII in Figs. 1 and 2, it can be seen that the 6707·45 Å SmII line, with laboratory intensity 3·0, cannot account for more than a minor part of the strong feature at 6707·8 Å. As there are no other strong rare earth lines at this wave-length we conclude that LiI is the main contributor.

With the aid of an intensity calibration of the photographic plate the equivalent width of the LiI line (partly dotted in Fig. 1) was found to be 340 mÅ. Following Przybylski's assumption that the atmosphere of *HD* 101065 is physically similar to that of the Sun, and allowing for the increased turbulence[1] in *HD* 101065 and the fact that LiI atoms would have a higher turbulent velocity than rare earth atoms, we deduce a lithium abundance, relative to the Sun, of $\log (N^*(\mathrm{Li})/N^{\odot}(\mathrm{Li})) \sim 2{\cdot}4$ (a correction for line saturation has been made assuming the shape of the curve of growth to be similar to that of the Sun, and we adopt $\log W^{\odot}(\mathrm{Li}) = -6{\cdot}15$).

Accurate measurements of the wave-length of the Li 6708 line can indicate the $^6Li/^7Li$ isotopic abundance ratio in a stellar atmosphere[3]. The extreme positions are 6707·81 Å for pure lithium-7 and 6707·97 Å for pure lithium-6. With the interference from the SmII 6707·45 Å and TbII 6706·79 Å lines (Fig. 1), it is not possible to give a very precise value for the wave-length in *HD* 101065. However, my measurements, with an attempt to allow for interference, give 6707·85 Å for the position of the LiI line, suggesting that at least some of the lithium in *HD* 101065 is in the form of lithium-6.

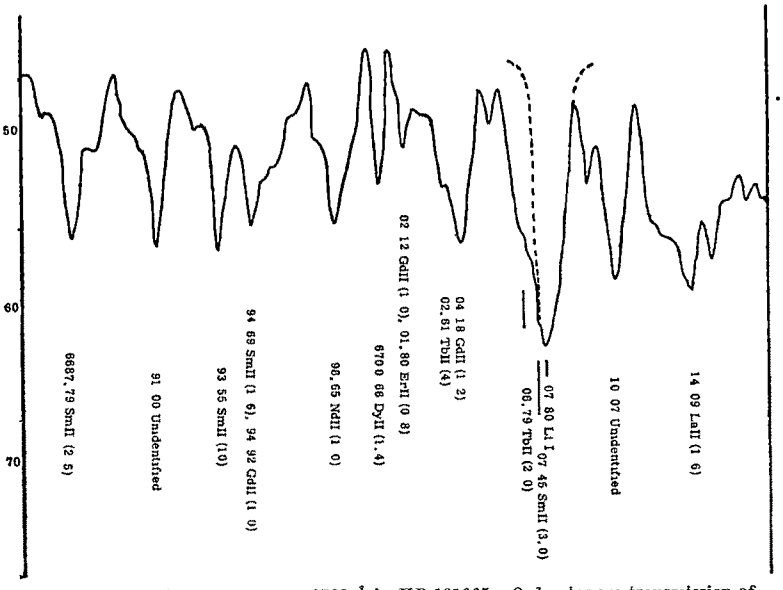


Fig. 1 The spectrum region near 6708 Å in *HD* 101065. Ordinates are transmission of plate

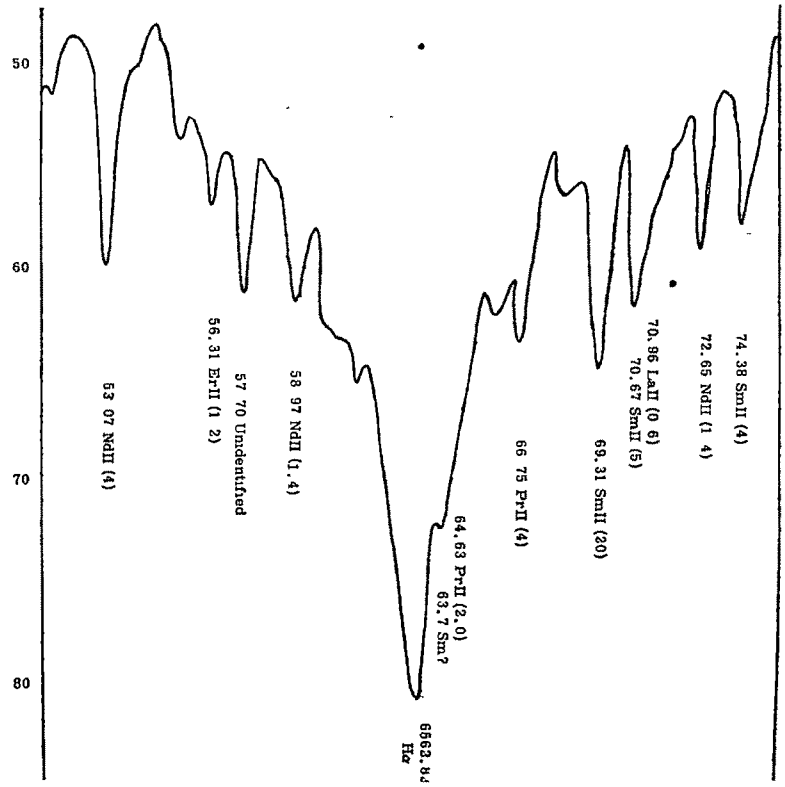


Fig. 2. The spectrum region near Hα in *HD* 101065

From a study of the photographic region, Przybylski was unable to find evidence for the occurrence of lutetium and ytterbium in *HD* 101065. The plate studied here shows the LuII lines at 5476·69, 5983·9, 6221·87 and 6463·12 Å to be present in considerable strength, so lutetium probably has an abundance similar to that of the other rare earth elements. No ytterbium lines can be identified with any conviction. The resonance line at 3694·19 Å should attain great strength if the abundance of ytterbium were similar to that of the other rare earths, but a feature observed near this wave-length is probably attributable entirely to SmII 3694·30 and GdII 3694·03.

The peculiar composition of the atmosphere of *HD* 101065 indicates that high-energy nuclear reactions (in particular the *r*-process of neutron capture) have occurred in the interior of the star and the products mixed to the surface. During such a process all lithium would be destroyed. The lithium observed at present is therefore likely to have been produced from a spallation process on the surface of the star; the probable presence of some lithium-6 is in agreement with this conclusion. Furthermore, if the highly turbulent atmosphere (Przybylski finds a turbulent velocity of 5 km/sec) indicates that the outer parts of the atmosphere are convective, lithium may be transported into the interior and destroyed, so that the observed abundance must represent an equilibrium value. These arguments suggest that *HD* 101065 has a strong magnetic field, by means of which the spallation process is energized. A search for other spallation products (for example, beryllium) and a magnetic field in *HD* 101065 should be undertaken when very large telescopes become available in the southern hemisphere.

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Abundance of Boron in the Sun

IN a recent paper, Waddell and Slaughter[1] suggested that the composite feature at 8668·43 Å in the solar spectrum includes a high excitation line of B I. This line is one of a number first reported in the laboratory spectrum of B I by Gunnvald and Minnhagen[2] (Table 1). From the observed central depth of 0·015, a boron: hydrogen ratio of $4{\cdot}0 \times 10^{-7}$ was deduced. The terrestrial ratio[3] is $7{\cdot}6 \times 10^{-10}$, while Russell's[4] photospheric upper limit is $1{\cdot}1 \times 10^{-7}$. This figure was later reduced by Babcock[5] to $2{\cdot}8 \times 10^{-8}$.

Lines from the other multiplets listed in Table 1 should have easily measurable central depths if Waddell's identification is correct. In particular, the lines at 11,660 Å, with a lower excitation potential of 4·96 eV and a total *gf* of 2·14, should be strongly present. A photoelectric record of the solar spectrum in this region taken at the Jungfraujoch[6], with wave-lengths and identifications from Babcock and Moore[7], is shown in Fig. 1. The stronger line at 11,660·05 Å is not well placed. The weaker line, however, falls in an apparently clear region. At 11,662·47 Å the trace shows no feature with a central depth greater than 0·01.

The other multiplets are not so useful, as they have the same lower level as the ^2P- ^2D multiplet. Moreover, the published traces[8] of 15,000–17,000 Å are rather noisy. For the most favourable case, 16,244·66 Å, any feature with a central depth of more than 0·02–0·03 can be excluded.

We have calculated the central depth of B I lines for various values of the abundance using the average column

Table 1

Wave-length (Å)	Transition		*gf* (Coulomb approx.)	*E.P.* (eV)
16,244·66	$2s^2 3p^2 P_{3/2}$–	$2s^2 3d^2 D_{5/2}$	5 462	6·03
16,240 32	$^2P_{1/2}$–	$^2D_{3/2}$		
15,629·01	$3p^2 P_{3/2}$–	$4s^2 S_{1/2}$	1 129	6·03
15,624·66	$^2P_{1/2}$–	$^2S_{1/2}$		
11,662·47	$3s^2 S_{1/2}$–	$3p^2 P_{1/2}$	2·141	4·96
11,660 05	$^2S_{1/2}$–	$^2P_{3/2}$		
8668 570	$3p^2 P_{3/2}$–	$5s^2 S_{1/2}$	0 110	6·03
8667·223	$^2P_{1/2}$–	$^2S_{1/2}$		

of the Utrecht reference photosphere. At the level of formation of a high-excitation B I line ($\tau_{5000}=0{\cdot}1$, $T=5100°$ K, $\xi=1{\cdot}6$ km/sec) the half width, assumed to be wholly Doppler, is 0·207 Å. Waddell's abundance would produce features with central depths of about 0·30 (11,660 Å) and 0·18 (16,244 Å). From the observations at 11,660 Å an upper limit of $4{\cdot}0\times10^{-9}$ can be set with considerable certainty. Weak-line calculations set a limit of $2{\cdot}1\times10^{-9}$ for an equivalent width corresponding to a boron line of central depth 0·01.

A smaller, though less certain, value for this upper limit can be obtained from far ultra-violet observations[9]. Lines of Be I, with a lower excitation potential of 2·71 eV, have been identified at 2495 Å. In laboratory spectra, Clearman[10] has reported two ground state lines of boron at 2496·72 Å and 2496·77 Å. The Coulomb approximation gf values for the two multiplets are 1·42 (Be I) and 0·12 (B I). The product $10^{-\chi}\times gf$ is then $2{\cdot}8\times10^{-3}$ for Be I and $8{\cdot}3\times10^{-2}$ for B I. Allowing for the fact that boron is about half ionized and that the B I lines would be partially masked by other features, it seems very unlikely that boron can be more abundant than beryllium. The Goldberg, Müller and Aller abundance[11] for this latter element is $2{\cdot}3\times10^{-10}$, although the far ultra-violet spectra indicate that this may be too high a value (Kacalov *et al.*[12]). Tousey[13] reports no certain identification of B I and B II lines.

Babcock[5] has reported lines of the Q and R branches of the $A^1\Pi$–$X^1\Sigma$ (0,0) band of BH in sunspots, but has not given any equivalent widths. Using the Zwaan[14] sunspot model, the boron abundance would be given by:

$$\frac{N_B}{N_H}=2\times10^{-8}\,\frac{1}{f_{00}}\cdot\frac{W_J}{S_J}$$

where W_J is the equivalent width, S_J the line strength and f_{00} the band oscillator strength. For the Q branch, lines near the band head will coalesce. The total line strength for the observed head is about 65. Assuming that Babcock detected a feature with $W_J=5$ mÅ:

$$\frac{N_B}{N_H}=1{\cdot}5\times10^{-7}\ (f_{00}=0{\cdot}01)\ \text{or}\ 1{\cdot}5\times10^{-9}\ (f_{00}=1{\cdot}0)$$

Compilations of band oscillator strengths indicate that f_{00} is unlikely to exceed 0·01 by a significant amount. This would yield an abundance near that of Waddell and Slaughter, in disagreement with our upper limit from the B I infra-red lines. Repetition of Babcock's observations is therefore highly desirable.

Waddell's identification of the line at 8668·42 Å as arising from S I seems sound. One other line from the same multiplet is almost certainly present at 8633·18 Å. The strengths of these lines are in the ratio 2 : 1 in LS coupling. Their measured equivalent widths[6] are 8·7 mÅ (8668 Å) and 4·6 mÅ (8633 Å). The calculated central depth for the 8668 Å line is 0·08, which is in fair agreement with observation. The calculated oscillator strength is subject to some uncertainty as the multiplet belongs to the displaced series in S I.

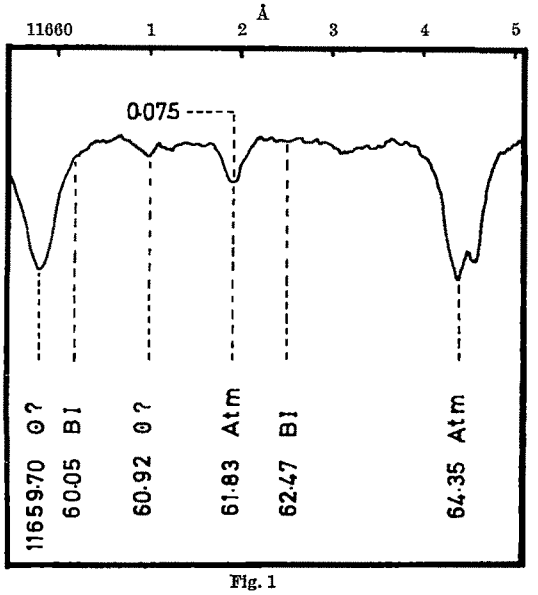


Fig. 1

Davis and Phillips[15] list two CN lines in this region: 8668·567 Å ((3,1) band) and 8668·555 ((5,3) band). Their calculated equivalent widths[16] are 1·5 mÅ and 0·35 mÅ, respectively. The former may in fact be the line Waddell identified as B I λ8668·570 Å. The wave-length and intensity agreement is good. If the line is formed in the region of the temperature minimum ($\tau_{5000}=0{\cdot}01$, $T=4500°$ K, $\xi=2{\cdot}6$ km/sec) its half width would be 0·127 Å. The fact that Babcock and Moore, by not giving any spot intensity, imply that the feature E–F disappears over a sunspot is not a fatal objection to this identification. The S I line would certainly disappear, as it has a lower excitation potential of 8·42 eV. In the 8660 Å region, Rowland intensity-3 corresponds to equivalent widths of 3–4 mÅ. As no weaker lines are listed by Babcock and Moore[7] the E–F feature could still disappear despite considerable enhancement of the CN line. Against this argument must be set Babcock's observation[5] that the number of CN molecules "above one sq. cm of the photosphere" is enhanced by a factor 3 (violet CN)–10 (red CN) over a sunspot.

We conclude that the upper limit to the boron abundance in the Sun can be set about 4×10^{-9}; however, at present there are strong indications that it may be an order of magnitude smaller. Fresh observations of the 11,660 Å region are to be undertaken at Oxford in an effort to obtain more precise values.

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Spectra of Nine Discrete Radio Sources in the 20–40 Mc/s Range

During October–November 1964 and April 1965, measurements were made with the 1,000-ft. radio telescope of the Arecibo Ionospheric Observatory[1] for the purpose of determining the spectra in the 20–40 Mc/s range of some of the stronger radio sources having declinations between $-1{\cdot}5°$ and $+38{\cdot}5°$. It should be of interest to compare these measurements, in which a filled aperture pencil beam was used, with other recent measurements[2–4] made below 40 Mc/s using a Mill's cross (T shaped) or spaced interferometric arrays.

With the 1,000-ft. spherical reflector illuminated by a log-periodic feed, the antenna temperature contributions of the various radio sources were measured at 22·30, 26·70, 33·45, and 38·75 Mc/s using four Ryle-Vonberg type radiometers. The antenna has a half-power beamwidth varying between about 2·0° at 38·75 Mc/s and 3·2° at 22·30 Mc/s. A number of runs (some drifts, but mainly driven scans in right ascension and declination) were taken for each source to help separate the source from the background and to lessen errors in measurement which could be statistically averaged out. The use of a fully steerable pencil beam enabled us, in general, to make several measurements per source on the many sources investigated during each night's observational period.

Since the Arecibo Ionospheric Observatory radio telescope consists of a fixed reflector with moving feed, the effective area varies as a function of the zenith angle. Rather than try to derive effective area theoretically, we tracked and measured the antenna temperature contribution of our calibration source Taurus *A* from zenith angles of 3 6° (its nearest approach) to 20° (the telescope limit). For our calculations a flux density of $2{,}300 \times 10^{-26}$ W m^{-2} (c/s)$^{-1}$ at 38 Mc/s with a constant spectral index of 0·27 was assumed[5] and effective area curves were plotted for the four operating frequencies. For small zenith angles, effective areas of 30,000–40,000 m^2 (41–55 per cent aperture efficiencies) were obtained between 38·75 Mc/s and 22 30 Mc/s with effective areas decreasing with increasing zenith angle.

The measured flux densities of the radio sources at the four observational frequencies and their anticipated errors are given in Table 1. Flux densities are given in units of 10^{-26} W m^{-2} (c/s)$^{-1}$. The spectra in the 20–40 Mc/s range of the nine discrete sources observed at Arecibo are shown in Fig. 1. A sample record of an observational scan of the source 3*C*157 is shown in Fig. 2.

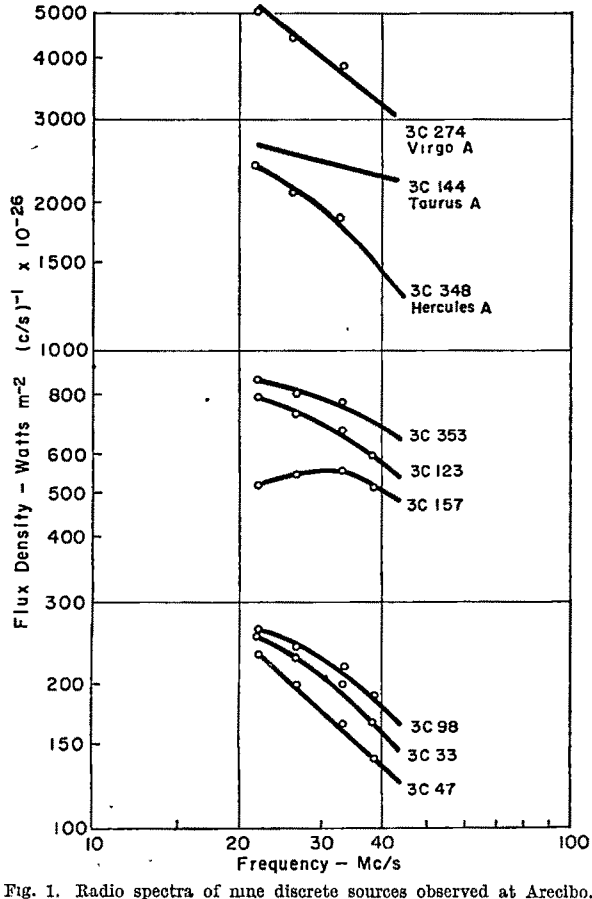


Fig. 1. Radio spectra of nine discrete sources observed at Arecibo. Experimental values are shown as open circles. For calibration purposes, flux density of Taurus *A* was assumed

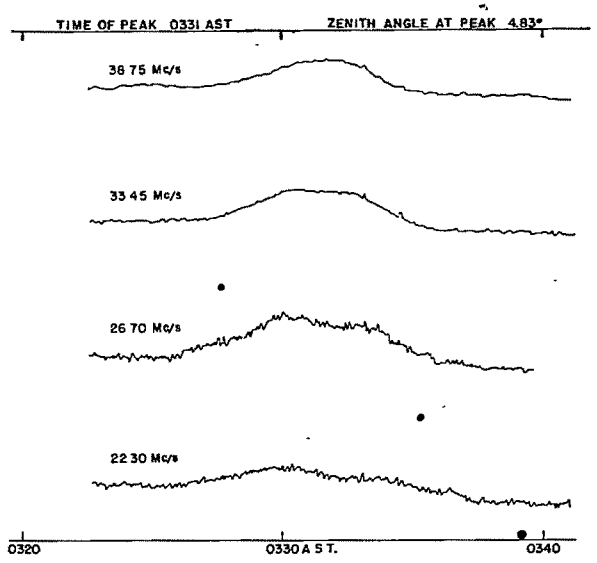


Fig. 2. Declination cut of 3C157 obtained on November 6, 1964. Note the absence of scintillation for this extended source (29′ angular diameter)

Limitations to our work included interference, ionospheric absorption and scintillation, confusion with other sources, and calibration errors. The effects of ionospheric absorption were minimized by operating at night (mainly from 2300 to 0500 h L.T.) during a period of sunspot minimum with small zenith angles. The averaging out of many observations helped to minimize errors due to scintillation. Errors due to confusion were minimized by confining our results to strong, isolated sources which gave clear and repeatable observations. As a check on the consistency of our effective area calibration, a number of observational runs were made on Taurus *A* similar to those made on the other sources and its flux densities calculated using the effective area curves. The difference between the flux densities thus calculated and the assumed values were about 3 per cent.

In Table 1, the error associated with each flux density value is given as the sum of the random measurement error and the precalculated systematic error for each frequency. The systematic errors were calculated as the square root of the sum of the squares of the calibration, confusion, and ionospheric errors. The random measurement errors were determined, under the assumption of normal distribution, from the expression used by Bazelyan[6] and ranged in the order of 1–4 per cent. The errors given do not make allowance for any error in the assumed flux densities of Taurus *A*; however, this should be less than 5 per cent.

Table 1

Source	$S_{22\cdot30}$	$S_{26\cdot70}$	$S_{33\cdot45}$	$S_{38\cdot75}$
3*C*33	259 ± 40	234 ± 35	198 ± 24	169 ± 21
3*C*47	225 ± 35	199 ± 28	168 ± 21	139 ± 20
3*C*98	264 ± 43	241 ± 38	215 ± 28	185 ± 22
3*C*121	795 ± 78	746 ± 69	683 ± 59	616 ± 54
Taurus *A**	2670	2550	2410	2300
3*C*157	521 ± 43	544 ± 39	560 ± 33	517 ± 29
Virgo *A*	5060 ± 500	4458 ± 375	3814 ± 319	—
Herc *A*	2393 ± 245	2124 ± 198	1838 ± 170	—
3*C*353	865 ± 113	818 ± 85	762 ± 79	—

* Assumed flux density on which the flux density determinations of the other sources are based.

Of the nine sources observed, the supernova remnants Taurus *A* and 3*C*157 (*IC*443) have the flattest spectra, with 3*C*157 actually reaching a turnaround point near 30 Mc/s. The source 3*C*157 gave the most consistent results of any source measured; it had random measurement errors of only about 1 per cent and showed no scintillation (because of its 29′ angular diameter[7]). The experimentally observed relationship between scintillation and the angular diameter of the radio source and its application in the determination of the size of night-time ionospheric irregularities has been discussed recently[8].

The steep spectra of the core and halo sources 3*C*47 and 3*C*274 show no appreciable curvature at lower frequencies, while the five other sources, all elliptical or double galaxies, show some degree of convex (downward) curvature. This downward curvature can be explained by the synchrotron self-absorption phenomenon with its increasingly greater effect at lower frequency. The apparent lack of curvature of the core and halo sources may be explained as the counter-balancing result of two effects, synchrotron self-absorption and halo predominance—an effect most prominent in the case of *NGC* 1275[8].

I thank C. Ferioli, W. Kidd, H. Strick and Dr. J. Aarons of the Air Force Cambridge Research Laboratories and the staff of the Arecibo Ionospheric Observatory for their assistance. The Arecibo Ionospheric Observatory is supported by the Advanced Research Projects Agency and is operated by Cornell University under contract with the Air Force Office of Scientific Research.

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PHYSICS

Shock Wave from a Release of Gas at 110 km Altitude

AT evening twilight on May 25, 1965, 4·5 kg of gaseous nitrogen dioxide was released at a height of 110 km from the Australian *Aero-High* sounding rocket. Two new observations are reported here. There was a visible glow cloud and, very surprisingly, a wave was observed expanding outwards from the glow. This wave is shown in Fig. 1, which is reproduced from a photograph taken by the Baker–Nunn camera at Woomera 13 sec after release of the nitrogen dioxide. At this time, the diameter of the wave was 11·8 km.

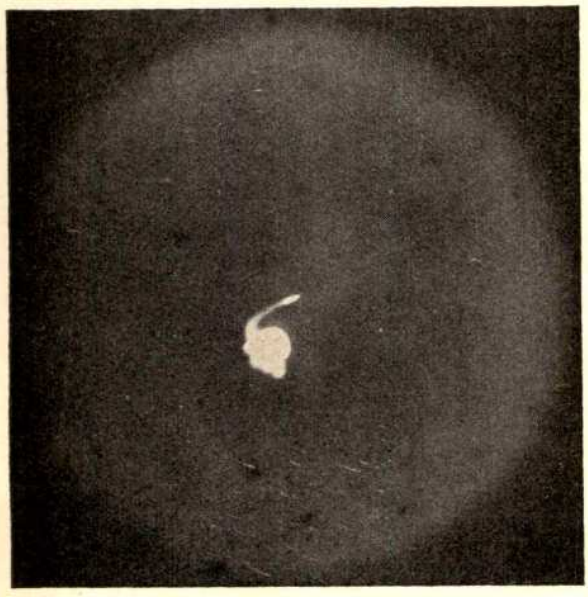


Fig. 1. Glow cloud and wave at +13 sec

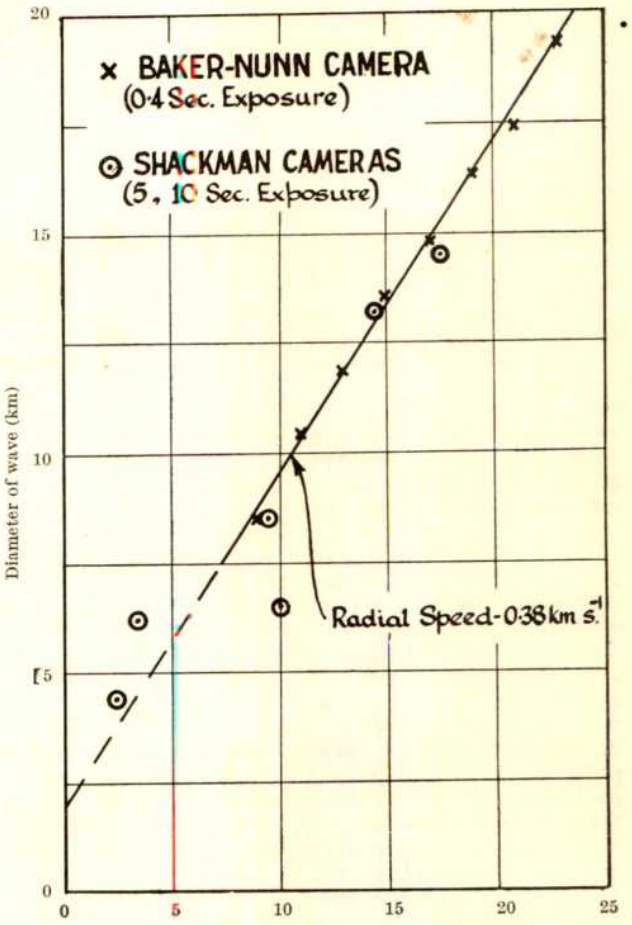


Fig. 2. Variation of wave speed with time

Photographs of the wave enabled diameter measurements to be made for 23 sec and the results are given in Fig. 2. These show that the wave speed became constant, at about 0·4 km sec^{-1}, a few seconds after release of the nitrogen dioxide. The wave is identified as the shock wave because it behaved in the same way as a shock wave formed by the sudden energy release of about 2×10^6 joules, which is the energy arising from this particular gas release. It is interesting to note that similar energy is released by the detonation of a grenade containing 0·2 kg of explosive.

A possible explanation for the detection of the shock wave by our cameras follows from a consideration of sky illumination at the time of observation. The Earth's shadow was at 65 km altitude, which corresponds to an effective shadow height of 90–100 km. In these circumstances, only molecules above 90–100 km altitude scatter significant sunlight at the wave-lengths of interest, and so a weak shock wave of about 10 km diameter near 110 km altitude could produce small, but significant, changes in the distribution of scattering molecules. If this occurred, there would be a detectable change in the radiance of the twilight sky. It follows that a shock wave formed well above the effective position of the Earth's shadow would not be detected because the density decreases by one order of magnitude in a height of about 20 km. Thus shock wave observations over a range of altitudes are possible only if a number of sounding rockets are launched at different times, corresponding to a range of Earth shadow heights.

When the shock wave becomes weak, its speed is constant and equal to the local speed of sound. From Fig. 2 the speed of sound is 380 m sec^{-1}, giving the temperature at 110 km altitude as 340° K.

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Nuclear Reactor Operation in Space

RECENTLY, Carpenter, Denfeld and Kronlund[1] estimated that the gamma-rays from a 1 MW (thermal) nuclear reactor operated in space could produce about 5×10^{12} electrons/sec by Compton scattering in the spherical shell of a large heat radiator placed around the reactor; a reactor of radius 0·3 m inside a shell of radius 3·1 m was taken as a possible arrangement. They suggested that these electrons would produce on the shell a positive potential of some 0·8 MV which might affect certain experiments.

We wish to make two comments on their proposal:

(1) At the altitude of 1,000 km, which they assume in their example, the ambient plasma will provide a powerful mechanism for neutralizing the charge on any body within it. Probe measurements with the *Ariel* 1 satellite[2] at altitudes close to 1,000 km have shown electron densities well in excess of 10^3/cm^3 and indeed in many regions the densities exceeded 10^4/cm^3. The effective electron temperature of the plasma is about 2,000° K and hence a flux ($n\bar{v}$) of $2{\cdot}8 \times 10^{10}$ electrons/cm^2 sec will be present even at the low density of 10^3/cm^3. On the elementary model of diffusion, an electron current density of $7{\cdot}0 \times 10^9$ electrons/cm^2 sec would flow on to an uncharged spherical surface, corresponding to a current on to the whole radiator surface (120 m^2) of $8{\cdot}4 \times 10^{15}$ electrons/sec. This is many hundred times greater than the outgoing Compton electron current quoted.

(2) The Compton current of about 0·8 μamp is quite a modest one to generate with a positive ion source which could be used to prevent the build-up of charge, even for a mission into regions of space where the plasma densities are negligible. The ions could be of low energy so that even if they were emitted in excess of the electrons, the potential on the shell would be small. It is also of interest that fast neutrons could be allowed to leak from the reactor and thus produce recoil ions from the shell—an effect which could be enhanced by an external coating of hydrogenous material if energetic protons could be tolerated and if the material could withstand the ambient conditions. A rough estimate of a possible yield of protons can be obtained by assuming that the reactor is designed to give a 10 per cent leakage of fast (fission) neutrons and by allocating an average energy of 1 MeV to them. The recoil protons will have a uniform distribution of energies up to this value and at a particular scattering angle, θ, relative to the neutron direction, the proton energy is given by:

$$Ep = \frac{En}{2}(1 + \cos 2\theta)$$

Thus for $\theta = 30°$, $Ep = 0{\cdot}75$ MeV and the associated range in a material such as polyethylene is about 1·5 m.gm/cm^2. For these protons to escape, irrespective of their points of origin in the shell, the effective shell thickness would have to be $1{\cdot}5 \cos 30° = 1{\cdot}3$ m.gm/cm^2 (the neutrons would arrive approximately radially for the geometry given).

For $\theta = 60°$, $Ep = 0{\cdot}25$ MeV and the associated range is 0·37 m.gm/cm^2. In such conditions the protons must be produced within 0·18 m.gm/cm^2 of the surface to escape and thus, for a converter thickness of 1·3 m.gm/cm^2 only about a seventh of them would do so. At 45° the fraction would be about a half. These values suggest that in this case a reasonable estimate of the proton escape probability will be given by taking it as unity for angles up to 30°, ½ for angles from 30° to 60° and zero above 60°. Thus overall, the fraction escaping would be given, for isotropic scattering in the centre of mass system, by

$$\frac{\int_0^{\pi/6} \sin 2\theta \, d\theta + \frac{1}{2}\int_{\pi/6}^{\pi/3} \sin 2\theta \, d\theta}{\int_0^{\pi/2} \sin 2\theta \, d\theta} = \frac{1}{2}$$

The polyethylene (or similar) converter would have a mean-free-path of about 2·5 gm/cm^2 for n–p scattering at 1 MeV and the scattering rate would thus be about 5×10^{12}/sec for 1-MW operation and 10 per cent leakage. Thus the proton emission would be about $2{\cdot}5 \times 10^{12}$/sec, which is of the same order as the Compton electron emission and it should therefore be possible to design a system to give nearly equal balance between the two and to have a small ion or electron source to produce exact equality. All this assumes that the neutrons, their decay products, the ions and the electrons could be accepted in the space experiments.

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Adhesion of Atmospheric Dustfall Particles to a Glass Slide

PARTICLES of atmospheric dust adhere strongly to solid surfaces, as can be judged by the persistence of their presence on automobiles, window ledges and other familiar surfaces. In our laboratory we have for several years been investigating the adhesion of solid particles to various surfaces with the view of clarifying the pertinent influential physical and chemical characteristics of the particle, substrate and ambient air[1–3]. In recent years, several different groups have reported results of measurement of small particle adhesion[4–6]. We were unaware of any previous efforts to measure the forces of adhesion between atmospheric dustfall particles and solid surfaces, or of any investigation that compared the adhesion forces of these particles to other common particle systems of comparable size.

In our investigations two methods are used to evaluate particle adhesion[3]. In the first, particles settle by sedimentation on to a substrate material and the substrate with particles is then mounted in a special holder which fits into the head of an ultracentrifuge. The holders were designed so that centrifugal force on the particles acted perpendicular to and away from the substrate, or parallel to the substrate. In the second apparatus, the substrate with particles was exposed to a high velocity air stream with a well-defined velocity profile. The basis for comparison of strength of particle adhesion was the efficiency of particle removal after exposure to a given centrifugal acceleration or to each bulk stream air velocity. With either technique, it was necessary to examine with a microscope the particle field before and after application of the removal force, that is, centrifugal or air drag. (These procedures have already been described elsewhere[3].)

Clean glass microscope slides were set on the roof of the Graduate School of Public Health in Oakland, Pittsburgh, for 4 h to collect atmospheric dustfall. The slide and deposit were later conditioned for 24 h at 35 per cent or 75 per cent relative humidity (20° C) in a glove box. The results obtained in centrifuge and re-entrainment tests are presented in Table 1 and Table 2, respectively, and are expressed as percentage of particle removed ("Efficiency of Removal") at any stated test condition. The last two columns in Table 1 indicate the centrifugal

Table 1. ACCELERATIONS REQUIRED TO DISLODGE ADHERING PARTICLES OF ATMOSPHERIC DUST, FLY ASH AND GLASS BEADS (RELATIVE HUMIDITY, 35 PER CENT, AND 20° C)

Acceleration, (g)	Size* (μ)	Efficiency of removal (per cent) Glass beads	Fly ash	Atmospheric dust	Centrifugal force (dynes) Glass beads	Fly ash
6,000	1·3	–	0·0	8·1	–	$1·25 \times 10^{-5}$
24,100	1·3	–	0·0	19·1	–	$5·01 \times 10^{-5}$
97,000	1·3	–	0·0	–	–	$2·00 \times 10^{-4}$
6,000	3·8	–	0·0	2·8	–	$3·40 \times 10^{-4}$
24,100	3·8	–	1·8	2·8	–	$1·36 \times 10^{-3}$
97,000	3·8	–	2·8	–	–	$5·44 \times 10^{-3}$
6,000	6·4	–	12·1	0·0	–	$1·57 \times 10^{-3}$
24,100	6·4	–	9·1	3·2	–	$6·31 \times 10^{-3}$
97,000	6·4	–	42·4	–	–	$2·51 \times 10^{-2}$
6,000	8·9	–	18·2	16·7	–	$4·33 \times 10^{-3}$
24,100	8·9	–	27·3	16·7	–	$1·74 \times 10^{-2}$
97,000	8·9	–	72·8	–	–	$6·93 \times 10^{-2}$
6,000	11·5	–	12·5	0·0	–	$9·18 \times 10^{-3}$
24,100	11·5	–	50·0	0·0	–	$3·68 \times 10^{-2}$
97,000	11·5	–	100·0	–	–	$1·47 \times 10^{-1}$
1,900	5·3	50·0	–	–	$3·63 \times 10^{-4}$	–
12,500	5·3	52·2	–	–	$2·39 \times 10^{-3}$	–
19,200	5·3	75·4	–	–	$3·67 \times 10^{-3}$	–
1,900	15·9	77·8	–	–	$9·80 \times 10^{-3}$	–
12,500	15·9	85·2	–	–	$6·45 \times 10^{-2}$	–
19,200	15·9	89·5	–	–	$9·90 \times 10^{-2}$	–

* Projected area diameter.

Table 2. BULK AIR VELOCITY REQUIRED TO DISLODGE ADHERING PARTICLES OF ATMOSPHERIC DUST, FLY ASH, AND GLASS BEADS (RELATIVE HUMIDITY, 35 PER CENT, AND 20° C)

Bulk air velocity (m/sec)	Size* (μ)	Efficiency of removal (per cent) Glass beads	Fly ash	Atmospheric dust
30	1·3		0·0	0·4
90	1·3		6·0	2·5
150	1·3		21·3	4·9
30	3·8		0·0	0·0
90	3·8		20·1	4·6
150	3·8		57·4	7·9
30	6·4		4·0	5·9
90	6·4		68·0	15·7
150	6·4		100·0	31·4
30	8·9		30·8	6·7
90	8·9		76·9	20·0
150	8·9		100·0	40·0
30	11·5		0·0	0·0
90	11·5		83·3	25·0
150	11·5		100·0	50·0
30	5·3	0		
90	5·3	0		
30	15·9	0		
90	15·9	0		
117	15·9	32		

* Projected area diameter.

forces which were responsible for the noted degree of removal of the spherical glass beads and fly ash particles. These forces were calculated from equation (1) for spherical particles:

$$F_c = m.a.$$
$$F_c = 1/6\pi\, d^3\ \rho\, w^2 R \qquad (1)$$

where F_c is the centrifugal force (dynes), w is the angular speed of rotation (radians/sec), d is the particle diameter (cm), R is the distance from centrifuge axis of rotation to substrate (cm), and ρ is the particle density (1·98 g/cm³ for fly ash and 2·50 g/cm³ for glass beads).

It was not possible to calculate the air drag or adhesion forces for these particles, because the density and shape of atmospheric particles vary over a wide range. For comparison purposes, results for glass beads (A. S. LaPine and Co., Chicago, Illinois) and particles of a Cottrell precipitated fly ash are also shown in Tables 1 and 2. In presenting these results we recognize the great variability in the composition of suspended particulates in urban atmospheres[7] and would anticipate large differences in the adhesiveness of particles collected in different cities, or even at different sites in the same city.

It required bulk stream air velocities as large as 150 m/sec to remove 50 per cent of adhering atmospheric dust particles of 11·5μ projected area diameter. However, it was even more difficult to remove glass beads, but easier to remove fly ash particles, of the same size. In the centrifuge tests, where removal depended on particle weight at any given acceleration, the atmospheric dust particles adhered more strongly than fly ash or glass beads of the same size. Even this generalization is not strictly valid, for with the two smallest sizes of particles (1·3μ and 3·8μ) fly ash adhered most tenaciously. The latter result could be explained by an increase in density of atmospheric particles in the two smallest sizes studied or by alterations in surface properties of particles in this size range. Adhesion of solid particles to solid surfaces is dependent on substrate and particle composition, surface microstructure, and the presence or absence of surface contamination, as well as on other factors[1–6].

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Snow and Rain Washout Coefficients for Process Plant Radioiodine Vapour

VERY little is known about the effectiveness of snow in scrubbing the atmosphere, and in the past there have been no reported measurements of the efficiencies with which atmospheric constituents are removed by snow. Theoretical calculations of the collection by raindrops of soluble gases have been reported by Chamberlain[1]; however, similar calculations for snow would require a knowledge of the electrostatic capacitance of the snow crystals and their number per unit volume in air.

A knowledge of the removal efficiencies of atmospheric constituents by precipitation is essential in the investigation of many basic problems in atmospheric physics[2], and the washout coefficients of both gaseous and particulate materials by both rain and snow are being studied. This information is of particular importance in explaining the fall-out rates and mechanisms of airborne radionuclides.

Several experiments to determine the washout coefficients of radioiodine vapour by snow and by rain have been conducted during the past year using the minute amounts of ^{131}I which are present in some stack gas effluents at the Hanford Atomic Plant. The radioiodine in these effluents is all in the vapour state and is about one-half inorganic (hydrogen iodide and $iodine_2$) and half in the form of organic iodides (apparently alkyl iodides)[3].

Experiments were designed to determine the removal of the ^{131}I from the stack plume by comparing the total ^{131}I leaving the stack to the ^{131}I carried down by precipitation in a sampling arc which subtended the plume. The data from these studies were treated in a manner similar

to that used by May[4] in his study of the washout of *Lycopodium* spores.

The quantity of ^{131}I washed out is used to determine the washout coefficient (fraction scavenged per time) for the rain or snowfall, using the following formula:

$$\Lambda = Vy\Sigma c/QtA$$

where Λ is the washout coefficient; V, the wind speed at plume height; y, the distance between samplers; Σc, the total material collected in all samplers, less background; Q, the source release rate; t, the sampling time; A, the area of one sampler.

The sample collectors are 1-sq.-m dishes formed by attaching polyethylene sheets, with some slack, to large hoops made of plastic hose. Fifty to seventy of these are exposed on a line or arc transverse to the stack plume overhead. The collected rain or snow is gathered after a few hundredths of an inch have fallen. There appear to be no splash or residue problems with these simple basins.

The organic and inorganic radioiodine are separated from the melted snow or rain by standard chemical techniques which were modified to accommodate large volumes[5] and are counted in a high sensitivity γ-ray spectrometer[6].

Fig. 1 shows five coefficients for inorganic iodine measured in snowfall, three of these for columnar crystals and two for large, wet agglomerates of spatial dendritic crystals. If crystal size and type were constant in a snowfall, then washout should be linearly related to water-equivalent precipitation rate. Lines of unit slope have been drawn through the data. We assume that there is a family of lines, one for each crystal type, with the position of each line being determined by the velocity and surface area of the crystal.

Also shown are two coefficients measured in rainfall. These values are ten times the theoretical values for iodine vapour washout at these rainfall rates[2]. These measurements were made at different times and processed independently, thus providing a dual verification of the high values. Near the data points are drawn the theoretical washout rates when rain falls through plumes of 20μ and 40μ diameter water drops[2]. Electrical charges on the rain would increase the expected washout by an unknown amount.

Organic forms of radioiodine were scavenged only about 1 per cent as effectively as the inorganic forms, but there was too little captured to allow calculation of coefficients. The rapid scavenging of airborne inorganic radioiodine has been independently observed in condensate from air coolers[7]. Also, the scrubbing efficiency of spray towers for inorganic radioiodine increases with humidity, and Owe Berg attributes this to condensation phenomena[8].

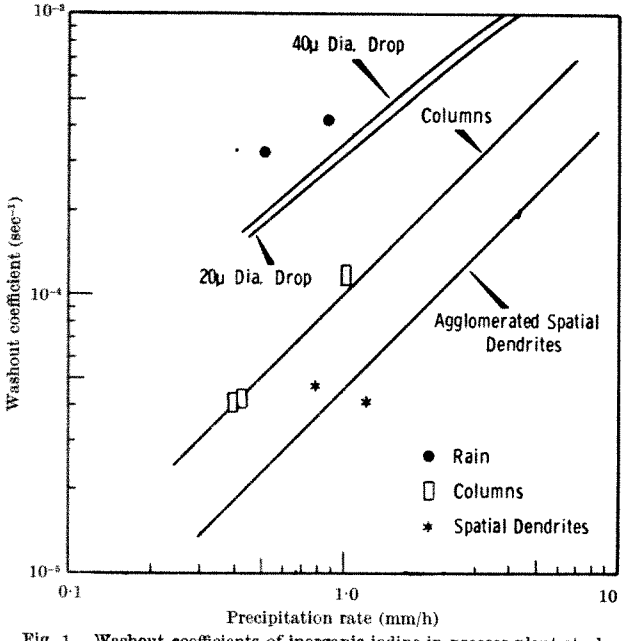


Fig. 1. Washout coefficients of inorganic iodine in process plant stack gases for rain and snow

These process stack plumes at the Hanford Atomic Plant contain sufficient water vapour to produce small water droplets when mixed with cold atmospheric air. Because there appears to be no alternative explanation, we conclude that nearly all the inorganic radioiodine gas is being captured during the water condensation process, as the stack gas mixes with the atmosphere, and that subsequent scavenging occurs at rates appropriate to tiny water drops.

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Sub-millimetre Maser Amplification and Continuous Wave Emission

IN our investigations of molecular stimulated emission, we have recently observed amplification by direct current discharges in CN at a wave-length of 0·337 mm. We have also constructed continuous wave oscillator sources of the same wave-length using similar direct current discharges in an apparatus with a plane mirror resonant cavity and beam-splitter coupler as was described earlier[1]. The oscillator discharge tubes were 90 cm long and 5 cm in diameter and were operated under flow conditions, as with pulsed CN masers, at an equilibrium pressure of about 0·1 mm. Typically, the potential drop across the tube carrying a current of 1 amp was 800 V and output powers of the order of 0·1 W have been recorded. Similar results have been obtained using discharges run at 50 c/s. Amplification was observed by means of a similar discharge tube of length 60 cm in the resonant cavity of an independent pulsed CN maser. When a direct current discharge is used in this way, increases of up to a factor of two have been observed in the output power of the pulsed maser.

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Pinched Lightning Discharges

THE suggestion contained in my recent letter[1] that pinching out of the discharge may occur on a wide variety of scales from lightning to quasars led to a search of the literature to see if lightning current waves existed which

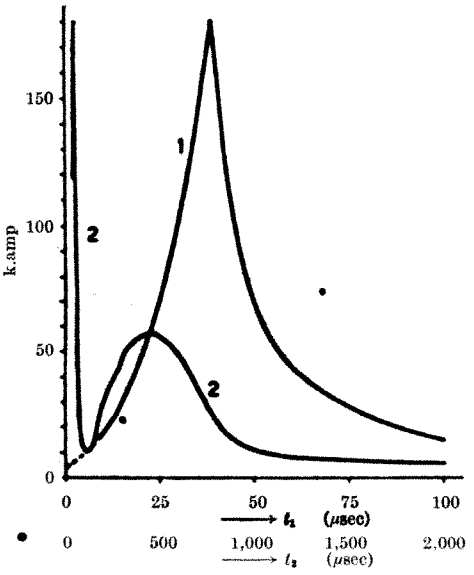


Fig. 1

supported the evidence of the one photograph available of a pinched lightning discharge[2]. The figure shows an oscillogram of the largest lightning current obtained by Berger and Vogelsanger[3] in their investigation of lightning striking their masts on the top of Mount San Salvatore. The similarity to the light curve of Nova Herculis 1934 referred to in the earlier note[1] is quite striking. After rising rapidly to a peak value of 180 kamp, the current fell still more rapidly to 10 k.amp, that is, the discharge was practically extinguished. It then rose again more slowly as the pressure generated by the sudden increase in temperature and by the magnetic pinch effect was released and reached a second peak of 50 k.amp.

A fuller account of the pinching process on this scale is given in a note in the June issue of *Electronics and Power*, in which the light curves of two novae are reproduced for comparison (Fig. 1).

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Vibrational Relaxation of Carbon Dioxide in Mixtures with Noble Gases

MEASUREMENTS have been made, using a spectrophone, of the relaxation time of the 4·3μ fundamental vibration of carbon dioxide diluted with the five noble gases—helium, neon, argon, krypton and xenon. The apparatus and experimental procedure have already been described[1].

Calculations based on the Swartz, Slawsky and Herzfield theory (S.S.H.) of collision induced vibrational energy transfer[2,3] show that the asymmetric valence mode of carbon dioxide should relax by a complex process, involving the two lower energy modes, the most likely decay scheme being:

$$h\nu_3 \rightarrow h\nu_2 \;(\text{translation}) \quad + \quad h\nu_1 \rightarrow 2h\nu_2 \;(\text{translation}) \qquad (1)$$

where the frequencies ν_1, ν_2 and ν_3 refer to the symmetric valence, bending, and asymmetric valence vibrations of carbon dioxide respectively, and h is Planck's constant. Because the process $h\nu_1 \rightarrow 2h\nu_2$ is very nearly a resonance exchange it should be very rapid, and the relaxation may effectively be described as the two-step process:

$$h\nu_3 \rightarrow 3h\nu_2 \rightarrow \text{translational energy} \qquad (2)$$

Each of these steps will have its associated rate constant and relaxation time.

For the present results, assuming this model for the relaxation, the relaxation phase lag, γ, between the modulated radiation input and the resulting optic-acoustic pressure fluctuations in the spectrophone can be shown to be approximately[4]:

$$\gamma = \tan^{-1}\left\{\omega\left[\frac{\frac{1}{\tau_1^{AA}}[\chi + g_1(1-\chi)] + \frac{1}{\tau_2^{AA}}[\chi + g_2(1-\chi)]}{\frac{1}{\tau_1^{AA}\tau_2^{AA}}[\chi + g_1(1-\chi)][\chi + g_2(1-\chi)] - \omega^2}\right]\right\}$$

Here τ_1 and τ_2 are the relaxation times of the first ($h\nu_3 \rightarrow h\nu_2$), and second ($h\nu_2 \rightarrow$ translation), steps of the energy transfer process respectively:

$$g_n = \frac{\tau_n^{AA}}{\tau_n^{AB}}$$

The superscripts AA and AB respectively refer to energy transfer in carbon dioxide self-collisions, and in collisions with diluent molecules. χ is the mol fraction of carbon dioxide in the mixture, and ω is the radiation modulation frequency.

Fig. 1 shows typical results of γ versus χ for the five mixtures, at a total mixture pressure of 60 torr and a modulation frequency of 800 c/s. The results strongly support the two-step relaxation scheme, in some cases phase shifts $> \pi/2$ being obtained, which is impossible for a single relaxation process.

Perhaps the most interesting feature of the results is the collision reduced mass dependence of the relaxation time. To analyse this, it is necessary to know the relative values of g_1 and g_2 for the first and second steps of the relaxation. Because the latter involves the de-excitation of the bending mode, values of τ^{AA}/τ^{AB} obtained from ultrasonic absorption and velocity measurements, or from spectrophone investigations using the 15-μ vibration band, should correspond to g_2. Values for carbon dioxide–helium and carbon dioxide–neon mixtures are available from Cottrell and Day[5], for carbon dioxide–argon from my own spectrophone results. Using these values for g_2, corresponding values of g_1 were calculated from the data. The results are given in Table 1.

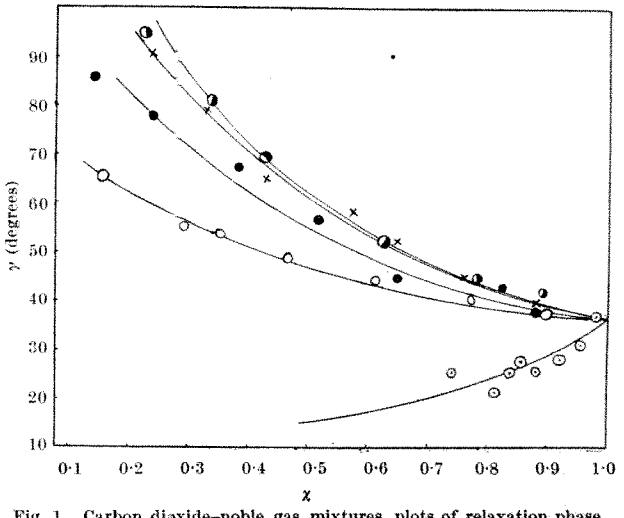


Fig. 1. Carbon dioxide–noble gas mixtures, plots of relaxation phase lag (γ) against carbon dioxide mol fraction (χ). Solid lines represent best fit of equation 2 to data for g_1 equals g_2. ⊙, Helium; ○, neon; ●, argon; ×, krypton; ◑, xenon

Table 1

Diluent	g_1	g_2
Helium	14	2
Neon	0·5	0·3
Argon	0·1	0·14
Krypton	0·028*	0·067*
Xenon	0·0167*	0·055*

* Values obtained by extrapolation of plots in Fig. 2.

If $\bar{P}_{10}$ is the probability of de-excitation/molecule/collision, simply related to the relaxation time by:

$$\bar{P}_{10} = \frac{\tau_c}{\tau(1 - e^{h\nu/kT})}$$

where τ_c is the average time between collisions, then the S.S.H. theory gives the dependence of $\bar{P}_{10}$ on μ as:

(Mass dependent pre-exponential factor) $\times$ exp.$(-B\mu^{\frac{1}{3}})$ where:

$$B = \frac{3}{2}\left(\frac{16\ \pi^4\ \Delta E^2}{\alpha^2 h^2\ kT}\right)^{\frac{1}{3}}$$

Here ΔE is the energy to be exchanged with translation, and α is the intermolecular force range parameter. The pre-exponential factor varies with the type of colliding molecule, but basically has the form of the product of μ^2 and a factor mainly dependent on the masses of the atoms constituting the relaxing molecule. This factor (A) will remain fairly constant for all the mixtures investigated. Thus taking the reduced mass dependence to be of the form:

$$\bar{P}_{10} = A\mu^2 \exp\left(-B\mu^{\frac{1}{3}}\right) \qquad (3)$$

where A is independent of μ. Fig. 2 shows a plot of:

$$\left[\log_e \frac{(\bar{P}_{10}^{AB})}{(P_{10}^{AA})} - 2\log \mu\right]$$

against $\mu^{\frac{1}{3}}$ for the present results. Agreement is excellent for the limited data. Extrapolation of the graphs, to obtain g_1 and g_2 for krypton–carbon dioxide and xenon–carbon dioxide mixtures, yields values which when substituted into equation (2) give a good fit to the experimental results for these mixtures.

The results show that the relative inefficiency of the noble gases at de-exciting carbon dioxide vibrations, compared with carbon dioxide itself, already noted in measurements on the bending vibrations[5,6] is confirmed for both stages of the relaxation of the asymmetric valence mode. The noble gases, as a group, give a reduced mass dependence of the carbon dioxide relaxation in good agreement with present-day theory. Making the assumption that α is constant for the five mixtures, and taking values for ΔE of 500 k and 960 k for the first and second steps of the relaxation, the slopes of the Fig. 2 plots give values for α of $5{\cdot}2 \times 10^8$ cm^{-1} and $5{\cdot}8 \times 10^8$ cm^{-1}, respectively.

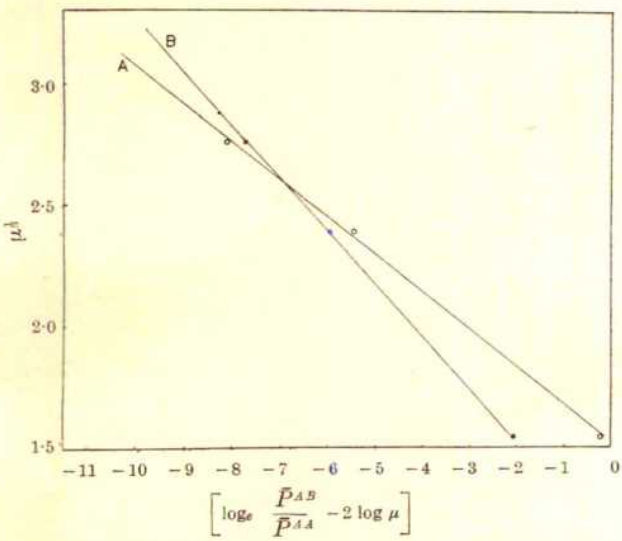


Fig. 2. Plots of $\log_e \frac{\bar{P}^{AB}}{\bar{P}^{AB}} - 2\log \mu$ against $\mu^{1/3}$ for the first (plot B) and second (plot A) steps of the relaxation of the ν_3 mode of carbon dioxide in mixtures with helium, neon and argon

As can be seen from Fig. 2, self-collisions between carbon dioxide molecules do not follow this simple mass dependence. The relatively simple collision between an atom and a triatomic molecule would seem more likely to agree with the predictions of approximate theories like the S.S.H. than that between two triatomic molecules, where unknown orientation or rotational factors may be important.

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CHEMISTRY

Structure and Formation of Magnesium Oxychloride Sorel Cements

THE hardening process by which a cement is formed from a polycrystalline matrix has been investigated using a cement of relatively simple composition and structure—Sorel cement[1]. This cement is formed by the addition of magnesium chloride solution to a fine powder of magnesium oxide[2]. The viscous paste hardens on drying and sets to a cement consisting of a mass of needle-like crystals of $Mg_3(OH)_2Cl{\cdot}4H_2O$. The appearance of the cement is shown in Fig. 1, which was taken with a scanning electron microscope (by A. Rezanowich and I. Frazer at the Pulp and Paper Research Institute, Montreal).

Figs. 2 and 3 are transmission electron micrographs of a cement specimen prepared in a dilute solution. These

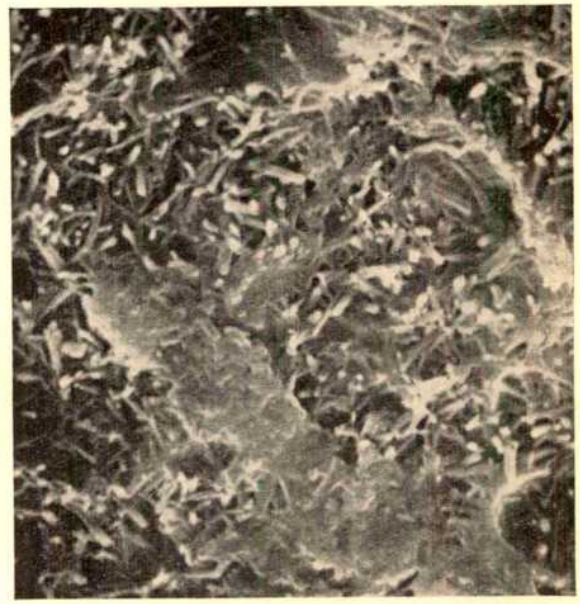


Fig. 1. A micrograph of a fractured surface of magnesium oxychloride cement taken on a scanning electron microscope. (×2,975)

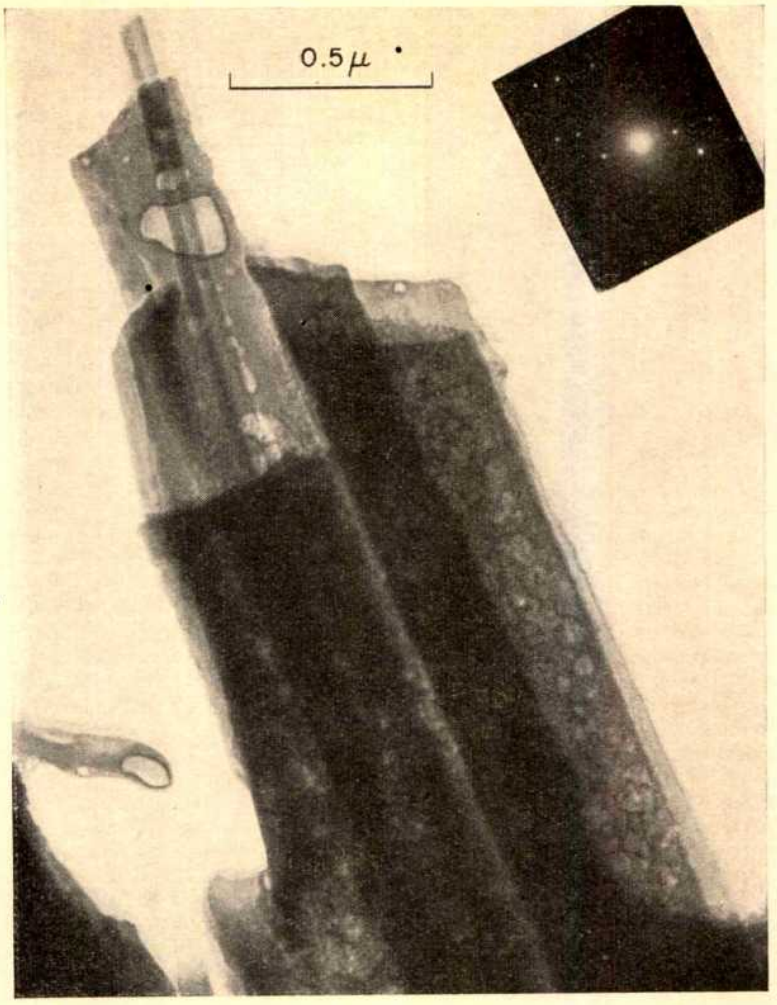


Fig. 2. Typical scroll-whiskers of magnesium oxychloride. The diffraction pattern displays layer lines typical of a rotation pattern, indicating the cylindrical nature of the crystals. The crystals have undergone some thermal etching in the electron beam in the microscope

micrographs show the needles of the oxychloride to be scroll-tubular whisker crystals, similar in many ways to those of graphite[3]. The structure of magnesium oxychloride[4] consists of puckered planes of Mg-O octahedra parallel to the needle or *b*-axis, separated by (100) planes of water and chlorine, so that these (100) planes are weakly bonded and stack without complete periodicity. Dehydration studies in the electron microscope also emphasize the scroll-tubular whisker nature, and indicate that the other hydrates of the oxychloride, in particular the $3H_2O$, have a similar structure (to be reported elsewhere).

In Fig. 3, two crossing magnesium oxychloride crystals are seen which either interpenetrate or have enhanced growth at the point of contact[3]. Partially unwound scrolls are frequently seen as well as highly curved whisker and tape crystals. In Fig. 4, where the specimen stood several days in the laboratory on the electron microscope grid, thin leader crystals can be seen in the micrograph similar to those observed from sodium chloride[3]. These leader crystals curve and interweave from one crystal group to another.

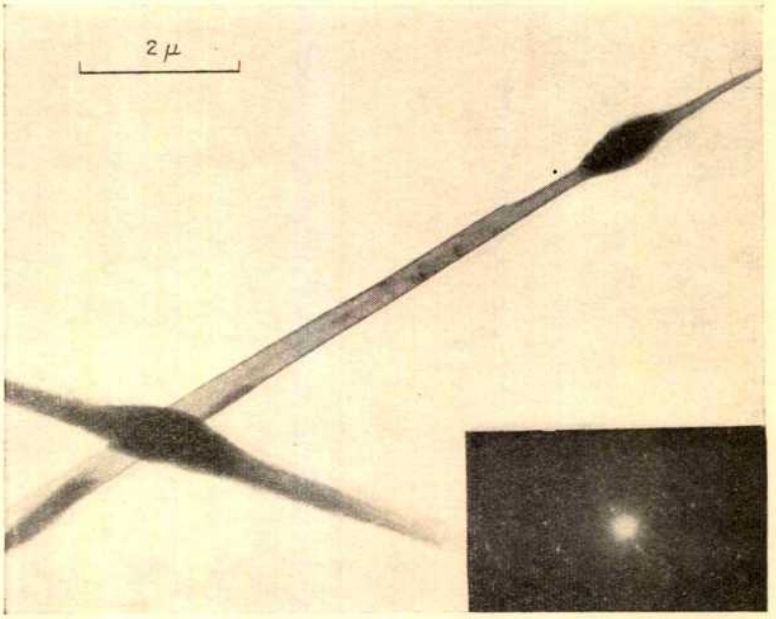


Fig. 3. Two needle-crystals of magnesium oxychloride crossing, possibly interpenetrating, with enhanced growth at the point of contact. Both crystals display features of scroll-type whiskers

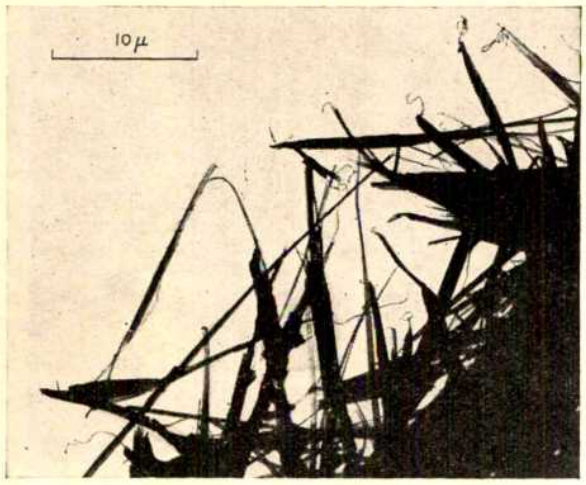


Fig. 4. Very thin crystals grow from the original crystals in the magnesium oxychloride cement after several days

The hardening process and formation of a cement can be interpreted in terms of these structural features as follows. Whisker crystals of the hydrates grow on the magnesium oxide crystals, which are prepared by a particular heat treatment of magnesium carbonate[2] and are thus likely to contain many crystal imperfections as sites for whisker growth. The whisker crystals are very strong and bond together mechanically. Further bonding occurs by the presence of unwound scroll crystals, as well as by the interpenetration of crystals, enhanced growth where crystals touch, and winding leader crystals.

A relationship can be seen to the scroll nature postulated and observed for tobermorite crystals in Portland cement pastes[5]. Some fine needle crystals have also been observed in some zinc phosphate preparations, which indicates that a similar structural mechanism may apply for zinc phosphate (dental) cements.

Other cements of structures related to magnesium oxychloride hydrate are suggested and cements have been formed from NH_4CdCl_3 and $K_2SnCl_6 \cdot 2H_2O$. Many other compounds containing infinite chain ions may also form cement materials, provided they crystallize in a suitable whisker form. In general, knowledge of whisker crystal growth and behaviour, such as growth promotion from porous substrates[3,6], can be used with advantage to develop properties and formation of cements.

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Examination of Surfaces by scanning with Charged Particles

A STUDY of the distribution of elements in inhomogeneous samples has assisted many chemical and metallurgical investigations, and in particular the electron probe microanalyser has proved a valuable tool for examining small areas at high resolution. In certain cases, however, a less costly analytical instrument of lower resolution is required for scanning larger areas, and for this purpose an X-ray milliprobe analyser has been designed, using a collimated beam of X-rays 1 mm in diameter[1].

We have described recently a number of analytical methods based on the measurement of γ-radiation emitted during the rapid decay of excited nuclear states formed as the result of charged-particle bombardment[2]. This procedure lends itself readily to the analysis of localized areas of a sample, since the low depth of penetration of charged-particles limits reaction to that part of the target near to the surface, and measurement of prompt γ-radiation permits simple use of a scanning technique. The method is particularly suitable for measurement of light elements, which are the most difficult to determine by X-ray methods.

The feasibility of examining surfaces by charged-particle scanning has already been briefly discussed[3,4], but we now report specific results obtained from scanning several test samples. Synthetic samples were chosen so that their known structure could be compared with that indicated by the scanning results.

A beam of protons accelerated to 1·80 MeV by a 2-MeV Van de Graaff electrostatic generator was collimated by an aperture 0·66 mm in diameter in a tantalum stop, and the beam current maintained at about 0·008 μamp. Targets were secured to a table which could be traversed in two directions at right angles to each other and the proton beam. Adjustment in one direction was relatively coarse, and once set was not changed during a scan; a micrometer-screw was used to move the target a known distance in the other direction between irradiations.

γ-Rays emitted by the target during irradiation were detected by a 3 in. × 3 in. thallium activated sodium iodide scintillator, and after amplification the output was fed to a 100-channel pulse–height analyser, controlled from the beam current integrator to permit the count to be totalled while the sample was irradiated to a pre-set proton dose. After each irradiation, which lasted about 5 min, the channel contents of the analyser, real and live times, were recorded, the micrometer adjusted and another irradiation started.

Targets were sheets of aluminium sandwiched between copper. One face of each target was machined flat, polished and etched, and mounted in the specially constructed target holder in order to traverse a copper–aluminium–copper section with the proton-beam.

The most intense prompt γ-ray from the reaction of 1·80-MeV protons with thick targets of aluminium has an energy of 0·84 MeV, corresponding to the decay of the first excited state of aluminium-27 produced by the inelastic scattering reaction $^{27}Al(p,p')^{27}Al$. Fig. 1 shows the variation in the detected yield of the 0·84-MeV γ-ray as each of three samples was passed across the proton-

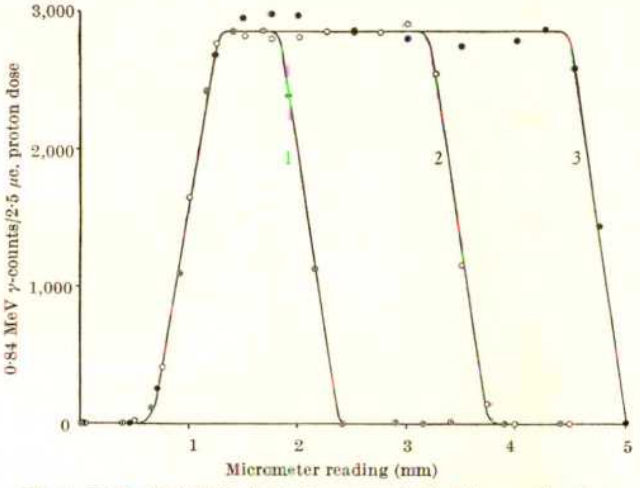


Fig. 1. Yield of 0·84-MeV aluminium γ-rays obtained by scanning three different copper–aluminium–copper targets with 1·80-MeV protons

beam. As the copper–aluminium interface was traversed, the count-rate increased to reach a constant value when the entire beam was collected on the aluminium section. The length of the plateau was determined by the thickness of the aluminium sheet, and in Table 1 widths calculated from half-height are compared with values measured optically with a travelling microscope. It can be seen that the agreement is good.

Table 1. WIDTH OF ALUMINIUM SHEETS IN TARGETS

Sample	Optical measurement (mm)	Proton scan (mm)
1	1·14	1·13
2	2·52	2·50
3	3·87	3·75

In these experiments, the minimum time required for an irradiation was governed by the long dead-time of the multi-channel analyser (900 μsec). If a faster analyser had been available, it would have been possible to reduce the irradiation time by using a higher beam-current, and taking advantage of the higher yield of 0·84-MeV γ-rays obtained on increasing the proton-energy.

Aluminium was scanned in this work because it can easily be incorporated in targets as sheets of known thickness, but particle scanning can equally well be applied to the determination of any element which can be measured by a prompt-radiation technique similar to that described for aluminium. By careful choice of bombarding-particle and energy, and with the aid of γ-ray spectrometry, methods are available for most of the light elements.

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Iron Enneacarbonyl

AT various times[1] doubt has been expressed as to the authenticity of iron enneacarbonyl, $Fe_2(CO)_9$, owing to its low solubility in all solvents and apparent lack of volatility. We have therefore carried out an investigation of this carbonyl, which was made by exposing iron pentacarbonyl, sealed under high vacuum in 'Pyrex' vessels, to sunlight; tri-iron dodecacarbonyl, which also formed in small amounts[1], was removed by repeated washings with petrol ether. A series of analyses on different samples of iron enneacarbonyl clearly indicates that the empirical formula is $Fe_2(CO)_9$. (Found (per cent): carbon, 29·9, 29·7, 30·2, 29·6; oxygen, 39·2, 38·0, 39·4. Calculated (per cent) for $C_9O_9Fe_2$: carbon, 29·7; oxygen 39·6.)

Although it is stated that iron enneacarbonyl is involatile[1], we have observed that under a good vacuum (10^{-5} to 10^{-6} mm) it will sublime at about 70° C to form hexagonal-shaped crystals. The sublimation occurs cleanly if the enneacarbonyl has been dispersed, by crushing and rubbing, against the apparatus walls, but attempts at the sublimation of bulk samples cause rapid decomposition and the formation of an iron mirror; volatility comparisons to the other metal carbonyls, including tri-iron dodecacarbonyl, suggest a molecular weight higher than that corresponding to $Fe_2(CO)_9$. However, the mass spectrum, obtained under conditions such that the enneacarbonyl was introduced into the electron beam at room temperature to minimize any possible thermal decomposition, definitely shows the molecular ion $Fe_2(CO)^+_9$ centred at mass 364 and has no peaks of higher molecular weight; furthermore, none of the ions formed on fragmentation contains more than two iron atoms. An interesting fact which emerges from a study of the mass spectrum is that the peak due to $Fe_2(CO)^+_3$ is the most intense of the $Fe_2(CO)^+_x$ ions. An attractive explanation for this is that the three carbon monoxides represent the bridging carbonyl groups in the original iron enneacarbonyl because it seems reasonable to assume these particular carbonyls (which are bonded simultaneously to the two iron atoms) will be less readily lost on fragmentation of the molecule than the terminal carbonyl groups. This work, therefore, substantiates the formulation of iron enneacarbonyl, but in no way explains the unusually low solubility and volatility for a compound of such low molecular weight.

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Decomposition of Ferrosic Sulphide to Pyrrhotite in an Electron Microscope

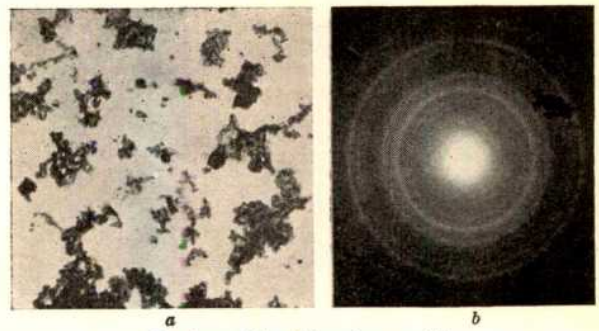
FRAGILE compounds undergo thermal decomposition in an electron microscope in which specimens are exposed to a high vacuum and to electron bombardment[1–5].

Ferrosic sulphide with a spinel structure has just become recognized as a well-defined compound since Skinner *et al.*[6] recently discovered mineral greigite. They have observed the thermal breakdown of greigite to pyrrhotite and pyrite at 320° C in a sealed, evacuated silica tube. In contrast with their result, the decomposition of ferrosic sulphide to pyrrhotite and sulphur vapour occurred when the specimen was heated above 300° C in a vacuum with the pump operating.

It was interesting to see if the same experimental condition could be achieved in an electron microscope, enabling one to follow the crystallographic and morphological changes during the decomposition.

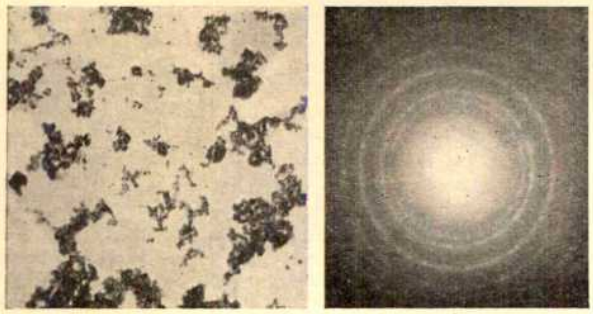
In a previous paper[7], a synthetic method of ferrosic sulphide is described in some detail. The powder particles of the ferrosic sulphide were mounted on a specimen grid covered with a collodion film. The crystals were examined in a Hitachi *HS-7* electron microscope operated at 50 kV. The $L\lambda$ values (L is the effective camera length and λ is the electron wave-length) were determined with a gold foil standard.

Electron micrograph and diffraction patterns at the initial stage were recorded, care being taken not to subject the specimen to more than the minimum beam intensity.



a *b*

Figs. 1*a* and 1*b*. Before decomposition



a *b*

Figs. 2*a* and 2*b*. After decomposition. Wave-length of the electron beam was 0·0548 Å. (×15,000)

In order to decompose the specimen, the beam intensity was gradually increased by controlling a condenser lens. A set of micrographs and diffraction patterns taken before and after decomposition are shown in Figs. 1*a*–2*b*. The original diffraction pattern is identified with that of ferrosic sulphide with a spinel structure of $a = 9{\cdot}90$ Å. The final pattern can be indexed for a hexagonal lattice with $a = 3{\cdot}43$ and $c = 5{\cdot}68$ Å corresponding to the value expected for pyrrhotite. The original particles of ferrosic sulphide are visible in Fig. 1*a*, but the contrast between them and the background is small. During decomposition the shape and the size of the particles remain almost invariable, though the contrast and transparency of the specimen increase in some degree. This is presumably because the ferrosic sulphide loses sulphur as vapour and thereby alters its form to pyrrhotite, the density of which is greater than that of ferrosic sulphide.

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Ion Exchange Method of estimating Strontium-90 and Other Radionuclides in Rainwater

THE collection of bulk samples for the assay of radioactivity in rainwater involves the handling and preparation of large volumes of water. The need to reduce this work, together with a desire to achieve greater sensitivity and more specific data on the distribution of radionuclides in rainwater, has led to the development of the following method of collecting rainwater.

The apparatus is based on an idea of Boni[1], and incorporates a technique for separating caesium-137 described by Morgan and Arkell[2]. It is contained in a wooden box, 5 ft. high × 1 ft. square section, on top of which is a rainwater collection pan 18 in. square with a hole 1 in. in diameter in the centre. The components of the apparatus are mounted vertically and centrally in the box, which is fitted with a door which is normally closed. To obviate freezing of the columns during cold weather, two electric light bulbs (100 W) are placed beside the ion exchange columns.

The column components (see Fig. 1) from top to bottom are: asbestos pad (1½ in. diameter × ¼ in. thick) to remove particulate matter; ammonium-12 molybdophosphate (AMP)/asbestos pad (1½ in. diameter × ½ in. thick) for caesium-137 estimation (the ammonium-12-molybdophosphate used is prepared according to the method of Smit *et al.*[3], which gives a fine crystalline product, and this is mixed with an equal weight of asbestos fibre to obtain satisfactory porosity); anion resin column (1 in. diameter × 6 in. long, 'De-Acidite *F.F.*' *S.R.A.* 69. −14 + 52 mesh $[OH]^-$); cation resin column (1 in. diameter × 6 in. long, 'Zeo-Karb 225'. *SRC* 19. 100–200 mesh $[H]^+$). All columns are glass and the connexions between them are made by rubber bungs and sleeves.

The effluent from the cation column is passed into a 10-l. storage bottle through a constant head device to prevent the columns running dry. The purpose of the storage bottle in this initial study was to check for radionuclides which may have escaped retention on the columns. In fact, no such activity was detectable. During normal operation the effluent from the columns runs to waste and a standard rainwater gauge is used to measure rainfall.

The various components of the apparatus were separated at the end of each sampling period (1 month) and analysed for strontium-90. The ammonium-12-molybdophosphate/asbestos pad was washed with 2 N ammonium nitrate solution followed by 0·01 N nitric acid to remove the strontium-90, which was then precipitated as carbonate from the wash solution together with added stable strontium carbonate carrier (85 mg), then chemically separated and purified by the standard fuming nitric acid technique[4]. The equilibrated yttrium-90 daughter was separated and counted by anti-coincidence low background technique.

The washed ammonium-12-molybdophosphate/asbestos together with the two ion exchange resins and the asbestos particulate filter were counted by γ-spectrometry.

The strontium-90 was removed from the ion exchange resins by leaching with 8 N nitric acid, precipitated as carbonate together with added stable strontium carbonate carrier (85 mg); and then treated identically as described for ammonium-12-molybdophosphate wash solution.

The γ-ray spectrometer analyses of the activity on column components showed that most of the caesium-137 was retained by the ammonium-12-molybdophosphate/asbestos pad, although in some cases part of it was etained by the particulate filter.

Table 1 summarizes the results of the strontium-90 analysis of the column components over the period June 1964–February 1965. Also included are the comparative results for rainwater analysed on a routine basis over the same period.

As additional information, Table 2 shows a typical distribution of various γ-emitting radionuclides in 1 month

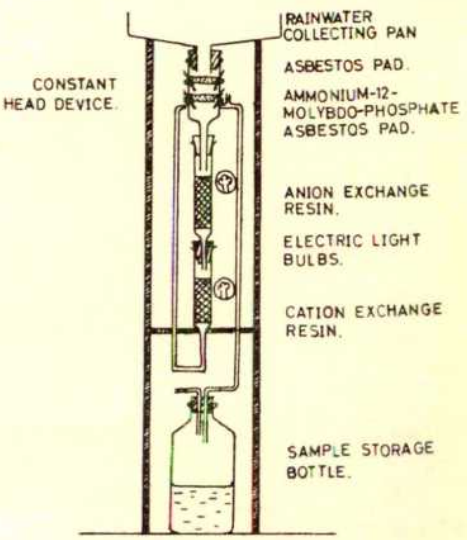


Fig. 1

Table 1. DISTRIBUTION OF STRONTIUM-90 BETWEEN COLUMN COMPONENTS

Date	Sample volume (l.)	Strontium-90 μμc./l. Particulate filter	AMP/ asbestos pad	Anion resin	Cation resin	Column effluent	Total	Comparison figures ^{90}S μμc./l.
June 1964	18·5	0·9	14·6	0·5	7·4	0·3	23·7	27·2
July 1964	8·3	0·4	15·9	0·6	4·0	0·0	20·9	22·6
August 1964	19·7	0·8	6·5	0·8	3·3	0·1	11·5	12·7
Sept./Oct. 1964	35·4	0·1	1·6	0·1	3·6	0·1	5·5	7·0 Sept. 5·4 Oct.
Nov. 1964	10·2	0·2	2·0	0·2	2·7	0·0	5·1	5·3
Dec. 1964	10·0	0·0	0·3	0·04	0·9	0·0	1·24	1·17
Jan. 1965	10·0	0·1	4·9	0·2	0·9	0·0	6·1	6·6
Feb. 1965	2·0	0·8	8·5	0·3	9·4	0·0	19·3	20·6

Table 2. TYPICAL γ-SPECTROMETRY ANALYSES OF RAINWATER

Isotope	Particulate filter	AMP/ asbestos pad	μμc./l. Anion resin	Cation resin	Column effluent
Ruthenium-106	70	—	15	—	—
Cerium-144	141	—	—	—	—
Caesium-137	18	40	—	—	—
Zirconium-95/ niobium-95	3	—	24	—	—
Mercury-203	—	—	Trace	—	—
Manganese-54	—	—	—	3	—

during the same period. Ruthenium-106 was distributed between the particulate filter and the anion column; caesium-137 between the particulate filter and the ammonium-12-molybdophosphate/asbestos pad; zirconium-95/niobium-95 on the anion resin.

It is interesting to note that all the cerium-144 and a high percentage of the other radionuclides were retained on the particulate filter.

It can be concluded that: (1) Caesium-137 was retained specifically by the ammonium-12-molybdophosphate/asbestos pad, provided it passed the asbestos particulate filter. (2) Some strontium-90 was loosely held by the ammonium-12-molybdophosphate/asbestos pad, but most of the remainder was retained by the cation resin. (3) The total strontium figures compare favourably with the routine district survey figures, the differences in most cases being accountable for by the precision of the counting procedure.

If the ammonium-12-molybdophosphate stage were omitted, it seems reasonable to suggest that almost all the strontium-90 would be retained by the cation resin. In these circumstances the method would be specific for strontium-90.

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Lewis and Bronsted Acid Sites on Silica–Alumina

SOME years ago, Mapes and Eischens[1], Nicholson[2] and Roev, Filimonov and Terenin[3] studied the infra-red spectrum of ammonia chemisorbed on silica–alumina cracking catalysts. Mapes and Eischens[1] reported absorption bands at 3,330 cm^{-1} and 1,640 cm^{-1} which were assigned to ammonia adsorbed on Lewis (electron acceptor) acid sites. In addition weak bands were observed at 3,030 cm^{-1} and 1,450 cm^{-1}, which were attributed to NH_4^+ ions formed by chemisorption of ammonia on Bronsted (proton donor) sites. From the relative intensities of the bands, Lewis sites were thought to predominate on dehydrated silica–alumina although adsorbed water was observed to cause conversion of Lewis to Bronsted sites. Nicholson[2] only investigated the spectrum in the region of NH stretching vibrations and reported approximately equal numbers of Lewis and Bronsted sites. However, Roev *et al.*[3] observed only ammonia on Lewis acid sites for their sample of silica–alumina.

Since that time Parry[4] and Basila, Kantner and Rhee[5] have investigated the chemisorption of pyridine and have established conclusively the existence of both Lewis and Bronsted sites on silica–alumina. However, as the results for the chemisorption of ammonia have been widely quoted we have repeated the experiment using an improved technique. Our results, which differ significantly from those of the earlier workers, are presented here.

The sample of silica–alumina (a commercial Esso cracking catalyst) had an alumina content of 25 per cent and a surface area of about 250 m^2/g. It was ground to a fine powder and compressed to a thin disk under a pressure of 10 tons/in.^{2}. The disk was mounted in a vacuum cell and all spectra were recorded without disturbing the alignment in the infra-red beam of the spectrometer. The spectra shown in Fig. 1 were recorded on a Perkin Elmer model 521 spectrometer using the linear absorbance attachment. The sloping backgrounds of the spectra were caused by scattering by the sample disk. An attenuator was placed in the reference beam to compensate for these radiation losses. It was periodically adjusted to maintain the trace from the spectrometer on the chart paper. This procedure caused the discontinuities in the spectra.

Fig. 1*a* shows the spectrum of a disk of silica–alumina after evacuation at 450° C. It is very similar to those given by Basila *et al.*[5]. The spectrum of Fig. 1*b* was obtained after the disk was exposed to 5 cm of ammonia gas and then evacuated for 1 h at 25° C to remove physically adsorbed ammonia. We interpret these spectra as follows. Ammonia chemisorbed on surface Lewis sites has absorption bands at 3,335 cm^{-1}, 3,280 cm^{-1} and 1,610 cm^{-1}. Some ammonia is also chemisorbed on Bronsted sites as NH_4^+ ions which absorb at 3,270 cm^{-1} and 1,440 cm^{-1}. This means that in the region of NH stretching vibrations the bands of both adsorbed species overlap. No bands are observed near 3,000 cm^{-1} where both Mapes and Eischens[1] and Nicholson[2] reported absorption by NH_4^+ species. However, our assignment of a band at 3,270 cm^{-1} to an ammonium species is in agreement with the assignment of Mortland, Fripiat, Chaussidon and Uytterhoeven[6] for ammonium ions on montmorillonite.

Addition of water vapour to the sample followed by evacuation for 1 h at 25° C (Fig. 1*c*) resulted in removal of the bands at 3,335 cm^{-1}, 3,280 cm^{-1} and 1,610 cm^{-1}, and an increase in the intensity of bands at 3,270 cm^{-1} and 1,450 cm^{-1}, that is, ammonia adsorbed on Lewis sites has been converted to ammonium ions. From the intensity changes of the bands at 1,610 cm^{-1} and 1,440 cm^{-1} following the addition of water, it is estimated that for dehydrated silica–alumina the ratio of the number of Lewis to Bronsted acid sites lies between 1 : 1 and 3 : 2. Basila *et al.*[5] estimated a similar ratio from their results for pyridine chemisorption. The weak band at 1,680 cm^{-1} in Fig. 1*c* may be due to the ν_2 bending mode of NH_4^+

which Uytterhoeven, Christner and Hall[7] observed at 1,680 cm^{-1} for ammonium ions on decationated *Y*-type zeolites.

After completing the adsorption of ammonia the silica–alumina sample was evacuated at 450° C and a spectrum similar to Fig. 1*d* obtained. Admission of water vapour to the sample followed by evacuation at 25° C gave the spectrum of Fig. 1*e*. Absorption bands appear at 1,630 cm^{-1} due to the deformation vibration of water molecules and at about 3,500 cm^{-1} due to water stretching vibrations. The exact frequencies of the latter vibrations are uncertain because of the contribution of hydrogen bonded surface hydroxyl groups to absorption in this region.

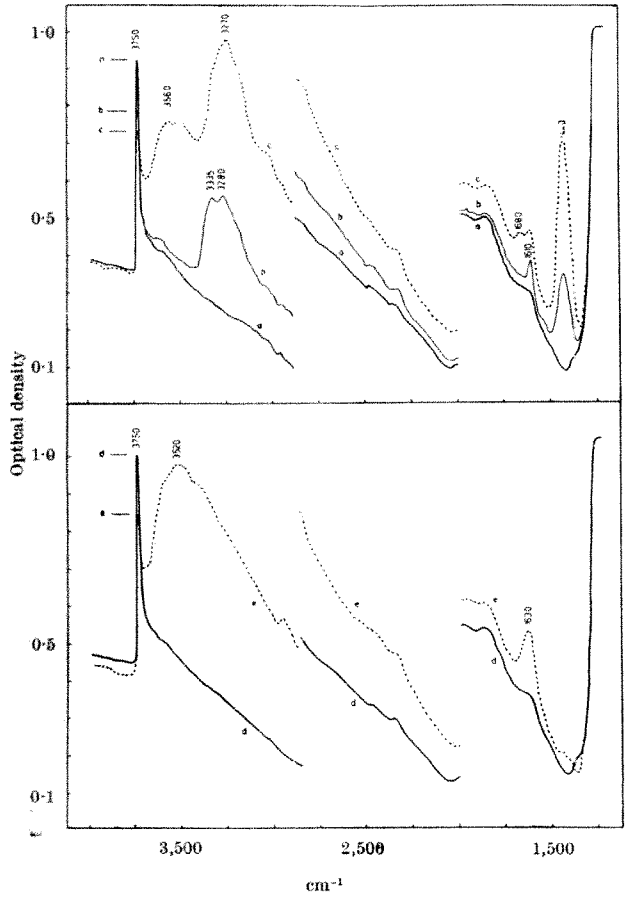


Fig. 1. The infra-red spectrum of silica–alumina with adsorbed ammonia and water: *a*, after evacuating the silica-alumina at 450° C; *b*, after adsorbing ammonia and evacuating at 25° C following *a*; *c*, after adding water vapour and evacuating at 25° C following *b*; *d*, after evacuating at 450° C following *c*; *e*, after adding water vapour and evacuating at 25° C following *d*. The horizontal lines on the spectra *a* – *e* mark the optical density of the 3,750 cm^{-1} band due to isolated surface hydroxyl groups

Fig. 1*c*, which was obtained after evacuation of water from the ammonia covered sample, shows a weaker band at 1,630 cm^{-1} than that given in Fig. 1*e*. This observation is consistent with the following reaction:

$$H_2O + H_3N : \text{Lewis acid} \rightarrow OH^- + NH_4^+ \text{ (ads)}$$

Since the water molecule is no longer present, no band due to its deformation vibration should be expected. The band of reduced intensity at approximately 3,500 cm^{-1} in Fig. 1*c* then originates from the OH$^-$ ion produced by the foregoing reaction, from some water molecules contributing to the weaker band at 1,630 cm^{-1} and from surface hydroxyl groups involved in hydrogen bonding.

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CRYSTALLOGRAPHY

Crystal Structure of Caesium Permanganate

IN investigations of the effect of high-energy radiation on crystals of the permanganates of the alkali metals, a knowledge of the crystal structure of caesium permanganate is required. The only previous investigation of this substance was by Muthmann[1], who, using optical goniometric methods, determined the axial ratios as: $a : b : c = 0{\cdot}8623 : 1 : 0{\cdot}6853$.

We obtained crystals (0·1 × 0·2 × 0·4 mm) of caesium permanganate from an aqueous solution containing stoichiometric amounts of caesium nitrate and potassium permanganate. The axial ratios were determined by optical goniometry as $a : b : c = 1{\cdot}734 : 1 : 1{\cdot}370$. Muthmann's values must have been obtained by using incorrect Miller indices, since with our results, and his indexing, we obtain the values of $a : b : c = 0{\cdot}867 : 1 : 0{\cdot}685$. These compare reasonably well with his.

The Laue symmetry was confirmed by X-ray methods as *mmm*. The unit cell dimensions were obtained from Weissenberg zero-level photographs using filtered CuK_α radiation. The method of Farquhar and Lipson[2] was used, except that the lattice parameter under investigation was plotted against $\cot\theta \cos^2\theta$ because of the high absorption of the crystals. The parameters obtained were:

$$\begin{aligned} \text{Orthorhombic: } a &= 10{\cdot}0692 \pm 0{\cdot}0005 \text{ Å} \\ b &= 5{\cdot}8080 \pm 0{\cdot}0005 \text{ Å} \\ c &= 7{\cdot}9470 \pm 0{\cdot}0005 \text{ Å} \end{aligned}$$

The axial ratios computed from these values are:

$$a : b : c = 1{\cdot}7337 : 1 : 1{\cdot}3683$$

The number of formula units per unit cell, using $d = 3{\cdot}597$ g/c.c., is four.

An investigation of systematic absences in zero- and upper-layer Weissenberg photographs taken with the crystal oscillating about the three principal axes gave the diffraction symbol as *mmmPna*-. This indicates two possible space groups, namely, *Pnma* or $Pna2_1$. By analogy with the corresponding potassium compound[3], and considering the consequent successful structural analysis, the correct space group is *Pnma*.

The Patterson projection on (010) was evaluated and the minimum functions M_2, M_4 and M_8 were set up. The preliminary positions of the manganese and caesium atoms obtained from these were used to calculate successive difference Fourier syntheses. For the third and fourth (final) difference synthesis the obvious anisotropic motion of the caesium atom was allowed for by using the fractional atom method[4]. The caesium atom was split into two half atoms along the x direction. The final electron density map, $\rho(x,z)$, is shown in Fig. 1.

Table 1

	x	y	z
Cs_1	0·296	0·750	0·345
Cs_2	0·322	0·750	0·348
Cs (mean)	0·309	0·750	0·347
Mn	0·049	0·250	0·298
O_1	0·060	0·479	0·180
O_2	0·060	0·021	0·180
O_3	0·160	0·250	0·440
O_4	0·387	0·250	0·157

The calculated values of the interatomic distances are

$Mn–O_1$	= 1·63 Å	$O_1–O_2$	= 2·66 Å
$Mn–O_2$	= 1·63 Å	$O_1–O_3$	= 2·66 Å
$Mn–O_3$	= 1·59 Å	$O_1–O_4$	= 3·60 Å
$Mn–O_4$	= 1·67 Å	$O_2–O_3$	= 2·66 Å
Cs–Mn	= 3·93 Å	$O_2–O_4$	= 3·60 Å
Average Mn–O	= 1·63 Å	$O_3–O_4$	= 3·21 Å

The O–Mn–O interatomic angles were as follows:

$\widehat{O_1MnO_2}$	= 109° 10′	$\widehat{O_2MnO_3}$	= 111° 10′
$\widehat{O_1MnO_3}$	= 111° 10′	$\widehat{O_2MnO_4}$	= 100° 56′
$\widehat{O_1MnO_4}$	= 100° 56′	$\widehat{O_3MnO_4}$	= 122° 02′

The space group *Pnma* projects on to (001) as the plane group *pgm*. The map of $\rho(x,y)$ was computed and plotted as for $\rho(x,z)$. Caesium and manganese peaks were found at $y = 1/4$ and confirmed the correctness of the space group chosen, namely, *Pnma*, rather than $Pna2_1$, since the latter projects as *pgg*.

The co-ordinates of the atoms, as shown in the lower left quarter of the (010) projection (Fig. 2), are shown in Table 1. The two half caesium atoms are recorded separately, and the mean caesium position is also given. For convenience the origin was taken at point O.

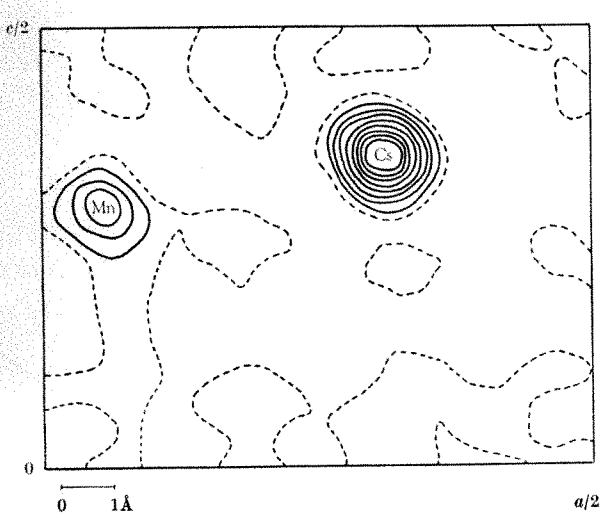


Fig. 1. Final two-dimensional Fourier projection, $\varrho(x,z)$. Contours at equal but arbitrary intervals. Zero contour broken

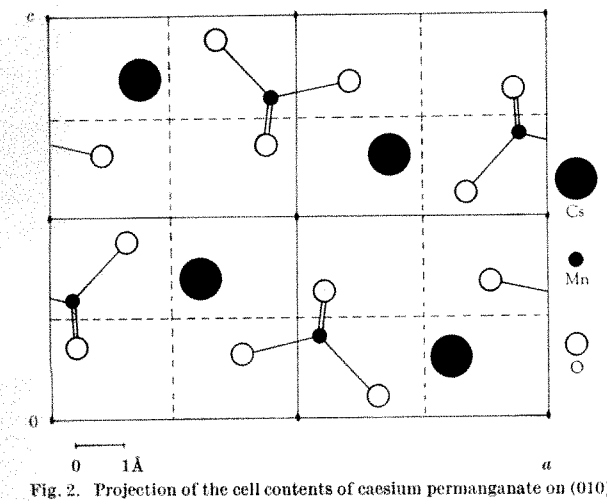


Fig. 2. Projection of the cell contents of caesium permanganate on (010)

The caesium atom is surrounded by eight manganese atoms at a mean distance of 4·38 Å. Four manganese atoms have the same y co-ordinate as the caesium atom, that is, they lie in the same plane. Their distances from the caesium atom are 3·86 Å, 4·58 Å, 4·59 Å and 6·26 Å. The other four manganese atoms lie in planes the y co-ordinates of which differ from the caesium y co-ordinate by $\pm$ 1/4. Their distances from the caesium atom are two at 3·93 Å and two at 3·95 Å. The manganese atom is surrounded by four oxygen atoms at an average distance of 1·63 Å arranged at the corners of a slightly distorted tetrahedron.

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Interstitial Dislocation Loops in Beryllium Oxide irradiated at High Temperatures

The presence of large numbers of prismatic dislocation loops parallel to the hexagonal {0001} basal planes of beryllium oxide after neutron irradiation at high temperatures (greater than 700° C) has been well established by transmission electron microscopy[1–3]. In addition, relatively low concentrations of loops lying on $\{11\bar{2}0\}$ prism planes have also been observed[3]. However, the nature of the defects comprising these loops has not previously been determined, primarily for two reasons: (*a*) Beryllium oxide does not lend itself to controlled chemical thinning, and specimens have therefore been prepared by crushing or fracture techniques. Prominent $\{10\bar{1}0\}$ cleavage in beryllium oxide causes nearly all chips suitable for study to be cleavage fragments perpendicular to the basal plane, and the abundant basal loops are therefore usually viewed edge-on and appear as lines perpendicular to the *c*-axis. (*b*) Most specimens so far available for investigation have received rather high neutron dosages ($\sim 10^{21}$ *nvt*, $E_n \geqslant 1$ MeV), and have usually contained such high densities of basal loops that overlap prevented the investigation of individual images.

Tilting experiments on chips from two specimens of beryllium oxide irradiated to 3·0 and 5·3 × 10^{20} *nvt* ($E_n \geqslant 1$ MeV) at 1,000° C and one irradiated to 3·5 × 10^{20} *nvt* ($E_n \geqslant 1$ MeV) at 1,200° C have now been carried out to determine the nature of the basal and prism plane loops. These specimens contained low enough basal loop concentrations ($\sim 10^{15}$–10^{16}/cm^3) to allow the investigation of individual loop images. The loops range from about 200 to 2000 Å in diameter, the average size being approximately 1000 Å. Most loops analysed were near the average size. The method of analysis has been described by Edmondson and Williamson[4], and involves large-angle tilting of the specimen to observe the geometrical change in projected loop widths, coupled with observation of the effect on the loop images of a change in sign of the quantity $(\bar{\mathbf{g}}\cdot\bar{\mathbf{b}})s$, where $\bar{\mathbf{g}}$ is the operating diffraction vector, $\bar{\mathbf{b}}$ is the Burgers vector of the dislocation, and s is the deviation from the reflecting position. To determine the Burgers vectors of the two types of loops, diffraction contrast conditions were noted for which the loops were at extinction; that is, $\bar{\mathbf{g}}\cdot\bar{\mathbf{b}} = 0$.

These experiments have established that both the basal and prism plane loops are interstitial in nature, and are both of pure edge character with Burgers vectors perpendicular to their habit planes. Observations of stacking fault contrast within the basal loops for certain $\bar{\mathbf{g}}$ vectors indicate that they are composed of an extra layer inserted into the *C* stacking positions in the normal *ABAB* hexagonal stacking sequence. Such an extra layer is probably composed of beryllium oxide pairs, as Sabine[6] has suggested, and results in a Burgers vector of $c/2 <0001>$. The prism loops are believed to consist of two extra $\{11\bar{2}0\}$ layers, presumably of beryllium oxide pairs, inserted between successive $\{11\bar{2}0\}$ lattice planes, and have Burgers vectors of $a/3 <11\bar{2}0>$.

This analysis represents the first direct identification of the nature of the dislocation loops in neutron-irradiated beryllium oxide, and in particular, the interstitial nature of the abundant basal loops agrees with models of the damage state previously proposed on the basis of X-ray diffraction data[5–7]. A more complete account of this work is being prepared for future publication.

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BIOCHEMISTRY

Heat Denaturation of β-Galactosidase: a Possible Approach to the Problem of Catabolite Repression and its Site of Action

Two varieties of enzyme repression have been observed in micro-organisms. In one, addition of the end-product of a biosynthetic pathway to the growth medium specifically prevents synthesis of enzymes concerned with the formation of the product in question. In the other, synthesis of a degradative enzyme is repressed by an end-product (or a closely related metabolite) of the action of the enzyme. Several authors[1,2] have suggested that a repressing metabolite may exert its effect by combining with newly formed ('nascent') enzyme and, thereby, somehow preventing its release from the site of synthesis. Extending this notion, it could be supposed that enzyme formed under non-repressed conditions might still retain 'recognition' sites accessible to a repressing metabolite. Binding of the metabolite to these sites might then be expected to alter certain properties of the enzyme, such as heat stability. It is commonly observed that the heat stability of an enzyme is altered when it is in combination with a substrate or an inhibitor. As an approach to testing for the possible existence of "repression recognition sites", we have examined the effects of metabolites derived from glucose on the heat stability of *Escherichia coli* β-galactosidase. The synthesis of this degradative enzyme, as well as several others, in *E. coli* is repressed when glucose is present during growth and can be similarly affected, though to a lesser extent, by any compound that can be used as a carbon source by the cells[3]. The active repressor is not glucose itself but some compound, as yet unidentified, that is derived from it.

We found that β-galactosidase in cell-free extracts of *E. coli K* 12 became remarkably heat labile in the presence of fructose-1,6-diphosphate (FDP). This could be readily demonstrated either by the usual type of test in which enzymatic activity was assayed at 30° C after heat treatment at some higher temperature, or by determining the time course of enzymatic activity at temperatures of 45° C or more, in the presence and absence of FDP. Partially purified β-galactosidase showed the same response to FDP as the crude preparation, and this is illustrated in Fig. 1. (This effect has been confirmed by Dr. H. V. Rickenberg with crystalline enzyme from both *E. coli K* 12 and *ML* 309.)

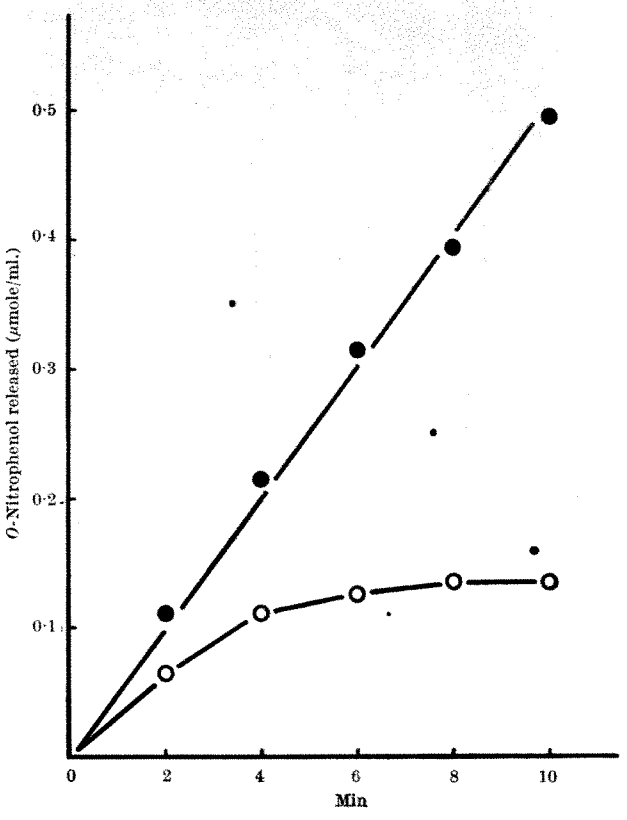


Fig. 1. Effect of fructose-1,6-diphosphate (2·5 mM final concentration) on thermostability of β-galactosidase of *E. coli* at 45° C. The enzyme was prepared from *E. coli K* 12 (grown on lactose) and purified about ten-fold by fractionation with ammonium sulphate. It contained 18 mg protein/ml. and was diluted, as required, with sodium phosphate buffer (0·1 M, *p*H 7·0) to 6 μg/ml. Diluted enzyme (6·0 ml.) at 45° C was added to a solution (12 ml.) at the same temperature containing the substrate (*o*-nitrophenyl-β-D-galactoside, 0·5 mg/ml.) (●), or FDP (○). The mixture was incubated at 45° C and at intervals samples (3·0 ml.) were removed, added to 1·0 M potassium carbonate (2 ml.) to stop the reaction and the extinction of the liberated *o*-nitrophenol measured

In the presence of 2·5 mM FDP and under the other conditions specified for Fig. 1, the enzyme was almost completely inactivated within 3 min at 45° C. It appeared that the effect of FDP was quite specific. Thus, little or no influence on heat stability was observed with lactose, glucose, glucose-1-phosphate, galactose, fructose, fructose-6-phosphate or 3-phosphoglyceric acid. Other compounds that had no effect on heat stability included the following enzyme complexants, methyl-β-D-thiogalactoside, isopropyl-β-D-thiogalactoside, and melibiose. Glucose-6-phosphate, however, was found to increase the heat lability significantly, but not nearly as effectively as FDP. The 'critical' temperature required for observation of the FDP effect is, as anticipated, determined by a number of factors, notably the concentrations of protein and FDP. Control experiments showed that at the usual assay temperatures (30° C) FDP did not measurably affect enzyme activity.

Although *E. coli ML* 308 forms β-galactosidase constitutively (that is, in the absence of inducer), synthesis of the enzyme in this organism is repressed by addition of glucose to the growth medium, as in the wild-type inducible strain[4]. FDP was found to increase the heat lability of the cell-free enzyme from *ML* 308 in the same way.

Of interest, also, is the finding that 10 mM β-mercaptoethanol, which stimulates β-galactosidase activity[5], protects the *K* 12 enzyme against the FDP effect. In this connexion it is noteworthy that replacement of phenylalanine residues in the β-galactosidase of *E. coli* by β-2-thienylalanine leads to pronounced heat lability, and that enzyme modified in this fashion is completely stabilized toward thermal denaturation by a combination of β-mercaptoethanol and Mn^{++} (ref. 6).

These observations do not, of course, prove that FDP is the 'real' repressor through which the glucose effect is exerted, nor do they show that the repressor acts by combination with the nascent enzyme. It is, however, probably significant that β-galactosidase should have a recognition site for FDP, a compound which, in other systems, is a key substance in the regulation of carbohydrate metabolism[7]. Our findings lead to the prediction that two distinct types of mutants possessing resistance to the glucose effect should be obtainable: those damaged in their ability to synthesize fructose diphosphate and those which make altered β-galactosidase molecules which are no longer able to recognize it.

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Defence by Smear: Supercooling in the Cornicle Wax of Aphids

THE cornicles of aphids, despite their definition in the *Shorter Oxford Dictionary* as the source of honey-dew, in fact secrete a lipid substance, and have a defensive function. Busgen[1] seems to have been the first to have proposed their defensive function, but misconceptions as to their role have persisted. Hottes[2], for example, considered their function to be excretory, and suggested that they released volatile substances derived from the plant sap on which the animals feed. Their defensive function has not been generally recognized; for example, Roth and Eisner[3] neglected them in their review of arthropod defensive mechanisms.

Deflexion of the cornicle or a light touch on the aphid's body elicits the release from the cornicle of a fluid composed of lipid droplets in a water vehicle. The lipid droplets coalesce, and when in contact with a solid surface, rapidly crystallize to form a hard waxy plaque. A small predator, such as a coccinellid, or syrphid larva, or an hymenopteran parasitoid receiving such a droplet is promptly fixed in a cast from which, at least in many instances observed in the laboratory, it is unable to extricate itself. That this unlikely form of defensive mechanism does operate in nature was proven while collecting *Aphis fabae* (Scop.) in the field. Among several thousand aphids examined, two were found with the shrivelled bodies of the hymenopteran parasitoid *Aphidius* sp. fixed to their backs by a plaque of cornicle wax.

The release of cornicle wax is under reflex control. Touching the aphid on one side, or deflecting one cornicle, will elicit the waxing response on that side only. Release is achieved by retraction of the cornicle muscle, a slender fibre which is inserted on a valve-like flap at the top of the cornicle (Fig. 1) and originates on the integument postero-dorsal to the base of the cornicle. Expulsion is evidently effected by abdominal turgor, for there is no contractile structure surrounding the base of the cornicle. Dehydrated aphids cannot be induced to release their cornicle wax. When the cornicle valves are opened, the aphid is in effect releasing material from the haemocoel, the border of the valve is a discontinuity in the integument. It is curious that the nearest parallel among the insects should be the reflex bleeding[4] of the coccinellid—an important predator of aphids.

The cornicle wax arises from cells which may be modified oenocytes. The secretion is stored in large globules enclosed by a membrane, sometimes together with residual cytoplasm and nucleus, sometimes without, which lie in the cornicle stalk and in the haemocoel, below the cornicle (Fig. 2).

The nature of the cornicle wax. The property of the cornicle wax of remaining fluid during the entire life of an undisturbed aphid, and yet of hardening when released by appropriate stimuli, is of some interest, and the following data have been collected on its properties. An aphid may be induced to release its cornicle wax under water. An immersed droplet, retained within a small inverted clean glass cup, will remain as a clear liquid for many hours before crystallizing to form an opaque sphere. On the other hand, contact with a solid surface in air causes rapid crystallization. These observations suggested that the wax may be kept in a liquid state by a solvent which evaporates rapidly in air, but dissolves slowly in water. Octanol, the solvent postulated by Beament[5] as a carrier

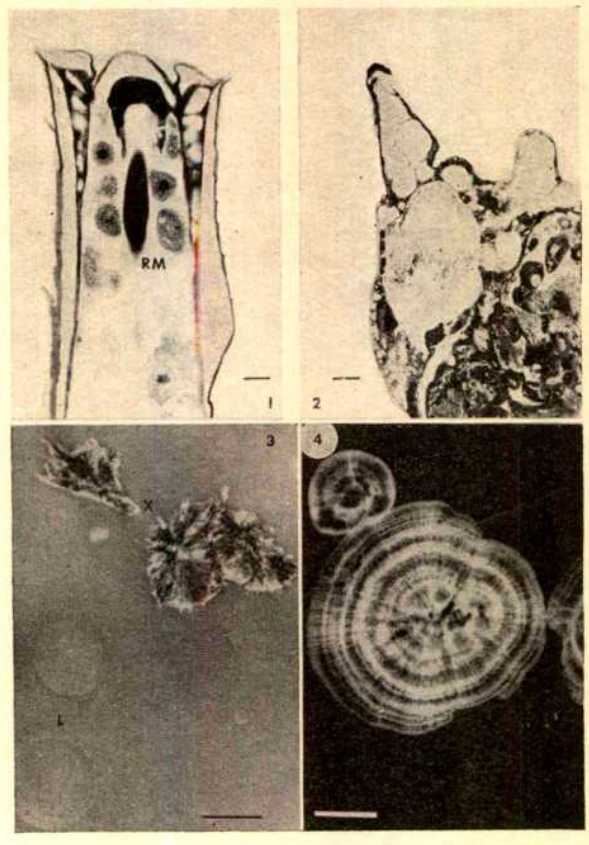


Fig. 1. Longitudinal section of cornicle of *Macrosiphum rudbeckiae* (Fitch.) wall of cornicle, terminal valve, and valve retractor muscle (*RM*). Haemocytes are present in the lumen of the cornicle. Scale, 25μ

Fig. 2. Oblique section through base of cornicle and postero-lateral abdomen. The large open cells in the cornicle and below it contain cornicle wax, part of which has been fixed by osmium and later treated with ethyl gallate. Scale, 100μ

Fig. 3. Cornicle wax of *Macrosiphum rudbeckiae* on surface of glycerol, showing both phases, liquid (*L*) and crystalline (*X*). Direct print from 'Kodachrome' photomicrograph using polarizing microscope. Scale, 1 mm

Fig. 4. Wax plaque on water surface. Plate prepared as in Fig. 3. Scale, 1 mm

of cockroach grease, would fulfil these requirements. However, no solvent of this type was detected by gas chromatography of water samples in which wax had been allowed to crystallize. The liquid state does not depend on an aqueous component, for droplets were maintained on the surface of absolute ethanol and on absolute glycerol for up to 6 h. Droplets of liquid cornicle wax on water and on dehydrating agents remain fluid only under clean conditions and in the absence of vibration.

An alternative explanation of the rapid change of cornicle wax from the liquid to crystalline state is that the fluid is in a stable liquid–crystalline state[6] within the aphid and changes to the solid crystal phase on contact with a seeding nucleus. Certain liquid–crystalline states exhibit birefringence, but no such property was detected in wax droplets on water examined under a polarizing microscope, although birefringence immediately appeared when a crystalline plaque formed (Fig. 3). The persistence of fluid wax droplets on the surface of dehydrating agents also argues against a liquid–crystalline structure involving an aqueous phase.

The rapid crystallization on contact with solid surface, a hair, or dust suggests that the liquid wax is in a supercooled state and that foreign material provides a seeding nucleus for the rapid crystallization. The supercooling property of water is evidently involved in the cold hardiness of insects[7,8], but the aphids appear to be unique in their exploitation of a phase change from the supercooled state.

The melting point of the wax of the golden-rod aphid *Macrosiphum rudbeckiae* (Fitch.) is about 48° C, that of *Myzus persicae* (Sulz.) about 42° C, and of *Aphis fabae* (Scop.) about 37·5° C. The waxes of these three species should therefore crystallize on seeding at normal summer temperatures.

The chemistry of the wax is as yet unknown. Drops of the liquid wax placed on the surface of a 1 per cent osmium tetroxide solution in 0·1 M phosphate buffer, *p*H 7, did not darken until after crystallization, when it gained access to unsaturated bonds. Chibnall *et al.*[9] examined a wax extracted from the aphid *Pemphigus xylostei* and found it to resemble that of the cochineal insect, which is composed of *n*-triacontanoic acid ($C_{30}H_{60}O_2$) and a corresponding keto-acid and keto-alcohol.

I am grateful to Dr. P. A. T. Swoboda, Low Temperature Research Station, Cambridge, for gas chromatographic examination of samples, and to Dr. David S. Barmby, Marcus Hook Research Laboratory, Sun Oil Company, for directing my attention to the supercooled state.

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Stabilization of Factor VIII by Glycerol

THE lability of the blood coagulation substance, factor VIII (antihaemophilic globulin, AHG, AHF), has hindered its investigation and has thus led to a search for techniques to stabilize it. Additions of glycerol[1,2], gelatine[3,4], human serum[4], and human serum albumin[4] were all found to stabilize factor VIII activity, but the degree of effectiveness of such additions has not been defined. The investigation reported here was an attempt to evaluate the action of glycerol in quantitative terms.

Factor VIII stability was tested in platelet-poor citrated normal plasma using the 'silicone' technique throughout[5]. All samples were sterile. Factor VIII was assayed by a one-stage procedure[5]. Fresh plasma from the same normal donor was used as standard throughout the investigation.

It was found that glycerol effectively preserves factor VIII activity of refrigerated plasma for at least 8 weeks (Fig. 1). Fig. 2 illustrates the extent to which glycerol protects against loss of factor VIII activity following *p*H changes. With prolonged storage and at extreme *p*H's, the protective effect of 50 per cent glycerol (v/v) was slightly superior to 30 per cent.

Addition of glycerol to aged untreated plasma did not restore factor VIII activity. Glycerol also failed to increase factor VIII activity in haemophilic plasma, either immediately or when assayed after storage of the mixture.

The results indicate that glycerol is effective in stabilizing factor VIII activity in human plasma. Because factor

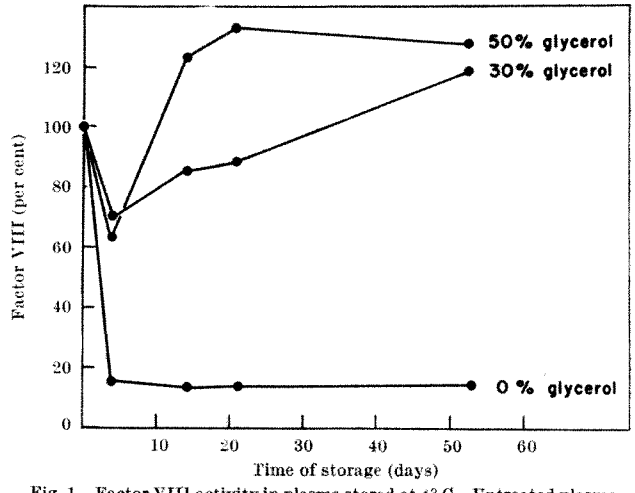


Fig. 1. Factor VIII activity in plasma stored at 4° C. Untreated plasma is compared with the same plasma mixed with glycerol to yield final glycerol concentrations (v/v) of 30 and 50 per cent glycerol. Levels of factor VIII are given as percentages of the original activity of the plasma and correction is made for dilution by glycerol

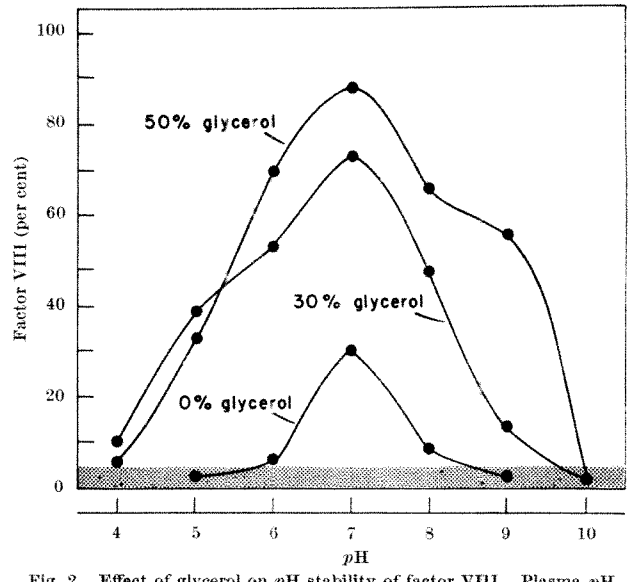


Fig. 2. Effect of glycerol on *p*H stability of factor VIII. Plasma *p*H was adjusted with hydrochloric acid or sodium hydroxide. After storage for 8 days at 4° C, *p*H was readjusted to 7 before assay. The assay method did not permit measurements of factor VIII at levels below 5 per cent. This range is indicated by the shading

VIII is even more labile in purified preparations[2,4], addition of glycerol may facilitate further purification of this coagulation factor as it has done in the case of certain labile enzymes[6].

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Dependence of Relative Sweetness on Hydrophobic Bonding

MANY differing types of sweet compounds have been known for some time, but it is only in recent years that an extensive investigation of the subject has been undertaken. Ferguson and his co-workers[1–5] examined the physiological aspects of taste and attempted to correlate relative sweetness with certain physical properties.

We have been able to correlate biological activity in many systems[6] using the following equation:

$$\log BR = -k\pi^2 + k'\pi + k''\sigma + k''' \qquad (1)$$

BR is the biological response which could be applied in this case to RS, the measured relative sweetness, σ is the Hammett constant and π is the hydrophobic bonding constant[7]. π is defined as $\log P_X/P_H$ where P_H is the partition coefficient of the parent compound between octanol and water, and P_X that of a derivative.

The relative sweetnesses of some derivatives of 2-amino-4-nitrobenzene with respect to cane sugar were measured by Blanksma and Hoegen[8] and are shown in Table 1. The following equations were calculated from their data by the method of least squares:

$$\log RS = 1{\cdot}214\pi + 1{\cdot}970 \qquad 0{\cdot}766\,(r)\ 0{\cdot}476\,(s) \quad (2)$$

$$\log RS = 1{\cdot}610\pi - 1{\cdot}831\sigma + 1{\cdot}729 \qquad 0{\cdot}936\,(r)\ 0{\cdot}282\,(s) \quad (3)$$

$$\log RS = 0{\cdot}119\pi^2 + 1{\cdot}485\pi - 1{\cdot}848\sigma + 1{\cdot}742 \qquad 0{\cdot}936\,(r)\ 0{\cdot}308\,(s) \quad (4)$$

r is the multiple correlation coefficient and s is the standard deviation. Equation (2) shows the high dependence of relative sweetness on hydrophobic bonding, and equation (3) confirms the observation of Lawrence and Ferguson that sweetness increases as the basicity of the derivative rises. F tests show that the π term in equation (2) is significant at $> 0{\cdot}975$ and that the addition of the σ term is significant at $> 0{\cdot}99$. Equation (3) accounts for 88 per cent of the variance in the data. Since the correlation is not improved by the addition of a π^2 term in equation (4), we can assume that the π values investigated are relatively low and fall in the "linear" portion of the parabolic curve.

Table 1. RELATIVE SWEETNESS OF 2-AMINO-4-NITROBENZENES (Ref. 8)

Group	σ	π	log RS Obs.	log RS Calc.*	Δlog RS
H	0	0	1·602	1·729	−0·127
1-OMe	−0·27	−0·02	2·519	2·192	0·327
1-OEt	−0·24	0·48	3·146	2·942	0·205
1-OPr	−0·24	0·98	3·699	3·746	−0·047
1-F	0·06	0·14	1·602	1·845	−0·243
1-Cl	0·23	0·71	2·602	2·451	0·151
1-Br	0·23	0·86	2·903	2·693	0·120
1-I	0·28	1·20	3·097	3·149	−0·052
1-Me	−0·17	0·56	2·519	2·942	−0·424

* log RS was calculated from equation (3).

The substituents methoxy, ethoxy and propoxy are an excellent example of the dependence of relative sweetness on π. It can be seen from Table 1 that their σ values are approximately constant, whereas their π values parallel the increase in sweetness. However, if π is further increased by the addition of CH_2 groups, the compounds will become less soluble in water and eventually tasteless. Kaufmann and Schweitzer[9] report the sweetness of the butoxy derivative as much less than that of the propoxy.

An important prerequisite for sweetness is high anionic character or the ability to form hydrogen bonds with water. Once the compound is in the aqueous phase, its sweetness will then be determined by favourable partitioning on to the receptor sites on the tongue. This step may depend on hydrophobic bonding, degree of polarity, distance of charge separation in the molecule, electron density at a specific point in the molecule as measured by σ, and steric effects.

It can be inferred from equation (3) that the receptor site consists of an area for hydrophobic bonding coupled with a site for electronic bonding.

Sugars are known to be very soluble in water, but are rather low on the relative sweetness scale, so that their attachment to the receptor sites is weak and critically dependent on their stereochemistry. Saccharin is used commercially as the sodium or calcium salt so that it will be readily soluble in water. Its sweetness decreases if the dissociation is inhibited by increasing the concentration in water or by the addition of sodium ions[10]. Any substitution on the isothiazole nucleus which causes loss of solubility also causes loss of sweetness[11]. The benzene ring presumably plays a part in the hydrophobic bonding to the receptor site. The sweetness of the 2-amino-4-nitrobenzenes is very dependent on the position of the substituent and its effect on the electrons of the ring. A distribution of charge occurs when an electron donating group is in a *para* position to the nitro group, as in the derivatives in Table 1, or when an electron attracting group is in a *para* position to the amino group. Examples of this type are the substituents —COOH and —SO_2NH_2 which Ferguson and Childers[5] reported as being sweet. Other sweet compounds such as sucaryl, 5-benzyl-2-furfuraldoxime and dulcin also fall into the general pattern of requiring charge separation for solubility and sufficient hydrophobic bonding power.

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Precursor–Product Relationship between Nuclear and Cytoplasmic Ribonucleic Acid

IN a previous communication[1] a simple kinetic model for the incorporation of radioactive adenine into the purine bases of nuclear and cytoplasmic ribonucleic acid (RNA) of exponentially growing cells was proposed. This was used in the evaluation of kinetic data reported by Harris and Watts[2]. Harris[3], in questioning the validity of the model, raised objections based on his interpretation of an equation derived from considerations of our model.

Harris[3] correctly points out that equation (1) of our communication[1] may be written as:

$$\frac{d(^aSc)}{dt} = \gamma\,(^aSn - {}^aSc) \qquad (a)$$

Where ^aSn is the specific activity of adenine in the nuclear RNA, and ^aSc is the specific activity of adenine in the cytoplasmic RNA.

This equation (a) does not state that ^aSc should decrease with $(^aSn - {}^aSc)$ as Harris[3] concluded. It does suggest that a decrease in $(^aSn - {}^aSc)$ should lead to a decrease in $\frac{d(^aSc)}{dt}$. This implies that the rate of change of specific activity of adenine in the cytoplasmic RNA should decrease with time. This is shown more clearly by equation (2) in our earlier communication[1], which represents a saturation type curve with $\frac{d(^aSc)}{dt}$ decreasing with time. Thus, the increase in ^aSc with time as observed experimentally (ref. 2, Fig. 1) is in no way contradictory to equation (a). If it were, the validity of equations (2), (3) and (4) (ref. 1) which are derived from equation (1) should be in question, and they are not.

An "almost linear" increase in ^aSc with time as observed by Harris *et al.*[2] does not raise any serious objection to our hypothesis. It is noticed that for $\alpha t \ll 1$, $(1-e^{-\alpha t})$ is approximately equal to αt and equation (2) in ref. 1 is reduced to a linear relationship, with the slope given by $\gamma\,{}^aSn(0)$. In fact, this suggests a convenient method for the evaluation of γ directly from the slope of the straight line, ^aSc against time. The value of γ, $0{\cdot}34 \times 10^{-3}$/min, estimated by this procedure is comparable to $0{\cdot}43 \times 10^{-3}$/min as obtained by the more rigorous method[1].

Recently, Harris *et al.*[4] reported more data on the incorporation of [8-^{14}C] adenine into RNA fractions of HeLa cells. These investigations differed from earlier ones in having a shorter labelling period (10 min) and in the pattern of incorporation of radioactivity. The following analysis, made at the request of Dr. Harris, shows that the new data are also consistent with the model proposed.

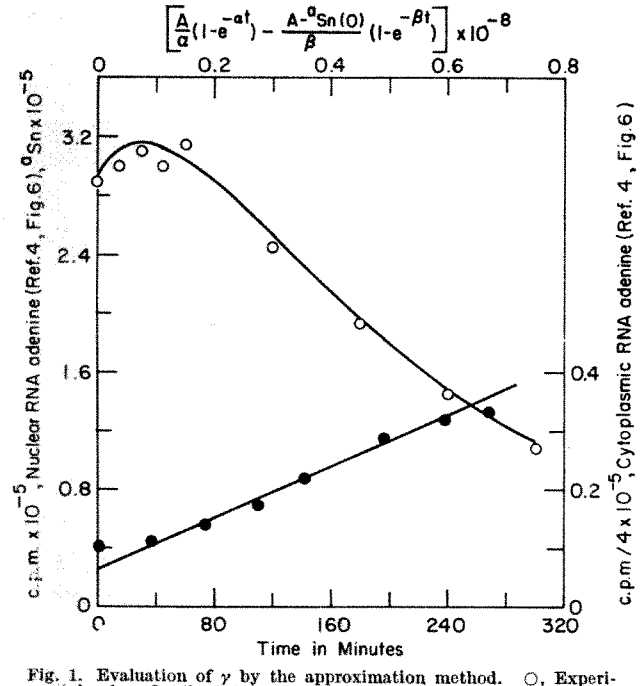


Fig. 1. Evaluation of γ by the approximation method. ○, Experimental values for the total amounts of radioactivity in nuclear RNA adenine reported by Harris *et al.* (ref. 4, Fig. 6). The curve through these points is the theoretical curve calculated from equation (b) for A, $5{\cdot}0 \times 10^5$, $^aSn(0)$, $2{\cdot}9 \times 10^5$, α, $5{\cdot}0 \times 10^{-3}$ min and β, 20×10^{-3} min. ●, Data reported by Harris *et al.* (ref. 4) plotted in accordance with equation (d)

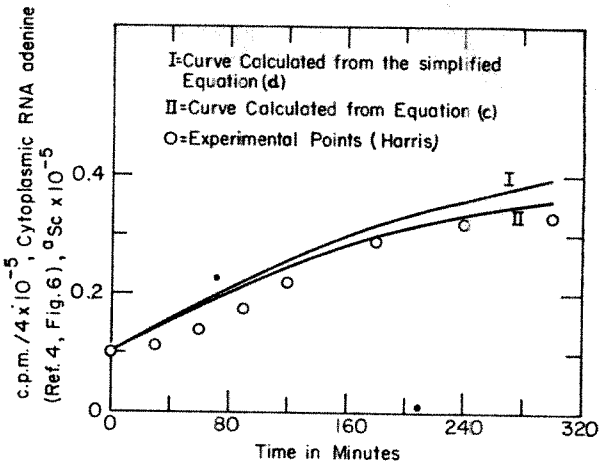


Fig. 2. Comparison between experimental and calculated values for the total amounts of radioactivity in cytoplasmic RNA adenine

In these studies (ref. 4, Fig. 6) the total amounts of radioactivity in purine bases are reported instead of specific activities. In order to maintain a continuity with the earlier treatment we have preferred to use the equations containing terms for specific activity. These expressions can be directly applied to the data reported by Harris *et al.*[4], provided the values for total amounts of radioactivity are multiplied by an appropriate factor so that the ratio of the total amounts of radioactivity in adenine of nuclear and cytoplasmic RNA is equal to the ratio of the corresponding values for specific activity. (After the completion of analysis of these data we have noticed that Fig. 7 in a recent communication by J. W. Watts[5] essentially represents a part of Fig. 6 in ref. 4. A comparison of the two figures indicates certain inconsistencies in the units used by the authors. However, it is gratifying to note that this does not affect our analysis due to a fortunate choice of 'correction factor' used in these calculations. The values in Figs. 1 and 2 ($10^{-5} \times$ c.p.m./μmole) of this communication should be regarded as specific activities.)

The specific activity of adenine (^aSn) in nuclear RNA (total amount of radioactivity, ref. 4, Fig. 6) can be represented by:

$$^aSn = Ae^{-\alpha t} - (A - {}^aSn(0))e^{-\beta t} \qquad (b)$$

where $A = 5{\cdot}0 \times 10^5$, $^aSn(0) = 2{\cdot}9 \times 10^5$, $\alpha = 5{\cdot}0 \times 10^{-3}$/min, and $\beta = 20 \times 10^{-3}$/min as shown in Fig. 1 of this communication. (The time scale in these equations is essentially the same as that used in Fig. 6 of ref. 4. Labelled cells are transferred to non-radioactive medium at '0' time which corresponds to the second point in Fig. 6 of Harris *et al.*[4]). On integration, equation (a) can then be written as:

$$^aSc = \left[{}^aSc(0) + \frac{\gamma A}{\alpha-\gamma} - \frac{\gamma(A - {}^aSn(0))}{\beta-\gamma}\right]e^{-\gamma t} + \frac{\gamma(A-{}^aSn(0))}{\beta-\gamma}e^{-\beta t} - \frac{\gamma A}{\alpha-\gamma}e^{-\alpha t} \qquad (c)$$

for $^aSc = {}^aSc(0)$ when $t = 0$. For a quantitative evaluation of equation (c) we need the value of γ. Since $^aSn \gg {}^aSc$ during the early period of incorporation, we can use the approximation applied earlier[1] to estimate the value of γ. The simplified equation in this case is given by:

$$^aSc = {}^aSc(0) + \gamma\left[\frac{A}{\alpha}(1-e^{-\alpha t}) - \frac{(A - {}^aSn(0))}{\beta}(1-e^{-\beta t})\right] \qquad (d)$$

Since the amount of cytoplasmic RNA obtained by the phenol method has been shown to be four times the amount of nuclear RNA, the quantities estimated from Fig. 6 in

ref. 4 are divided by four. In Fig. 1 one-fourth of the total amount of radioactivity in cytoplasmic RNA (ref. 4, Fig. 6) is plotted in accordance with equation (*d*). γ is estimated to be $0{\cdot}44 \times 10^{-3}$/min from the slope of the straight line in Fig. 1. It may be noted that the value of γ, $0{\cdot}44 \times 10^{-3}$/min, estimated from these data is in excellent agreement with one ($0{\cdot}43 \times 10^{-3}$/min) obtained from the experiments reported earlier by the same authors[1,2]. The deviation of experimental points from the straight line during the early period of incorporation (Fig. 1) is primarily due to the presence of low molecular weight RNA, which is known to undergo rapid turnover[6]. As shown in Fig. 2 the deviation of experimental values from the theoretical values derived from equation (*d*) during the later period of incorporation is partly due to omission of the second term in the right-hand side of equation (*a*).

The behaviour of guanine (ref. 4, Fig. 6) is similar to that observed in our studies and has been discussed in detail elsewhere[6]. Suffice to say that the parabolic nature of the curve for guanine in cytoplasmic RNA can be readily explained on the basis of the saturation type curve obtained for the same base in nuclear RNA.

The discrepancies (emphasized by Harris[3]) in the amounts of radioactivity lost from the nuclear RNA and those gained by the cytoplasmic RNA do not contradict the hypothesis of the nuclear origin of cytoplasmic RNA. It should be noted that equation (*a*) does not indicate the mechanism involved in the transfer of radioactivity from the acid-soluble precursor pool to nuclear RNA, and for that reason it does not exclude the possibility of its degradation. Recent investigations (not yet published) suggest that such degradation is more prominent in non-growing cells. Equation (*a*) is merely an expression for the behaviour of cytoplasmic RNA in relation to that of nuclear RNA acting as an immediate precursor. The general applicability of the analysis over a wide range of variations in the relative distribution of radioactivity in nuclear and cytoplasmic RNA appears to be amply demonstrated by the data reported by Harris *et al.*[1-4] and those obtained in this laboratory[6].

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Provitamin A_2: Electroretinographic Measurements of its Effect on Photopic Sensitivity in Chicks

RADIANT energy absorption in chickens normally occurs in the molecules of the vitamin A_1-based rod and cone pigments rhodopsin and iodopsin. The participation of rhodopsin and iodopsin in scotopic and photopic vision, respectively, has been demonstrated by the Purkinje shift, both in behavioural[1] and in electroretinographic experiments[2].

That a displacement of the spectral sensitivity as the result of dietary manipulation is possible is shown by the work of Shantz *et al.*[3], who succeeded in modifying the vitamin A_1-based retinal pigment of rats by feeding vitamin A_2 instead of vitamin A_1. Since the rat retina consists almost exclusively of rods, the vitamin A_2-based pigment porphyropsin takes the place of rhodopsin, which implies a shift in the absorption maximum (λ_{max}) by about 20 mμ toward the red end of the spectrum.

Recently, Budowski *et al.*[4] showed that 3′-hydroxy-3,4-dehydro-β-carotene (anhydrolutein) is converted in the chicken to vitamin A_2. The feeding of this carotenoid as the sole source for vitamin A should, therefore, result in the appearance of porphyropsin and cyanopsin in the chicken retina. Cyanopsin is a vitamin A_2-based cone pigment which has been produced *in vitro*[5], but which has not yet been extracted from retinae. Its absorption maximum is at 620 mμ while that of the vitamin A_1-based cone pigment iodopsin is at 562 mμ. We have tried to demonstrate electroretinographically the effect of anhydrolutein on the photopic sensitivity of the chicken.

One-day-old cockerels (New Hampshire crossed with White Leghorn) were kept on a basal vitamin A-deficient diet (ground sorghum, 65 per cent; solvent-extracted soybean meal, 25 per cent; fish meal, 3 per cent; wheat bran, 4 per cent; vitamins B and minerals, 3 per cent). At the end of 20 days, chicks exhibiting ataxia and loss of appetite were selected. Controls were given the basal diet supplemented with 400 I.U. vitamin A_1 as gelatin beadlets per 100 g feed. The experimental animals were fed the basal diet supplemented with 2 per cent refined soybean oil containing 200 μg anhydrolutein[4] per g oil; they thus received 400 μg anhydrolutein per 100 g feed. The refined soybean oil used was found in previous tests to be devoid of vitamin A activity.

The electroretinographic measurements were carried out on four control and six experimental animals when they were between 13 and 22 weeks old. During this period, the vitamin A_1 storage in the livers of the control chickens amounted to 1,100–1,400 μg per liver. The supply of anhydrolutein, although supporting normal growth, produced only a very small amount of liver storage of vitamin A_2 (20–47 μg per liver). A larger supply would have been desirable, but the amount available was limited.

After an initial ether anaesthesia, necessary for fixing the animal in a head holder and removing the nictitating membrane, only pantocaine was used locally. The tip of a silver-ball electrode, 1 mm in diameter, was applied at the cornea; as indifferent electrode a stainless-steel needle was inserted into the comb. Equal-energy monochromatic flash stimuli (grating monochromator, dispersion ± 3·3 mμ) of 20 msec duration were focused on the pupil of the chicken by an optical arrangement. The electrical responses of the retina (ERG) were recorded by means of a cathode ray oscilloscope through an a.c. amplifier (frequency range between 0·16 c/s and 75 c/s). However, there was not enough energy in the existing light source for superimposing the stimuli on a constant adapting light. This would have been preferable for the determination of the photopic sensitivity. The measurements were, therefore, carried out on dark-adapted chickens after the stimulus strength necessary to elicit a primarily photopic response had been determined. This was done by recording the response to stimuli at 570 and 630 mμ the intensity of which was gradually increased by means of a calibrated neutral density wedge with a range of five log units. Thereafter, the ERG recordings were made. They were elicited by equal energy stimuli over the range from 500 to 700 mμ.

The ERGs obtained were of a complex nature but similar in shape for the two dietary groups. A typical series of ERG recordings obtained from a chicken fed provitamin A_2 is shown in Fig. 1.

The findings of the measurements over the wave-length range tested are presented in Fig. 2. This shows the average photopic spectral curves obtained in the control and the experimental animals. Despite the relatively small amount of anhydrolutein supplied, the spectral sensitivity

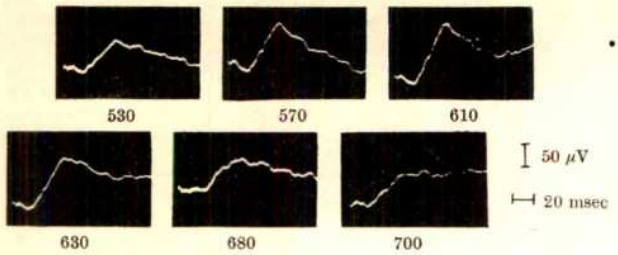


Fig. 1. Typical ERG recordings at different wave-lengths obtained from an animal fed provitamin A_2

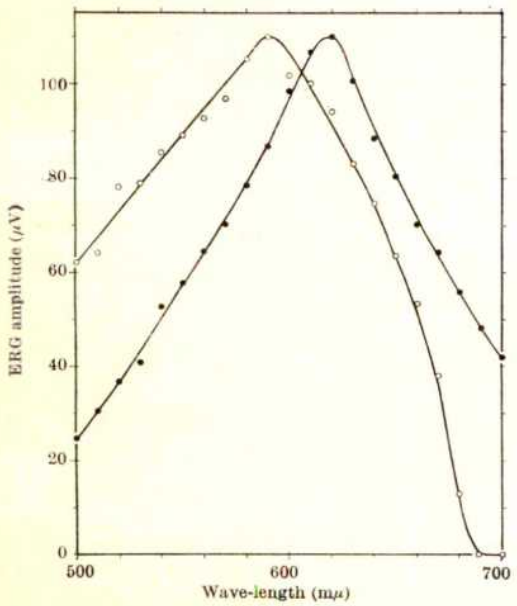


Fig. 2. Photopic sensitivity curves. Open circles: vitamin A_1-fed controls (average of four animals). Filled circles: provitamin A_2-fed chickens (average of six animals)

of the experimental animals shows a distinct shift toward the red as compared to the controls. The difference in photopic sensitivity becomes particularly striking at the red end of the spectrum: at 690 mμ there is practically no measurable response in the controls while a fairly large ERG can still be recorded at 700 mμ in the experimental animals (Fig. 1).

The spectral sensitivity peak at 620 mμ obtained in the experimental animals agrees well with the λ_{max} of cyanopsin[5]. On the other hand, the photopic sensitivity peak at 590 mμ in the controls differs from the λ_{max} at 562 mμ found for iodopsin by Wald *et al.*[6], and from the ERG measurements of Armington and Thiede[2], which have a maximum at around 570 mμ. It agrees, however, with the results from behavioural tests in chickens reported by Honigmann[1].

The most likely explanation for the shift in photopic sensitivity is the substitution of the retinene$_1$ moiety of iodopsin by retinene$_2$, resulting in a cyanopsin-like receptor pigment. The actual nature of the visual pigments obtained under these conditions remains, however, to be established.

The possibility of altering the visual system by dietary means, as evidenced by the modification of the receptor pigment in rats[3] or by the shift in photopic sensitivity in chickens described here, contrasts with the idea[7] that the pattern of visual systems is determined genetically, rather than by environment.

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Liver Transplantation Effects on Canine Plasma Lipids

IN order to achieve long-term success of the hepatic homograft, satisfactory metabolic function of that organ in the host is essential. Since the liver is directly and/or indirectly involved in the synthesis and metabolism of the lipid fractions, it is important to know the effect of transplantation on the host's 'lipid profile'.

Orthotopic hepatic homografts were performed on a series of thirteen healthy dogs, of which three survived more 100 days[1,2]. The data used in this communication were obtained from these three dogs. Azathioprine ('Imuran', an immunosuppressant), methionine and antibiotics were administered daily post-operatively. The diet consisted of commercial dog food fed *ad libitum*. Fasting, peripheral venous bloods were collected pre- and post-operatively twice a week for clinical and lipid analysis.

All three dogs had some evidence of early liver injury as judged by changes in standard liver-function tests. There were, in each, rises in alkaline phosphatase, SGOT and SGPT. Only one of the three animals developed hyperbilirubinaemia. The changes in these dogs, as well as a much larger series of animals, have been reported in

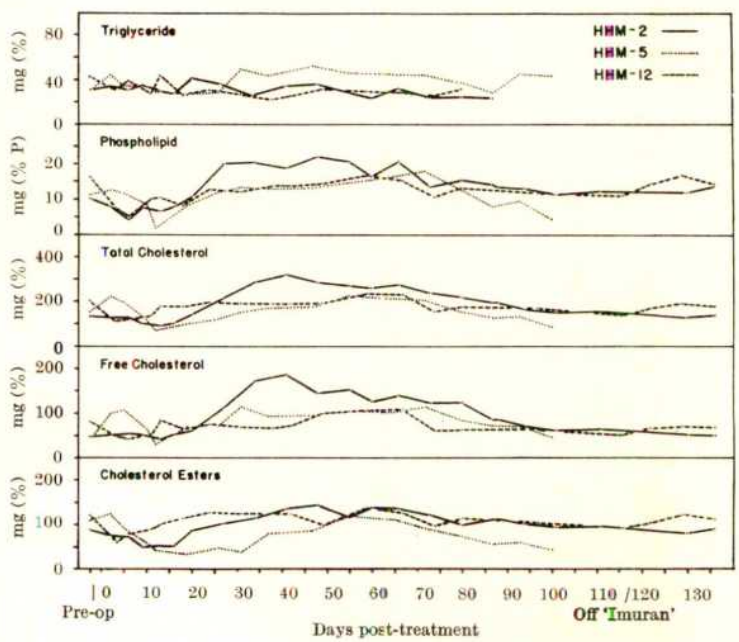


Fig. 1. Plasma 'lipid profile' in the canine hepatic homograft

detail[2]. The dog which became jaundiced during the period of the present investigation died after 100 days. The other two were still alive with good liver function 285 and 295 days post-transplant.

The 'lipid profile', consisting of total, free and esterified (by difference) cholesterol, phospholipid, non-esterified fatty acid (NEFA), and triglycerides (Fig. 1), was analysed by standard chemical procedures[3–6]. Of the lipid classes, cholesterol and phospholipid fractions changed the most. Total cholesterol dropped to about 50 per cent of the pre-operation value in the first ten days post-operative and returned to the pre-operation value in thirty to forty days. Esterified cholesterol also dropped to about 50 per cent of the pre-operation level and returned to the pre-operation value within forty to fifty days post-operative. Here again, we saw the adverse effects on the liver enzyme system, as demonstrated in the liver-function tests. Phospholipid dropped by the tenth day post-operation to about 50 per cent of pre-operation value, then returned to the pre-operation value or above by the thirtieth day. Triglyceride did not change much, remaining in the 30–40 mg per cent range. The NEFA values fluctuated between 430 and 1,800 μequiv./l., exhibiting no obvious trends.

The phospholipids were further analysed to ascertain whether one particular phosphatide was responsible for the change within the first thirty days. After extracting the total lipid[7], separation by thin-layer chromatography[8] yielded four phosphorus-containing fractions. These fractions were tentatively identified as lecithins, sphingomyelin, lysolecithin and phosphatidylethanolamine. Phosphorus determinations were performed[4] on these four fractions. The four samples analysed were from the fifth, tenth, twentieth and thirtieth days post-operation. The results indicated a uniform change of all four phosphatide fractions even though there was a three-fold change in the total phospholipid.

Azathioprine is known to be a hepatotoxic agent[2]. To be sure that the lipid changes that took place were not caused by the medication, a control series of dogs were administered the same medication ('Imuran', methionine, antibiotics), diet, and care as the experimental dogs, but no surgical operation was performed. Transient rises occurred in SGOT, SGPT and alkaline phosphatase but without the development of jaundice. In these control dogs, the plasma lipids remained unchanged (Fig. 2), indicating that the altered lipid pattern seen in the experimental dogs is not the result of 'immunosuppressant therapy' but is a physiological adjustment by the host to the hepatic homograft or the operational shock.

In the canine hepatic homograft host, acceptable plasma lipid metabolism was obtained after 30–50 days. To properly evaluate these data, we must keep in mind that the peripheral plasma values are an expression of extra- as well as intra-hepatic function, and differentiation between mobilized, synthesized and ingested lipids in the lipid pool is difficult. Extra-hepatic synthesis can compensate for differences to a point, but a liver must be present with reasonably normal function to have a proper 'lipid profile' since the majority of lipid metabolism either takes place in or is controlled by the liver.

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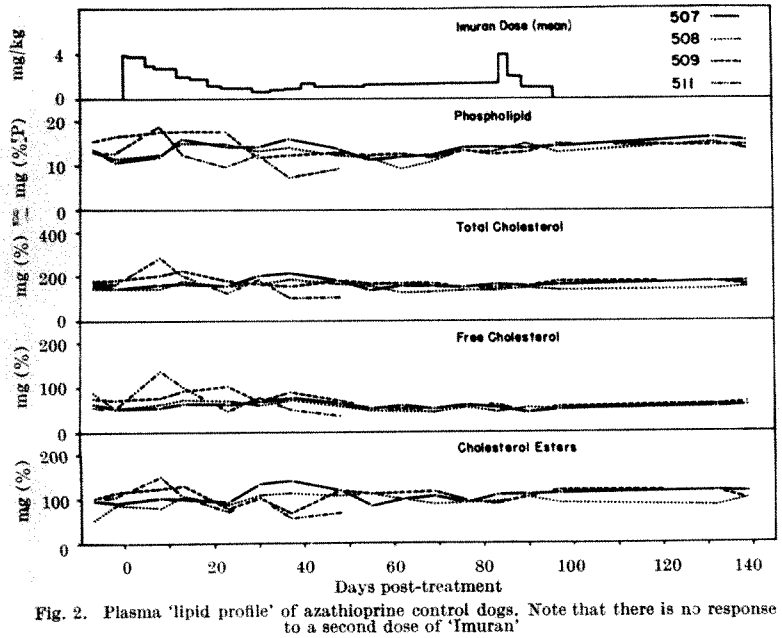


Fig. 2. Plasma 'lipid profile' of azathioprine control dogs. Note that there is no response to a second dose of 'Imuran'

Comparison of Physicochemical Properties of Mitochondrial and Nuclear Deoxyribonucleic Acid from Rat Liver Cells

THE discovery of deoxyribonucleic acid (DNA) in mitochondria[1–9] has opened the way for investigations of the long-standing problem of extra-genome cytogenetic determinants. DNA fibres have been found in mitochondria from tissues belonging to various different classes of animals[1]. The principal difficulty to be dealt with in the isolation of mitochondrial DNA is caused by the necessity of separating the soluble nuclear DNA, adsorbed on the surface of mitochondria. For this purpose we used a method of purification of mitochondria in 'Urographin' density gradient[8] or treatment of the mitochondrial fraction with DNase (150 μg/ml. for 1·5 h at 0° C). Fibres of high-polymer deproteinized DNA were obtained by means of the phenol method after Kirby[10] and Marmur[11]. The average DNA content in liver mitochondria amounted to 0·65 μg/mg protein.

In an attempt to determine how far mitochondrial DNA is identical with nuclear DNA from rat liver cells, both these nucleic acids were submitted to physico-chemical analysis. Comparison of the hydrodynamic properties of mitochondrial and nuclear DNA and of their modification on exposure to DNase has shown that both the DNAs display high viscosity, although their response to addition of DNase proved to be different. While, as early as 4 min after addition of the enzyme, nuclear DNA was found to have lost 40 per cent of its viscosity, the same effect was only attained after 60 min in the case of mitochondrial DNA. The mitochondrial DNA sedimentation constant obtained by centrifugation in ultra-violet light has also

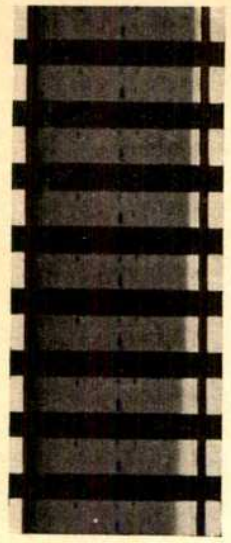


Fig. 1. Determination of sedimentation constant of mitochondrial DNA, using ultracentrifugation in ultra-violet light. Velocity, 30,000 r.p.m.; time, 30 min; DNA concentration, 30 μg/ml. in 0·15 M sodium chloride

shown the high-polymer structure of this nucleic acid and its distinction from nuclear DNA. Thus, the sedimentation constant of mitochondrial DNA, purified on a 'Sephadex *G*-75' column in 0·15 M sodium chloride, was $18{\cdot}6 \times 10^{-13}$ sec, giving a molecular weight of $4{\cdot}8 \times 10^6$ (Fig. 1), while in the case of nuclear DNA, the S_{20} amounted to $22{\cdot}2 \times 10^{-13}$ sec, or to a molecular weight of 8×10^6.

Differences between properties of mitochondrial and nuclear DNA were also shown by determinations of the hypochromic effect, which was found to amount to 38 per cent for mitochondrial DNA and to 53 per cent for nuclear DNA. The transition temperature of the DNA helix for mitochondrial DNA was found to be within a higher range, indicating the prevalence of guanidine–cytosine pair couple bases.

The descending paper chromatogram method[12] was used for establishing the composition of mitochondrial DNA and for its comparison with that of nuclear DNA. Analysis of the spots revealed the composition of nitrogen bases shown in Table 1. This showed the presence of a minor component of DNA 5-methylcytosine.

Table 1. BASE COMPOSITION OF MITOCHONDRIAL AND NUCLEAR DNA

(Mol. per cent)

	G	*A*	*C*	*T*	*G*+*A*/*C*+*T*
Mitochondrial DNA	31	21	24	24	1·08
Nuclear DNA	25	26	21	28	1·04

Rabinowitz *et al.*[13] obtained somewhat different values for the guanidine–cytosine content of nuclear and mitochondrial DNA from chick embryos. Our experiments were made on adult rats, which may explain this difference. Nevertheless, further investigations with larger quantities of mitochondrial DNA are necessary for this question.

One of the properties peculiar to mitochondrial DNA is its capacity to undergo structural modification on exposure to ultra-violet light. The mechanism of ultra-violet irradiation injury has been shown to depend on the formation of thymine dimers between two thymine molecules in a single DNA chain[14]. This results in a loss of the replication capacity of DNA and in arrest of its synthesis. It appeared to be of interest to establish whether such a mechanism actually occurs on irradiation of mitochondrial DNA, that is to find whether in this case errors in structure of the DNA molecule were really due to the formation of thymine dimers. To this end, DNA preparations from liver cell mitochondria and nuclei were exposed to ultra-violet irradiation at 2537 Å, total dose 960 ergs/mm² ('*BUV*-30', ultra-violet output 95 per cent). After DNA hydrolysis, the composition of nitrogen bases was studied. The chromatogram revealed the appearance of new spots with R_F 0·83, conforming to the R_F of dimerized thymine. Non-irradiated thymine is known to have an R_F of 0·72. The percentage of thymine dimers (compared with total thymine) amounted to 16 per cent for mitochondrial DNA and to 25 per cent for nuclear DNA.

The possibility of extra-chromosomal determinants of heredity in mitochondria raises the problem of their partial genetic autonomy. Calculation of the possible information content inherent to the DNA in a single rat liver mitochondrion gives a value equivalent to 120 proteins with a molecular weight of 20,000. The amount of DNA present in mitochondria is thus sufficient for the reproduction of mitochondrial protein structure.

Mitochondrial DNA may be assumed to contain information for synthesizing proteins of the electron transport chain and coupled phosphorylation and, consequently, for determining one of the universal mechanisms of energy generation, inherent to all cells, irrespective of their specialization. On the other hand, nuclear DNA may be responsible for controlling the rate of protein synthesis, thus determining the specificity of the cell. Such a hypothesis is supported by evidence from the use of the method of radiation inactivation of nuclei[15], which shows the respiratory function of mitochondria to be independent of the nucleus, the latter being responsible for only activation of respiration. The possibility of a genetic system located in mitochondria does not invalidate the existence of genetic control from the nucleus.

To sum up, investigation of a mitochondrial DNA preparation in an open system in the presence of RNA-polymerase (made by Dr. Markosian and Dr. Shmerling) has shown that RNA synthesis occurs.

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Prediction of the Relative Passive-transfer Rates of Short-chain Fatty Acids across Lipid Membranes

A VAST quantity of literature has accumulated concerning the mechanism of lipid transfer across the intestinal wall, yet the process still remains largely unclear[1]. One point which seems definitely established is the difference between the transfer of short-chain fatty acids, which appear free in the liver portal blood, and the long-chain acids ($> C_{10}$), which appear mainly in lymph in the form of triglycerides[2]. Whereas the latter transfer system is complex, involving emulsification, micelle formation and

enzymatic esterification, the former may be relatively simple, involving a combination of a fatty acid permease and passive penetration. Smyth[3] and Barry and Smyth[4] have demonstrated that fatty acids containing from two to six carbons are actively transported from the mucosal to the serosal side of intestinal sacs *in vitro*. Higher concentrations of fatty acids suppress *in vitro* active transport, the suppression being more pronounced with increasing chain length, but this inhibition does not seem to influence the rate of disappearance of fatty acids from inside the intestine *in vivo*[4]. The possible contribution of passive transport is therefore of some interest.

Carr[5] has proposed that the decreasing capacity of fatty acids to be absorbed at higher chain lengths might be explained on the basis of molecular size and has shown[6] that the permeation of a fatty acid salt through artificial porous membranes decreases sharply when its concentration is increased above the critical micelle concentration. Since critical micelle concentration in turn decreases with increasing chain length, it is reasonable to assume that some selectivity would be obtained by means of penetration through aqueous pores. Micelle formation would be limited with the smaller fatty acids, however, and passive penetration would therefore be determined either by diffusion through aqueous channels or by transfer across lipid membranes.

The observed *in vivo* rate of absorption at 38° C increases only about 20 per cent as the chain length is increased from two to six carbon atoms[4]. On the basis of the benzene : water partition coefficient alone, the passive penetration rate through lipid membranes should increase nearly 300-fold with a change from two to six carbon atoms (see Table 1), which would imply that passive transfer through lipid is a minor contributing factor below C_6. This would suggest either that active transfer tends to increase with decreasing chain length to compensate or that passive transfer by aqueous pathways is a major factor. However, it is also possible that passive transport across lipid membranes may be much more prominent for the lower fatty acids than would be expected from the simple oil : water partition coefficients.

Table 1. COMPARISON OF RELATIVE RATES OF FATTY ACID TRANSFER: OBSERVED TOTAL RATES AND THEORETICAL PASSIVE RATES ACROSS LIPID MEMBRANES

Acid	No. of carbon atoms	*In vivo* absorption rate	log *P*	log *C*	*pK*	Passive rate (R/R_A)
Acetic	2	1·00	+1·49	−1·26	4·75	1·00
Propionic	3	1·09	+0·95	−0 55	4·87	1·20
Butyric	4	1·09	+0·37	+0·03	4 81	0·80
Valeric	5	1·17	−0·12	+0·57	4·82	0 68
Caproic	6	1·22	−0·85	+1·21	4·83	0·46
Refs.		(4)	(8)	(10)	(9)	

P = Vapour pressure of pure acid in mm of mercury at 38° C; C, benzene: water partition coefficient, R/R_A, $P^{0.6}C^{0.4}K_A/P_A{}^{0.6}C_A{}^{0.4}K$, where subscript A indicates values for acetic acid. The *in vivo* absorption rate from rat intestine was reported for 20 mM acid over a 20-min period at 38° C and *p*H 7·4.

The hypothesis has recently been advanced[7] that the vapour pressure of the solute may be an important factor in the rate of penetration of biological membranes. For similar organic solutes, the permeability constant is nearly proportional to $P\gamma C^{(1-\gamma)}$, where P is the vapour pressure of the pure solute at a given temperature, C is the oil : water partition coefficient, and γ is a number between 0 and 1·0 which is related (in this hypothesis) to the extent of dissociation of solute from both lipid and aqueous phases during passage into the membrane. γ was found empirically to be near 0·60 for alcohol and ester penetration into a variety of animal cells. In order to apply this relationship to fatty acid penetration, the following assumptions will be made: (*a*) Passive transfer through a lipid membrane involves the passage of un-ionized fatty acid. At *p*H 7·4, as used by Barry and Smyth[4], about 0·2 per cent of the fatty acid will be un-ionized, and the amount of un-ionized acid can be approximated as $m[H^+]/K$, where m is the total concentration of acid and its salt and K is the acid dissociation constant. Since m and $[H^+]$ are held constant, the quantity of un-ionized acid will be inversely proportional to K. (*b*) The value of γ for the un-ionized acid will be the same as for alcohols and esters, or 0·60.

The relative rate of passive transfer will then be proportional to $(P^{0.6}C^{0.4}/K)$ or, in logarithmic form:

$$\log R = 0{\cdot}60 \log P + 0{\cdot}40 \log C + pK + \text{constant},$$

where R is the rate of passage. The values for log P, log C, and pK are given in Table 1 along with the calculated value of R/R_A, or the rate for a given acid divided by the rate for acetic acid (R_A). It may be seen that the passive-transfer rate through a lipid membrane could actually decrease with increasing chain length. On this basis, it would be difficult to distinguish between aqueous and lipid pathways. In either case, active transfer would increase with increasing chain length.

It should be emphasized that both the observed *in vivo* absorption rate and the calculated passive rate are given relative to acetic acid and not relative to each other. Further elaboration on this prediction must await more information about the magnitude of the passive-transport contribution to total transport, the extent of transport through aqueous pores, and the value of γ for fatty acids.

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Purification and Chemical Constitution of the Phospholipids from *Corynebacterium diphtheriae* P.W.8

PREVIOUS investigations on the ether-soluble lipids from *Corynebacterium diphtheriae* have revealed the presence of saturated and unsaturated fatty acids with 16 and 32 carbon atoms[1–3].

The fraction extracted with ethyl ether and precipitated by acetone consists mainly of complex lipids[4–6]. Working with this fraction, Asselineau[6] obtained, after column chromatography on silicic acid, a fraction with the following analytical characteristics: melting point 185° C $\alpha_D^{20\,\circ} = +20°$; phosphorus, 3·0 per cent; nitrogen, 0·8 per cent. On acid hydrolysis he obtained an ether-soluble and a water-soluble fraction. The ether-soluble fraction contained palmitic acid and corynomycolic acid; and in the water-soluble fraction he detected inositol, mannose and glycerol.

From an acetone-insoluble lipid fraction we purified a glycolipid which we showed to contain corynomycolic and corynomycolenic acid[7] esterified to trehalose. Further purification of the acetone-insoluble lipids yielded three phosphoglycolipids. The present communication describes the purification, analyses and chemical characterization of these substances.

Isolation of the phospholipids. The fraction of *C. diphtheriae* P.W.8 lipids insoluble in boiling acetone[5] was used as the starting material in this work. This fraction was dissolved in chloroform and passed through a column of cellulose powder (Whatman No. 1). After evaporation of the eluate to 1/10 its volume an equal volume of methanol was added. The precipitate that formed was centrifuged and dried in a desiccator under vacuum.

The dried material was redissolved in a small volume of chloroform and chromatographed on a column of silicic acid (Mallinckrodt) using increasing concentrations of methanol in chloroform: 1/19, 1·5/8·5 and 1/1. The phospholipids were eluted with chloroform–methanol 1/1. After removal of the solvent this fraction was rechromatographed on silicic acid. The following concentrations of methanol in chloroform were used: 2·5/7·5, 3·5/6·5 and 1/1. Three peaks were obtained that we called, respectively, *F* 25, *F* 35 and *F* 50 in accordance with the percentage of methanol used. The homogeneity of the peaks was confirmed by thin-layer chromatography on silicic acid[8]. The results are shown in Table 1. The analytical characteristics of the three fractions are presented in Table 2.

Table 1. THIN-LAYER CHROMATOGRAPHY ON SILICIC ACID OF THE PURIFIED PHOSPHOLIPIDS*

Solvent systems	R_F Fraction 25	R_F Fraction 35	R_F Fraction 50
Chloroform–methanol–water (65 : 25 : 4)	0·46	0·36	0·31
Chloroform–methanol (4 : 1)	0 35	0·31	0·29
Chloroform–methanol–ammonia (80 : 20 : 0·8)	0 28	0·42	0·15
Diisobutylketone–acetic acid–water (40 : 20 : 5)	0·14	0·21	0·07

* The spots were detected by ammonium molybdate-perchloric acid reagent for phosphorus and an alcoholic solution of rhodamine *B* for lipids.

Table 2. ANALYTICAL CHARACTERISTICS OF THE PURIFIED PHOSPHOLIPIDS

	$a_D^{\lambda°}$ C (c = 0·7 in $CHCl_3$)	Melting point	Phosphorus (per cent)	Reducing sugar (per cent)*	Nitrogen (per cent)
F 25	+26°	195–202° C	2·5	10·4	—
F 35	+20°	214–217° C	2·0	7·8	—
F 50	+25°	240–246° C	2·0	13·8	—

* Measured as mannose.

Characterization of the carbohydrates. The three phospholipid fractions were saponified separately by exposure to 15 per cent methanolic potassium hydroxide solution for 45 h at room temperature. After acidification with acetic acid, we recovered 54 per cent, 48 per cent and 41 per cent of *F* 25, *F* 35 and *F* 50, respectively, as ether-soluble fatty acids. The aqueous fractions were desalted by passage through an Amberlite *MB*3 column and evaporated under vacuum, and found to consist of 26 per cent, 20 per cent and 32 per cent of neutral constituents. The hexose content of the latter fractions was estimated with anthrone and was found to be 60 per cent, 58·5 per cent and 71 per cent, respectively. The neutral residue was hydrolysed for a further 8 h with 5 per cent sulphuric acid in a boiling water bath and neutralized by passage through a column of 'Amberlite *IR* 4*B*'. Paper chromatography of the three hydrolysates was carried out on Whatman No. 1 with the following solvent systems: isopropanol–acetic acid–water (6 : 3 : 1); *n*-butanol–acetic acid–water (120 : 30 : 50) and *n*-propanol–ethyl acetate–water (7 : 1 : 2). Glycerol, mannose and inositol were detected in the three hydrolysates by the use of periodic acid–benzidine reagent. The presence of mannose was confirmed by the preparation of the phenylhydrazone (melting point 195°C). There was no depression of the melting point on mixture with authentic phenylhydrazone of mannose. Inositol was further confirmed by preparation of the hexa-acetate with acetic anhydride in pyridine. The crystalline compound has a melting point of 223°–225° C. The mixture with authentic hexa-acetyl inositol gave no depression on the melting point.

Characterization of the fatty acids. The ether-soluble fatty acids from the saponification were obtained as a crystalline product with an average molecular weight of 240 by titration, and a melting point of 44°–55° C. By paper chromatography on Whatman No. 1 paper saturated with 'Nujol'[9] and developed with acetic acid–water (9 : 1) at 45° C for 20 h (descending), two spots were detected by spraying with mercuric acetate and diphenyl carbazide, corresponding to myristic and palmitic acid. They were further characterized by thin-layer chromatography on silica gel *G* (Merck) impregnated with 'Nujol'[10] in the following solvent systems: acetic acid–acetonitrile (1 : 1) and hexane–acetic acid–ethyl ether (83 : 1 : 16) and detected by spraying with an alcoholic solution of rhodamine *B*.

In conclusion, our results confirm those of Asselineau[6] in regard to the non-saponifiable fraction of the phospholipids but differ in regard to the analytical data and the constitution of the fatty acids.

The chemical constitution of these phospholipids of *C. diphtheriae* is similar in many respects to the glycerophosphoinosito mannoside compounds of *M. tuberculosis*, studied by Vilkas and Lederer[11], which was shown to contain palmitic and stearic acid as the fatty acid moieties.

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Effect of Urethan on Nucleic Acids

BIOCHEMICAL investigations have not so far been successful in elucidating the mechanism of action of urethan carcinogenesis. The problem has been studied extensively from a biological standpoint and there are excellent reviews on the subject. Boyland *et al.*[1] and Kaye[2] have investigated its metabolism thoroughly, but its mode of action in the organism is still not understood. The present communication reports preliminary observations on the effect of injected urethan on the nucleic acid metabolism of mice.

Two inbred strains of mice, Swiss and *L*(*P*), were used for experimental purposes. Swiss strain mice are resistant to spontaneous and induced breast carcinomata, while *L*(*P*) strain mice are resistant to spontaneous breast cancer but highly susceptible to chemically induced breast cancer[3]. Swiss strain mice are reported to be susceptible to induced lung and skin cancers, but a carcinogenic effect of urethan on *L*(*P*) mice has not yet been reported. Therefore *L*(*P*) strain mice were used to see if urethan exerted any strain-specific effect.

The following experimental groups were used for investigation.

(1) 10–12-week-old male mice of both strains were injected with 1 mg/g body weight of urethan intraperitoneally and were killed 4 h later.

(2) 10–12-week-old normal males of Swiss and *L*(*P*) strain were injected with 1 mg/g urethan daily for 4 days and were killed 96 h after the first injection.

(3) Swiss males bearing transplanted fibrosarcomata were injected with 1 mg/g urethan intraperitoneally on

the sixth day of transplantation. These were then divided into two groups.

(*a*) Killed 4 h after a single injection of urethan.

(*b*) Injected daily for 4 days and killed 96 h after the first injection.

Animals were killed by decapitation and lung, liver and kidney tissues were used for estimation of RNA and DNA. RNA was estimated by Rosen and Ogur's method[4]. DNA was estimated by Indole's method[5]. Protein was estimated by Folin's method[6]. The content of both the nucleic acids is expressed per μg protein.

Fig. 1 shows the effect of urethan injection on the RNA and DNA content of liver, kidney and lung tissues of normal males (10–12 weeks old) of Swiss and *L(P)* strains. It can be seen at a glance that a single injection of urethan affects the levels of both the nucleic acids of all the three tissues of normal Swiss males. The decrease in the nucleic acid level was 75–80 per cent. Repeated injections of urethan have not caused further decrease in nucleic acid levels. In contrast to Swiss mice, *L(P)* strain mice have not shown any change at all in both the nucleic acid levels even after repeated injections of urethan.

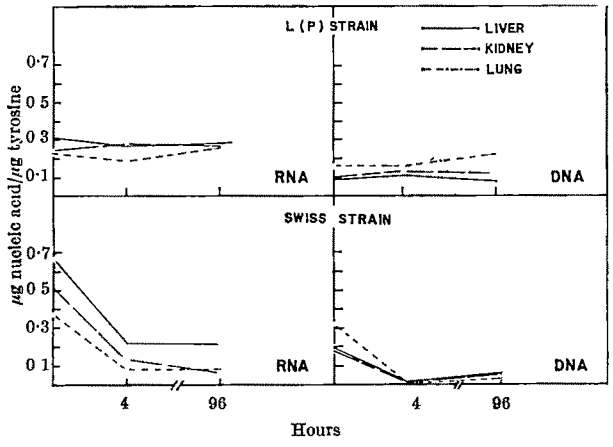


Fig 1. Effect of urethan injection (intraperitoneal) on the nucleic acid content of different tissues of two strains of mice

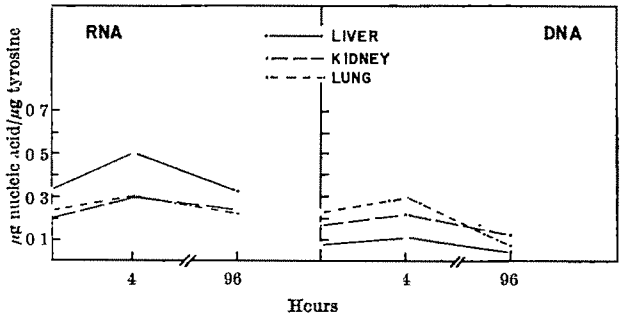


Fig. 2. Effect of urethan injection (intraperitoneal) on Swiss mice bearing fibrosarcomata

Fig. 2 shows the effect of a single injection as well as of repeated injections of urethan on different tissues of Swiss mice bearing fibrosarcomata.

It can be seen that there is an increase in both the nucleic acid levels of all the tissues in the 4-h group. However, in the group receiving repeated injections, there is no significant difference between the treated and untreated animals.

A 75–80 per cent decrease observed in the nucleic acid levels of all the tissues of Swiss mice within 4 h of single injection of urethan is a significant observation. A possible effect of urethan on nucleic acid metabolism was suggested first by Rogers[7] and then by Elion *et al.*[8]. These authors were able to inhibit tumour formation by giving nucleic acid hydrolysates or by thymine but not by uracil. They postulated that urethan inhibits DNA synthesis at the site of thymine biosynthesis. In the present experiments, the inhibitory effect of urethan on RNA and DNA levels is comparable. It may be mentioned here that the effect of urethan on RNA has not been reported previously. The decrease in nucleic acid contents of the tissues of urethan-treated normal Swiss mice is still more significant, as no changes are seen in urethan-treated *L(P)* mice. It has been proved that urethan causes lung adenomata and skin tumours[9] in Swiss mice and thus even its immediate biochemical effects seem to be strain specific.

It is also interesting to observe that the mode of action of urethan in normal Swiss mice is different from that in tumour-bearing mice. It may be stated here that urethan is known as a carcinogenic as well as a carcinostatic compound[9] and it may thus have different modes of action in normal and tumour-bearing mice. These observations, however, are of a preliminary nature and it is essential to study the mechanism of action of urethan in further detail before formulating its role in carcinogenesis. Investigations on these lines are in progress and will be reported later.

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Effect of Cortisol on the Synthesis of Chondroitin Sulphate by Embryonic Cartilage

CORTISOL, in low concentrations, profoundly affects the growth of the cartilaginous limb-bone rudiments from embryonic chicks, cultivated in a synthetic medium[1–4]. One aspect of this effect is a decrease in the degree of hydration of the tissue and, because much of the bound water of cartilaginous tissues may be associated with mucopolysaccharide, it seemed possible that cortisol might be acting on the synthesis of the polysaccharide component of the intercellular matrix. This possibility was supported by the observation that rudiments cultivated in the presence of cortisol ($2{\cdot}1 \times 10^{-7}$ M, or less) exhibit particularly intense toluidine blue staining[3], although there are now several reports (reviewed by Dziewiatkowski[5]) of an inhibition of polysaccharide synthesis in connective tissue, with reduced metachromasia, by high levels ($1–2 \times 10^{-4}$ M) of cortisol. Whitehouse and Lash[6,7] have also described inhibition of sulphation without interruption of polysaccharide synthesis.

Experiments were made to decide whether cortisol, at physiological concentrations, has any effect on the synthesis of polysaccharide by cartilaginous limb-bone rudiments cultured *in vitro*.

In each of a series of six experiments tibiae and femora from two 7-day-old chick embryos were cultivated on stainless steel grids[8] at 37·5° C. A chemically defined medium (*BGJ* 4) (ref. 9), modified by the inclusion of sulphur-35-labelled sodium sulphate, was used. Four bones from each set were cultivated in medium which also contained cortisol (0·1–0·001 μg per ml., that is, $2{\cdot}1 \times 10^{-7}$ M–$2{\cdot}1 \times 10^{-9}$ M), and several bones from each batch of eggs were immediately dried as zero-time

Table 1. RESULTS OF TWO EXPERIMENTS IN WHICH PAIRED GROUPS OF FOUR BONE RUDIMENTS WERE COMPARED AFTER CULTURE IN THE PRESENCE OR ABSENCE OF CORTISOL

	Conc. of cortisol (μg/ml.)	Tissue weight (mg) Fresh	Dry	Fresh/ Dry	Radioactivity (c.p.s.) Total	Free	Bound	Uronic acid (μg) Total	Bound	Formed *in vitro*	Polysaccharide formed *in vitro* C.p.s./ μg uronic acid	Atom sulphur/ mol. uronic acid
Expt. 1	—	14·65	1·59	9·2	2,670	201	2,400	94	95·2	75·7	31·7	0·97
	0·01	10·45	1·36	7·7	2,230	177	1,860	88	79·0	59·5	31·4	0·96
Expt. 2	—	16·55	1·88	8·8	2,810	203	2,500	110	102·2	82·7	30·3	0·93
	0·1	8·45	1·32	6·4	1,970	176	1,660	82	75·3	55·8	29·8	0·91

The medium contained sulphur-35 labelled sulphate giving 5,460 c.p.s. per ml., or 182 c.p.s. per μg sulphur, as 30·1 μg sulphur atoms were present per ml. Quantities of uronic acid are expressed as glucuronolactone, and the calculation of the quantity incorporated during the culture period is based on the presence of 19·5 μg in a group of zero-time control bones

controls. The explants were re-fed and gassed (with air + 5 per cent carbon dioxide) at intervals of 2 days.

After 6 days the rudiments were removed from the grids, rinsed in balanced salt solution, blotted and weighed. Each set of explants was reweighed after drying at 60° C *in vacuo*, and was then digested with papain[10]. The digests were diluted to 5·0 ml., 0·1 M with respect to sodium sulphate, and sampled for estimation of uronic acid[11]. Total radioactivity also was measured, after plating out, by means of a windowless scintillation counter, and appropriate corrections were made for self-absorption and decay.

Acidic polysaccharide was precipitated from the digests (3·5 ml.) by the addition of cetylpyridinium chloride solution[12] (0·2 ml., 1 per cent) and, after centrifugation, the clear supernatants were sampled for measurement of non-polysaccharide sulphur. The detergent–polysaccharide complexes were washed with sodium sulphate solution (0·1 M), and then redissolved in 1 M sodium chloride containing a little ethanol. The free polysaccharide was precipitated with ethanol (four volumes). A solution of the polysaccharide was taken for measurement of uronic acid content and radioactivity. The results of two typical experiments are presented in Table 1.

It can be seen that the presence of cortisol in the culture media did not have any effect (within the limits of accuracy attainable) on the ratio of sulphate to uronic acid in newly synthesized polysaccharide. The ratio of sulphur atoms to residues of glucuronic acid was close to unity, as would be predicted from the composition of chondroitin sulphate.

Cortisol inhibited polysaccharide synthesis as a whole, however; the extent of inhibition was 35 per cent at 0·1 μg per ml. (the mean of two experiments), 10 per cent at 0·01 μg per ml. (the mean of two experiments) and 3 per cent at 0·001 μg per ml. (in a single experiment).

Whitehouse and Lash[6,7] described the production of an incompletely sulphated chondroitin sulphate by chick somites in an *in vitro* system, when cortisol ($1·5 \times 10^{-4}$ M) was present, but in experiments with slices of bovine cartilage and cornea, Whitehouse and Borström[13] found cortisol to inhibit polysaccharide synthesis and sulphation equally. This latter finding is in agreement with work on skin[14] and other tissues[5].

It has already been reported by Reynolds[3] and by Dingle, Fell and Lucy[4] (in apparent contradiction of Schryver[2]) that total polysaccharide synthesis is inhibited by 0·1 μg per ml., or less, of cortisol, and this is confirmed by the present work. It is interesting that Whitehouse and Lash[6] detected no inhibition of uronic acid incorporation in their system, with cortisol at 720 times this concentration.

The possibility that the kind of sulphated polysaccharide synthesized by the chick limb-bone rudiments might be altered by cortisol was investigated in some unpublished work[15]. The glucosamine to galactosamine ratio in polysaccharide from bones cultured in the presence or the absence of cortisol, or directly *ex ovo*, was determined by a modification of Scott's method[16], and in each case only 3–5 per cent of the total hexosamine was glucosamine—the remainder was galactosamine. This suggested that the polysaccharide of the cartilaginous rudiments from each group was almost exclusively chondroitin sulphate. This finding is in contrast with that of Glick *et al.*[17], who used an *in vitro* system based on that of Lash, Holtzer and Holtzer[18].

In summary, this work shows that cortisol, at near-physiological concentrations, can reduce the synthesis of sulphated polysaccharide in cartilaginous bone rudiments in culture. This represents an extension of the many previous reports of inhibition at much higher concentrations of steroid.

No substantial disturbance of the expected ratio of sulphate to uronic acid was detected, and the results do not explain why the cortisol-treated rudiments were less hydrated than their paired controls.

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In vivo Complex Formation of Actinomycin *D* and Deoxyribonucleic Acid

ACTINOMYCIN *D* (AMD) has been shown to inhibit the ribonucleic acid (RNA)-polymerase activity of bacterial cells. This inhibition is considered, at present, to represent the sole mechanism by which this agent inhibits cell division[1]. It appears to result from the formation of complexes between the antibiotic and deoxyribonucleic acid (DNA)[2]. Following parenteral administration to animals the antibiotic appears to possess similar activity, but differs in that high doses are required and the effects are reversible[3]. When administered to rats which have undergone partial hepatectomy, the expected subsequent mitotic activity can be eliminated[4].

It remained, therefore, to demonstrate that the mammalian cell activity of AMD is mediated by the same mechanism as in bacteria, and to explain these differences we have isolated DNA from the livers of rats previously treated with AMD and measured the priming activity of the DNA in a bacterial RNA-polymerase system.

This activity was significantly reduced when compared with DNA isolated from untreated rats and was restored by separation of the AMD. Similar parenteral doses demonstrated their expected inhibition of mitotic activity. The priming activity of liver DNA from untreated rats was inhibited after *in vitro* addition of AMD to a degree similar to that previously reported using thymus nucleic acid.

AMD was dissolved in sterile water at a concentration of 200 μg/ml. and the solution injected by way of the femoral vein. Animals which were investigated for the effect of AMD on mitosis were subjected to 70 per cent hepatectomy as described by Higgins and Anderson[5] and the antibiotic was administered simultaneously. Colchicine was administered intraperitoneally (1 mg/kg) at 25 h and the animals were killed at 31 h. Sections of liver were prepared and studied by standard histological techniques for mitotic activity. Liver nuclei were prepared by the triton extraction of Hymer and Kuff[6] and were free from cytoplasm when examined under phase microscopy. These were layered above caesium sulphate solutions and the DNA obtained by ultracentrifugation[7]. Such preparations were tested in an RNA synthesizing system utilizing the polymerase of *M. lysodeikticus* as described by Nakamoto, Fox and Weiss[8]. The incorporation of tritiated uridine triphosphate (UTP-^{3}H) was utilized as a measure of RNA production. The AMD–DNA complexes were extracted by precipitation in 3 : 1 isopropanol, followed by centrifugation. This process was repeated when sufficient DNA was available and the DNA finally dissolved in 0·001 M sodium citrate–0·01 M sodium phosphate buffer.

DNA isolated from liver cell nuclei of rats which had received AMD had a decreased ability to prime the polymerase system. This decrease was roughly proportional to the dose of the antibiotic administered (Table 1). The DNA isolated as described above was a highly hyperchromic material, apparently free of protein contamination as determined by ultra-violet spectrum analysis. A maximal inhibition was achieved with a dose of 300 μg/kg as complexes isolated from animals receiving doses as high as 1 mg/kg failed to show a significantly increased inhibition. Histological examination indicated that 300 μg/kg was also the minimal dose which invariably inhibited the mitotic response in rats following 70 per cent hepatectomy.

Table 1 RNA-POLYMERASE PRIMING UTILIZING *in vivo* FORMED COMPLEXES OF AMD AND DNA

Dose (μg AMD/kg)	Incorporation* (per cent)	Extracted* (per cent)	Expected mitosis† (per cent)
100 (3)‡	52–64		‡(6) 85–100
200 (2)	33·7–37·7	§(1) 56·9	(6) 36–86
300 (4)	23 4–29	(1) 42·0	(6) 0
1,000 (2)	20·1–25·4		(3) 0

* Percentage incorporation of UTP-^{3}H into RNA synthesized as compared with the priming by untreated liver DNA.
† Compared with average mitotic counts of (twelve) partially hepatectomized, untreated rats.
‡ Number of animals tested.
§ Number of extractions performed.

Extraction of these *in vivo* formed complexes by isopropanol precipitation reduced the degree of inhibition of priming ability. The number of extractions was limited by the amount of material available and complete restoration was not achieved as with *in vitro* formed complexes (Tables 1 and 2). No residual damage to DNA could be demonstrated by measurement of hyperchromicity.

The DNA isolated from rat liver nuclei proved to be highly active in RNA–polymerase systems, and was at least as active as standard thymus nucleic acid preparations. Addition of AMD to this DNA before its addition to the polymerase system resulted in inhibition of RNA production proportional to the quantity of the antibiotic added (Table 2). Extraction of this system with propanol precipitation restored the priming ability of the DNA.

It has been possible to isolate the complex which is formed between parenterally administered AMD and rat liver DNA, and we have demonstrated an inhibition of its priming ability. The use of the triton method of nuclear preparation and caesium sulphate gradient ultracentrifugation limits the exposure of the complex to extraction by numerous solvents or by cytoplasmic components. The maintenance of the complex throughout the preparation, however, indicates considerable binding strength of the AMD to DNA since the centrifugation itself is capable of freeing the nucleic acid of its tightly complexed proteins. Our inability to detect the AMD on spectroscopic examination of the complex fits well with the concept that the binding sites to which the antibiotic is complexed are of high functional activity, but of low capacity. These would represent, therefore, the tight binding sites as described by Gellert *et al.*[9].

That the inhibition is based solely on the presence of AMD was demonstrated by the return of priming ability of the isolated DNA on extraction of the antibiotic. More extensive extraction of the *in vitro* formed complexes in several instances completely restored priming ability. This indicates a lack of residual damage to the DNA.

Table 2. RNA–POLYMERASE PRIMING UTILIZING AMD–DNA COMPLEXES FORMED *in vitro*

mμM AMD/ml.	Incorporation* (per cent)	Extracted* (per cent)
0 500	2 5–4 7	†(2) 29 4–37 8
0·250	12 7	
0 125	30·2	
0 100	28 3–38 7	(2) 62·5–62·8
0·071	71 0	
0·056	79·1	
0 050	72 0–73·5	(2) 99 5
0 010	84·5	(2) 101
0 005	87·0	(2) 102

* Percentage incorporation of UTP-^{3}H into RNA synthesized as compared with priming by untreated liver DNA.
† Number of extractions performed.

It has previously been reported that the mitotic activity which normally follows partial hepatectomy is eliminated by high doses of AMD[3]. It has been indicated that this effect results from the inhibition of synthesis of RNA and the failure of the subsequent production of the enzymes involved in the synthesis of DNA. Support for this concept of inhibition of RNA synthesis *in vivo* is offered by the present data, but the type of RNA synthesis so effected is not indicated. The dose of antibiotic optimal for inhibiting mitotic response in liver cells, 300 μg/kg, also represents approximately the maximal binding capacity of DNA for this agent *in vivo*. This may reflect the maximal capacity of AMD to compete with nuclear proteins for binding sites or the maximal number of binding sites of high affinity for the antibiotic. Our results show that AMD acts within the animal in a fashion similar to that manifested in bacteria and isolated cells. The mechanism of differences between the two systems has not yet been clarified.

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Reduced Benzo(a)pyrene and Phenolic Content of Smoke from Experimental Cigarettes

THE properties of tobacco and non-tobacco leaves can be modified by subjecting these leaves to a series of chemical extractions which strip the whole leaf, or shredded leaf, to almost pure cellulose. This extraction process removes unhealthy substances and improves the flavour and aroma of such leaves when they are used as a smoking product. The extracted leaf is reconstituted, preferably to its original weight, by adding back non-toxic chemical agents which enhance the burning qualities, flavour and aroma.

In recent years increasing statistical evidence has associated cigarette smoking with increased death rates from lung cancer and other diseases, notably coronary heart disease, emphysema and chronic bronchitis[1]. Recently, an advisory committee to the Surgeon General of the United States claimed to have established a causal relationship between cigarette smoking and lung disease.

Nearly 500 different chemical compounds have been isolated from cigarette condensate, including numerous polycyclic, aromatic, and other organic and inorganic compounds. Many of these compounds are carcinogenic and toxic when assayed on animals.

Polycyclics generally initiate the carcinogenic process and several polycyclic hydrocarbons are complete carcinogens. Because only relatively small amounts of polycyclics are present in the unburned leaf, they must be formed during the burning process from materials inherent in the leaf[2]. Polycyclics may be formed on pyrolysis of cellulose, lignin, pectin, starch, sucrose, glucose, and fructose and of citric, malic, and oxalic acids. Pyrolysis of tobacco paraffins at temperatures higher than 600° C yields polycyclics[3]. Incomplete pyrolysis of sterols may also produce polycyclics[4]. An efficient extraction process should remove sterols, paraffins, and other possible precursors of polycyclics. Recent evidence indicates that polonium-210 may also initiate the carcinogenic process[5]. Polonium-210 should also be removed with an adequately designed extraction process.

The polycyclic fractions from cigarette smoke condensate account for less than 10 per cent of the carcinogenic activity of cigarette tar. It is believed that co-carcinogenic compounds are responsible for more than 90 per cent of the carcinogenic activity in the smoke condensate[6]. Several phenols are known to be co-carcinogens[7]: phenol, dimethylphenol, ethylphenol and *ortho-*, *meta-*, and *para-*cresol are all present in cigarette smoke condensate[8]. Other types of substances as yet unidentified may also be promoting substances. Co-carcinogens have been demonstrated in the alkaline extracts of unburned tobacco[9].

The burning process may release co-carcinogens by either dry distillation or by incomplete destruction of usually non-carcinogenic compounds. (Dry distillation is the volatilization of substances which arises because of high temperatures near the burning zone of an ignited cigarette.) These substances are transmitted unchanged to the mainstream smoke. A second way in which co-carcinogens may arise is through incomplete pyrolysis of materials inherent in the leaf. Rearrangements, oxidations, cracking, dehydrogenation and condensation reactions may convert usually non-carcinogenic compounds into carcinogens.

Sun-cured soybean leaves were first shredded with a model 00 Himhoff-cutter at a standard cut of 30 cuts/in. The shredded leaves were placed in stainless-steel wire-mesh baskets, and these baskets were raised or lowered frequently out of larger stainless-steel drums containing the solvents. The proportions of dry leaf to solvent were kept constant. Twenty millilitres of extracting solvent were used per gram of initial unextracted shredded dry leaf. All the extractions were carried out for 50 min at 55° C. The leaves were first rinsed twice with water under the aforementioned conditions. These two rinse solutions were discarded. Fresh water was heated to 55° C and added to the stainless-steel containers. Sodium chlorite was added until its concentration was 1 per cent. The *p*H was adjusted to 4 with glacial acetic acid and the leaves were again lowered into the solution. After discarding the bleach solution, the leaves were rinsed twice with water to remove the bleach. Acetone was the final extracting solvent. The baskets were then raised and allowed to drain. The shredded leaves were removed from the baskets and air dried.

Table 1. RESULTS OF PHENOL, BENZO(*a*)PYRENE, AND TAR ANALYSES OF EXPERIMENTAL CIGARETTES AND CONTROL AMERICAN TOBACCO CIGARETTES

Cigarette	Tar/cig. (mg)	Phenol/cig. (mg)	BP/cig. (γ)
Non-tobacco	10·6	0 046	1·50
Tobacco	38·6	0·27	6·15

The shredded leaves were then flavoured with several ingredients to ensure the proper hydroscopic and burning qualities. In this experiment the compounds used were: 10 per cent invert sugar, 5 per cent glycerol, 2 per cent honey, 1·5 per cent St. John's bread (that is, ground meal from the ripe fruit of *Ceratonica siliqua*), 1 per cent cocoa and 3 per cent potassium nitrate (selected on the basis of their low benzoid content). The ingredients were sprayed on to the shredded leaves in a rotary drum. To ensure a good tobacco flavour, various non-carcinogenic fractions of tobacco extract could be added to the flavouring mixture[10]. 50 lb. completed leaves was adjusted to a humidity content of 13 per cent. Cigarettes of the thus prepared flavoured shredded leaves were made in an American Machine and Foundry cigarette maker.

85-mm cigarettes were smoked on a standard Ecusta smoking machine. Standard American cigarettes were used as controls. Smoking parameters were set at a 35 ml. puff of 2 sec duration at 1 min intervals. The average butt length was 22 mm. Tars were collected on a previously weighed Cambridge filter. After the tar had been collected, the filter was weighed again. Phenols were determined by the method of Oakley *et al.*[11]. Benzo(*a*)pyrene analyses were made by the method of Wynder[12]. Results of the phenol and benzo(*a*)pyrene analyses are given in Table 1.

The extraction process reduces the tar content per cigarette to only 27·4 per cent of a standard American cigarette (Table 1). Phenols were also reduced to 17 per cent of the control. The reduction of benzo(*a*)pyrene suggests that it arises during the burning process through re-arrangement of complex polyphenols, steroids or other precursors inherent in the leaf. The extraction process also almost completely removes amino-acids, other nitrogen compounds and sulphur compounds. Toxic compounds, such as nitrogen dioxide, methyl nitrite, hydrogen cyanide and hydrogen sulphide are also probably considerably reduced in concentration in the mainstream smoke.

The great reduction of tar, phenols, benzo(*a*)pyrene and toxic substances indicates that it is possible to design a relatively safe cigarette.

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PHARMACOLOGY

Goitrogenic Activity of Onion Volatiles

GOITRE is known to be prevalent in the Lebanon, mainly in the high mountain valleys[1–5]. In an investigation of the population of an endemic area in the Lebanon, Cowan *et al.*[6] found that iodine deficiency was the primary cause of the endemic goitre. This deficiency could be intensified by the consumption of goitrogenic foods which cause either an increased iodine excretion or an inhibition of the biosynthesis of the thyroid hormone, thyroxine.

Two sulphur-containing substances, thiourea and thiouracil, have been found to inhibit the iodization process of thyroxine[7]. In addition, thiocyanate and thiooxazolidone-forming substances, in plants mainly of the Cruciferae family, have been shown to be goitrogenic[8–11]. However, there have been no reports on the possible goitrogenicity of onions, although sulphur compounds are known to be the principal components of their oils[12,13].

Onions are grown extensively in the Lebanon and are consumed in great amounts by the Lebanese people; thus an investigation of the possible goitrogenic activity of this plant is important. Saghir *et al.*[14] found that propyl disulphide is the main volatile constituent of certain food onions (common onion, *Allium cepa*; leek, *A. ampeloprasum*; Japanese bunching onion, *A. fistulosum*; and chive, *A. shoenoprasum*). This compound was therefore selected to be tested for its goitrogenic effect. Preliminary experiments indicated that 5 μl. and 10 μl. doses of the chemical slightly inhibited thyroid function in rats. The present work was initiated to investigate the effects of high levels of this sulphide.

Synthetic *n*-propyl disulphide (PDS) was obtained in pure form from Eastman Organic Chemicals; previous work had shown this compound to be identical to that found in onions[12,13]. For the assessment of goitrogenicity, the "acute" test described by Langer and Stolc[15] was used. The experimental animals were young female albino rats of the Sprague–Dawley strain, weighing about 150 g. Because the test was based on short-period (3 h) values for radioiodine uptake, iodine-deficient animals were used so that the control values would be relatively high. The rats were rendered deficient in iodine by maintaining them on a low iodine diet for 6 weeks. This diet was complete except for iodine, and the animals grew normally during the period of iodine depletion.

Five animals were assigned to each of seven test groups, according to a randomized block design, based on body weight. "Normal" uptake values were obtained from a control group which received only corn oil (since the test material was an oil). To check the validity of the test by demonstrating definite inhibition of thyroid function, two groups of rats were treated with two levels of a known goitrogen, propyl thiouracil (PTU).

The animals were fasted overnight before the experiment; the appropriate amounts of corn oil, PTU or PDS (Table 1) were then administered by stomach tube. 1 h later, each rat received, by intraperitoneal injection, 1 μc. of carrier-free sodium iodide labelled with iodine-131 in 0·5 ml. of 0·9 per cent saline solution. Exactly 3 h later, the animals were killed with chloroform and the thyroids were removed and weighed. Each gland was digested overnight at 50° C in a tube containing 2 ml. of alcoholic potassium hydroxide (potassium hydroxide, 5 g; water, 50 ml.; ethanol, 100 ml.). Total radioactivity was measured in each tube using a well-type crystal scintillation counter (Baird-Atomic). Percentage uptake was calculated by comparing the counts per mg thyroid tissue with those obtained from a standard dose counted at the same time as the thyroid digests.

The results are shown in Table 1. It is clear that at all levels used, *n*-propyl disulphide inhibited thyroid function significantly as is shown by the low values for uptake of iodine-131 in the treated animals. These low values were similar to those obtained by treatment with PTU. These observations point to the possibility that the consumption of food onions high in PDS may contribute to the incidence of goitre in countries such as the Lebanon where iodine intake is low. Further work is planned to investigate the possible goitrogenic effects of other sulphide radicals known to be constituents of other *Allium* species.

Table 1. EFFECT OF VARIOUS LEVELS OF *n*-PROPYL DISULPHIDE AND PROPYL THIOURACIL ON THE UPTAKE OF IODINE-131 IN RATS

Treatment	Dose	Average percentage uptake of iodine-131 per mg tissue*
Corn oil	100 μl.	1·176
PTU	1 mg	0·060
PTU	2 mg	0·080
PDS	60 μl.	0·070
PDS	120 μl.	0·080
PDS	180 μl.	0·087
PDS	240 μl.	0·103
LSD†		0·053

* Average of five values.
† Least significant difference (5 per cent level).

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6-Hydroxyskatole Glucuronide, a Skatole Metabolite

THE metabolism of 6-hydroxylated indoles is of interest in view of their possible psychotoxicity as reported by Szara[1] and the finding, originally by Leyton[2], that 6-hydroxyskatole sulphate is excreted in larger amounts by schizophrenic patients.

Hydroxylation and subsequent conjugation with either sulphate or glucuronic acid has been shown to be a pathway for the detoxification of a number of indoles. Skatole has been shown to undergo hydroxylation primarily in the 6 position and subsequent conjugation with sulphate[3]. Melatonin, another 6-hydroxylated indole, is excreted both as the sulphate and the glucuronide[4]. This report presents evidence that 6-hydroxyskatole is also excreted as the glucuronide.

6-Hydroxyskatole was prepared according to the method of Heacock[5] from 6-benzyloxygramine by catalytic hydrogenation. The compound was administered to 150-g Sprague-Dawley male rats in doses of 0·1, 1, 10, 25 and 50 mg/kg. Urine was collected at 2-h intervals

and subjected to paper chromatography and analysis for 6-hydroxylated indoles. Chromatography was ascending on Whatman No. 1 paper using isopropanol–ammonia–water, 10 : 1 : 1. Compounds were located with the acidic diazo reagent of Jepson[6] and with Ehrlich's reagent.

Quantitative estimations were carried out using 0·1 ml. urine, 0·9 ml. water and 5 ml. acidic diazo reagent prepared as described by Sohler *et al.*[7]. The colour was extracted into butanol and read at 415 mμ for the conjugated compounds and 530 mμ for free 6-hydroxyskatole. 'Unknowns' were compared with standard curves prepared from 6-hydroxyskatole sulphate for the conjugates and 6-hydroxyskatole for the free compound.

Chromatography of urine samples showed three 6-hydroxylated indoles to be present in the urine after the rats received 6-hydroxyskatole. The first compound (R_F 99) is free 6-hydroxyskatole[2]; the second (R_F 73) is the sulphate conjugate, while the third (R_F 15) proved to be the glucuronide of 6-hydroxyskatole. The R_F 15 material was isolated from urine by paper chromatography on Whatman 3 *MM* paper using the isopropanol–ammonia–water system. It was then eluted and chromatographed once more on Whatman 3 *MM* paper using a butanol–pyridine–water (60 : 60 : 60) solvent system. Acid hydrolysis of the purified material yielded a new spot which reacted positively with naphthoresorcinol reagent and on subsequent chromatography proved to be glucuronic acid. This spot was also obtained when the purified material was treated with 'Glusulase'—an enzyme preparation having both glucuronidase and sulphatase activity.

Fig. 1 illustrates the effect of 'Glusulase' on the 6-hydroxylated urinary metabolites. On 'Glusulase' treatment both sulphate and glucuronide spots tended to disappear while the spot of free 6-hydroxyskatole increased. 6-Hydroxyskatole would seem to be eliminated quite rapidly in the rat in the form of the free compound and the sulphate and glucuronide conjugates—the rate of excretion is maximal during the first 4 h, then levels off and is almost complete within 12 h.

Trace amounts of the glucuronide conjugate were found in human subjects who excreted large quantities of 6-hydroxyskatole sulphate. The presence of the glucuronide was established by concentrating urine samples tenfold. The concentrate was treated with urease and afterwards 200 lambda of the concentrate was spotted on Whatman 3 *MM* and run in the isopropanol–ammonia–water system. The glucuronide was detected with Ehrlich's and diazo reagent and its identity as the glucuronide was established with naphthoresorcinol reagent.

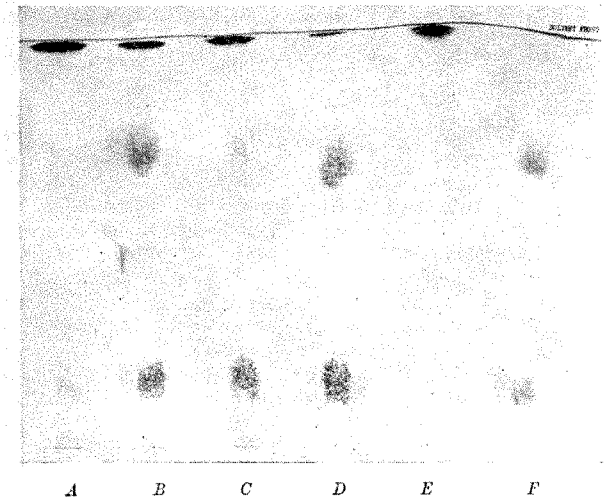


Fig. 1. Effect of 'Glusulase' on 6-hydroxylated indole metabolites in rat urine after administration of 6-hydroxyskatole. Urine samples were collected at 2-h intervals, 30 lambda of urine or its 'Glusulase'-treated equivalent was spotted. *A*, 2-h urine treated with 'Glusulase'; *B*, 2-h urine; *C*, 4-h urine treated with 'Glusulase'; *D*, 4-h urine; *E*, 6-h urine treated with 'Glusulase'; *F*, 6-h urine. Solvent system, isopropanol–ammonia–water (10 : 1 : 1)

Conjugation with glucuronic acid would seem to be an alternate pathway for the elimination of 6-hydroxyskatole acting in both rat and man.

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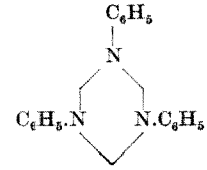
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Oral Hypoglycaemic Activity of an Anhydroformaldehyde Aniline–Insulin Mixture

TWENTY-FIVE to thirty per cent of diabetic patients require insulin to remain alive and well. In the main these patients have the juvenile-onset type of diabetes mellitus and treatment for many years is normal. So far, the insulin they require can only be administered parenterally, despite many attempts in the past 40 years to circumvent the injections needed and the physical risks and psychological trauma involved[1].

It was of interest, therefore, when Ferguson[2] reported that the oral administration of a mixture of insulin and an anhydroformaldehyde–aniline (AFA) compound caused hypoglycaemia in rabbits, but the AFA compound alone was without effect. The implication that this compound allows insulin to be absorbed in a physiologically active form is of such importance that the work was repeated in our laboratories.

The AFA compound is formed by the reaction between aniline and formaldehyde, the primary product being the Schiff's base. This immediately polymerizes and under suitable conditions yields 1,3,5,-triphenylhexahydro-*s*-triazine[3], which Ferguson refers to as "anhydroformaldehyde–aniline" and gives the formula as



This can be isolated as a white crystalline material with a melting point of 141° C (ligroin).

In the present experiments, male rabbits of the New Zealand White strain (3–3·5 kg) were used, either fed or fasted for 18 h, as shown in Tables 1–3. All drugs were administered by stomach tube. Blood was taken from the marginal ear vein for measurements of the sugar-levels ('Autoanalyser' method). Samples were taken in duplicate before and at intervals after the AFA-insulin mixture or control solutions. Three separate experiments were performed.

AFA was prepared as described by Ferguson from aniline hydrochloride and commercial formalin, dried, powdered and mixed with commercial insulin ('Iletin' regular, 80 U. per ml.). This mixture was administered by stomach tube to both fed and fasted rabbits. Control solutions of

insulin, AFA alone and water alone were also given. No signs of illness were observed in any animal. Results are shown in Table 1. No hypoglycaemic effect of the mixture could be demonstrated.

Table 1. Effect of Oral AFA–Insulin Mixture and Control Solution on Blood Sugar of Fed and Fasted Rabbits

Drug	No. of animals		Blood sugar mg per 100 ml. Hours after administration 0	1	2	3	4	5
AFA 200 mg	4	fed	97	107	109	102		91
	4	fasted	81	77	81		76	81
Insulin 400 U.	4	fed	97	102	102	98		94
	4	fasted	89	87	85		82	90
Water 10 ml.	4	fed	99	99	97	95		92
	4	fasted	74	75	79		76	90
AFA 200 mg plus	4	fed	91	101	95	91		88
100 U. insulin	4	fasted	80	80	80		74	85
AFA 200 mg plus	4	fed	90	91	87	84		85
200 U. insulin	4	fasted	72	76	79		76	86
AFA 200 mg plus	4	fed	88	94	91	87		81
400 U insulin	4	fasted	78	81	81		80	86

In the second experiment, aniline was made to react with formalin in 65 per cent ethanol and the commercial insulin added after the formalin, as described by Ferguson. This suspension was administered to the rabbits without further processing. This method of preparation had been shown to be as effective as the first. The results in fasted rabbits are shown in Table 2. Thirty per cent ethanol was used as control, as this is required in the preparation to keep the reactants in solution. No signs of illness were observed in any animal. No hypoglycaemic effect of this preparation could be demonstrated.

Table 2. Effect of Oral AFA–Insulin Mixture (Method 2) and Controls on Blood Sugar of Fasted Rabbits

Drug	No. of animals	Blood sugar mg per 100 ml. Hours after administration 0	1	2	3	4	6
AFA plus 200 U. insulin	6	80	80	79	81	82	81
AFA alone	4	80	81	81	81	83	86
30 per cent ethanol 7 ml.	4	74	73	72	73	75	76

In the third experiment a pure sample of the anhydroformaldehyde–aniline compound was prepared according to the method of Miller and Wagner[4]. Measurements of its melting point and microanalysis give results which are similar to those with the compound prepared by the first method of Ferguson. Results are shown in Table 3 of the effect of this compound on the blood sugar-levels of fasted rabbits.

Table 3. Effect of Oral AFA–Insulin Mixture (Method 3) and Controls on Blood Sugar of Fasted Rabbits

Drug	No of animals	Blood sugar mg per 100 ml. Hours after administration 0	1	2	3	4	6
40 mg AFA plus 800 U. insulin	4	68	73	67	71	69	72
200 mg AFA plus 400 U. insulin	4	79	80	80	82	81	83
200 mg AFA alone	4	75	77	78	78	74	80
2 g AFA alone	4	72	71	70	71	70	72

These results clearly show that under our experimental conditions, the anhydroformaldehyde–aniline compound as described by Ferguson does not allow insulin to be absorbed in a physiologically active form. There are two aspects of the work of Ferguson, however, which require amplification.

First, he states that fasting rabbits, before administration of the AFA–insulin complex, have blood sugar-levels of 120–140 mg per 100 ml. This is far in excess of any levels we have seen in starved rabbits. Secondly, in the second method for the preparation of AFA–insulin, ethanol was administered with insulin. It has already been shown that 20 per cent ethanol facilitates the absorption of insulin[5,6]. In a small pilot experiment in four fasted rabbits, we were able to show that ethanol in the concentration used in our second experiment (30 per cent), when given with insulin by stomach tube, causes a small but significant fall in blood sugar-levels after 1 h ($P < 0{\cdot}02$). This is not seen with insulin or ethanol alone. However, this effect of ethanol could not explain the reported hypoglycaemia produced by the first preparation of the AFA compound.

Our results do not support the claim that anhydroformaldehyde–aniline compounds facilitate the absorption of insulin in a physiologically active form.

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PHYSIOLOGY

Velocity and Force of Some Karate Arm-movements

A special aspect of Karate (a Japanese technique of self-defence and, in competitions, a game with very strictly regulated rules in which the partners are not allowed to strike each other) can sometimes be seen as a demonstration: the Karateka breaking bricks, tiles or blocks of wood with a strike of his bare hands, feet, fingers or forehead[1], the object being supported only at its ends.

The principles of the dynamics of a suddenly applied force on a supported beam are applicable here. When a falling body strikes a beam supported at its ends with a certain velocity the beam will break if the force per unit area due to the collision is greater than the maximum stress-resistance of the material of the beam. Thus one might expect that the man who breaks a brick or other solid object with a stroke of his bare hands or feet will: (1) Develop a great velocity of movement of the mass of his arm or leg at the moment of the impact. (2) Keep a very hard and relatively small contact area between hands or feet and the object so as to concentrate the force of the impulse on a small surface. (3) Have a great determination and courage to strike powerfully at a hard object with his bare hands or feet. These three 'actions' may be singled out or work together.

We performed some studies on the velocity of some Karate movements (striking) and on the reaction force when breaking objects with the bare hand. The velocity of movement was calculated from photographs taken with the aid of a calibrated stroboscopic lamp. The movements, the velocity of which was measured, were principally accomplished in a plane parallel to the photographic plate. The force was measured with a strain-gauge device on which the objects and their supports were placed. The objects were supported at their short ends. The subjects were well-trained Karatekas (subject No. 2, for example, is one of Japan's best Karate men). They performed their movements in the usual way, without being impeded by the measuring devices. Special attention should be given to the way the Karateka holds his hand when he is going to break a brick or a plate. Breaking a brick is done with a hyper-dorsiflexed and pronated hand, the fingers almost stretched. During the impact the place of contact is the area over the pisiform bone of

the wrist-joint. A plate is struck either with the tops of the forefinger, the middle finger and the ring finger of the semi-stretched hand and the fingers held tightly together—the tops of the fingers almost forming one plane—or with the fist; in this case the knuckles of the forefinger and the middle finger are the places of contact. In these experiments the fist was used and one plate was broken. However, usually more plates are broken at one time when striking with the fist. In order to offer some idea of the velocity of movement and the breaking force of the Karateka in comparison with subjects not experienced in Karate we shall give some data of two control subjects (both moderately trained) who were asked to make the same movements.

Table 1 presents the maximal linear velocity of the fingers during a stroke through the air as well as the force with which bricks and plates were hit and/or broken with the hand and the fist. It must be noted that the data of the velocity do not apply to the experiments in which the bricks or plates were broken.

From these data it can be said that: (1) The velocity of the arm movement of the Karatekas is about 25 per cent higher than that of the control subjects. (2) The force of the Karatekas and control subjects with which the bricks were hit did not differ much. In some cases the Karatekas could not break the brick even with greater forces probably because of the inhomogeneity of the material. The data give a good idea of the magnitude of the force that can be exerted with a stroke of the bare hand on a hard object. (3) The forces necessary to break one plate are not large. Also in this case one of the control subjects broke one plate (we did not measure the force which he exerted).

As to the hard contact surface, the following is tentatively suggested: usually the knuckles of the forefinger and the middle finger are covered with a large amount of callosity, which is, in our opinion, less hard than the knuckles covered only by the skin. This finding seems to be in conflict with the principles of the dynamics of an impulse which state that with a definite change in momentum the force is greater the shorter the time of the impulse. On the other hand, the powerful strokes will probably damage the hands when these are not covered with compressible material (tissues). Therefore this callosity can be considered to offer protection against knuckle damage (the Karatekas mostly have special training to develop this callosity). This idea is supported by the fact that the skin over the pisiform bone was not very callous in any of the Karate subjects, nor were the tops of the fingers, although these parts of the hand were also used during the breaking of objects at other events. Probably at those places the skin with the underlying tissues is sufficiently thick to protect the underlying bone. It is also supported by the fact that the Karatekas often put a rather thick cloth on the pile of the objects, especially when there are a number of objects to be broken and they must strike very hard. It is probable that the factor of courage is of great importance because it can be seen that the two control subjects who were unfamiliar with Karate dared to strike a brick with about the same force as the Karate specialists. Furthermore, as already reported, control subject No. 1 broke one plate of the same size with his fist.

Table 1. MAXIMUM VELOCITY AND FORCES OF SOME KARATE MOVEMENTS (STRIKING)

Maximum velocity of the fingers during a stroke through the air

Karate subject	Velocity (km per h)
1	51
2	49
3	46
Control subject	
1	40
2	39

Forces during breaking of objects with a stroke of the bare hand

Karate subject	Object	Force (kg)		Object	Force (kg)	
1	One brick*	58	(broken)	One plate†	29	(broken)
1	,, ,,	89	(not broken)			
2				,, ,,	13	(broken)
3	,, ,,	47	(broken)			
3	,, ,,	58	(not broken)			
Control subject						
1	,, ,,‡	60	(broken)			
2	,, ,,	60	(broken)			

* Size of the brick: 200 × 105 × 55 mm. Material, red baked clay.
† Size of the plate: 270 × 240 × 20 mm. Material, deal.
‡ Sandstone brick (softer than red baked clay).

Finally, we performed some additional measurements, using laboratory apparatus, of isometric muscle forces during arm bending and knee stretching, as well as of maximal velocity of arm bending. The results for the Karatekas were not significantly different from those of the controls.

In conclusion, we believe that Karatekas develop a greater speed of movement than is normal and effect a hard and relatively small contact area between their hand or foot and the object they are striking. However, it is probable that good technique and a large amount of courage to strike powerfully a hard object are of equal or even greater importance.

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Deposition of Inhaled Particles in Human Lungs

DURING the past 25 years, six independent groups of workers have measured the decrease in concentration of particles in exhaled air during steady breathing, while their subjects inhaled various aerosols, and have expressed their findings as a relationship between particle size and lung deposition. These results fall into two ranges, three of the groups of workers obtaining much higher values for deposition than the other three for particles between 0·5 and 6μ diameter. The difference cannot be explained by the nature of the particles or the method of breathing[1].

The techniques used in such work have recently been investigated, and it has been decided that there may be a substantial overestimate of lung deposition in the work of Van Wijk and Patterson[2] and of Dennis and Sawyer[3].

In these experiments, the sampling of the exhaled air proceeded continuously, with a thermal precipitator by the former and a conifuge by the latter, even while the subject was inhaling. Since the first part of the exhaled air comes from the dead space of the tidal airways, it will have deposited fewer particles in the lungs than the last part of the exhaled air, which has approached the lung alveoli. The concentration of particles towards the end of an exhalation is therefore much less than at the beginning and may even be zero. If sampling is continued during inhalation it is clear that about half the volume sampled may contain particles in a concentration below the average, so that an overestimate of lung deposition will arise. Among other factors, the extent of this error depends on the thoroughness of mixing of the exhaled air before reaching the sampling point; if complete mixing of the entire volume of each exhalate were possible, before the sample was taken, no error would result.

It is difficult to assess how much mixing took place in the box into which the exhalate of the subjects of Van Wijk and Patterson was passed, but a baffle was used to direct what was probably a laminar stream across the orifice of the thermal precipitator, which would therefore tend to receive sequential concentrations.

Dennis and Sawyer fed the exhalate through a glass bulb, which was in many cases smaller than the tidal volume, and sucked a sample through a conifuge from the

Table 1. ERROR DUE TO SAMPLING DURING THE PERIOD OF INHALATION

Tidal air volume (cm³)	Apparent average concentration in exhaled air divided by true value: Particle diameter (microns) 0·25	1·4	3·8	6·3
450	0·89	0·82	0·72	0·69
900	0·82	0·68	0·55	0·50
1,350	0·79	0·64	0·54	0·50

base of the bulb; it is therefore likely that the air sampled at the end of each breath and during the succeeding period of inhalation was substantially deficient in particles.

Estimates of the error which can arise in this way have been calculated from the experimental data of Landahl, Tracewell and Lassen[4] for human inhalation of aerosols of triphenyl phosphate. They give the percentage concentrations exhaled in four successive quarters of a breath; from these figures the ratio of the apparent average exhaled concentration to the correct value has been derived and is given in Table 1. The apparent value results when the average is taken over the entire breathing cycle with the ultimate, deficient part of the exhalate being sampled during inhalation.

The maximum error is 0·5 when the last part of the exhalate is free from particles.

This error probably accounts for the high values for lung deposition obtained by Dennis and Sawyer and by Van Wijk and Patterson. It cannot be the cause of an overestimate of total deposition by Brown, Cooke, Ney and Hatch[5], because they sampled fractions of exhaled breath with electrostatic precipitators which were ventilated only during exhalation.

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Development of Neuronal Activity in Neocortex of the Kitten

MAMMALIAN cerebral cortex undergoes rapid structural changes during the postnatal period. Dendritic connexions increase in number and complexity, the volume of neurone somata increases, and myelination occurs in subcortical and cortical fibres[1]. Little is known about the functioning state of cerebral neurones during this period of rapid development. In kittens, spontaneous and evoked activity has been recorded from the cortical surface during the 'new-born' period, but such records have been difficult to interpret[2,3]. For example, it is not possible to be certain whether the activity recorded from surface originates in cortex itself, or is transmitted from subcortical structures. In the present investigations, recordings were obtained from single cortical neurones and from subcortical fibres in unanaesthetized, unrestrained kittens. The results indicate that large parts of neocortex in the kitten are electrically silent at birth, but that neuronal activity rapidly develops in the postnatal period. The early patterns of neuronal discharge show striking differences from those seen in adult animals.

The technique used was similar to that described by Hubel and by Evarts for recording from unanaesthetized, unrestrained adult cats[4,5]. A metal cylinder, to which a hydraulic microdrive could be attached, was implanted on the skull under general anaesthesia. Attachment of the cylinder to skull was secured by use of dental cement. The animals were allowed to recover from the anaesthesia before recordings were obtained. The microelectrodes used were glass-insulated platinum–iridium wires[6]. A stroboscopic light was used to evoke flash responses in visual cortex and in optic radiations. The electrocorticogram was recorded from stainless steel wires placed on the dura. Recordings were obtained from lateral and suprasylvian gyrus, and from geniculate radiation fibres. Twelve kittens, aged from 1 day to 2 weeks, were used in the investigations.

Activity was already occurring in subcortical fibre tracts at birth. In optic radiations, responses to flash could be recorded in neonatal animals, indicating that retina, optic nerves, and lateral geniculate neurones were capable of some degree of function at this age. The flashes were presented through closed eyelids. The latency from flash to unit discharge was considerably longer than in the adult, varying between 120 and 250 msec. In addition to evoked discharges, subcortical fibres in neonatal animals showed occasional spontaneous activity. This consisted of brief bursts, with intervening periods of silence which lasted for many seconds. Neighbouring fibres discharged synchronously during a burst of activity. Bursts were often associated with muscular twitches, and such twitches, which involved muscles of extremities, neck, face and ears, were very prominent when the kittens were asleep.

In contrast to the findings in subcortical fibres, cortical areas from which recordings were obtained showed no evidence of either spontaneous or sensory evoked neuronal activity during the first three days after birth. Occasional positive spikes of rather long duration (up to 20 msec) were observed as the electrode penetrated cortex. These persisted for only a few seconds, and showed a rapid decrease in amplitude. Such spikes probably represented injury discharges due to mechanical distortion of otherwise silent cortical neurones by the recording electrode. They differed from injury discharges in adult cortex in that rapid repetitive firing did not occur.

Both evoked and spontaneous activity appeared in cortical neurones at about the same time—between ages of 4 days and 2 weeks. Active cells were quite rare in 4–7-day-old kittens, and at times several penetrations of a whole thickness of cortex had to be made before an active neurone was encountered. Flash responses had a latency of 100 msec or longer. Inhibition of spontaneous firing by flash, which is a frequent finding in adult cortex[5], has not been observed in kittens less than 2 weeks old. However, the failure to see inhibition may be due to the very low rates of spontaneous discharge in immature neurones, rather than due to the absence of inhibitory connexions. Mean rates of spontaneous activity during the first two postnatal weeks ranged from only 1 spike/min to 1 spike/sec, as compared with a mean discharge rate of 7–8 spikes/sec in the same cortical areas in adult cats[7]. The pattern of spontaneous activity also showed differences from adult cortex. Cortical neurones in animals less than 2 weeks old characteristically discharged in brief bursts of 2–10 spikes, followed by prolonged periods of inactivity, which at times lasted for several minutes. More prolonged bursts, or regular, sustained spike discharges, were not seen in immature cortex. Unit activity in immature cortex often was synchronous with muscular twitches, similar to the findings in subcortical fibres.

Most of the recordings in this investigation were obtained during sleep; the reason for this is that records in waking kittens tend to be obscured by movement artefact. A few units were investigated after arousal of the animals, but these showed no obvious changes in discharge patterns from those found in sleep. In kittens less than 10 days old it was at times difficult to be certain whether an animal was asleep or awake, since the electroencephalogram patterns associated with sleep and waking in adults were as yet absent.

The present observations indicate that cerebral cortex in the areas investigated is not in a functioning state at the time of birth. Impulses in afferent fibre tracts are reaching cortex at this stage of development, but cortical neurones are not activated by the afferent volleys. The earliest activity in cortical neurones shows little of the

complexity seen in adult cerebral cortex. It consists of discharges in brief bursts which appear to occur synchronously in large neuronal populations, and which correlate with muscular twitches in the animals.

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Polarized Light Responses from Crab Retinula Cells

LIKE many other arthropods, crabs can orientate themselves to plane polarized light[1], and it has been shown that single retinula cells of the fly can respond differentially to the plane of polarization[2–4].

Evidence from vertebrate rods indicates that the photopigment chromophore is strongly dichroic and highly orientated only in the plane parallel to the rod disk membrane[5]. It is an attractive possibility that the pigment chromophores in the arthropod rhabdom are arranged both parallel to the membrane and in line with the axis of the regularly arranged tubules which make up the rhabdom structure. This would provide a polarization analyser, in contrast to the vertebrate arrangement.

The decapod Crustacea provide an opportunity for investigating this conjecture in that they have only two alignments of rhabdom tubules at right angles to one another, in a regular array across the eye[6–8].

Freshly excised eyes of *Carcinus maenas* were mounted in sea water in a small chamber, looking out through a glass coverslip. An optical perimeter allowed a small bright source (0·3° at the eye) to be located on the visual axis of a cell. Care was taken to see that the emerging light was at this stage non-polarized. A 'Polaroid' sheet that could be rotated through 360° in 15° steps, and an interchangeable set of neutral density filters, were interposed in the beam.

The procedure was to locate a retinula cell through a hole in the cornea using conventional intracellular microelectrode techniques, find its optical axis, and rotate the 'Polaroid' through successive positions in between repetitive 200 msec light flashes. This produces a differential response with a minimum sensitivity at 'Polaroid' positions 2 and 14 in the example shown (Fig. 1*a*). Adaptation during a series of flashes is usually negligible as can be judged by the symmetry of the responses. Measurements are taken from the 'Polaroid' positions at minimum sensitivity where adaptation is least. The angle of the 'Polaroid' for maximum stimulation relative to a particular row of ommatidial facets in the region of interest is noted so that measurements on different eyes can be related.

Relative to this co-ordinate, cells fall into two well-defined groups with maximal polarization-sensitivities 90° apart (Fig. 1*d*).

Should recording conditions be stable enough, it is possible to plot the intensity-response curve (Fig. 1*b*), and the response to constant flash at different angles to the optical axis (Fig. 1*c*). The equivalent angular sensitivity curve is then calculated[9], and in this case, when corrected for refraction and a scale error of the stimulator, the acceptance angle at half sensitivity in the vertical plane for the animal is 2·5°. This is representative for the few accurate measurements made.

Likewise, data in Fig. 1*b* can be used to measure the actual sensitivity to light polarized in different planes. Fig. 1*a* represents a 7·7-fold change in sensitivity between response extremes: it is equivalent to a change in light intensity from 100 per cent to 13 per cent, or 0·89 log unit. This would seem to be representative for the few cells accurately measured so far. It contrasts with a change of about 0·3 log unit in the fly[3] and 0·1–0·7 (mean 0·37) in the locust[10], where all the cells appear to respond to some extent. Other data from the fly[4] are more difficult to interpret, especially as only half the cells are claimed to be selective and yet no figures for the lower limit of detectability are given. A conservative estimate of 0·05 log unit would be appropriate in the present work, which would be difficult to distinguish from Fresnel artefacts. Cells differ considerably in their intensity-response curves so that it is necessary to determine each individually if accurate comparisons are to be made or response saturation avoided. The large differences in polarization sensitivity between cells and between animals presumably reflect a greater degree of alignment of rhabdom tubules or of pigment within them.

Neither the polarization plane for maximum response nor the actual sensitivity to polarized light changes measurably on altering the angle of incident light either in crab or locust, although this can necessarily be varied only over a small range because of narrowness of receptive field. For the locust, removal of the experimental chamber glass window does not affect the response, and simultaneous recordings from two electrodes in one ommatidium on one occasion showed that two different cells had peak responses at polarization planes 60° apart. In this case the rhabdom is the only structure in the optical path which is not shared by both cells. This evidence militates against the alternative that the polarization responses reported here are artefacts due to extrinsic Fresnel reflexion effects at some interface, producing intensity differences on the receptors. Such effects would in any event be much too small to explain the response magnitudes observed unless multiple reflexions were involved, and these are certainly not present here.

Taken with this evidence, the finding in the crab of two groups of cell with maximal polarization sensitivities at right angles to one another (Fig. 1*d*) reinforces the belief

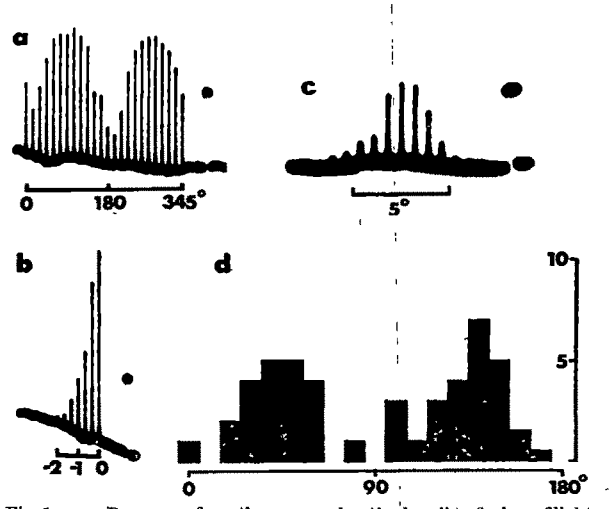


Fig. 1. *a–c*, Responses from the same crab retinula cell to flashes of light. Calibration pulses are 10mV and interflash intervals 2·5 sec throughout. (*a*) Response to flashes at different planes of polarization of incident light. A 'Polaroid' sheet is rotated 15° between flashes. (*b*) Approximate response-log intensity curve plotted directly from the response by removing neutral density filters from the beam From the right, successive log attenuations are 0, 0·3, 0·7, 1·0, 1·3, 1·7, 2·0 (*c*) Response to the light source attenuated by 0·7 log unit when moved across the receptive field in equal 0·7° steps (corrected, see text) (*d*) Plane of polarization relative to the eye (abscissa), against numbers of cells with each orientation of maximal sensitivity (ordinate) Forty-seven cells

that the photopigment orientated in the rhabdom tubules constitutes the polarization analyser. This view is strengthened by recent work on selective adaptation of *Octopus* electroretinogram with polarized light[11].

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Dissociation between Antidiuretic and Vasopressor Activity in Rat Posterior Pituitaries on Incubation *in vitro*

IN an investigation designed to investigate whether the release of vasopressin (ADH) from rat posterior pituitaries *in vitro* would be a reliable index of the discharge of this hormone *in vivo*, a dissociation between antidiuretic and vasopressor activity was found on incubation in the posterior lobe as well as in the medium.

Male white rats weighing from 170 to 270 g were anaesthetized with an intraperitoneal injection of 4·25 mg of sodium pentobarbitone per 100 g. After 30 min the animals were decapitated and the posterior pituitary was quickly removed and weighed to the nearest 0·05 mg on a torsion balance. The gland was either submerged in 0·1 ml. of 0·1 N hydrochloric acid for extraction of peptide material or it was placed in flasks containing 2 ml. of Krebs–Ringer-bicarbonate glucose (200 mg per cent) at 37° C and shaken for 1 h in a Dubnoff metabolic shaker under 95 per cent oxygen and 5 per cent carbon dioxide. At the end of the incubation the posterior pituitary was removed and extracted in the same way as the fresh posterior lobes. Glands were ground with a glass rod and were diluted with saline after complete destruction before assay. The material present in the incubation medium and in the posterior lobes was immediately assayed for its antidiuretic and its vasopressor activities.

Antidiuretic potency was determined in the female rat anaesthetized with alcohol[1] and the vasopressor potency was measured in the male rat pretreated with dibenzyline and anaesthetized with urethane[2]. A 4·2 dose assay was carried out using purified lysine vasopressin (Organon, *N.V.*, Oss, Holland) as the standard.

Table 1 summarizes the results. The amount of antidiuretic and vasopressor activity before incubation varies considerably from gland to gland. The two activities did not, however, differ significantly. The data are in agreement with those of others[3–5]. After 1 h incubation, the posterior lobe had lost nearly all its antidiuretic activity, while the vasopressor activity was reduced only slightly. The antidiuretic activity seemed to have been released into the incubation medium, as the amount present in the medium did not differ significantly from that which had disappeared from the posterior lobe during incubation. Accordingly, incubation of the posterior lobe gave rise to a dissociation between the two activities of the posterior lobe principle.

Table 1. ANTIDIURETIC AND VASOPRESSOR POTENCY OF POSTERIOR PITUITARY TISSUE AND OF INCUBATION MEDIUM

	Posterior pituitary extract		
	Before incubation	After 1 h incubation	Incubation medium
Antidiuretic activity	1·29* ± 0·49†	0·03 ± 0·01 $P < 0·01$	1·13 ± 0·19 $P < 0·01$
Vasopressor activity	0·53 ± 0·10	0·62 ± 0·28	0·04 ± 0·01

* U/mg fresh posterior pituitary.
† Mean of six assays ± standard error of the mean.

Since the isolation, structure-analysis and synthesis of vasopressin by Du Vigneaud *et al.*, it has been taken for granted that this peptide (with a vasopressor/antidiuretic activity ratio of unity) is the principle formed and stored in the supraoptic-neurohypophysial system. Recent data from Sachs and Takabatake[6] suggest, however, that vasopressin may be formed in the form of a precursor which is transformed into the active principle on transportation to the posterior lobe.

The present experiments reveal that at least two principles are present in the posterior lobe on incubation, one with mainly vasopressor and one with mainly antidiuretic activity. It may be that changes have taken place under the present artificial conditions with the consequent formation of two factors with different diffusion characteristics. It is also possible that the precursor molecule is transformed into peptides of different biological activities. This may occur not only *in vitro* but also *in vivo*, since Hild and Zetler[7] showed that the antidiuretic activity of the hypothalamus was lost before the vasopressor activity when dogs were dehydrated for several days.

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IMMUNOLOGY

Suppression of Homograft Immunity in Chickens grafted *in ovo* with Allogeneic Thymus

NO convincing evidence exists which demonstrates that mammalian thymus cells can directly express their immunological activity. It has been assumed, therefore, that the immunological role of the thymus is an indirect one, that is, that it supplies the 'executive' part of the immune machinery of the body with cells the replication of which may well be required to establish normal immune function. Nevertheless, the relationship of thymus cells to the plasma cells as well as to the population of small lymphocytes (which has been considered to be linked with several immune manifestations including tolerance[1,2]) is still obscure. A similar concept could be applied to the immunological function of the thymus in birds. However, some observations made in chickens have suggested that cells derived from the thymus might be properly qualified to induce directly an immune response or at least to participate in it[3–7]. This report provides some evidence to show that the thymus is capable of inducing a state of tolerance to skin homograft in the chicken.

Non-inbred Rhode Island Red embryos and chickens of unknown blood group genotype and parentage were used in this investigation. Thymic lobes were aseptically removed from 8-week-old donor animals and cut into pieces varying in size from 2 × 3 mm to 2 × 4 mm. One piece of thymus was placed on the ectodermal layer of the chorioallantoic membrane of 12-day-old embryos. The method of implantation was essentially that developed by Ballantyne[8]. Grafted embryos were divided into four groups. One group was killed on the fifteenth, another

on the eighteenth, and the third group on the twenty-first day of incubation. Thymus grafts, chorioallantoic membrane and spleen were taken for histological examination. The fourth group of embryos receiving grafts was allowed to hatch, and 3-week-old chickens from this group were used as recipients of skin homografts. For this purpose, the same chicken which served previously as the thymus donor was now used as the skin donor. Skin grafts 1·5 mm × 1·5 mm were taken from the thorax of the donor and transplanted to the corresponding place on the chest of recipient chickens. Six days after transplantation the adhesive dressing was removed and daily observations made of the skin grafts. The intensity of the reaction was graded grossly. No attempts were made to find haemagglutinins for donor red blood cells in the sera of chickens grafted *in ovo* with thymus.

Three days after transplantation on chorioallantoic membrane, histological examination of thymus grafts revealed various degrees of vascularization. There were no signs of necrosis, and the cellular architecture of the implants was well preserved; however, grafts taken from 18- and 21-day-old embryos showed cell depletion of varying intensity.

In 15-day-old grafted embryos the spleen sinuses were filled with mononuclear cells of different types, whereas such cells were rare in the sinuses of non-grafted embryos (Table 1). Occasionally, some of these cells exhibited the earliest characteristics assigned to the plasmacellular elements. Six days after grafting, necrotic foci surrounded by multi-nuclear giant cells appeared in the spleen. No necrotic lesions were observed in the control embryos. In a number of spleen sections, a well-defined empty reticular structure was seen 9 days after transplantation. At that time of incubation (21 days) the spleen of embryos receiving grafts was larger than in the intact controls (Table 2).

Table 1. HISTOLOGICAL FINDINGS IN SPLEEN AND CHORIOALLANTOIC MEMBRANE OF 15–21-DAY-OLD EMBRYOS GRAFTED WITH THYMUS

Group	Spleen			Chorioallantoic membrane	
	Mononuclear cells in sinuses	Empty reticulum	Necrotic lesions	Cellular infiltration	Necrotic lesions
Control	±	0	0	0	0
Grafted	+++	++	+++	+++	++

Table 2. SPLEEN WEIGHT OF EMBRYOS GRAFTED WITH ALLOGENEIC THYMUS

Group	Spleen weight (mg)*		
	15-day-old	18-day-old	21-day-old
Control	11·2	10·2	13·2
Grafted	10·6	10·9	31·0

* Mean represented by five embryos.

Table 3. SKIN HOMOGRAFT REJECTION IN 3-WEEK-OLD CHICKENS GRAFTED *in ovo* WITH ALLOGENEIC THYMUS

Group	Gross rejection at					
	6 days		9 days		18 days	
	Not rejected	Rejected	Not rejected	Rejected	Not rejected	Rejected
Control	0	14				
Grafted	13	0	10	3	4	9

Extensive cell infiltration of the chorioallantoic membrane was observed in embryos receiving grafts (Table 1). Cell aggregates consisted mainly of mononuclear cells, medium and large lymphocytes, a few small lymphocytes, reticulocytes, blasts and some unidentified cells. This cell population showed a marked deficiency in ribonucleic acid. Cellular formations resembling the Hassall's corpuscles in the thymus were noted in the chorioallantoic membrane. Six and nine days after transplantation necrotic lesions occurred in the chorioallantoic tissue.

The results are presented in Table 3 of skin homograft rejection in 3-week-old chickens. All chickens which were not grafted at the embryo stage with thymus material rejected skin homografts in a manner indistinguishable from the rejection observed in an additional group of control chickens hatched from sham-operated eggs. The rejection was complete 6 days after grafting. At that time, the skin grafts in chickens which had been implanted *in ovo* with thymus material were in excellent condition. Four of thirteen chickens of this group showed no signs of homograft rejection even at 18 days.

Our observations indicate that implantation of allogeneic thymus on the chorioallantoic membrane of 12-day-old embryos induces a graft-versus-host reaction, thus providing further evidence for the immunological competence of chicken thymus cells. Splenomegaly is presumably produced by proliferating host cells as well as by colonization of the spleen with cells coming from the implant[9]. The same could be said for aggregates of cells in chorioallantoic membrane[10,11].

Another important feature of this investigation is the induction of partial tolerance to skin homograft following *in ovo* transplantation of thymus from adult chicken. This state of tolerance is probably due to the lymphocytes of a number of embryos being immunologically immature at the time of grafting[12]—assuming that skin and thymus cells share common isoantigens. It can also be argued that cells deriving from the thymus graft are 'accepted' by recipient animals, and that such cells continue to multiply and differentiate in lymphoid tissues of the host resulting in partial suppression of homograft immunity. Both explanations imply the persistence of antigen in the host[13]—although the second assumption assigns a more functional role to donor thymus cells in the initiation and maintenance of tolerance. The latter seems acceptable when the part played by thymus-dependent lymphoid tissue in delayed hypersensitive responses of the chicken is taken into account[14–17].

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Anti-ribonucleic Acid Factors in the Sera of Germ-free Animals

WHEN an animal is subjected to prolonged immunization with ribosomes (of whatever origin), its serum becomes progressively endowed with NG I antibodies*, which specifically precipitate various types of RNA and all the synthetic polyribonucleotides so far tested[1,2,4]. On the other hand, these sera and the sera of animals not immunized against ribosomes both contain NG II globulins[3]: these factors precipitate poly-I and poly-U only; however, the reaction is specifically inhibited by various RNAs (and by either poly-A or poly-C) but not by DNA. We have reason to believe that NG II factors in equine serum are antibodies which belong to the 7*S* globulin

* NG, anti-nucleic acid globulins.

fraction recently described by Sandor *et al.*[5]. However, we have previously shown that the sera of animals not immunized against ribosomes contain these factors in amounts of from ten to twenty times less than those found in immunized animals[3].

We were faced with the question of whether the presence of NG II in the sera of animals not immunized by ribosomes represents a response to an exogenous stimulus, that is, to ribosomes of bacterial origin.

To answer this, we undertook a comparative investigation of the sera of germ-free and conventional animals

The germ-free animals were Wistar rats descended from rats received from the Lobund Institute in June 1960. The control rats were also from this source.

The polyribonucleotides, obtained from Miles Chemical Co., were re-purified and the fraction containing products of higher molecular weight used. The RNA was a ribosomal RNA prepared from *Escherichia coli K* 12. Precipitation reactions, either in serum or in 0·14 M sodium chloride, 0 005 M *tris*, at *p*H 7·4, and the analysis of the precipitates were carried out by methods already described[6].

In the first series of experiments, immunological reactions were carried out with the sera of germ-free and conventional animals. The results are shown in Table 1 (each mean value represents three to twelve animals).

(*a*) Sera from either conventional or germ-free rats precipitated the poly-I. The age of the rat made no difference to the percentage of poly-I precipitated.

(*b*) For the same dose of poly-I, the quantities of conventional serum proteins present in the precipitate increased between the first and third months, but did not vary noticeably thereafter.

(*c*) For sera of both germ-free and conventional animals, the quantity of protein present in the precipitates increased with the quantity of poly-I added.

(*d*) This reaction has the same specificity character as has been observed in rabbit, goat, horse and human sera[3,7], as the precipitation is inhibited by RNA and by both poly-A and poly-C, but not by DNA or poly-U.

It is possible to utilize, instead of the sera, the euglobulin fractions obtained during dialysis against a medium of low ionic strength at *p*H 6·3 (refs. 4 and 7). These fractions contained all the NG II factors. The quantity of euglobulins present in conventional sera was 10–90 per cent higher, depending on age, than that present in germ-free sera. For conventional rats, the concentration increased continuously with age; in the case of germ-free sera, the concentration remained unchanged after the third month (Table 2).

Furthermore, the results listed in Table 3 (mean values obtained for three to fourteen animals) indicate that the percentage of poly-I precipitated increased regularly with the decrease in the quantity of poly-I added. The percentage of poly-I precipitated reached about 15 per cent for a ratio "euglobulin added/poly-I added" greater than 40, whereas the ratio "protein precipitated/poly-I precipitated" stayed about unity.

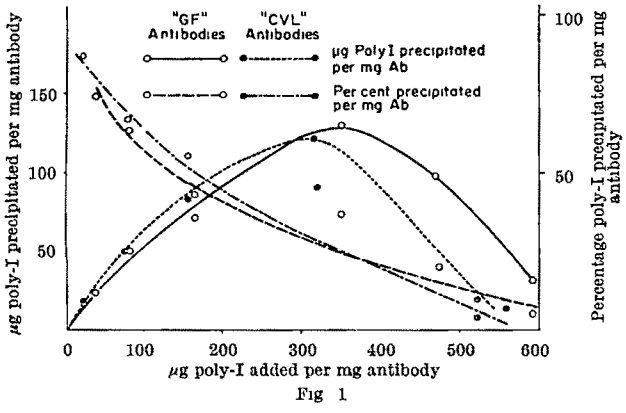


Fig 1

These results show that an important proportion of the euglobulins did not represent NG II factors. It was possible to purify and to concentrate NG II by a previously described method[3,4]: dissociation of the specific precipitate obtained from the euglobulins by 1 M magnesium chloride or 2 M lithium chloride. The proteins went into solution and behaved as NG II factors, similar to those obtained from the sera of animals of other species.

In one experiment, we prepared NG II from two euglobulin pools obtained from conventional (thirty-two rats) or germ-free (twenty-eight rats) sera. In the case of conventional rats, 15 ml. of serum produced 21 mg of euglobulins and this in turn gave 2·2 mg of NG II. Thus about 140 μg NG II was produced per ml. of serum. In the case of germ-free rats, 12 ml. of serum yielded 9 mg of euglobulin, which in turn gave 0·8 mg of NG II, that is, 66 μg/ml. of serum.

The conventional or germ-free factors showed an identical activity (Fig. 1). Moreover, the precipitation curves exhibited the usual aspects of immunological precipitation, and characteristic zones of equivalence and of inhibition in excess of antigen were present.

Finally, poly-I precipitation, in both conventional and germ-free NG II, was inhibited by poly-A, poly-C and RNA, but not by poly-U or DNA.

A second purification by dissociation of the new precipitates yielded about 50 per cent values. After a third purification, this quantity remained unchanged.

The results presented here suggest a physiological explanation of the synthesis of NG II factors. Accordingly, these factors result from a normal globulin synthesis which could be due to autologous immunization (for example, by the animal's own ribosomes liberated as a result of endogenous cytolysis). On the other hand, it is also possible that the properties of NG II reflect an intrinsic quality of certain normally synthesized globulins.

Table 1. POLY-I PRECIPITATION (MEAN VALUES) WITH RAT SERA

	Conventional rats				Germ-free rats			
Age of rats	μg Poly-I added/ml. serum	Protein precipitated (μg)	Poly-I precipitated (μg)	Poly-I precipitated (%)	μg Poly-I added/ml. serum	Protein precipitated (μg)	Poly-I precipitated (μg)	Poly-I precipitated (%)
1 mth.	205	108	22	10·7	205	127	20	9·7
	502	177	69·8	13 9	380	152	42	11
3–4 mths.	221	160	24	10 8	218	141	27	12·4
	552	325	58	10 5	550	212	59	10 7
8–10 mths.	218	157	22	10·1	216	118	16·7	7·7
	550	331	59·4	10·8	300	165	27 8	9 2
12 mths.					218	111	16	7 3
					552	252	44 5	8

Table 2. "EUGLOBULIN" CONCENTRATION IN CONVENTIONAL AND GERM-FREE RAT SERA

	mg Euglobulin/ml. conventional serum				mg Euglobulin/ml. germ-free serum			
		Protein values (mg/ml)				Protein values (mg/ml.)		
Age of rats	No. of rats	Maximum value	Minimum value	Mean value	No of rats	Maximum value	Minimum value	Mean value
1 mth.	3	1 8	1·2	1 5	4	1·6	0 98	1 3
3–4 mths.	9	6·9	1·08	2 58	13	2 96	1 4	2 3
6–8 mths.	22	4·2	1 54	2 9	15	3·8	1·4	2 4
9–12 mths.	4	9 1	2 5	4·8	6	2 8	1 4	2 54

Table 3. POLY-I PRECIPITATION (MEAN VALUES) BY EUGLOBULINS

	Conventional rat euglobulins				Germ-free rat euglobulins			
Age of rats	μg Poly-I added/mg euglobulin	Protein precipitated (μg)	Poly-I precipitated (μg)	Poly-I precipitated (%)	μg Poly-I added/mg euglobulin	Protein precipitated (μg)	Poly-I precipitated (μg)	Poly-I precipitated (%)
1 mth.	39	5 9	4 8	12	44	6 8	5 2	11·8
	240	13·8	14 1	5 9	396	18 7	21 5	5·4
	407		18 6	5 6	640	21	24·2	3·8
3–4 mths.	30	6 1	4 4	14 7	54	5 9	4·9	9
	146	9 2	11 5	7 8	230	8	8 2	3 5
	260	11	11·8	4·5	769	8	9	1 1
6–8 mths.	58	8·3	6 4	11	52	9·2	6·5	12 5
	210	19	21 3	9	210	19 3	24·8	11 7
	640	23	35·1	5 4	460	24 2	31 4	6·8
10–12 mths.	63		4 2	6 7	38		5 2	13 7
	195		8	4·1	205		16 4	8
	640		23	3 6	350		21	6

The precipitation of poly-I is, indeed, only an indication of the presence of factors which have the biologically important property of combining with RNA to give soluble complexes[3]. It is possible that, in certain cases, such a combination could maintain, or even reinforce, the functional value of the RNAs. Although auto-antibodies are usually referred to as negative (unfavourable) because of their pathological consequences, we suggest, on the basis of our results, that some auto-antibodies exist as positive (favourable) elements in the organism, which are synthesized by it and necessary to it.

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RADIOBIOLOGY

Photosynthesis and Uptake of Strontium-85 in Freshwater Plankton

AMONG the radioisotopes which are assimilated differentially in light and dark by phytoplankton or macrophytes are phosphorus-32[1], zinc-65[2], strontium-89 and 90[3], molybdenum-99[4] and caesium-137[5]. This communication considers this effect for strontium-85 in relation to primary productivity.

Rice's work with radioactive strontium[3] indicated assimilation by twelve species of marine algae (mostly planktonic) to be primarily by ion exchange, but at different rates for different species. Cells killed by acid or heat took up strontium by adsorption or exchange, and the process was complete within minutes. In a light–dark experiment living cells kept in the dark for 72 h did not concentrate strontium to significantly greater levels than killed cells, but illuminated cells accumulated strontium with increasing population size and in proportion to the rate of cell division. Most of this radioactivity was exchangeable.

Fig. 1 shows light and dark uptake of strontium-85 by organisms in water obtained from a depth of 2 ft. in Melton Hill Lake, Oak Ridge, Tennessee, on August 26, 1964, and incubated in a controlled environment

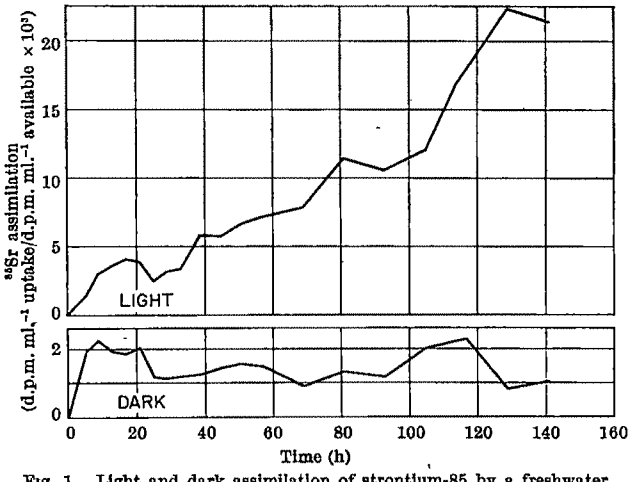


Fig. 1. Light and dark assimilation of strontium-85 by a freshwater plankton aggregation as a function of time

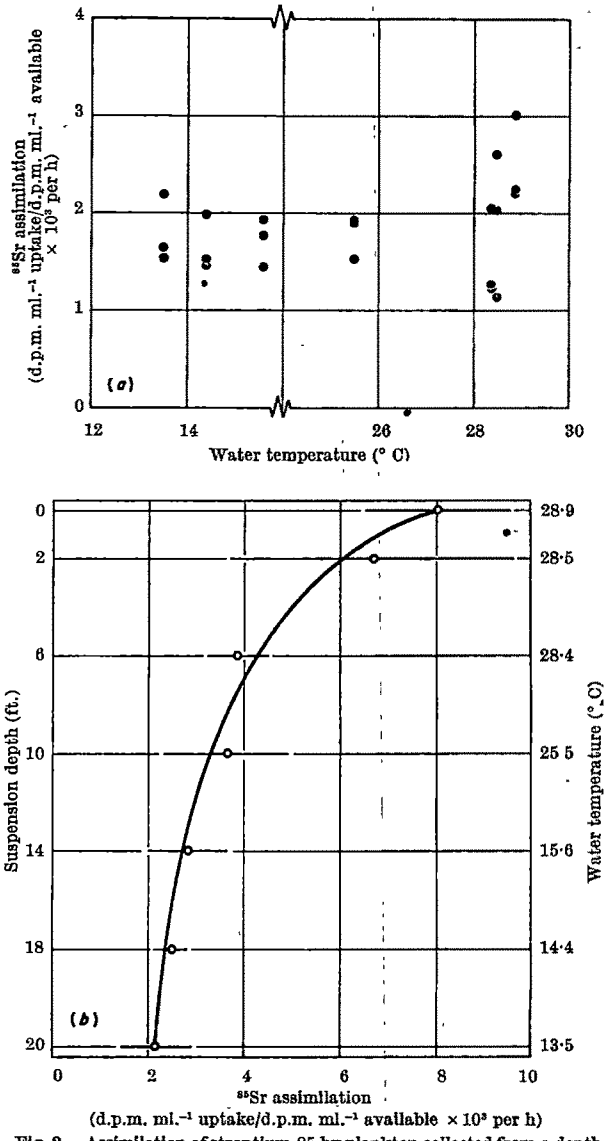


Fig. 2. Assimilation of strontium-85 by plankton collected from a depth of 8 ft. in Melton Hill Lake on July 29, 1964, and suspended *in situ* for 6 h at various depths. Initial phytoplankton concentration was 4·1 × 10^3 cells/ml. Principal genera were *Synedra* and *Navicula*; minor species were from *Achnanthes*, *Chlamydomonas*, *Oocystis*, *Scenedesmus*, *Peridinium* and *Euglena*. (*a*) Assimilation in dark as a function of temperature (three replications at each suspension depth). (*b*) Assimilation in light as a function of suspension depth (means of three replications, with two standard errors on either side of each mean)

chamber (temperature about 25° C, light about 1,000 ft.-candles, photoperiod 12 h). Initial phytoplankton concentration was 36·6 × 10^3 cells/ml., and principal forms were *Achnanthes*, *Navicula*, *Synedra* and an unidentified green alga with lesser concentrations of *Chlamydomonas*, *Chlorella*, *Oocystis*, *Peridinium*, *Scenedesmus* and *Synura*. Initial uptake of strontium-85 proceeded rapidly in both light and dark, but assimilation in light became greater after 6 h. Both light and dark samples showed a decrease in cell radioactivity for about 24 h, after which the dark material remained approximately constant while the illuminated samples showed a linear increase with time.

The results in Fig. 1 are typical of our experience with natural populations from Melton Hill Lake. Not all species in a mixed population may be responsible, however, as is shown by an experiment with cultured *Spongiochloris excentrica* Starr, which gave no differential assimilation in light and dark during 48 h.

To relate the results of Fig. 1 to light and temperature levels, a series of 6-h field experiments were performed *in situ* in Melton Hill Lake. Aliquots of a composite sample

of natural material from the lake were suspended in dark and light '*BOD*' bottles at different levels in the water column. The lake was thermally stratified during the summer, when this work was performed, providing a vertical gradient of ambient temperatures. Assimilation under dark conditions in a typical experiment is given as a function of temperature in Fig. 2*a*. The data indicate no change in strontium-85 uptake over a temperature range of 13·5°–28·5° C; at 28·9° C, however, uptake was significantly greater. This was the surface water temperature and dark bottles suspended at this level were exposed to fairly direct sunlight which may have caused internal heating above ambient temperature. From Fig. 2*a* and similar data from other experiments it is concluded that strontium-85 assimilation by natural plankton material is independent of temperature except possibly at abnormally high temperatures. Thus, metabolism does not appear to play a significant part in the uptake of this radioisotope. On the other hand, assimilation in light is dependent on light intensity. This is shown in Fig. 2*b*, where the vertical profile of strontium-85 uptake shows an exponential decrease with depth which must be related to the pattern of light extinction since temperature is not a factor.

Data such as those of Fig. 2*b* raise the possibility that strontium-85 uptake may be related to primary productivity. If it were possible to quantify such a relationship over a wide range of organisms and environments, this radionuclide could prove useful in the estimation of aquatic production because assay of its gamma-radioactivity is easy compared with carbon-14. Preliminary comparisons of Melton Hill Lake productivity as determined by the oxygen method[6] and by strontium-85 assimilation gave results typified by Fig. 3, which shows the same experiment as Fig. 2. As shown, a linear relation exists between net oxygen production and strontium-85 uptake at low and intermediate light intensities (suspension depths of 6 ft. and below), but at high light levels (0 and 2 ft. suspensions) oxygen production was inhibited while strontium-85 accumulation continued to increase. Photosynthetic inhibition under high illumination is commonly observed when production is determined by oxygen and carbon methods. The continued increase of strontium-85 assimilation with increased light (Fig. 3) has two possible explanations: (i) accumulation of the radioisotope is related to photosynthesis and photoinhibition of the latter is therefore an artefact of the usual methods of measuring productivity, or (ii) uptake is dependent on light but the mechanism is unrelated to photosynthesis.

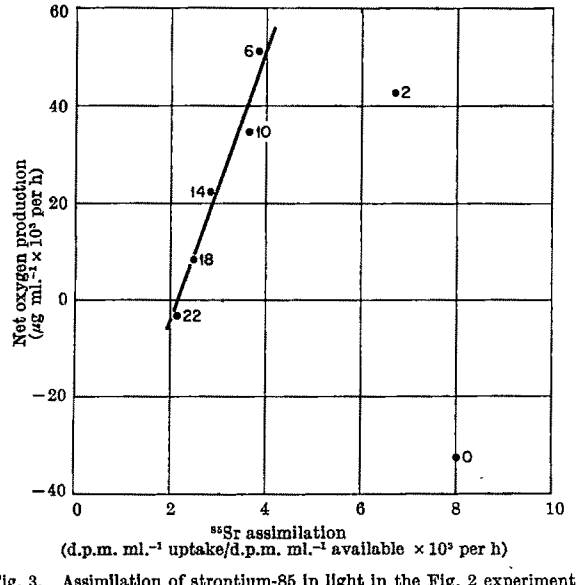


Fig. 3. Assimilation of strontium-85 in light in the Fig. 2 experiment as a function of net oxygen production (dissolved oxygen determined by the Pomeroy–Kirschman–Alsterburg modification of the Winkler method). Suspension depths in ft. are indicated beside each data point

The first possibility ostensibly requires both decreased oxygen production and decreased carbon assimilation since photoinhibition has been amply documented by both carbon and oxygen methods. However, recent evidence indicates that phytoplankton excrete significant quantities of photoassimilate into the medium as dissolved organic matter[7]. If this process was accelerated under intense illumination while assimilated strontium-85 were retained, then a significant lowering of the photosynthetic quotient (oxygen produced/carbon dioxide assimilated) in high light, involving both reduced oxygen output and increased carbon uptake with subsequent elimination, could explain the results in Fig. 3.

The second possibility suggests several alternative mechanisms. First, Rice[3] has indicated that strontium-85 uptake occurs by adsorption and exchange. The former process is a function of surface area so that acceleration of cell division by light[8] could produce increased uptake of the radioisotope even though biomass production was photoinhibited. Secondly, phytoplankton cells of many kinds contain light-sensitive organelles other than chloroplasts, such as stigmata and non-green chromatophores. Stigmata are usually thought to function in phototaxis and chromatophores in photon capture by accessory photosynthetic pigments, but it is conceivable that they may also in some way serve in the regulation of mineral metabolism. One or both of these mechanisms could explain how the assimilation of strontium-85 can be dependent on light yet independent of photosynthesis. There may be others to consider as well.

These conjectures indicate that new problems in primary production can be brought to light through investigations using radioisotopes, and that further work to establish the basis of differential light–dark assimilation of strontium-85 and other radionuclides may lead to additional insights and clarifications of productivity processes.

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Effect of Ultra-violet Irradiation on Skin Collagen

Bottoms and Shuster[1] have shown that when hairless mouse skin is exposed to ultra-violet irradiation *in vitro* there is a decrease in the saline and citrate soluble collagen fractions; however, the insoluble collagen remains unchanged. At the same time, there is an increase in the toughness of the pelts. It was suggested that these effects of ultra-violet irradiation were due to increased cross-linkage between the collagen fibrils. In the experiments described here, we attempted to measure the degree of

cross-linkage of mouse skin and kangaroo tail tendon collagen before and after ultra-violet irradiation.

Harwell homozygous hairless albino mice were used throughout. Their necks were broken, and after a longitudinal incision made dorsally and ventrally the approximately equal half-pelts were removed to the paws, snout and tail. Subcutaneous fat was dissected away; the skin was dried with filter paper and weighed. One half-pelt was exposed to ultra-violet irradiation—the other half from the same mouse served as the control.

Salt-extracted, acetone-dehydrated kangaroo tail tendons[2] were given by the Research Station, Department of Agriculture and Stock, Warwick, Queensland, Australia. They were re-hydrated by soaking in distilled water for 24 h and in 0·9 per cent sodium chloride for a further 24 h. The tendons were then divided into two equal lengths, one half being exposed to ultra-violet irradiation and the other half of the same tendon being used as control.

The tendons and skins were placed in glass dishes, such that the epidermis of the skin was uppermost. In some experiments the tendons and skins were placed directly on the glass, in other experiments they were placed on a filter paper. The rims of the dishes were layered with silicone grease in order to make an airtight joint when they were covered by a quartz plate. The dishes containing the control tissues were covered with black paper to exclude the ultra-violet irradiation. Irradiation was carried out in a refrigerator so as to minimize bacterial decomposition, the dishes being placed at the bottom of a refrigerator at $-20°$ C. The ultra-violet source was from a medium pressure mercury arc (Hanovia lamp, ultra-violet *S*.220 arc tube type 504/4) and it was directed downwards from a point 24 in. above the pelts and tendons. Exposures were for 19 or 44 h. At the end of the exposure, the temperature adjacent to the tissues was never $> -4°$ C.

The tendons and pelts were weighed before and after irradiation. They were then treated in several changes of acetone, blotted and the residual acetone allowed to evaporate.

Comparison of the number of cross-links present in mouse skin and kangaroo tail tendon before and after irradiation was by load-extension and volume fraction measurements on the hydrothermally denatured materials, using experimental procedures already detailed[2,3] in conjunction with the Flory-Rehner equation[4]. Even if the absolute values of *Mc* and *C* are not given correctly by the calculation, the relative values for irradiated and unirradiated samples are certainly essentially correct.

The strip of mouse skin (6·0 × 0·5 cm) or piece of tendon (c. 15 cm in length) was soaked in distilled water overnight and then transferred to distilled water at 98° C for 30 sec. On removal its temperature was immediately reduced by quenching in cold water. For each experimental condition duplicate strips of mouse skin were prepared. All physical measurements were in distilled water at 65° ± 0·1° C after an equilibration period of 2 h at that temperature.

The results indicate that ultra-violet irradiation produces an increased molecular cross-linkage between the collagen fibrils both of hairless mouse skin and kangaroo tail tendon (*Mc* and *C*, Tables 1 and 2). The reduced capacity of the irradiated collagen to take up water (*V* in Tables 1 and 2) is presumably related to this increase in cross-linkage. The present results are in keeping with our previous observations that the saline and citrate soluble collagen fractions of hairless mouse skin are decreased by exposure to ultra-violet irradiation, because the more cross-linked collagens are insoluble in saline and citrate.

These findings may be pertinent to the changes which occur in the collagen of ageing human skin. There is a loss of collagen with age both in exposed and unexposed skin[5], but the collagen which remains is less soluble than in younger skin[6,7] and is more cross-linked[8]. It is now clear that ultra-violet irradiation *in vitro* produces collagens with properties resembling those found in ageing skin, which result from the production of intermolecular cross-links. The precise mechanism of this effect of ultra-violet irradiation will need further elucidation.

Table 1. THE EFFECT OF ULTRA-VIOLET IRRADIATION ON KANGAROO TAIL TENDON COLLAGEN. CONTROL AND IRRADIATED TISSUES ARE HALVES OF THE SAME TENDON

Control			Ultra-violet irradiated		
Mc	*C*	*V*	*Mc*	*C*	*V*
641,000	0·23	0 047	90,200	1·7	0·140
600,000	0·25	0 034	130,000	1·2	0·114
501,000	0·30	0·044	103,000	1·5	0 135
490,000	0·31	0·061	167,000	0·90	0·129
470,000	0·32	0·046	264,000	0·57	0·085
431,000	0·35	0 062	375,000	0·40	0·084
155,000	1·0	0·132	83,700	1·8	0·153
111,000	1 4	0·153	39,400	3·8	0 153
99,400	1·5	0·155	36,500	4·1	0·194
97,400	1·9	0·159	50,900	2·9	0·175
95,200	1 6	0·141	32,900	4·6	0·206
60,100	2·5	0·167	32,700	4·6	0·215

Mc, Mean molecular weight per cross-linked chain segment; *C*, number of cross-links per molecular weight unit of 300,000; *V*, the volume fraction, that is, $\frac{\text{volume of dry material}}{\text{volume of water wet material at 65° C}}$

Table 2. EFFECT OF ULTRA-VIOLET IRRADIATION ON HAIRLESS MOUSE SKIN. THE CONTROL AND IRRADIATED HALF-PELTS ARE FROM THE SAME MOUSE AND THE SYMBOLS ARE AS IN TABLE 1

Control			Ultra-violet irradiated		
Mc	*C*	*V*	*Mc*	*C*	*V*
160,000	0 9	0·125	36,500	4·1	0·205
151,000	1·0	0·138	25,700	5·8	0·212
102,000	1·5	0·189	48,800	3·1	0·190
96,700	1·6	0·165	49,000	3·1	0 219
95,900	1·6	0·152	51,700	2·9	0·180

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ENTOMOLOGY

Radiation Sterilization of Sugar-cane Leafhoppers of the Family Delphacidae

WE are at present investigating the possibility of eradicating sugar-cane leafhoppers of the genus *Perkinsiella* by the sterile male technique as suggested by Knipling[1]. Insects of this genus are economic pests, because they are the vectors of Fiji disease[2] of sugar-cane.

We have found that the fifth instar nymph is the most suitable stage to irradiate, and all irradiations mentioned here are those of fifth instars. These irradiations were effected by gamma rays from a cobalt-60 source.

With *Perkinsiella saccharicida* (Kirk) we obtain sterility at 3,500 (females) and 10,000 rads (males). At these dose-levels, adult longevity and mating behaviour are not significantly affected. Their sperm is motile and thus sterility is presumably caused by lethal mutations. A dose of 12,000 rads renders a percentage of the sperm inactive, but adult longevity and mating behaviour are

not sufficiently affected to prevent the use of this dose in a sterile eradication programme. A dose of 23,000 rads renders the sperm inactive, shortens adult longevity and adversely affects wing development.

In preliminary release experiments using normal fifth instars caged with irradiated fifth instars we have been able to reduce progeny production according to the ratio of sterile to fertile insects.

The results so far indicate that we have a large safety margin within which we can produce sterility, without significantly affecting sperm motility, mating behaviour or adult longevity.

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BIOLOGY

Morphogenetic Effects of Acidic Glycoproteins on the Development of Sea Urchin Eggs

IT is known that certain chemical agents can influence the differentiation of the ectoderm and the entomesoderm in the larva of the sea urchin. Thus certain acidic organic derivatives which carry sulphonic groups tend to favour the differentiation of ectodermic structures (animalization). The animalizing activity of these agents increases with their affinity for proteins[1].

It was considered to be of interest to determine whether acidic proteins, such as glycoproteins, are also able to induce the animalization of larvae. This article reports some investigations of the effect of the glycoproteins of fraction VI of ox serum (prepared by the method of Cohn *et al.*[2]). This fraction contains concentrated acidic glycoproteins such as orosomucoid and α2-glycoprotein[3,4], and the acidity of these is linked with the presence of acetylneuraminic acid in their molecule.

Fertilized eggs of the sea urchin *Paracentrotus lividus* were cultivated in sea water containing from 2 to 1 mg/ml. of Cohn fraction VI (obtained from the Nutritional Biochemicals Corporation) and a sodic derivative of sulphadiazine (M/1,000). Each culture contained in the region of 1,000 eggs/ml.

The larvae which developed under these conditions showed the morphological characteristics of animalization and radialization. The animalized larvae, however, predominated in cultures containing 2 mg/ml. of fraction VI, and were hyperciliated blastulae corresponding to type 3/4 in Hörstadius's[5] classification. The radialized larvae predominated in cultures containing 1 mg/ml. of fraction VI; these showed a typical radial symmetry, a body elongated parallel to the animal–vegetal axis and a well-developed pre-oral lobe covered with long, movable cilia. The radialization exhibited an attenuated aspect of the animalization when eggs were treated with animalizing agents at a concentration slightly less than that necessary to induce animalization.

In the control cultures prepared with the complete lyophilized ox serum (2 mg/ml.), the pluteus developed normally. The larvae also showed a normal morphology with acetylneuraminic acid at the same concentration.

It has been known since 1892 (ref. 6) that lithium ions tend to favour the development of entomesodermic structures (vegetalization). Eggs cultivated in the presence of lithium chloride ($6{\cdot}5 \times 10^{-3}$ M and $3{\cdot}25 \times 10^{-3}$ M) and fraction VI material (2 and 1 mg/ml., respectively) produced larvae more strongly vegetalized than did those cultivated in the presence of lithium chloride only.

These experiments thus revealed two new activities: (1) the animalizing action of acidic glycoproteins of the fraction VI of ox serum; (2) the reinforcement of the vegetalizing effects of the lithium ions by such acidic glycoproteins.

In the animalized larvae, the differentiation of an ectodermic type extends to territories which, in a normal larva, would have formed entomesodermic structures. This phenomenon can be interpreted when it is considered that, in territories the differentiation of which is thus modified, the genes responsible for the ectodermic differentiation are derepressed.

Histones (basic proteins) are able to repress the activity of the genes[7]. According to Frenster[8], nuclear polyanions by combining with histones can derepress the activity of the genes

Acidic glycoproteins form compounds with histones[9]. I therefore suggest that, in these experiments, acidic glycoproteins, as well as polysulphonic derivatives, by combining with basic proteins 'derepress' the activity of the genes responsible for the differentiation of the ectodermic structures. This derepression can be observed at the level of the genes, at the time of passing on the genetic message, and at the level of the ribosomes when this genetic message is translated. Concerning the first sphere of activity, it will be recalled that a hormone of a glycoproteic nature, gonadotrophin, has been shown to be capable of stimulating the synthesis of RNA within the nucleus[10]. Regarding the second sphere of activity, the cytoplasm of the sea urchin egg contains basic proteins of the histone type associated with ribosomes[11].

The second result which was brought to light by these investigations is the reinforcement by the acidic glycoproteins of the vegetalizing action of lithium ions. This effect suggests an increase in permeability to the lithium ions; such a phenomenon could be attributed to a modification of the physicochemical properties of the cellular membrane, or to the carrier role of the glycoproteins at the time when the lithium ions penetrate into the cells.

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Exposure of Cut Flowers to Ethylene in the Presence and Absence of Carbon Dioxide

FLOWERS produce ethylene[1,2], and ethylene in low concentration has a marked effect on development and senescence of the cut bloom[2]. With gas chromatography and flame ionization detection[3], it has been possible to examine critically the effect of exposure to a concentration of 0·05 p.p.m. ethylene on carnations over a range of temperature. The time interval between cutting and the first appearance of petal wilt can be readily determined.

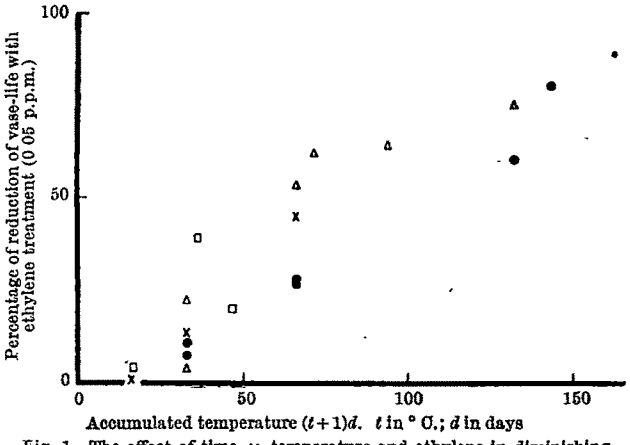


Fig. 1. The effect of time × temperature and ethylene in diminishing the vase-life of carnations. ×, 60° F (15·6° C); ●, 50° F (10·0° C); △, 40° F (4·4° C); □, 35° F (1·7° C)

The number of days between cessation of treatment and petal wilt is here termed 'vase-life' and has been determined at 16°–21° C.

The results of experiments in which carnation blooms were kept at different temperatures for various periods of time, in the presence and absence of ethylene at 0·05 p.p.m. concentration, in a continuous stream, have been brought together in Fig. 1. The percentage reduction in subsequent vase-life induced by exposure to ethylene has been plotted against accumulated temperature $(t+1)d$, that is, the product of the number of degrees difference between treatment temperature and the approximate freezing temperature (−1° C) and the number of days exposed to treatment.

Thus, an equivalent reduction of vase-life can be achieved by longer exposure at a lower temperature or shorter exposure at a higher temperature. It can be seen that there is a pronounced drift of the plotted points with increase of accumulated temperature.

Carbon dioxide at 5–10 per cent concentration was shown by Kidd and West[4] to delay the onset of the climacteric in apples, for the initiation of which it has been adduced that ethylene is responsible. Carbon dioxide in concentrations less than 10 per cent has also been shown to reduce the efficiency of ethylene in checking the elongation of pea seedlings[5].

We have observed a further inhibiting effect of carbon dioxide in that a concentration of the order of 5 per cent is sufficient to counteract the effect on the ageing of flowers of ethylene at 0·05 p.p.m. Carnation blooms, var. 'Red Sim', standing in water ('wet') or without water ('dry') were subjected to constant flows of air, air + 0·05 p.p.m. ethylene, 5 per cent carbon dioxide in air and 5 per cent carbon dioxide with 0·05 p.p.m. ethylene for 3 and 4 weeks at −0·4° C. The vase-lives recorded after treatment and removal to 16°–21° C are shown in Table 1.

A parallel experiment with cut narcissus blooms, var. 'Zurich', gave the results shown in Table 2.

Thus storage in 5 per cent carbon dioxide in the presence or absence of ethylene resulted in longer subsequent vase-life than storage in air, but storage in air with

Table 1

Treatment	Air	Air + 0·05 p.p.m. ethylene	5 per cent carbon dioxide	5 per cent carbon dioxide + 0·05 p.p.m. ethylene
3 weeks 'wet'	6·0	4·7	7·4	7·2
3 weeks 'dry'	5·2	3·0	7·1	6·7
4 weeks 'wet'	6·5	5·0	7·6	7·4
4 weeks 'dry'	5·5	2·9	7·1	7·4
Mean	5·8	3·9	7·3	7·2

Main effects: Carbon dioxide significant at 0·1 per cent level. Ethylene significant at 0·1 per cent level
Interaction: Carbon dioxide × ethylene significant at 1·0 per cent level.

Each figure is the mean of two samples of five flowers.

Table 2

Treatment	Air	Air + 0·05 p.p.m. ethylene	5 per cent carbon dioxide	5 per cent carbon dioxide + 0·05 p.p.m. ethylene
7 days −0·4° C				
'wet'	9·0	8·2	9·0	8 8
'dry'	8 8	8·3	9·2	9·0
14 days −0·4° C				
'wet'	6 0	5 6	6·6	7·2
'dry'	6·4	5·8	7 2	7·1
Mean	7·6	7·0	8·0	8·0

Main effects: Carbon dioxide significant at 0 1 per cent level. Ethylene significant at 1·0 per cent level.
Interaction: Carbon dioxide × ethylene significant at 1·0 per cent level.

Each figure is the mean of two samples of ten flowers.

added ethylene reduced life. Concentration of carbon dioxide of this order is unlikely to occur unless composition of the storage atmosphere is purposely controlled, for example, in so-called 'gas-packs'. Concentrations of ethylene of 0·05 p.p.m. with carbon dioxide less than 1 per cent may arise, as has been reported earlier[6], when cardboard containers are stacked in large bulk or when flowers are placed in proximity to a source of ethylene emanation, for example, ripening fruit, leakage of illuminating gas or when fog occurs in built-up areas.

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Prevention of Ethylene Injury to Carnations by Low Concentrations of Carbon Dioxide

THE finding that the reduction of the life of carnations (that is, the time interval between cutting and first appearance of petal wilt) which results from exposure to 0·05 p.p.m. ethylene is completely counteracted by the addition of 5 per cent carbon dioxide to the atmosphere[1] raises the question of what is the threshold concentration of carbon dioxide for achieving the effect.

The threshold concentration of ethylene which will produce a significant reduction of life of the cut carnation bloom has not been determined. A concentration of 0·05–0·07 p.p.m. was, however, chosen as one which may occur under conditions of normal handling of cut flowers[2], and is also within the lower limit for accurate determination, in which gas chromatography and flame ionization detection are used[3]. Carnations were kept in an air stream of 20 l./h with or without ethylene and with a range of concentrations of carbon dioxide from 0·1 per cent (the amount present in the emergent stream without addition of the gas) up to a maximum concentration of 2·2 per cent when carbon dioxide was added. The requisite atmosphere was passed through a polythene film envelope enclosing the flowers standing in water with 0·003 per cent silver nitrate as bactericide. Treatments for 2 and 4 days were given at a temperature of 18·3° C and, after removal, observation of subsequent behaviour was made at 18·3° C in air at 60 per cent relative humidity. The results of

Table 1. TOTAL LIFE (IN DAYS) OF CUT CARNATION BLOOMS

Period of exposure days	Ethylene-free air*	Air + ethylene 0 05 p.p.m *	Treatment Air + ethylene 0 05 p.p.m. + 2 2 per cent carbon dioxide	Ethylene-free air + 2·2 per cent carbon dioxide	*L.S.D.* at 5 per cent level = 1·91
2	8·6	5·8	8·9	8·1	
4	8·0	5·3	9·7	8 5	

* 0·1 per cent carbon dioxide in emergent air stream.
Each figure represents the mean of two samples of five flowers each.

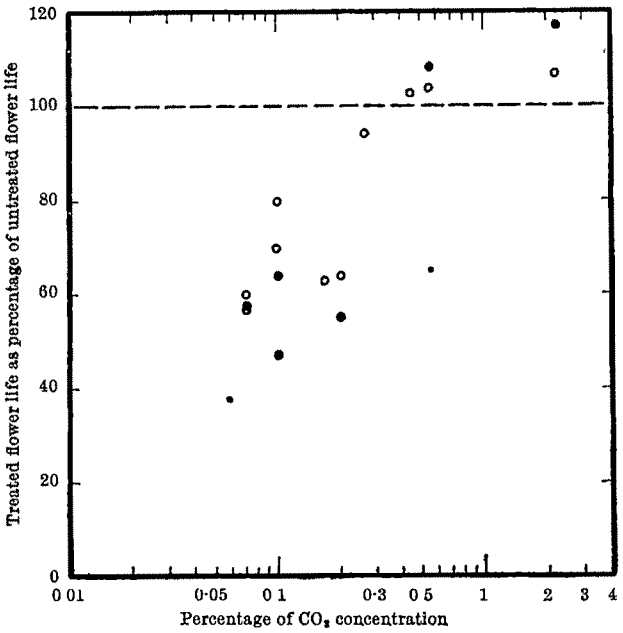


Fig. 1. Effectiveness of carbon dioxide in checking the reduction of life of cut carnation blooms when exposed to 0 05 p.p.m. ethylene; O, 2-day treatment; ●, 4-day treatment

two such experiments made on successive batches are given in Table 1.

Fig. 1 brings together the results of six experiments on carnations, in which different concentrations of carbon dioxide were used for 2- and 4-day treatment. For convenience, carbon dioxide concentration is plotted on a log scale. In one experiment the treatments were given on flowers not standing in water, but the responses to ethylene and carbon dioxide were unaltered.

It will be seen that, in the presence of ethylene (0·05 p.p.m.), the total life of the flowers was only reduced below that in ethylene-free air when carbon dioxide concentration was less than 0·35 per cent. Above this concentration the presence of carbon dioxide resulted in slightly increased life over that of flowers in an air stream with no detectable ethylene and about 0·1 per cent carbon dioxide. This increase in life with carbon dioxide in the presence of low concentrations of ethylene has been reported before[1].

It now seems evident that a relatively low concentration of carbon dioxide is sufficient to overcome the effect of ethylene at ordinary room temperature. Partial restriction of ventilation, which may result, for example, from close stacking of flower boxes, in which concentrations of 0·05 p.p.m. ethylene and 0·4 per cent carbon dioxide occur is not likely to cause reduction of life[2].

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VIROLOGY

Outbreak of Influenza *A/equi*-2 in Switzerland

EQUINE influenza virus was first isolated in 1956 in Czechoslovakia[1]; this original strain was called *A/equi*-1. A different type (*A/equi*-2) was recognized in 1963 in the United States[2]. For a number of years the *A/equi*-1 virus was apparently the only type responsible for horse influenza in Europe[3,4].

At the middle of March 1965, an epidemic of a serious febrile respiratory disease in horses broke out and quickly spread to the whole of Switzerland. Investigations were carried out on conjunctival and nasal swabs and on lung and on blood specimens which had been collected in the Federal Army Remount Depot (EMPFA) in Berne and in a riding stable in Geneva.

Isolation assays were made by inoculation of the material into the amniotic and allantoic cavity of 11-day-old eggs. One out of twelve conjunctival and nasal swabs (No. 65-891, Berne) and one out of five lung specimens (No. 65-951, Geneva) yielded an haemagglutinating agent. These two agents were identified as influenza *A/equi*-2 viruses by both haemagglutinating inhibition (HAI) and complement fixing (CF) tests. Acute and convalescent horse sera, which had been treated with potassium periodate to remove non-specific inhibitors, were used for identification by HAI tests[5]. The increase in antibody titre shown by convalescent sera was found to be almost identical when tests were carried out with the same number of HAI units of both isolates and of a reference strain (*A/equi*-2/New York/63) (Table 1). CF tests were carried out with specific *A/equi*-2 *V*-antisera prepared in guinea-pigs using the method of Henle *et al.*[6]. Both isolates (Nos. 65-891 and 65-951) reacted as influenza *A/equi*-2 viruses (Table 1).

Acute and convalescent sera of twenty-four horses were tested by HAI and CF tests at dilutions of 1 : 8 and higher. After all sera had been treated with potassium periodate, HAI tests were carried out with two haemagglutinating (HA) units of *A/equi*-1/Prague/56 crude allantoic fluid and with the same number of HA units of *A/equi*-2/New York/63 ether-treated allantoic fluid[7]. CF tests were performed at the same time with two CF units of an influenza *A* soluble antigen and with equivalent amounts of *A/equi*-1/Prague/56 and *A/equi*-2/New York/63 pure particular antigens prepared by the method of Lief and Henle[8]. Three units of complement were used, since horse sera are naturally slightly anti-complementary.

In both tests, only a fourfold or greater increase in antibody titre has been considered as significant. Results are summarized in Table 2. They clearly show the evidence of a recent infection with an influenza *A/equi*-2 virus in nineteen out of twenty-four horses by CF test and in all horses by HAI test. They confirm that the outbreak of respiratory disease in the Swiss horse population was caused by influenza *A/equi*-2.

Table 1. IDENTIFICATION BY HAEMAGGLUTINATION-INHIBITION AND COMPLEMENT FIXATION TESTS OF AGENTS ISOLATED FROM HORSES AS INFLUENZA *A/equi*-2 VIRUSES

Antigens	Sera: HAI titres* Horse No. 1 acute	Horse No. 1 convalescent	Horse No. 2 acute	Horse No. 2 convalescent	CF titres* *A/equi*-2 anti-*V*† *A*	*B*
65-891 (nasal swab)	<8	32	<8	32	32	32
65-951 (lung)	<8	32	<8	32	32	32
A/equi-2/New York/63 particular	<8	64	<8	64	64	128
A/equi-1/Prague/56 particular	<8	8	<8	<8	<8	<8
Influenza *A S*-antigen	–	–	–	–	<8	<8

* Reciprocals of serum dilutions.
† *A/equi*-2/New York/63 *V*-antisera prepared in guinea-pigs.

Table 2. NUMBER OF HORSES SHOWING AN INCREASE OF FOURFOLD OR GREATER IN ANTIBODY TITRE AGAINST THE INFLUENZA *A* SOLUBLE ANTIGEN AND THE *A/equi*-1 AND *A/equi*-2 VIRUSES BY COMPLEMENT FIXATION AND HAEMAGGLUTINATION-INHIBITION TESTS

Antigens	Number of horses: CF 4*	8	16	Total	HAI 4*	8	16	Total
Influenza *A S*-antigen	8	4	7	19	–	–	–	–
A/equi-1/Prague/56 particular	1	0	1	2	5	1	1	7
A/equi-2/New York/63 particular	8	8	3	19	4	11	9	24

* Increase (4 = fourfold increase).

A significant increase in antibody titre against the *A/equi*-1 virus has been found in two horses by CF test and in seven by HAI test. Such results have been obtained only in animals which showed an increase of eightfold or greater in antibody titre against *A/equi*-2. This is more suggestive of a booster response to a common antigenic fraction in the *A/equi*-2 and in the *A/equi*-1 particles than of a recent or concomitant infection with the latter agent.

Our own unpublished investigations on horse sera collected in Switzerland in 1963 and 1964 show that at this time no animal had any antibody against influenza *A/equi*-2. It is therefore not surprising that the virus spread quickly and produced serious symptoms when introduced in such a receptive population.

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Oncogenicity of Bovine Adenovirus Type 3 in Hamsters

THE prototype strain (*WBR* 1) of bovine adenovirus type 3 (ref. 1) is pathogenic for colostrum-deprived calves[2], and serological evidence indicates that, in addition to bovine adenovirus types 1 and 2, this serotype is involved in natural outbreaks of respiratory disease in cattle[3]. It has now been shown that the *WBR* 1 strain can induce the formation of tumours in hamsters, a capacity shared by certain other adenoviruses including human types 3, 7, 12, 18 and 31 (refs. 4–8), the chick embryo lethal orphan (CELO) virus[9] and a number of simian adenoviruses[10].

Nine litters of Syrian hamsters were inoculated by various routes on the day of birth with different doses of virus (strain *WBR* 1) grown in primary bovine kidney (BK) cultures and, in a number of these animals, tumours later developed at the site of inoculation. These tumours were either of a relatively firm consistency, resembling tumours induced by other adenoviruses or, as the virus dose was increased, were of a cystic type which appeared more rapidly (see Table 1). The cysts were largely filled with blood, although they also contained nodules of firm tumour tissue. Neither type of primary tumour was progressive; the firm tumours did not appear to grow after reaching a size of approximately 2–3 cm but remained static, whereas the cystic types regressed within 10 days leaving small subcutaneous nodules. Primary tumours of the firm type were transplanted in weanling and adult hamsters; in all these animals growth of the transplant tumours was progressive. Hamsters inoculated with uninfected BK cells remained healthy and free from tumours.

Table 1. INDUCTION OF PRIMARY TUMOURS IN HAMSTERS WITH BOVINE ADENOVIRUS TYPE 3 (STRAIN *WBR* 1)

Dose (log_{10})	Route	No. with tumours/ No. in litter inoculated*	Type of tumour	Latent period (days)
3·9	s/c	1/4	Firm	67
4·6	s/c	4/9	Firm	60–62
4·9	s/c	3/5	Firm (1)	53
			Cystic (2)	26
5·0	s/c	3/4	Cystic	24–26
5·2	s/c	1/6	Cystic	26
5 2	s/c	4/7	Cystic	24–27
4 0	i/c	2/3	Firm	34
4·3	i/t	2/3	Firm	47
4·6	i/p	2/4	Firm	55
		Total 22/45		

s/c, Subcutaneous. i/t, Intrathoracic. i/c, Intracerebral. i/p, Intraperitoneal.
* Survivors 7 days after inoculation.

On histological examination[11] of fixed tissues, the firm tumours were seen to comprise sheets of polygonal cells with numerous mitotic figures interspersed with multinucleate giant cells, some with ten or more nuclei. The general appearance suggested an undifferentiated sarcoma, but origin from epithelial tissue could not be excluded. In the cysts, the wall contained foci of tumour cells and the histological appearance of the nodules resembled that of the firmer tumours.

Monolayer cultures of primary or transplant tumours were comprised of cells resembling the types seen in sections. Such cells grown *in vitro* were capable of inducing progressive tumours when inoculated into adult hamsters. Attempts to demonstrate infective virus in tumours or their cultured cells have been unsuccessful to date.

Sera collected at various intervals after the appearance of tumours were used in complement fixation tests with 10 per cent extracts of tumour tissues or with tissue culture medium containing infective homologous virus. Complement-fixing antibody to viral antigen could be demonstrated in all hamsters, often in high titre, but could not be shown in significant amounts in those with transplant tumours. These findings may account for the stasis or regression of the primary tumours and for the progression of the transplant tumours. Complement-fixing antibody to tumour antigen was detected in low titre in one hamster with a primary tumour but not in any of the other hamsters. Complement fixation tests for the presence of polyoma or *SV*40 antibodies in the sera, or for antigens in the tumours, yielded negative results[12]. Neutralizing antibodies to bovine adenovirus type 3 were demonstrated in the sera of a number of the hamsters bearing primary tumours.

Bovine adenovirus types 1 and 2 were not found to be oncogenic for hamsters within an observational period of 90 days[7], whereas the present experiments indicate that bovine adenovirus type 3 is the first non-primate adenovirus, other than the avian CELO virus, which can induce tumours in hamsters.

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Fractionation of Partially Degraded Tobacco Mosaic Virus Ribonucleic Acid

IN the step-wise analysis of nucleotide sequence in tobacco mosaic virus ribonucleic acid (TMV–RNA)[1], occasional random splitting of internucleotide bonds results in the production of partially degraded molecules. These

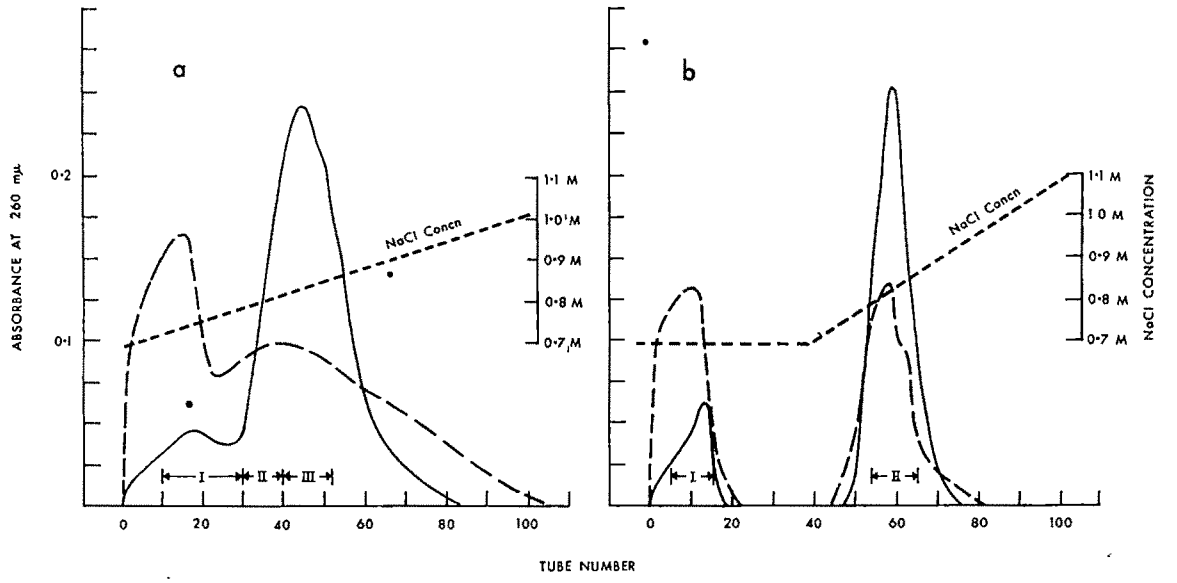


Fig. 1. Elution profiles of TMV–RNA from MAK columns. ——, Typical preparation of TMV–RNA. - - - -, Partially degraded TMV–RNA. (*a*) Gradient elution began immediately after RNA was loaded on to column. (*b*) Column was loaded and washed with about 100 ml. starting buffer before gradient elution was commenced

broken chains give rise to additional and, of course, false end-groups. In order that the sequence analysis be meaningful it is necessary that a method for the removal of broken chains be developed. We have looked into the possibility of separating undegraded from partially degraded TMV–RNA molecules by chromatography on methylated serum albumin–kieselguhr (MAK) columns[2]. Such a fractionation may also be of use in the reverse situation where it is desired to eliminate any intact molecules from a deliberately inactivated RNA preparation.

RNA was isolated from common strain TMV by a modified phenol technique[3]. As a source of partially degraded TMV–RNA we used both sonicated RNA and RNA recovered as a by-product from the isolation of TMV-protein[4]. Improvement, or otherwise, in the quality of the RNA as a result of fractionation was assessed on the basis of sedimentation behaviour and specific infectivity. Sedimentation velocity was determined at 4° C in 0·1 M phosphate buffer using ultra-violet optics. The infectivity of RNA samples (after reconstitution with TMV-protein[5]) was measured on *Nicotiana tabacum* L. var. Xanthi nc. by comparison with dilutions of a standard TMV preparation. Procedure for the MAK columns was as described by Mandell and Hershey[2], with the exception that the buffers were millimolar with respect to versene and 10 millimolar with respect to phosphate. The low solubility of phosphate in ethanol can be a problem during the recovery of the RNA from the column fractions.

Single-layer columns[6] with step-wise elution were used in the initial experiments to determine the optimum salt molarity at which the RNA should be applied and the sensitivity of the elution profile to variations in temperature and in the *p*H of the buffers. It was found that undegraded TMV–RNA is eluted from the column by 0·8 M sodium chloride, *p*H 6·7, at 2° C, but that, if either the *p*H of the buffer is 6·1 or the temperature is raised to 23° C, then 0·85–0·90 M sodium chloride is required to effect elution.

All further experiments were carried out using a three-layer column at 2°–4° C and a 250-ml. or 500-ml. linear salt gradient. Approximately 0·8 mg RNA was applied in 50 ml. 0·7 M sodium chloride and gradient elution started immediately. A typical elution profile for both normal RNA and partially degraded RNA is shown in Fig. 1*a*. The contents of the tubes were grouped into fractions as indicated and the RNA recovered by precipitation with ethanol. Recoveries from the column itself averaged 85 per cent of the starting material and precipitation of the RNA from the fractions was nearly quantitative. Sedimentation patterns and infectivity of the various fractions were compared with those of the unfractionated RNA.

In all columns we have run, the first fraction eluted appeared polydisperse in the ultracentrifuge and exhibited little, if any, infectivity (Table 1). Subsequent fractions showed varying amounts of the 31*S* component and considerable infectivity. Where the starting RNA was a partially degraded preparation it was possible to recover fractions from which, judging by their increased specific infectivity and sedimentation homogeneity in the ultracentrifuge, many of the degraded molecules had been removed. Where the original RNA was a good preparation (70–75 per cent homogeneous), then little improvement in quality was achieved. Flattening the elution gradient was without further benefit.

Our failure to remove completely the degraded molecules might have been due to tailing of the polydisperse, non-infectious material into the subsequent undegraded fractions. We examined this possibility by loading the RNA on to the column, washing with starting buffer

Table 1. ANALYSIS OF RNA FRACTIONS FROM MAK COLUMNS

Type of RNA	Unfractionated		Fraction I*		Fraction II*		Fraction III*	
	Infectivity†	Homogeneity‡	Infectivity	Homogeneity	Infectivity	Homogeneity	Infectivity	Homogeneity
Normal§	60	60	8	Polydisperse	59	66	63	71
Normal§	76	76	3	,,	77	71	91	82
Partially degraded§	21	28	0	,,	—	—	38	50
Normal‖	76	76	4	,,	80	83	—	—
Partially degraded‖	29	29	0	,,	53	53	—	—

* Fractions were obtained by pooling the contents of tubes as shown grouped in Fig. 1.
† Infectivity refers to the percentage of the RNA which reconstitutes with protein to form infectious virus.
‡ Percentage of the RNA which sediments as a homogeneous 31*S* component.
§ Elution pattern of type shown in Fig. 1*a*.
‖ Elution pattern of type shown in Fig. 1*b*.

until the absorbance at 260 mμ of the eluate returned to the base line and then beginning the gradient elution. Elution profiles for this type of procedure are shown in Fig. 1*b*. It can be seen, however (Table 1), that even with this variation not all the partially degraded molecules were eliminated from the RNA preparation.

It would seem, then, that it is possible by fractionation of MAK columns to remove many of the degraded molecules from a partially degraded TMV–RNA preparation. However, the efficiency with which this has been achieved is not so high as might have been expected, in view of the established resolving power of MAK-columns for other systems[7,8]. It is possible that greater resolution is obtained on the column itself but that, during the process of recovery of the RNA from the fractions, some degradation has taken place, thus cancelling some of the improvement in quality which might otherwise have been apparent.

We thank Miss F. Putney and Dr. H. K. Schachman for the sedimentation data, Mrs. B. Singer for infectivity assays and Dr. H. Fraenkel-Conrat for his continued interest.

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AGRICULTURE

Influence of Seed Source on the Date of Ear Emergence in Bread Wheat

THE growth of grain crops may in some cases be influenced by the conditions under which the crop which produced the seed was grown. Among the characters affected have been yield[1] and winter hardiness[2] of winter wheat, and yield of spring wheat[3], while similar differences have been reported in oats[4,5], maize[6–8] and rice[9]. In other cases and for some additional characters considered in these investigations no consistent differences in performance were found[5,9–11]. Some of these effects have been attributed to differences in seed size[3], condition and age of the seed[5,6], mechanical damage[2] or the presence of seed-borne pathogens[6], while the level of soil fertility of the parental environment as reflected in the quality of the seed has also been implicated[3]. However, there are some instances in which it was considered that much of the variation remained unexplained[1,2]. It has previously been noted that the time of ear emergence of wheat can be modified in this way[12], but the differences recorded were ascribed to the effects of selection for earliness in a mixed population. Small changes in heading date were also found in the investigation of the influence of seed source on winter wheat[1].

In the 1960 season, single plant progenies of a New Zealand bread wheat (*Triticum aestivum* emend. Thell. spp. *vulgare* MacKey variety Hilgendorf 61) from both Canadian and New Zealand seed sources were grown together in the experimental plots of the University of Manitoba, Winnipeg, and substantial differences in the heading date were found between the two groups. Six single plant progenies from the Canadian seed source averaged 75·8 ± 2·1 days from sowing to ear emergence, while twelve similar lines of the same variety from New Zealand-grown seed from autumn sowing headed after 59·9 ± 0·4 days. In addition to being considerably later, the Canadian seed lines were much more variable in their heading date, but there was no overlap between the two groups.

In a further investigation of this effect, the Hilgendorf 61 lines were grown under three contrasting environments: both autumn- and spring-sown at Lincoln, New Zealand, and spring-sown in Manitoba. The seed produced was then grown in small plots at each of the test environments and the date of ear emergence recorded. A plot was considered to have headed when half the ears had emerged. Similar observations were taken on short rows of plants grown in boxes in an artificially illuminated growth chamber held at 70° F with an 18-h day-length. Where the plots were replicated, a randomized block layout was used.

The data presented in Table 1 are derived from four Hilgendorf 61 lines which received the most extensive testing, but they are typical in their reaction of all the lines investigated. These results indicate that while the autumn sowing of the lines did not reveal any differences in the time to ear emergence, the spring-sown plots both in Canada and New Zealand, and particularly the material sown in the growth chamber, showed substantial differences in this character.

Table 1. INFLUENCE OF SEED SOURCE ON THE NUMBER OF DAYS TO EAR EMERGENCE OF FOUR LINES OF HILGENDORF 61 WHEAT SOWN UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

Seed source	Number of days to head in different test environments: Autumn sown (N.Z.) 1963–64	Spring sown (Canada) 1963	Spring sown (N.Z.) 1963–64	Growth chamber 1964
Autumn sown (N.Z.)	193·0	70·0	89·3	75·3
Spring sown (Canada)	193·0	76·5	94·6	92·2
Spring sown (N.Z.)	192·0	—	90·2	82·4
	Unreplicated, each value the average of determinations from four lines		Least significant difference 5% = 2·5 1% = 3·4	

A combined analysis of the results from replicated trials indicated that, even in cases where there was a similar average period of development, the conditions of growth affected the expression of the effect of the origin of the seeds. The interaction of the environmental conditions and the origin of the seed was very highly significant. The four individual lines of Hilgendorf 61 did not, however, differ significantly in their performance.

The use of the same lines of Hilgendorf 61 in the establishment of the seed lines from diverse sources was necessary in order to eliminate any genotypic variation which might have been present within the variety. The daily photoperiod under which the tests were conducted was longest in the growth chamber; the maximum photoperiod of the Canadian spring-sown trial was greater than that of the New Zealand spring-sown trial.

The main features of the effect of the seed source on heading date of Hilgendorf 61 are: (1) The effect of seed source had little or no influence under autumn sowing. (2) Seed derived from autumn-sown sources headed considerably earlier than other treatments under spring-sown or long-day conditions. (3) The effect was expressed as increasingly delayed ear emergence with increasing maximum day-length experienced for similar seed sources. (4) The effect was expressed as increasingly delayed ear emergence with increase in the maximum day-length to which the seed source had been exposed, under uniform test conditions.

The variety Hilgendorf 61 shows some response to vernalization treatment[13] and it could be suggested that this is satisfied by the low temperature and short-day conditions experienced during the autumn-sown cycle of seed production. If this is the case, however, the vernalization stimulus which may be acquired at the tillering stage[14] is expressed in the initiation of the reproductive stage of the progeny. Although a number of workers

have suggested that vernalization can take place in the developing grain[15–17], none have suggested that the vernalization requirement for the following generation can be satisfied at a stage earlier than fertilization. Vernalization after grain formation can be discounted, for after the time of heading of autumn-sown lines of this variety (mid-November at Lincoln) protracted cold periods are not experienced, and the day-length is in excess of 13·5 h. In addition, this explanation would not allow for the differences found between the different spring-sown seed sources. If the effect is one of photoperiodic induction alone, the nature of the mechanism and the manner by which the stimulus is transmitted through the seed stage are not known. The modifications induced are not, however, permanent changes, and the effect can be reversed by exposing the seed line to the alternative set of environmental conditions.

The profound influence of the parental environment on the performance of the progeny has important implications. It makes necessary the use of strictly comparable seed in investigations of vernalization or photoperiodic response and for the accurate comparison of varieties in field trials.

A practical application of this effect with suitable varieties would be the control of the date of heading in commercial crops. Earlier ripening of spring wheat would be an advantage in the south of New Zealand, and field trials to investigate this effect and associated differences in performance of wheat varieties are being undertaken.

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GENERAL

Algorithm to assist in finding the Complete Sub-graphs of a Given Graph

Let the operation M, applied to a graph G, have the effect of removing from G all lines which are not members of at least one K_3 (a complete graph or sub-graph having three points, that is, a triangle). The new graph MG consists entirely of triangles, and it will be unchanged by a second application of the operator M. $M^2G = MG$.

This is not true of the operation D, to remove from G all lines which are not members of at least two K_3's, as Fig. 1 shows. However, D^nG converges with increasing n either to an empty graph or to a graph $D^\infty G$ of which every line is a member of at least two K_3's. This is of interest in comparison with the convergency of Boolean relation matrices[1]. Fig. 2 shows the two simplest symmetrical examples of a $D^\infty G$. Form 2(*a*) is a K_4. In the graphs investigated so far (not including crystal structure), form 2(*b*) has never occurred and every line has been a member of at least one K_4.

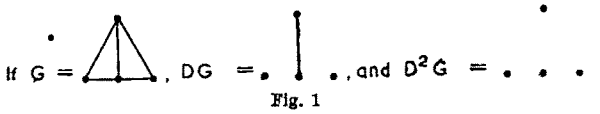


Fig. 1

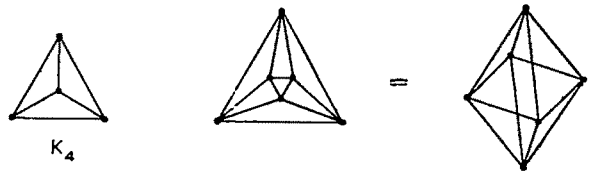


Fig. 2

Similarly, if TG is the graph left by removing all lines which are not members of at least three K_3's, all the lines in $T^\infty G$ were members of K_5's.

The time taken by a computer to find the K_4's and K_5's in a large graph is not long when the D^∞ or T^∞ algorithms are used. Applied to a graph of 1,000 points and 1,791 lines, D^4 was equal to D^8 and T^4, T^3 and T^2D were equal. After separation of the residue into its component sub-graphs, the original graph was seen to contain one K_6, twenty K_5's, and large numbers, not yet counted, of K_4's and K_3's. From an examination of the results, it seems unlikely that in this example there are any lines, like the twelve lines of Fig. 2(*b*), which belong to no K_4. Strictly, however, the algorithm should be supplemented by a test to distinguish the complete sub-graphs.

The graph-theoretical problems leading to our use of the foregoing algorithm arose out of an attempt to generate groups of words by purely mechanical operation on a large number of related documents. Words within groups were required to have strong associations with each other, and the groups were needed for research on the mechanical classification of documents. The presence or absence of each of 1,000 selected words or word-stems in each of 12,000 documents was recorded, as 1,000 binary vectors each having 12,000 bits. In comparing the vectors of every pair of words, an arbitrary test was made to decide whether the pair of words was "associated" or not. It was convenient to regard the results of the 500,000 tests as a graph on 1,000 points, each representing a word, in which a line joining two points represented an "association" between the words.

Thus, we were faced with the task of arranging the points of a large graph into groups, such that there were many lines within groups and few lines between groups. It was evident that groups of this kind would contain numerous complete sub-graphs; the algorithm provides one of several possible ways to find, first the complete sub-graphs, and then the groups.

The partitioning of graphs is becoming increasingly important in language studies, sociology, economics, network theory, logistics, automated computer design and many other situations where multiple association or correctedness is involved.

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Some Observations on the Great Fire of London, 1666

1966 marks the tercentenary of the Great Fire of London. In reading descriptions of this fire, I was impressed by the facts that only four people died directly as a result of the fire and that only 436 acres were burnt in 87 h. The London fire is dwarfed when compared, for example, with the Chicago 'Mrs. O'Leary's Cow' fire of 1871 when 2,124 acres and 17,450 buildings were burnt between October 8 and 10, 250 people were killed and about

100,000 left homeless. Although fire-fighting techniques had become more advanced and buildings more widely separated than they were in the London of 1666, the Chicago fire was a much faster spreading and more destructive fire.

The reason for this great difference in fire behaviour can be attributed to the generation of a fire storm over Chicago, while there is no indication that one was formed over London in 1666. The meteorological factors giving rise to a fire storm have been discussed in detail by Besson[1] and by Ebert[2] in connexion with the Hamburg fire storm caused by air raids during the Second World War. Ebert thought that a blanket of smoke from a previous raid gave rise to very high ground temperatures and large temperature gradients with altitude, thus generating a very unstable atmosphere above the city. A low velocity ground wind was present, but apparently it did not disturb the convective column above the fire and a high velocity vortex of hurricane force was thus formed. These conditions were amazingly similar to those existing in Chicago in 1871. A large fire that had begun on October 7 was actually still smouldering when the second and larger fire began. The first fire provided a smoke cover which absorbed excessive amounts of solar energy and reduced radiation losses at night, thus raising ground temperature. Compounded with a hot, dry summer spell and a low ground wind, the conditions were ideal for the generation of a fire storm.

The presence of a steady high velocity easterly ground wind during the London fire would seem to have saved London from a larger catastrophe. Apparently, it prevented the formation of a vortex above the fire and directed the fire towards the west making it easier for the inhabitants to predict its path and thus evacuate their houses in ample time. The increase in burning rate due to a steady ground wind does not cause as much damage as that resulting from a fire storm.

The map compiled by Bell[3] was used to obtain the cumulative area burnt on different days. A plot of the square root of area versus time gives a good linear relation (Fig. 1). This is consistent with a constant linear rate of propagation. Even the last point is, as expected, slightly below the line indicating a reduction in the rate of burning. By the third day, the fire had spread outside the walls of the city where the buildings were more widely separated. At the same time, the efficient use of fire breaks and a drop in wind velocity helped to reduce the rate of progress of the fire. Since the fire perimeter was approximately semicircular, then:

$$A = \tfrac{1}{2}\pi(\bar{R}t)^2 \qquad (1)$$

where A is the total area burnt (cm²), t is the time since the beginning of the fire (sec), and $\bar{R}$ is the average linear rate of burning (cm/sec). Equation (1) gave an average linear rate of burning of 0·36 cm/sec. However, because the fire spread mostly to the west and scarcely made any progress to the east, the maximum linear velocity can be calculated by dividing the farthest distance travelled to the west (that is, between Pudding Lane and the Temple) by the total time of duration of the fire. This gives a value of 0·59 cm/sec.

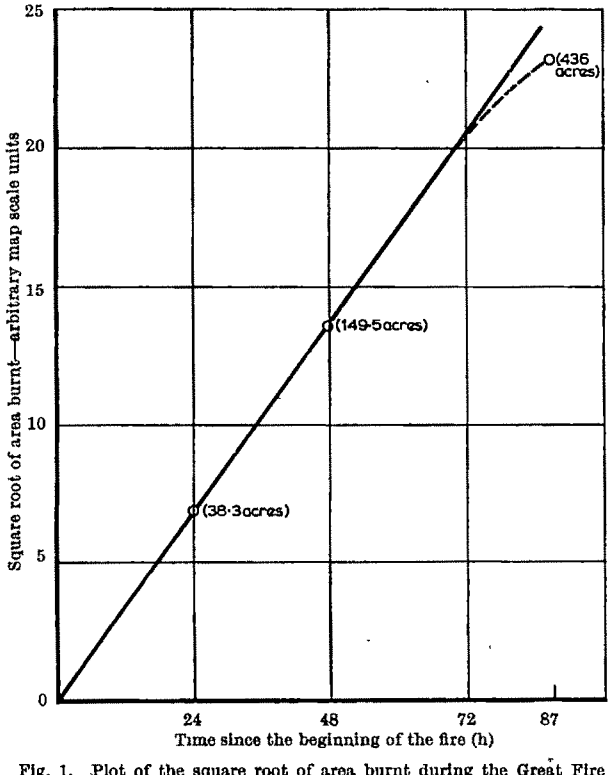


Fig. 1. Plot of the square root of area burnt during the Great Fire against time since the beginning of the fire

Since the spread of fire was more or less regular and along a relatively well-defined perimeter, it was considered of interest to compare its rate of spread with that predicted from laboratory experiments on small models.

Byram *et al.*[4] conducted several experiments in which wooden cribs of different spacings were burnt in the presence of wind and the rate of spread of fire measured. Thomas[5] showed that Byram's data could be correlated by the equation:

$$R\rho_b \cos\varphi = 0{\cdot}0085\ \mathrm{g\,cm^{-2}\,sec^{-1}} \qquad (2)$$

where ρ_b is the bulk density of the crib (g/cm³), φ is the deflexion of the flame from the vertical (degrees), and R is the linear rate of burning (cm/sec). A value of R for the Fire of London can be calculated from this equation provided appropriate values of φ and ρ_b are used.

The wind velocity during the London fire is not known, but the average wind speed at Kew Observatory varies between 10 miles per hour in January to about 7 m.p.h. in August[6]. An average wind speed of 10 m.p.h. will be assumed to have existed during the fire. Experiments on wooden crib fires in this laboratory with a wind speed of 10 m.p.h. gave an angle of deflexion, φ, of about 70°. Fire load values for several types of building have been given in the literature. Halpaap[7] gave a fire load of 125 kg/m² for a library and 200 kg/m² for a timber warehouse. Eisinger[8], in analysing the fire load of the old section of the town of Regensburg (a photograph of which showed heavy timber construction), gave a value of $11{\cdot}55 \times 10^5$ kcal/m² which is approximately equivalent to a fire load of 300 kg/m². Using this value for London (1666) and assuming an average building height of 7 m, a bulk density of 0·043 g/cm³ can be calculated. Substituting the preceding values of φ and ρ_b in equation (2) yields a value of R of 0·58 cm/sec. In view of the assumptions made, this is in excellent agreement with the maximum rate observed during the Great Fire.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Saturday, July 9

BIOLOGICAL ENGINEERING SOCIETY (at Mullard House, Torrington Place, London, W.C.1)—All-day meeting on "The British Biomedical Instrumentation Industry".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

PROGRAMMER (with previous programming experience) for data processing—The Deputy Secretary, The University, Southampton (July 8).

RESEARCH ASSISTANT (with an honours degree in geography, some graphic ability and an interest in the graphic arts, and preferably experience in some aspect of map production) IN CARTOGRAPHY IN THE DEPARTMENT OF GEOGRAPHY—The Secretary of the University Court, The University, Glasgow (July 8).

LECTURER (with experience in some aspect of civil engineering hydraulics, and prepared to teach and conduct research in this field) IN FLUID MECHANICS—The Secretary, University of St. Andrews, Queen's College, Dundee (July 9).

TUTORIAL ASSISTANT (graduate with qualifications in sociology, social anthropology or a related discipline) IN THE DEPARTMENT OF SOCIOLOGY AND SOCIAL ANTHROPOLOGY, to help in the teaching of sociology—The Registrar, The University, Hull (July 9).

RESEARCH FELLOW (preferably with some experience in either kinetics of reactions in solution or transition-metal or lanthanide chemistry) IN CHEMISTRY for work on the kinetics of fast inorganic reactions in collaboration with Dr. E. F. Caldin—The Registrar, University of Kent at Canterbury, Canterbury, Kent (July 11).

DEMONSTRATORS (with good honours degree and approximately one year's research experience) IN PHYSICAL, ORGANIC OR INORGANIC CHEMISTRY—The Registrar, University College of Wales, Aberystwyth (July 12).

DEMONSTRATOR (with a good honours degree in biochemistry or agricultural biochemistry and preferably with some postgraduate experience) IN AGRICULTURAL BIOCHEMISTRY—The Registrar, The University, Newcastle upon Tyne, 2 (July 12).

LECTURER or ASSISTANT LECTURER IN SOCIOLOGY—The Registrar, The University, Hull (July 12).

LECTURER or ASSISTANT LECTURER (preferably with qualifications in mathematical physics or astrophysics) IN APPLIED MATHEMATICS—The Registrar, The University, Leeds, 2 (July 12).

SENIOR LECTURER (preferably with a special interest in thermodynamics or fluid dynamics) IN THE DEPARTMENT OF ENGINEERING SCIENCE—The Secretary, University of Exeter, Northcote House, The Queen's Drive, Exeter, Devonshire (July 14).

BRITISH INDUSTRIAL PLASTICS RESEARCH SCHOLAR (with a good degree including chemistry) IN THE CHEMISTRY DEPARTMENT, for work concerned with cationic polymerization of oxygen compounds—The Registrar, The University, Keele, Staffs (July 15).

EXPERIMENTAL OFFICER (with a degree, H.N.C. or equivalent qualification) IN THE PEDOLOGY DEPARTMENT, to assist in research on soils—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/90 (July 15).

LECTURER IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, The University, Liverpool, quoting Ref. CV/118 (July 15).

SENIOR DEMONSTRATOR (graduate, preferably with a higher degree, and research experience in industry, a university or government research establishment) IN THE SCHOOL OF PHYSICS—The Registrar, The University, Newcastle upon Tyne, 2 (July 15).

SENIOR LECTURER (medically qualified) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/119 (July 15).

DIRECTOR OF THE COMPUTING CENTRE—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff, South Wales (July 16).

LECTURER and an ASSISTANT LECTURER IN THE DEPARTMENT OF BIOLOGICAL CHEMISTRY—The Secretary, The University, Aberdeen (July 16).

RESEARCH WORKER (with experience in applied respiratory physiology, and preferably a medical qualification, but this is not essential if an honours degree in physiology is held) IN THE MEDICAL RESEARCH COUNCIL'S CLINICAL RESPIRATORY PHYSIOLOGY RESEARCH UNIT—The Secretary, King's College Hospital Medical School, Denmark Hill, London, S.E.5 (July 16).

S.R.C. RESEARCH STUDENT IN THE DEPARTMENT OF PSYCHOLOGY—Head of the Department of Psychology, Queen's College, Dundee (July 16).

DEMONSTRATOR (preferably with a special interest in taxonomy or ecology) IN THE DEPARTMENT OF BOTANY—The Registrar, University College of Wales, Aberystwyth (July 18).

HOSPITAL BIOCHEMIST (Top-Grade) (appropriately qualified science graduate) IN THE DEPARTMENT OF CLINICAL BIOCHEMISTRY—The House Governor and Secretary, Royal Victoria Infirmary, Newcastle upon Tyne (July 18).

JUNIOR RESEARCH OFFICER (with, or about to obtain, an honours degree in botany, agricultural botany or a related subject) IN THE DEPARTMENT OF BOTANY to work on root competition and its relation to productivity—The Registrar, University College of Wales, Aberystwyth (July 20).

RESEARCH ASSISTANT (with a good honours degree in chemistry or biochemistry) for work on the enzymic degradation of collagen in the Rheumatism Research Centre in close collaboration with the Department of Medical Biochemistry—The Registrar, The University, Manchester, 13, quoting Ref. 162/66 (July 20).

LECTURER or ASSISTANT LECTURER (with good qualifications in astronomy with some research experience, or qualifications in mathematics or physics with experience in astronomy) IN ASTRONOMY—The Secretary, University College, Gower Street, London, W.C.1 (July 22)

RESEARCH ASSISTANT (with a first- or second-class honours degree in astronomy, mathematics or physics) IN ASTRONOMY—The Secretary of the University Court, The University, Glasgow (July 22).

LECTURER IN ELECTRICAL ENGINEERING—The Secretary, University of St. Andrews, Queen's College, Dundee (July 23).

LECTURER IN PSYCHOLOGY IN THE DEPARTMENT OF PHILOSOPHY—The Registrar, University College of Wales, Aberystwyth (July 23).

PROFESSOR OF SOIL SCIENCE; LECTURER or SENIOR LECTURER IN SOIL SCIENCE; PLANT PATHOLOGIST (Research Fellow/Senior Research Fellow); GROUNDNUT BREEDER (Research Fellow/Senior Research Fellow); and an AGRICULTURAL ENGINEER (Research Fellow/Senior Research Fellow) in the Institute for Agricultural Research and the Faculty of Agriculture, Ahmadu Bello University, Northern Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (July 26).

SENIOR LECTURERS, LECTURERS or ASSISTANT LECTURERS IN (*a*) Biochemistry and (*b*) PHYSICS at the University College of Rhodesia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (July 26).

CHAIR OF ORAL SURGERY at the Royal Dental Hospital of London, School of Dental Surgery—The Academic Registrar, University of London, Senate House, London, W.C.1 (July 29).

CHAIR OF MECHANICAL ENGINEERING at the University of Newcastle, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 30).

LECTURERS (2) IN PSYCHOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, July 30).

RESEARCH ASSISTANT (with a university degree with distinction in petrology) IN THE DEPARTMENT OF GEOLOGY to collaborate in research on West Greenland Tertiary rocks (particularly lavas)—Dr. H. I. Drever, St. Salvator's College, University of St. Andrews, St. Andrews, Fife, Scotland (July 31).

SENIOR TEACHING FELLOWS or TEACHING FELLOWS (preferably with a Ph.D. degree or completing the requirements for the degree, and with previous teaching or research experience) IN THE DEPARTMENT OF CHEMISTRY—The Academic Registrar, Monash University, Clayton, Victoria, Australia; or the Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, July 31).

RESEARCH ASSISTANT or TECHNICAL OFFICER (with several years experience in a physical laboratory and familiar with high vacuum techniques and electronics) IN THE DEPARTMENT OF ENGINEERING SCIENCE to be engaged in a research programme on low-frequency plasma oscillations and waves—The Administrator, Department of Engineering Science, The University, Parks Road, Oxford (August 1).

CHAIR OF PATHOLOGY at St. Mary's Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 5).

LECTURER/SENIOR LECTURER IN VETERINARY MEDICINE, at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 5).

HEAD (plant physiologist) OF THE CROP PHYSIOLOGY DEPARTMENT—The Secretary, Grassland Research Institute, Hurley, Maidenhead, Berkshire (August 8).

CHAIR OF EDUCATION at the University of Malawi—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 12).

LECTURER or ASSISTANT LECTURER (with interests in plant physiology and preferably research experience in plant metabolism, including radiotracer techniques) IN BOTANY—The Registrar, University College of North Wales, Bangor, North Wales (August 15).

PROFESSOR OF BIOMATHEMATICS—The Registrar, University Registry, Oxford (August 27).

ASSISTANT PHYSICIST (with a good honours degree in physics, but candidates graduating this summer will be considered) to work in the Regional Medical Physics Department on the application of physics to clinical problems in the hospital service—The Chief Physicist, Regional Medical Physics Department, 21 Claremont Crescent, Sheffield, 10.

DIRECTOR OF THE UNIVERSITY COMPUTING CENTRE—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

JUNIOR/SENIOR LABORATORY TECHNICIAN (with chemistry A-level) IN THE DEPARTMENT OF GROWTH AND DEVELOPMENT for a research team doing specialized steroid estimating in children—The Secretary (G-6), Institute of Child Health, 30 Guildford Street, London, W.C.1.

LECTURER (preferably with an interest in statistics and/or computing) IN MATHEMATICS—The Principal, Medway College of Technology, Horsted, Maidstone Road, Chatham, Kent.

NON-MEDICAL SCIENTIFIC OFFICER (Basic Grade) FOR THE RESPIRATORY FUNCTION UNIT with related Cardio-Thoracic Unit, for work which includes various research projects in the clinical and radiological fields—The Chief Administrative Officer, United Sheffield Hospitals, Central Office, Royal Hospital, West Street, Sheffield, 1.

PROFESSOR OF ENGINEERING PRODUCTION—The Registrar, Bradford Institute of Technology, Bradford 7.

PSYCHOLOGIST (with some experience of postgraduate research, preferably in the psychology of hearing) IN THE LABORATORY OF EXPERIMENTAL PSYCHOLOGY, to work on the problem of finding a method of converting print into a sound pattern intelligible to the blind—The Assistant Registrar (Establishment), University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

RESEARCH ASSISTANT (graduate in veterinary science) to join a team of workers who are concerned with research into the control of mastitis—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 66/23.

RESEARCH ASSISTANT (graduate or student about to graduate) IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY for research into the mechanism and control of nucleic acid synthesis—The Assistant Bursar (Personnel), University of Reading, Reading.

RESEARCH CHEMIST IN ANALYTICAL CHEMICAL TOXICOLOGY IN THE DEPARTMENT OF FORENSIC MEDICINE, to assist in a programme of research into the development of rapid methods of toxicological analysis—Dr. Harold V. Street, Department of Forensic Medicine, University of Edinburgh, Teviot Place, Edinburgh, 8.

RESEARCH DEMONSTRATORS (graduates or students graduating this summer) IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY, for duties which will include assistance in practical classes and research in physiology or biochemistry, or in their application to animal nutrition—The Assistant Bursar (Personnel), University of Reading, Reading.

RESEARCH STUDENTS IN THE DEPARTMENT OF APPLIED PHYSICS to work on crystal defects in semiconducting compounds—Prof. D. A. Wright, Department of Applied Physics, University of Durham, South Road, Durham.

SENIOR TECHNICIAN and TECHNICIAN (A) to supervise the establishment of new research laboratories and to assist in the research work that is being undertaken—Prof. N. R. Butler, Department of Child Health, Children's Hospital, St. Michael's Hill, Bristol, 2.

TECHNICIAN/SENIOR TECHNICIAN (preferably with a knowledge of histological techniques) for a small experimental laboratory; and a JUNIOR TECHNICIAN (with some knowledge of animal work) for an experimental biological laboratory, for duties which will include the maintenance of a small inbred mouse colony—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

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TECHNOLOGY AFTER COUSINS

MR. WEDGWOOD BENN is exceedingly lucky to have succeeded Mr. Frank Cousins as Minister of Technology at this critical time for the Ministry. Ministers in charge of new departments frequently find themselves spending much time and effort in gathering together the instruments of power, often by means of tussles with their colleagues. Mr. Benn will have none of that to do. Mr. Cousins has done it for him, and there is something in the view that Mr. Cousins was too much concerned with the accumulation of power—the people, the laboratories and the money—and too little concerned with using it. Whatever the truth may be, the accession of the core of the Ministry of Aviation to his Ministry in the autumn will mean that Mr. Benn will be able, if he wishes, to direct the largest single instrument of British expenditure on research and development. By good luck there are signs that the Ministry of Technology is beginning to find its feet at last; modest achievements now exist as counterpoise for the disappointments of the past two years (see p. 115 of this issue).

What, then, should Mr. Benn do? First he must decide what the Ministry of Technology can reasonably be expected to accomplish. At the beginning of the Ministry's existence, after the General Election in 1964, ambition tended to get the better of good sense, and there was a tendency to believe that the Ministry with its own resources would be able to make up for all the deficiencies of British industry, real and imaginary. It is therefore fascinating to see how often the Ministry has been converted from taskmaster into protector. Frequently it has turned out that the best service which the Ministry can provide for an important sector of industry is not research and development but intervention with another government department, or a hint to some well-placed merchant bank. It has also become clear that the Ministry cannot hope to cater for the whole of British industry. It has to do some things well, and hope that these will serve as examples to the parts of industry not directly affected. Even with the new accession of strength from the Ministry of Aviation, it will have to remain selective. Mr. Benn must keep a tight rein on his enthusiasm.

He must also reckon that the organization of his resources will take—and will deserve—much of his attention. The laboratories within the Ministry of Technology are not by any means the coherent force they could now become. There needs to be much closer co-ordination of their work, ideally by means of the frequent interchange of people from one place to another. The aim is not to eliminate duplication, which could well be a false economy, but to arrange for the fullest exchange of creative ideas. It should also be made much easier for scientists and technologists in the new organization to move from their laboratories into the universities (and back again) and into administration. Too many of the laboratories seem like backwaters. Providing them with certain obvious common services—economic analysis or a computer centre, for example—would probably pay well, but the over-riding need is an environment in which the members of the Ministry's huge skilled labour force can sense, more clearly than at present, how valuable its work could be to industry.

Deciding how and where to exert the efforts of the Ministry is another problem. One difficulty is that there is no case law to show how best to enact the optimism which sustains the British Government in its view that intervention with public funds can indeed improve the condition of industry. So far, the Ministry of Technology has set a great deal of store by the letting of contracts to companies willing to undertake development in certain fields, and has thus done something to reproduce in Britain some of the industrial benefits of expenditure on defence research and development in the United States. There has been a brave attempt to keep the British computer industry alive and forward-looking. Good works are also now apparent in fields as different as chemical engineering and water purification. Nevertheless, the impression remains that resources are too thinly spread to be really effective. More might be accomplished by the pursuit of excellence in a narrower field—the optics industry, for example.

Already enough is known about the hazards of public sponsorship of industrial innovation to make it clear that technology as such is only half the need. In shipbuilding, for example, the Geddes Committee showed, earlier this year, that reorganization may be more important than the acquisition of new techniques. Elsewhere, British companies are often uncompetitive for lack of an incisive appraisal of the market. This is why Mr. Benn should do more than his predecessor to encourage the spread of new management techniques. Technical retraining is another neglected opportunity. Somehow the Ministry must also win a better economic insight into the condition of technology in Britain, if necessary by letting research contracts to university departments.

To this list of formidable, but mercifully tangible, tasks to be attempted, there must be added the need somehow to bridge the gulf which has grown up, in the past two years, between technology and what is sometimes called pure science. This is in part a consequence of the administrative separation of public support for science and for technology in separate departments of the British Government. No amount of committee work can guarantee good co-ordination, and Mr. Cousins's sensitivity about the autonomy of his department had not helped to foster smooth relations. But the real trouble is that the Ministry of Technology has chosen to give itself a sense of identity by accentuating the distinction between technology and science. As a consequence, the Ministry has done virtually nothing to foster the reform of engineering education in British universities

and technical colleges. Complacency about all things technological has also diverted it from a critical appraisal of the engineering professions, and from the need to pay technologists more money. On the other hand, there has grown up the view that the work of the pure sciences is somehow irrelevant to the progress of technology and the health of British industry. The best that can be said of it is that this opinion is ill-informed. Nobody knows enough to know how best to strike a balance between technology and what is now called science, although there is no question that they are in some sense mutually dependent. The best service Mr. Benn could do his Ministry, and the industry it is meant to serve, would be to convince his acolytes that science and technology are really inseparable parts of the same entity.

DATA FOR PHYSICS

THE Institution of Electrical Engineers is right to ask itself what kind of service *Physics Abstracts* should provide (see p. 112 of this issue), but there is more at stake than mere modernity. The question of whether the world's principal physics abstracting journal should continue to be published from the United Kingdom is also involved. One difficulty is that *Physics Abstracts*, like other abstracting journals, is not a paying proposition. Costs keep rising, and more or less in tune with the steadily increasing volume of publications in physics. At the same time, there is a steady pressure for an ever faster service, which means high capital expenditure on data processing equipment and the like. Yet circulation is linked not with the volume of scientific activity, but with the number of laboratories and libraries, and there are obvious limits to the extent to which it can be taxed. These are the principal reasons why subsidies are necessary. In recent years, American sources have generously helped to keep *Physics Abstracts* on its feet.

More recently, however, there has been a tendency to believe the job could be done more efficiently in the United States. Though the service offered by *Physics Abstracts* has been steadily improving, and though this is now augmented by the new journal *Current Papers in Physics*, there is no question that American experience in running abstracting journals far outstrips that in the United Kingdom. *Nuclear Science Abstracts* and *Chemical Abstracts* are both masterpieces of the application of technology to the processing of scientific information. From time to time there has actually been talk of letting *Nuclear Science Abstracts* grow so as to include the whole of physics, though the prospect of this happening is to some extent restricted by the view of the United States Government that responsibility for nuclear abstracting should belong to some international authority—the International Atomic Energy Agency, for example. In any event, there seems to be an entirely sensible recognition within the community of physicists that an efficient abstracting service must inherently be international. The American Institute of Physics has been particularly keen, in recent years, to help the Institution of Electrical Engineers along by providing timely abstracts of material appearing in its own journals. In a sense, one object of the study on which the Institution of Electrical Engineers has now embarked is to find some way of matching American efficiency with British. With luck, there is no reason why the venture should not succeed, though there are signs that American critics of present arrangements would be more easily satisfied if the Institute of Physics in Britain were more closely concerned in producing *Physics Abstracts*.

Producing abstracts quickly is, however, only half the battle. Knowing how best to use them is another problem which is harder to resolve. The most obvious difficulty is the most serious, for ordinary people find it hard to accumulate information by ploughing through the abstracting journals. This is why large laboratories provided with efficient information services have taken to distributing extracts from the abstracts to those known to have an interest in them. By all accounts, the system works well. It is certain to be imitated as widely as circumstances and machines permit, and there is no reason why even small laboratories should not be helped with selected abstracts by some central service. But how, then, to browse? Evidently there is a need for ways of letting scientists know something of the contents of a wide selection of the literature, and this is where new types of journals may have an important part to play. In all the circumstances, the Institution of Electrical Engineers should not be too dismayed if its enquiries suggest that there is only a limited future for abstracting journals as at present known. It is more important to provide the kind of service which the professionals need.

NO MORE VISITS

THE Society for Visiting Scientists has announced that it will in future not collect scientific periodicals and books and that its present collection will be disbanded This is another step in the slow process by which the Society appears to be being driven out of existence. Ever since it left its cheap-rent premises in Old Burlington Street, the Society has found it hard to make ends meet. In its annual report published in December 1965, the Society said that the "news of our financial situation is bleak. We anticipated no substantial increase in membership involving full subscription, in view of the fact that we no longer are able to provide the club amenities we used to offer". In the event the Society has found that covenants signed by a number of companies several years ago have run out simultaneously. Though some support continues, it looks as if the British Council will be the only enduring source of funds.

In spite of the vigorous programme of lectures and discussions arranged for last year, officials of the Society are inclined to be as despondent as these hard facts suggest they should be. The trouble, of course, is that the Society for Visiting Scientists must be able to help people visit, and for that it needs some premises.

NEWS AND VIEWS

More Oxford Colleges

THE Report of the Franks Commission may already have borne some fruit. The Ford Foundation of America and the Wolfson Foundation in the United Kingdom have between them agreed to contribute a total of more than £4 million to support and endow two graduate colleges at Oxford. This is the largest single sum the University has ever received, and there is some reason to think that the generosity of the two foundations has been stimulated by the argument of the Franks Commission in favour of more postgraduate teaching at Oxford. The two colleges to benefit are St. Antony's, originally founded in 1950, and a new foundation to be called Wolfson College. The latter will concentrate its interests in the sciences.

After the public announcement of the double endowment, Mr. William Deakin, the Warden of St. Antony's, said that he hopes he will be able to increase the number of postgraduate students at the College and the number of scholarships available to people from abroad. The College specializes in studies of Eastern Europe and the Far East, and plans to increase the amount of research in those subjects. The College also needs a new building, having begun its career with the ascetic view that learning comes before living, and having recently found the discomforts of a converted house in north Oxford an impediment to thought, or at least to good administration.

Wolfson College has been endowed equally by the Ford Foundation and the Wolfson Foundation to the tune of roughly £3 million. It will absorb the graduate society hitherto called Iffley College. The intention is that roughly two-thirds of its fellows should be scientists or doctors, though there will be Sir Isaiah Berlin, professor of social and political theory at Oxford, as Master (in effect though not, perhaps, in name) to redress any imbalance there may be. Mr. McGeorge Bundy, now president of the Ford Foundation but until recently Special Assistant to the President of the United States, has let it be known that he hopes that these endowments of Oxford will help to counteract the drift of people from Europe to the United States.

No Immunity against Fall-out

No general methods of immunizing man against the hazards of radioactive fall-out are likely to be forthcoming for many years—if ever. Given as many as possible of the factors relating to a particular case, some remedial measures might now be possible, but even then it would be necessary to balance the anticipated risk from radiation against the risks and social disadvantages that might result from the implementation of particular countermeasures. Such are the conclusions of the Medical Research Council's Committee on Protection against Ionizing Radiation, under the chairmanship of Sir John Cockcroft, in its report *The Assessment of Possible Radiation Risks to the Population from Environmental Contamination* (H.M.S.O., 1*s*. 9*d*.). By predicting the magnitude of the doses that will arise many years hence, and coupling this with long-term studies of the survivors of Hiroshima and Nagasaki and with other data, risk estimates have been extrapolated in terms of the possible incidence of fatal malignancies and certain genetic defects.

As a result of the atmospheric tests of nuclear weapons which had taken place up to the end of 1965, it is estimated that in the United Kingdom, by the year 2000, three additional cases of leukaemia, two of all other types of fatal cancer and two of thyroid cancer per million of the population exposed may, at the worst, have been caused. These figures refer to the increased incidence over and above the natural incidence—the figure for all types of fatal cancer (including leukaemia) in Britain is at present about 2,200 cases per million per year. It has also been estimated that, in the case of the present generation, one additional case of visible genetic abnormality could be caused in the offspring of each million persons exposed. (The natural incidence is about 15,000 per million live births.)

The report directs particular attention to countermeasures against radioactive fall-out. Iodine-131 has a considerable effect on the thyroid glands of infants. Because fresh cow's milk is the dominant route of this isotope to the body, the substitution of dried for fresh milk in the diet of infants would provide a large measure of protection: storage of dried milk would certainly offer a high degree of protection, as the activity of this isotope decreases by a factor of about 15 in every month. High concentrations of strontium-90 have been found in the bones of children between 1 and 2 years old; again, cow's milk is known to be the main source of this isotope. Remedial measures at present under examination are those designed to inhibit selectively the uptake into the body of this isotope. Thus supplementation of the diet by calcium in various chemical forms has been considered. Protection against the genetic effects are more difficult, as these are attributable primarily to external radiation and ingested caesium-137. Except in the early months of life, a large fraction of the caesium-137 that enters the body is contained in components of diet other than milk and, as the Committee emphasizes, any attempt to decontaminate these would involve major dislocation of normal food supplies.

Is a Virus a Virus?

THE World Health Organization has made public a cautionary statement on the detection of viruses associated with leukaemic tissues—human and those taken from domestic animals.

The search for virus particles by electron microscopy has to be considered as a preliminary stage only and the finding of characteristic particles as a useful guide for further biological experimentation. The absence of virus does not exclude the viral nature of the disease and the presence of particles does not necessarily mean that they are causally related to the disease. The assumption that viruses like those of avian and murine leukaemia are responsible for the human disease may not be correct. Other types of viruses or other causative agents may be involved.

If it is assumed, however, that viruses exist in leukaemic tissues from man and domestic animals similar to the viruses associated with mouse and chicken leukaemia, the following criteria for detecting particles which have a high probability of being viruses (based on observations on known leukaemia viruses) should be taken into consideration in using the thin-section and

negative staining techniques for examining tissues, cells, and pellets from associated fluids.

(*a*) *Budding particles.* The maturation (budding) process is known in detail for leukaemia viruses in mice and chickens. Confirmation of similar processes in human tissue would be good evidence of virus formation.

(*b*) *Detached particles.* These particles should possess a dense central region or nucleoid separated by a clear zone from the envelope which should have unit membrane structure. The envelope may or may not exhibit an external fringe. The nucleoid is not uniformly dense and may have a filamentous structure. The boundary of the nucleoid is sharply delimited. The presence of particles possessing a double shell, the inner of which is more electron dense and thicker than the outer, is considered to be of significance. However, the presence of particles consisting of two unit membranes, one inside the other, is not considered significant.

Particles of uniform size and consistent morphology should be present (if only isolated particles can be found identification is less certain).

The characteristics described here are important in distinguishing viruses from secretion granules, specific granules of blood cells, elementary bodies of *Mycoplasma*, etc.

In addition to these criteria, evidence for the presence of nucleic acid (presumably RNA) in the nucleoid by cytochemical methods should be sought and if found would argue strongly in favour of the viral nature of the particles.

Great caution should be exercised in the use of negative staining in the search for virus particles and whenever possible results should be confirmed by studies with thin sections. To be significant a number of particles of uniform size and consistent morphology should be present.

The avian and murine leukaemia virus particles have not so far been shown to possess the characteristic symmetrical structures that allow many other viruses to be easily recognized by this method. Nucleoids can be observed in murine and avian leukaemia virus particles if the particles are fixed before negative staining. The consistent presence of similar structures in human material would be an indication of viral nature.

The 'tails' that may be observed in leukaemia viruses after negative staining are not sufficiently characteristic to be useful as a diagnostic criterion.

This statement is being published both in this journal and in the *Bulletin of the World Health Organization.*

Engineers by Television

A PILOT experiment in the use of television for training professional engineers is to begin in Poland in September. In the first year of the project, four television production centres at Warsaw, Gdansk, Wroclaw and Katowice will broadcast five half-hour programmes each week on the Polish television network. In 1967–68 and thereafter, there will be ten programmes a week covering the material normally included in the first and second years of the Polish engineering curriculum. The pilot scheme is being sponsored by Unesco, and will be conducted in such a way that other authorities wishing to use educational television for professional training will be able to do so. Students wishing to follow the television course in engineering will be required to pass an examination so as formally to enrol and to qualify for paid study leave and the travelling expenses that will from time to time arise in visiting university centres. Formally enrolled students will also have first call on a set of text-books. It is expected that some 9,000 students will be taken on in the first academic year of the project, and that there will be room for 12,000 in later years, when instruction in the core of the engineering course is properly under way.

Enthusiasm for television teaching is clearly as high in Poland as it is wherever else television educators meet. Mr. I. Waniewitz, director of education television in Poland, says that his next objective will be a television course for training teachers. But first there is to be a thorough study and evaluation of the engineering course, not only in educational terms but also from the point of view of those concerned with cost-effectiveness. That could be a valuable piece of work.

Abstracts for Physics

WHAT information do physicists want and what is the best way of making it available? These are the questions that *Physics Abstracts* is trying to answer. The British Institution of Electrical Engineers, which publishes *Physics Abstracts*, is using £54,000 from the Department of Education and Science to carry out a 3 year study into the problems of a physics information service. According to Mr. J. R. Smith, assistant director of the Institute and head of the project, there are really four problems to be solved: (1) What is physics and what fields should it cover? The area that is now covered by the abstracts could easily be doubled. (2) What should be in the literature—should books, theses, and patents, as well as journals, be included? (3) What services do physicists actually need and want? (4) What is the best way of mechanizing *Physics Abstracts* and also turning it into an efficient information retrieval service?

The American Institute of Physics, which works jointly with the I.E.E. on *Physics Abstracts* and is co-operating on the present project, has its own documentation research project and has recently started investigating a "community network for physicists"—the information network from primary journals to the data centres required for disseminating information. The I.E.E.'s study will form a middle section of this overall review.

The British project grew out of a private conference on physics abstracting held in London in October 1964, which concluded that *Physics Abstracts* should be mechanized and that a service for information dissemination and retrieval should be established. To answer these needs, the I.E.E. is attempting to prepare a 'unit record' of each piece of data; each record will contain enough information for a variety of uses, including the present abstract journal and the current-awareness journal, *Current Papers in Physics*—a bi-monthly tabloid giving brief references for nearly all physics literature that will later be abstracted. In addition, the 'unit record' could be used for machine retrieval—straight searches for particular information, for selective dissemination of information, or for printed indexes.

Mr. Smith is hopeful that within a very few years such a mechanized system could be used for the

Physics Abstracts and the *Current Papers* at no more than the present costs; any further uses of the system would be a bonus. While there is no pressing need at the moment for mechanization, Mr. Smith said that at the present 10 per cent growth rate, now 40,000 new abstracts per year, and with a redefinition of physics, within 10 years there would be 200,000 more articles a year, an impossible number to handle by present methods. He is hopeful that when the present study ends in 1967 the Department of Education and Science will agree that a mechanized information system must be established before the present stream reaches flood level.

Parliament in Britain

In the House of Lords on June 30, Lord Byers pressed hard for an immediate further Government contribution towards the cost of the *Atlas* computer at the University of London. Lord Shackleton replied that this had been considered as part of a general review of requirements for computer time in London in the light of the Flowers Report, which the Government had adopted. Until the regional centre recommended by the Flowers Committee comes into operation, the available computer capacity would be short of the stated needs of the schools and colleges of the University of London. While the Government and the University Grants Committee would in principle wish to see the computing power available to the University of London increased, Lord Shackleton firmly maintained that the Government could not provide from available resources an additional grant for this purpose in the current year. He hoped that certain other proposals, including the early introduction of an IBM machine at University College, might ameliorate the situation.

In answering a question from Lord Chorley regarding progress with research into the desalination of sea water on June 30, Lord Shackleton said that the programme being undertaken by the Atomic Energy Authority in conjunction with industry had now been operating a little more than twelve months. Experimental multi-flash distillation facilities were in operation at Winfrith and Harwell and an additional sea-water test establishment would shortly be completed at Troon. All these facilities would be used in efforts to improve design and to reduce capital costs. Recent work on reverse osmosis had opened up the possibility of producing an improved type of membrane which was to be tested, and engineering problems associated with the design and construction of plants using this process would be studied. A new prototype electrodialysis testing unit was nearing completion.

The present programme was based on a planned expenditure of about £1·5 million over 3 years and expansion would depend on the evaluation of the technical results of current work. In this work of the Atomic Energy Authority, research and development and engineering design were very closely integrated, and thus major design studies had been made of desalination plants one order of magnitude greater than built hitherto and the possible combination of such units with nuclear power plants. There were greater hopes that the programme would lead, especially in association with nuclear power plants, to an additional source of water which would be at least as cheap as fresh water, taking into account all the equipment required for pipes and reservoirs. In view of the pressing need for fresh water, however, it was too early to cancel any existing plans.

In moving the second reading of the Overseas Aid Bill in the House of Lords on June 28, Lord Beswick said that the Bill provides authority for the Ministry of Overseas Development to use funds for certain specific immediate purposes, and the opportunity had been taken to rationalize and regularize the authority previously vested in the Ministry of Overseas Development by statutory instruments. The debate followed on one opened by Lord Todd on technical assistance to developing countries in January, and Lord Beswick affirmed the Government's belief that both Government aid and private investment had important parts to play in developing countries and that private investment was directly dependent on the creation of an infrastructure of the basic public utilities.

Total British aid had increased from £81 million in 1957–58 to £151 million in 1960–61, £191 million in 1964–65, and was expected to exceed £200 million in 1965–66; in the current year the target was £225 million. The cost-effectiveness of aid was secured by the greater co-ordination under a single responsible Ministry and by a careful scrutiny of the development programmes of individual countries. The policy of devoting a large proportion of available resources to technical aid should also assist. Dispersements on technical assistance had risen from £25 million in 1962 to £32 million in 1965. Lord Beswick also referred to the increase in contributions to multi-lateral agencies. The clause relating to the Indus Basin Development Fund Agreement was specially welcomed in the debate. The Bill was generally welcomed.

University and College News:

Battersea College of Technology

Dr. K. E. Puttick, reader in metal physics, has been appointed to the second chair of physics as from June 1.

Cambridge

The following have been appointed to the three newly established chairs of engineering: Prof. J. H. Horlock (at present professor of mechanical engineering in the University of Liverpool); Prof. L. E. Goodman (at present head of the Department of Civil Engineering and Hydraulics in the University of Minnesota); A. H. Beck (at present reader in electrical engineering in the University of Cambridge).

Manchester

Prof. D. H. Valentine, at present professor of botany in the University of Durham, has been appointed professor of botany and director of the Experimental Grounds in the University of Manchester in succession to Prof. C. W. Wardlaw. When Prof. Valentine joined the University of Durham in 1945 as reader in botany, the Department had almost no accommodation, one lecturer, a research fellow and a few students. It grew slowly but steadily; in 1950 Valentine became its first professor and his first research student began her work. This led to a tradition of experimental taxonomy which was to give the Department a firm and well-merited reputation. His own work in this field, with its emphasis on evolutionary significance rather than mere classification, is well and widely known, and many botanists will think that it is fortunate that he is taking a prominent part in the production of *Flora Europaea*. Many of his former research students occupy university posts in various parts of the

world. The vigorous and forthright manner of argument engendered by Prof. Valentine's tendency towards dogmatism has enlivened many discussions in colloquia, at society meetings, and over the departmental tea-table. This is very much relished by students at all levels, who find him a very stimulating personality. With university expansion following the usual pattern, the Department of Botany at Durham had to be content to occupy accommodation left vacant as the non-biological sciences moved into new buildings. But Prof. Valentine has ensured that the Department has maintained a proper growth-rate. As new teaching posts were created, its interests and research activities widened greatly, and he will leave behind him a flourishing and lively Department. He will be much missed.

Massachusetts Institute of Technology

DR. JULE G. CHARNEY, one of the world's leaders in the application of computers to weather analysis, has been appointed Alfred P. Sloan professor of meteorology at the Massachusetts Institute of Technology. He will be the first to occupy this new chair, established through a fund pledged by the late Alfred P. Sloan, jun., before his death early this year. Dr. Julius A. Stratton, retiring president of the Massachusetts Institute of Technology, has been elected a life member of the Corporation, the governing body. Dr. Stratton has been an *ex-officio* member of the Corporation since 1951, when he became vice-president. He became acting president in 1957 and president in 1959. Prof. H. C. Hottel has been appointed the first Carbon P. Dubbs professor of chemical engineering.

Sheffield

WHEN Prof. G. Porter vacates the Firth chair of chemistry on August 31 the Firth professorship will cease to be linked specifically to one chair of chemistry, and a Firth visiting professorship in the Department of Chemistry will be established. This will enable distinguished chemists to be invited to participate in the work of the Department. The following have been appointed to chairs: Dr. G. W. Greenwood (metallurgy); Dr. T. R. Kaiser (physics); Dr. A. S. C. Lawrence (personal chair in the Department of Chemistry).

Sussex

DR. A. M. UTTLEY, at present superintendent of the Autonomics Division at the National Physical Laboratory, has been appointed research professor of experimental psychology in the University of Sussex, and he is to lead an important scientific project concerned with the application of pattern recognition theory to our understanding of the organization of nerve cells in the brain by using a computer to set up simulated models. The work is to be supported by a Science Research Council grant of £72,000 over a period of 5 years, and Dr. Uttley has also been awarded a grant by St. Dunstan's of £7,000 per annum for 5 years, to support research on reading aids for the blind. In addition, the Carnegie Institution of Washington has made a grant of £9,000 in support of Dr. Uttley's research work. The Science Research Council has also made an award of £19,000 to Prof. N. S. Sutherland, head of the Laboratory of Experimental Psychology at Sussex. He will be comparing models of perception and learning with data derived from psychological studies of human and animal subjects.

Announcements

DR. HENRY B. BIGELOW, founding director of the Woods Hole Oceanographic Institution, has been awarded the Compass Distinguished Achievement Award at the annual meeting of the Marine Technology Society in Washington, D.C. Dr. Bigelow has served as president of the Corporation of the Institution, as chairman of the board of trustees and as curator of the Museum of Comparative Zoology at Harvard University; he is now an honorary trustee of the Oceanographic Institution and professor emeritus of zoology at Harvard. The Award is given by the Marine Society "for significant contributions to the advancement of the science and engineering of oceanography and marine technology".

R. D. GILLARD has been awarded the Meldola Medal for 1965 in recognition of his work in the field of inorganic chemistry, with special reference to the chemistry of transition-metal complexes and their optical activity

DR. TOBIAS LASSER, director of the Botanical Institute, Caracas, Venezuela, was confirmed as president of the Association for Tropical Biology for 1966–67 at the meeting on the Biota of the Amazon Basin, held in Brazil during June 6–11. Dr. A. C. Smith of the University of Hawaii was elected president-elect.

SHELL research fellowships have been awarded to Dr. R. B. Homer, University of East Anglia, to support his research on the interaction of small molecules with biopolymers, and to Dr. Margaret Wright, University of St. Andrews, for kinetic and equilibrium studies of the hydrolysis of biologically important compounds; and some aspects of enzyme kinetics relevant to haemolysis. These awards are worth £1,300 per annum and are tenable for 2 years from October 1966. They are the first to be made from Shell International Petroleum Company's new series of post-doctoral research fellowships.

THE annual report of the Smith Kline and French Foundation for 1965 says that grants made by the Foundation in the first 4 years of its existence amount to £103,347. The Foundation specializes in small grants (less than £2,500 each) for research in medicine and related fields. Further information can be obtained from the secretary, Mr. A. T. Cousins, Mundells, Welwyn Garden City, Hertfordshire.

AN exhibition to commemorate the centenary of the second term of office of Sir Joseph Whitworth, arranged by the Institution of Mechanical Engineers in conjunction with the Whitworth Society, will be held at the Institution of Mechanical Engineers during July 19–August 26. Further information can be obtained from the Secretary, Organizing Committee, the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1.

A SUMMER institute on "Non-Linear Effects in Plasmas" will be held in the University of Paris during September 5–23. Further information can be obtained from Dr. Kalman, JILA, University of Colorado, Boulder, Colorado.

A JOINT meeting of Commissions II and IV of the International Society of Soil Science on "Soil Chemistry and Fertility" will be held in King's College, University of Aberdeen, during September 5–10. Further information can be obtained from Dr. J. Tinsley, Department of Soil Science, University of Aberdeen.

THE annual symposium on "Molecular Structure and Spectroscopy" will be held in the Department of Physics, Ohio State University, during September 6–10. Further information can be obtained from Prof. K. Narahari Rao, Department of Physics, Ohio State University, 174 West 18th Avenue, Columbus, Ohio.

AN advanced study institute on "Symmetry Principles in Elementary Particle Physics", under the directorship of Prof. B. Kursunoglu, will be held at the Cekmece Nuclear Research Center in Istanbul during August 8–27. Sponsored by the North Atlantic Treaty Organization, the summer institute will be directed primarily to advanced graduate and postgraduate students. Some funds will be available for those unable to obtain complete financial support elsewhere. Further information can be obtained from Prof. A. Perlmutter, Center for Theoretical Studies, University of Miami, Coral Gables, Florida.

THE SHORT HISTORY OF THE MINISTRY OF TECHNOLOGY

THE British Ministry of Technology was created in October 1964, after the General Election in that year. The plan for such a Ministry was itself an important part of the discussions within the Labour Party in the months preceding the election. The chief argument in its favour was that the government of a modern industrial nation requires a concentration of power in an organization capable of initiating change. To begin with, critics of the then arrangements for sponsoring and fostering industrial development and research were able to point to the diversity of the interests concerned—the Department of Education and Science, the Ministry of Aviation, the Board of Trade, the Ministry of Power, not to mention a number of independent public bodies such as the United Kingdom Atomic Energy Authority. The first objective, then, was to unite as many as possible of these entities in one organization.

In the event, the Ministry of Technology created in October 1964 was less grand. In the arrangements for organizing civilian science and technology, the Department of Education and Science has retained responsibility for the research councils whose chief function is to support scientific research in universities and elsewhere, but which also carry out some work of their own. (Thus the Science Research Council is directly responsible for running the Radio and Space Research Station at Slough.) The Ministry of Power remains formally responsible for fostering scientific research in power generation, while, for most of the past twenty months, the Ministry of Aviation has retained responsibility for a number of research establishments, some of which are directly concerned with defence and others with more civilian developments, and has thus been an ironic reminder that some of the earliest plans for the Ministry of Technology assumed that it would be constructed around the Ministry of Aviation.

To begin with, the Ministry of Technology was made responsible for ten research stations transferred from the old Department of Scientific and Industrial Research (Table 1), together with the United Kingdom Atomic Energy Authority. At the same time the Ministry of Technology was made responsible for the co-operative research associations, which were again transferred from the Department of Scientific and Industrial Research and which are financed partly by government grant and partly by contributions from the industries concerned. The Ministry of Technology was also, from its creation, given responsibility for the National Research and Development Corporation, whose job it is to invest public money in the development of new processes and devices (the most profitable of which has been the Bailey bridge and the best known the Hovercraft). The Ministry of Technology became formally responsible for these various institutions within a few months of the creation of the Ministry; the transfer of power was complete by the beginning of April 1965.

The Atomic Energy Authority consumes roughly ten times as much money as the laboratories and engages not merely in research and development but also in the production of nuclear materials and the processing of spent uranium fuel. Since the inception of the Ministry of Technology it has been assumed that the Atomic Energy Authority will somehow diversify its activities, taking on pieces of research and development on behalf of industry. As yet, there is little to show how successful this policy has been.

With its creation, the Ministry also assumed responsibility for the industrial research associations, now forty-eight in number and requiring annual expenditure

Table 1

	1966–67 Cost (£)	No. of people
Building Research Station	936,000	693
Fire Research Station	187,000	149
Forest Products Research Laboratory	239,000	177
Hydraulics Research Station	257,000	285
Laboratory of Government Chemist	570,000	395
National Engineering Laboratory	940,000	795
National Physical Laboratory	2,216,000	1,661
Torry Research Station	229,000	198
Warren Spring Laboratory	505,000	398
Water Pollution Research Laboratory	228,000	171
	6,307,000	4,872

estimated to amount to £3,570,000 in 1966. In addition, the Ministry is reckoning to spend £750,000 on research and development contracts, a roughly similar amount on a scheme to purchase from industry advanced machine tools and other devices where it is thought that development is held up for lack of customers, £700,000 on advice for industry, £290,000 in launching the National Computing Centre, and £400,000 on support for the British Standards Institution.

As yet, it is not clear how much of the work of the Ministry of Aviation will be transferred to the Ministry of Technology in the autumn of this year. One of the first tasks to be tackled by the new Permanent Secretary to the Ministry of Technology, Mr. Richard Clarke, will be to work out a rational division of the Ministry of Aviation. Some of the defence research establishments will no doubt be transferred to the Ministry of Defence, but it seems clear that the Ministry of Technology will also grow in the process. Certainly its capacity for research will be strengthened in solid-state physics, electronics, and aerodynamics.

In its responsibility to Parliament for the National Research and Development Corporation, the Ministry of Technology has been able to sponsor an increased budget. In the year ahead it plans to invest a further £5 million of public money in the work of the National Research and Development Corporation. At the same time it has been agreed that the Corporation should write off some £2·5 million of interest accumulated since 1947 on capital borrowings from the Treasury and unlikely to be paid in the near future. To the extent that this step represents an admission that an organization instructed to take chances on research and development projects cannot always make a profit, it is likely that the Corporation will now feel more free than it has done to engage in risky enterprises.

Apart from its direct responsibility for institutions like these, the Ministry of Technology has an avuncular relationship to British industry as a whole. Much of the energy of its officials has gone into detailed enquiries into the state—technical and economic—of particular sectors of industry. There have, for example, been enquiries into machine tools and optics. The Ministry is not merely concerned to find out; it also hopes that the process of enquiry will stimulate industries to seek remedies for problems which become apparent.

In recent months the Ministry has been particularly active internationally. For one thing, it is trying to introduce British manufacturers to standards (of measurement and of specification) in use elsewhere in the world, in the hope of making exports easier to come by. It is also spending an increasing amount of its energy in dealing with the difficulties which beset British manufacturers wishing to sell things abroad, sometimes using its influence to persuade another department of the British Government to remove some fortuitous obstacle to trade.

CONTINENTAL DRIFT, MESOZOIC CONTINENTS AND THE MIGRATIONS OF THE ANGIOSPERMS

By DR. R. MELVILLE
Royal Botanic Gardens, Kew

THE origin of the flowering plants, how they came to appear relatively suddenly in the northern hemisphere in the Cretaceous and how they attained their present distribution are different aspects of one of the outstanding problems of biology. In recent decades it has become increasingly evident that the angiosperms must have had a long evolutionary history before the Cretaceous. Hawkes and Smith[1] recently supplied evidence favouring the existence of the genera *Gossypium*, *Bromus* and *Solanum* early in the Cretaceous and suggested an origin much earlier and probably on the former continent of Gondwanaland. Stebbins[2], Thomas[3] and Camp[4] have reached similar conclusions from studies of living angiosperms, and Plumstead[5] has reviewed the fossil Glossopteridae, which dominated the flora of Gondwanaland, and discussed the evidence linking them with the angiosperms. In evidence drawn mainly from floral vascular systems, I have proposed[6–8] a new theory of floral evolution, which indicated the Glossopteridae as the probable ancestral group of the angiosperms. The problem is too complex to be solved by any single line of evidence, but requires the correlation of data from many fields including taxonomy, phylogeny, palaeobotany, palaeogeography, geomorphology and palaeomagnetism.

The critical period for this study extends through the Jurassic and Cretaceous when Mesozoic land masses were breaking up before their rearrangement into the present continental system. Before this upheaval, during the Palaeozoic and the early part of the Mesozoic, four great floral regions can be distinguished. Three of these were in the northern hemisphere and the fourth occupied the former Gondwana continent, which endured in the south from about the Silurian until it began to break up in the Jurassic. Palaeobotanical evidence does not indicate any contact between Gondwanaland and the northern continents during the whole of this long period[5,9]. Recent palaeomagnetic research has provided independent evidence for the continued existence of the Gondwana continent at this time[10].

By the Carboniferous, the Glossopteridae had developed as the characteristic flora of Gondwanaland. In the northern hemisphere, the coal flora occupied western Europe and eastern North America, which together formed a continent centred over the North Atlantic, which may be called Atlantica. A second distinctive flora, the *Gigantopteris* or Cathaysian flora, occupied a large part of China and South-east Asia and also the western part of North America[9]. These regions formed a continent centred over the North Pacific which may be called 'Pacifica'. In the region between, the Angara flora occupied an area covering Siberia southwards to about the present 40° parallel of latitude. At a later epoch the orogenesis which split up and dispersed Gondwanaland also cleft in two both Atlantica and Pacifica. Western Atlantica drifted into contact with Eastern Pacifica to form the present North American continent. The other two fragments, Eastern Atlantica and Western Pacifica, contributed to the modern Eurasiatic landmass. We must now seek evidence for the boundaries of these ancient continents and their positions at the beginning of the orogenesis.

Geological discontinuities along the sites of ancient geosynclines mark the boundaries of the former continental fragments, which came together to make the present continents. The distribution of earlier floras can be traced from the fossil record and can be correlated with the geological evidence to make a reconstruction of the former continents before their rearrangement. Following this line of research, we find that fossil Glossopteridae occur in South America only south of the Amazon, so that only the southern portion can have formed part of Gondwanaland. The Amazon itself is no ordinary river. Although it runs for more than 2,000 miles through country not more than 36 ft. above sea-level its channel is broad and deep. At 500 miles above Para the river is still 348 ft. deep[11]. These unusual features can be accounted for if the river follows a rift where continental blocks came together.

Crossing the Atlantic to the African shore, a deep trench is found in the seabed in latitude 8–9° N. off Sierra Leone[12]. This probably marks the junction between the north-west portion of Africa, which yields no *Glossopteris* fossils, and the remainder of the continent which formed part of Gondwanaland. From 10° N. on the coast, the boundary of the old Gondwana block skirts around the uplands of Futa Jalon and then follows roughly the line of the Niger to Timbuktu. Thence it runs northwards to skirt around the Ahaggar massif and follows the line of the high ground around to the mouth of the Nile. Arabia belongs with Africa to Gondwanaland, and the boundary then follows the coast of the Mediterranean to the head of the Gulf of Iskenderun. From this point it turns eastwards, following the southern boundary of the Anatolian Mountains and finally south-eastwards along the edge of the Zagros Mountains to the Persian Gulf.

Passing eastwards, India forms the next Gondwanic block. The boundary of the *Glossopteris* flora has been discussed by Sahni[13]. From Karachi it follows the valley of the Indus to the Himalaya, then along the line of the Himalaya to about long. 100° E., lat. 30° N. From this point it turns southwards along the line of the ranges between the Salween and Mekong rivers to Malaya. Sahni took the boundary along the spine of Malaya, but was uncertain whether or not to include Borneo as part of Gondwanaland. Some fossil Glossopteridae have been found in Borneo[9] which forms part of the Oriental faunal region[14]. The Wallace line, which passes along the Makasar Strait between Borneo and Celebes and through the deep strait between Bali and Lombok, marks the boundary of two distinctive faunal provinces. From what follows in this article, it is probable that it also is part of a Gondwanic boundary, the islands to the west, Sumatra, Java, Bali and Borneo, with the whole of Malaya forming part of the Indian block of the Gondwana continent.

The whole of the Australian continent belongs without question to Gondwanaland, but doubt remains about

New Guinea. The similarities between the floras are too slight for New Guinea to have been associated with Australia for a long period, as pointed out by Good[15]. It has been excluded in the present reconstruction except for the Merauke Ridge. The presence of the Upper Gondwana flora in New Zealand indicates its origin and with it must go the extensive ramified block on which it stands, the greater part of which is now submerged. The associated islands include New Caledonia, Fiji and Samoa, and to the south, Macquarie and Campbell. Finally, the whole of Antarctica belongs to Gondwanaland; fossil Glossopteridae are known from both east and west provinces[5].

The present positions of the fragments of Gondwanaland are shown in Fig. 1. Because the boundary line has been determined primarily from the distribution of the Glossopteridae, it will be convenient to call it the 'Glossopterid Line'. The general trend of this line is confirmed by the distribution of other fossil and modern gymnosperms. Florin[16] has established that different groups of gymnosperms occupied the northern and the southern (Gondwana) continents in the Palaeozoic and Mesozoic up to the end of the Jurassic. Taking Podocarpus as an example of the southern group, all its fossils are south of the line, and the living species only transgress the line in South-East Asia and in the North Amazonian province of South America and Central America, where they have migrated since the beginning of the Cretaceous. Conversely, fossil pines (*Pinus*) are only known north of the line and the modern species only cross the line into Syria, and small areas in Burma and Sumatra.

Any reconstruction of the Gondwana continent should take into account the physiography of the ocean basins and palaeomagnetic and biological data. In the solution offered by King and Downard[17], India is not in a position consistent with drift along the line of the two great transcurrent faults of the Maldive–Laccadive and Ninety-East degree. They also place the New Zealand block in contact with Eastern Australia, a position inconsistent with the absence of the large Australian genera *Eucalyptus* and *Acacia* from New Zealand. Creer's partial reconstruction[10] based on palaeomagnetic data also requires adjustment in the light of other evidence, while that of Ahmad[18] conflicts with the position of ocean rises and the Carboniferous glaciation. The mid-oceanic rises may be used as the starting-points for the reassembly of the dispersed fragments. The Gondwanic portions of Africa and South America can be fitted along the South Atlantic Rise in the positions worked out by Bullard *et al.*[19a]. The position of the combined block must have been somewhat to the south before the break-up of Gondwanaland and in contact with Antarctica. Menard's map of the Pacific basin[20] shows some remarkable coincidences between the shape of the submerged continental platform of east Antarctica and that of the Pacific–Antarctic Rise, which fixes a position for Antarctica. This would bring the coast east of the Weddell Sea in contact with Africa. The Malaysian part of the Indian block must have been in contact with peninsular India. The two parts fit together along the Ninety-East fault if the Malayan peninsula is flexed at its narrowest point to straighten it. By moving the Indian block so assembled southwards along the Ninety Degree and Maldive–Laccadive faults, it fits into the arc of the South Indian Rise in proximity to the new position of Antarctica. No palaeomagnetic data are available to check the position of India, but Irving and Green[21] have given a pole position for the older volcanics of Victoria, estimated to be of older Pliocene age, which would bring that part of Australia over the Indian–Antarctic Rise and considerably to the west of its present position. For this to be possible in relation to the remaining parts of Gondwanaland, Australia would need to be rotated through a right angle into the position shown in Fig. 2. This would imply very little change in latitude for Western Australia since the Jurassic, but considerable changes in latitude and climate for Eastern Australia. Such a sequence of events is consistent with the high proportion of endemics in the Western Australian flora and the survival of many relatively primitive forms in that area. Finally, the New Zealand block must have been to the south-east along the Pacific Antarctic Rise opposite Eastern Antarctica. Palaeomagnetic evidence is unfortunately wanting for this area.

The extent and direction of the Carboniferous glaciation as recorded by Holmes[22] may be used as an independent check on the validity of the reconstruction. With the proposed assembly the glaciated area would be nearly circular and the directions of the ice flow (Fig. 2) are in good agreement. The positions for Creer's South American poles 14 and 15 (ref. 10) lie close to the apparent geographical pole and are consistent with the reconstruction.

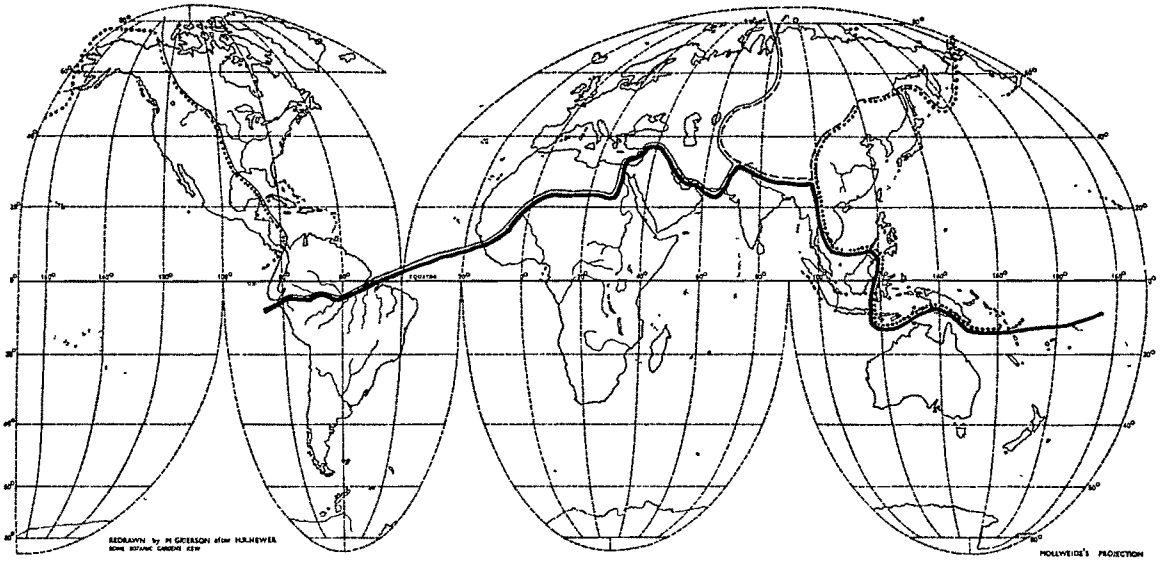


Fig. 1. Palaeozoic floral regions. Heavy line, the glossopterid line marking the northern boundary of the fragments of Gondwanaland; thin line, boundary of the Carboniferous coal flora; dotted line, boundary of the *Gigantopteris* flora; broken line, boundary of the Angara flora

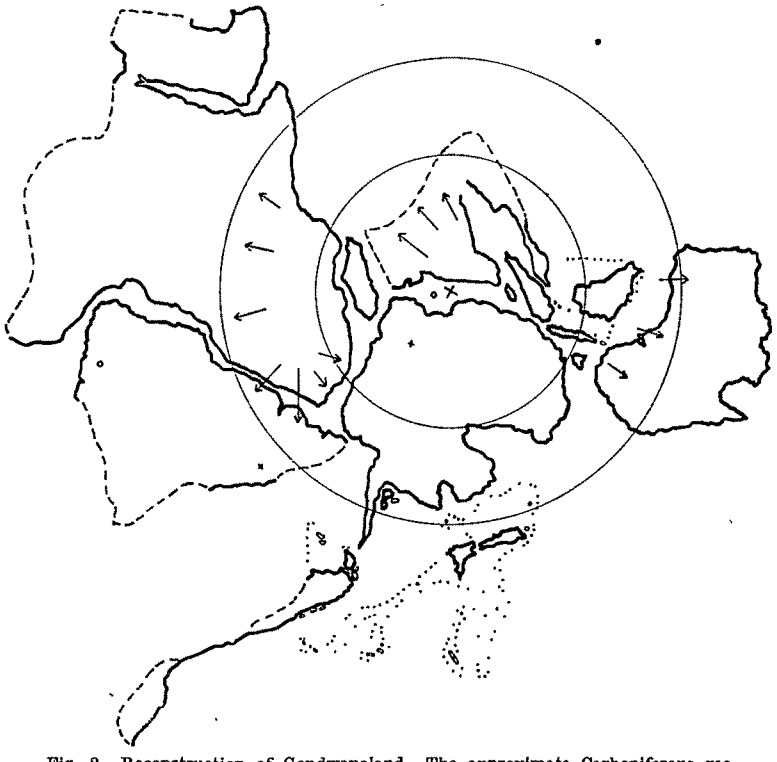


Fig. 2. Reconstruction of Gondwanaland. The approximate Carboniferous geographical south pole is marked, with the Antarctic circle and 50° circle of latitude which nearly coincides with the limits of the Carboniferous glaciation in South America, Africa, and Australia. Creer's South American palaeomagnetic poles Nos. 14 and 15 are shown with their conjugate points. Dotted lines, continental shelves; broken lines, severed continental boundaries; arrows, direction of Carboniferous iceflow

Turning now to the northern hemisphere, the fit of the two sides of the North Atlantic as calculated by Bullard[19a] can be accepted, except that further consideration must be given to the non-Gondwanic portions of Africa and South America. There is reason to think that north-west Africa formed part of Atlantica, as Wilson[19b] has directed attention to the fact that Hercynian fold belts of this region complete the southern part of the Newfoundland—Virginia belt of the same age. No such guide is available for the north Amazonian block of South America. Palaeomagnetic data are lacking for both areas. No fit can be made between the Caribbean shores of the Americas as they exist at present. Before the reconstruction of Atlantica can be attempted the part of North America which before the Jurassic formed part of Pacifica must be removed. The boundary as indicated by fossils of the *Gigantopteris* flora[9] runs southwards from the mouth of the Mackenzie River skirting east of the Rockies to a point on the Gulf of Mexico west of the Mississippi delta (Fig. 1). The presence of marine deposits of Permian to Jurassic age in the Mackenzie basin is consistent with this boundary[19b], which is confirmed by Schuchert's palaeogeographic maps for these periods[23]. The low-lying Florida peninsula formed of Cretaceous and later sediments did not exist at the period with which we are concerned. Jurassic seas pierced the central American isthmus in two places and an arm of the Jurassic sea must have penetrated as far as the Cape Verde Islands to account for the only marine Jurassic deposit along the whole of the Atlantic[19b]. Probably block faulting across the south-western part of Atlantica was responsible for these effects, separating the Greater Antilles, which can be fitted neatly along the northern coast of South America. If, now, the North Amazonian block is moved into position against North America, Atlantica will be complete.

By the end of the Jurassic the South America–African block must have drifted into contact with south-eastern Atlantica in the position shown in Bullard's reconstruction. A large bay then separated south-west Atlantica from South America, the eastern end of which is shown in Bullard's reconstruction as an anomalous triangular gap.

Little attention has been given to the possibility of a mid-Pacific continent in the Mesozoic, but the evidence for the existence of Pacifica is of the same kind as that for Atlantica. In both cases the present oceans are flanked by continents bearing the severed halves of a Palaeozoic flora—respectively the *Gigantopteris* and the coal floras. In both there is a mid-ocean rise which marks the line of the upwelling convection current that caused the severance of the former continents, that for the Pacific being called the Darwin Rise by Menard[20] to distinguish it from the East Pacific Rise. Over the significant areas of both oceans no fossils or datable material of pre-Cretaceous age have been found[19b,20]. Much evidence from geological coincidences[19d] and palaeomagnetic measurements[19c] supports continental drift in the Atlantic. The evidence for the Pacific is much less complete, primarily because far less attention has been given to it.

Two periods of regional metamorphosis are reported for Japan[19e], 200 and 100 million years ago, which would correspond with block faulting in the Permian and later volcanism accompanying drift in the Cretaceous. Western North America was geosynclinal in the late Mesozoic[19f] when drift was closing the gap between the western and eastern parts of the continent. Palaeomagnetic results for western North America are discordant with those from the east and indicate a former separation. No palaeomagnetic data for the Mesozoic of China have come to my notice, but the poles calculated by Deutsch and Watkins[24] for the Triassic of the Tunguska River region of Siberia indicate a position of the Angara block 25° south and 87° to the east of its present position. For this to be true, the Angara block must have been in contact with Cathaysia at that time, when the present coast of China was in the middle of the Pacific. The augmented Pacific continent must have been in existence at least from the Triassic until it broke up early in the Cretaceous, but the Cathaysian core extended back to the Carboniferous.

An attempt can now be made to reconstruct Pacifica. The fragments can be assembled along the line of the Darwin Rise in its present position. No difficulty arises in placing the western American portion, but the island arcs off the eastern coast of Asia must be closed up to form a compact mass against the continental shelves. In Fig. 3, the deeps of the enclosed seas of Okhotsk, Japan, and East and South China are eliminated and the peninsulas of Kamchatka and Korea have been folded against the continental margin; Borneo and the parts of Malaysia which do not form part of Cathaysia have been removed. New Guinea, which floristically and geologically is distinct from Australia[15], has been placed in a position to fit the curvature of the Darwin Rise, and so complete the continent.

From the Jurassic to the Cretaceous another land mass existed in the South Pacific. This consisted of the New Zealand block together with Antarctica assembled along the line of the South-east Pacific Rise in the position already indicated for the reconstruction of Gondwanaland. In addition, it is probable that the Andean provinces of South America, embracing Peru, Bolivia, Chile and Western Argentina, south of the Gulf of San Jorge with

Fuegia and the Falklands, were situated along the eastern edge of the rise. The eastern boundaries of these provinces are marked by a line of marine Jurassic sediments[25]. Allowance has been made in the reconstruction for the displaced section of the rise south of the Easter Island fault. Palaeomagnetic data are wanting for this South Pacific continent, but it is supported by the distribution of numerous plant genera and species and by the absence of Carboniferous glaciation in Patagonia.

For the biologist, the timing of the various episodes in the break-up of the Mesozoic continents is of the utmost importance. The moment of severance limits the migration of land organisms by normal methods and must be a major factor in determining modern distributions. Evidence for stages in the break-up of Atlantica is more complete than for any other continent. The initial faulting which demarcated Greenland occurred in the Carboniferous[19g]. Marine invasions in this area began in the upper Permian and continued with recessions through the Triassic. Progress of the rift was slow until the beginning of the Cretaceous and then proceeded rapidly southwards along the North American coast.

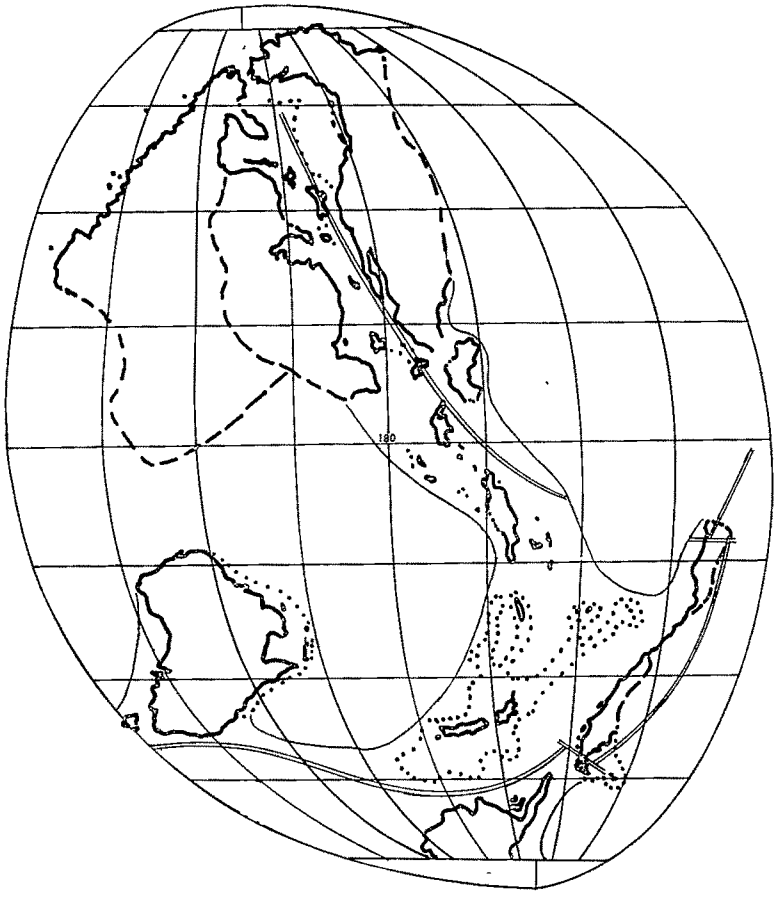


Fig. 3. The central Pacific continent, Pacifica, at the close of the Jurassic. A thin line marks the approximate limits of the isthmus joining Pacifica to southern Gondwanic lands. Double lines, the position of the Darwin and East Pacific Rises; dotted lines, continental shelves; broken lines, severed continental boundaries

Africa and South America began to drift away from the remainder of Gondwanaland in the upper Permian according to palaeomagnetic evidence[10]. Assuming this date to be accurate, the general trends of evolution in the angiosperms must have been determined by this time although their fertilization mechanism may still have been gymnospermous. Progenitors of such families as Proteaceae, Leguminosae and Restionaceae must have been dispersed through both African and Australian provinces of Gondwanaland to account for their modern distribution. The African province drifted towards Atlantica and probably made contact with south-east Atlantica during the Jurassic. Faulting along the line of separation between Africa and South America must have then been far advanced, as the separation followed rapidly on that of Europe and North America and was completed early in the Cretaceous. It is probable, therefore, that Africa was in contact with Atlantica for a large part of the Jurassic, time enough for a considerable migration of angiosperms before the Atlantic Ocean developed.

The faulting which heralded the break-up of Pacifica—the rift valley phase—was probably well advanced by the late Triassic. It was accompanied by metamorphosism in the Japanese region and followed by a second phase of metamorphosism in the late Cretaceous[19e], which must have marked the final stages of the drift. The uprising convection current under the continent raised the land surface 2–3 km along the line of the Darwin Rise. At the southern end this caused the formation of a land-bridge between Pacifica and the New Zealand block, linking the regions now forming New Guinea, New Caledonia and the western fragments of South America which then formed part of the southern Pacific land mass. This land bridge must have formed early enough to allow a few representatives of the *Glossopteris* flora to reach Kansu in the Triassic[9]. It was the major link between Gondwanaland and the northern continent and accounts for the high concentration of relatively primitive angiosperms which still survive in south-east Asia and New Guinea and New Caledonia. Contact between north and south continued longer before the drift started than that between Africa and Atlantica, but the onset of drift was probably early in the Cretaceous to account for sedimentation in the Pacific basin and the ages of atolls and guyots reported by Menard[20].

The next phase in the disruption of Gondwanaland was the development of the mid-Indian rise which continued south-eastwards through the junction between Australia and Antarctica. Evidence to date the northward drift of India is scanty, but it may have started in the mid-Cretaceous, and contact was made with the Angara–Cathaysia block by the Palaeocene, though folding of the Himalaya continued into the Oligocene[19b]. The drift of India was relatively rapid and, in its passage north, the resulting climatic changes caused the almost complete annihilation of its rich gymnosperm flora[18]. Perhaps for the same reason, India appears to have made a smaller contribution to modern floras than its size would warrant.

Concurrently with the drift of India, Australia was pushed eastwards along the line of the Diamantina fracture zone[19f]. The centre of activity moved eastwards along the Indian–Antarctic rise, driving eastern Australia northwards late in the Cretaceous. The combined movements caused the rotation of Australia, bringing it into contact with New Guinea which, by this time, had drifted to the south-east of its present position. New Guinea was forced northwards and the adjacent island arcs were curved into their present positions. The rift continued eastwards along the line of the Pacific–Antarctic Rise and then north-eastward along the East Pacific Rise, separating the New Zealand block from Western Antarctica and from the Chilean and Peruvian fragments of South

America at the end of the Cretaceous or Palaeocene. The final stages of the movement caused the raising of the Andes in the Miocene.

Before the break-up of Gondwanaland, continuous land connexion existed between India, Antarctica and eastern Australia (Fig. 3). With the development of the East Pacific Rise, the gap between Australia and New Zealand across the Ross Sea area must have been bridged. It is evident that during the Cretaceous the circum-Antarctic land bridge postulated by plant geographers from Hooker[26] to van Steenis[27] actually existed. The reason for such sub-Antarctic distributions as that of *Juncus scheuchzerioides* in Kerguelen, Tasmania, New Zealand, Fuegia and the Falklands is now clear. The separation of the New Zealand block from the Chilean fragments of South America was late enough to account for the identity of the small number of *Hebe* species in the latter area with their New Zealand congeners. The genus *Nothofagus*, at present with a disjunct distribution in Tasmania, Eastern Australia, New Guinea, New Caledonia, New Zealand, Chile and Western Antarctica (fossil), in the Cretaceous occupied a single land area. *Coriaria*, now inhabiting New Zealand, South America and China with one species in Europe, was able to migrate from its Gondwanic home to China in the early Cretaceous, and, after drift, across to Europe. Pleistocene glaciation probably accounts for its absence now in the rest of temperate Asia. The links between the floras of Japan and South America discussed by Maekawa[28] can be explained on the basis of pre-existing land connexions. So also can other distributions mentioned by Camp[4], and it may be found profitable to compare the maps published by Vester[29] with the disposition of land in the Jurassic and Cretaceous as revealed by the present synthesis.

Although parts of these reconstructions may appear to be somewhat speculative, every position mapped rests on evidence from two or more disciplines. It is hoped that the attention drawn to gaps in our knowledge will lead to further research on related problems. I am grateful to Dr. E. P. Plumstead for discussing the distribution of the *Glossopteris* flora and to colleagues at Kew and the British Museum for their interest and encouragement.

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(*e*) Sutton, J., 107;
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BOOK REVIEWS

UNBRIDLED RACISM

Racial Contours

The Factor of Race in Human Survival. By H. B. Isherwood. Pp. 423. (Douglas, Isle of Man: Times Press, Ltd., 1965.) 18*s*.

READING this book is a disheartening experience. It is scarcely credible that such congeries of misrepresentation, warped judgment, and just plain gibberish can be published in the Age of Science, and in Great Britain rather than in some underdeveloped country. Here are some samples: ". . . we have no alternative but to assume that from the statistical analysis there are fewer genes (or less complex gene combinations) in the lower primates than in, say, the Australoids, even though they may have the same number of chromosomes, that also there are fewer genes in the lowly Australoid than in, say, the Negroid, and that there are fewer genes in the Negroid than in the Mongoloid". The blue-blooded Nordics must be brimming with genes. This does not make them safe from dangers, because "Hybrids do not breed true and by miscegenatic multiplication inferior variants would be thrown off and the superior race as a biological entity would lose its place in the evolutionary scramble. In short, exogamy indulged in by a more advanced people inevitably tends to impair the intrinsic quality of that people. About this there can be no dispute". These, and many other equally "indisputable" facts, are the basis of many pages of unbridled racism. Pages 148–178 are an antisemitic tract, complete with the story, "believed by the learned as well as the ignorant", that Jews used the blood of children of other races in their paschal celebrations, and the *Protocols of the Learned Elders of Zion*. The political conflicts and the Cold War are explained very simply: "Americans believe themselves to be in direct ideological opposition to the U.S.S.R. The impossibility of reconciliation between these two great Caucasoid powers arises from roots deeper than ideas or social forms. Large percentages of the peoples of both countries are non-Caucasoid and the racial ratio will change in each country, coloring the collective character and ethos of the populations. In this respect the evolutionary advantage undoubtedly lies with the U.S.S.R. Yet both powers, indeed the whole Caucasoid race, may in the end be beaten in the struggle to survive by the more stable and homogeneous Mongoloids". There is not even hope that the Caucasoid thoroughbreds can be preserved by emigration: "Despite the rapid progress now being made in cosmic rocketry the feasibility of transporting men in effective numbers to another planet or star could be ruled out as not being realizable in time". Here one is tempted to conclude—if mankind really is what the author says it is—that other planets ought not to be contaminated by it. THEODOSIUS DOBZHANSKY

WORKING ON ANIMALS

Methods of Animal Experimentation

Edited by William I. Gay. Vol. 1, pp. xv+382. 96*s*. 6*d*. Vol. 2, pp. xv+608. 148*s*. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.)

DR. GAY is to be congratulated on undertaking a task of considerable magnitude, knowing well that it would be impossible to achieve perfection at the first attempt. As chief of the Animal Resources Branch, Division of Research Facilities and Resources of the U.S. National Institutes of Health, he is in a particularly good position

to compile a book on methods of animal experimentation. He has, indeed, produced a most useful encyclopaedia.

With so much to praise, I also have a duty to criticize, in the sure hope that the second edition will correct whatever deficiencies detract from the first. A chapter on drug administration is sketchy and contains little of practical value. In the chapter on hibernation there is no mention of the work of Parkes, Smith and others of the National Institute for Medical Research, London, on freezing and recovering warm-blooded animals. There is no account of methods of measuring spontaneous activity, although there is an excellent chapter on controlled exercise. There is no account of methods of pyrogen testing. Most surprisingly, the reader will look long for instructions about how to make simple injections, and will eventually track down the information in a chapter on methods of infection. There is no description of methods of skin transplantation, nor of the replacement of haemopoietic tissue following whole body irradiation.

The arrangement of the chapters follows no obvious system, which makes these volumes less easy to refer to than they should be. The index, which is particularly important in such a valuable work of reference, is incomplete, misleading, and even inaccurate in places. Moreover, each volume has its own index. It would surely have been possible to have had a comprehensive index for both volumes reproduced at the end of each volume as is done in so many other two-volume works of reference. It would also have been better to have numbered the chapters serially from the beginning of Volume 1 to the end of Volume 2, and to have done the same thing with the pagination.

Of the twenty-three authors, twenty-two are shown to work in American laboratories, and one in a British laboratory. This results in an inevitable bias, because even to-day only a few workers are as familiar with the literature and methods foreign to their own country as they are with indigenous material. But in one or two chapters this process seems to have gone a little too far. In one, 35 per cent of the references are self-citations, and in another, 30 per cent. In a third, the author cites two of his own "unpublished observations".

Despite these remarks, the two volumes are a great contribution to the literature of biological methods. There is a tendency to-day for the newly qualified biologist to be turned loose on a problem of which he has no experience. He reads the literature, and is impatient to get on with his first series of experiments. But who is to teach him how to manipulate the subject of his experimental methods, namely, the living animal? This work is therefore not only a necessary bench book for every biologist who uses animals, but is also bound to assist in the humane treatment of animals under experiment.

The presentation of both volumes is consistent with the very high standard that we have come to expect from Academic Press. Unfortunately, the price of the two volumes together may deter some laboratories from buying them. I am quite unable to recommend such a false economy.

W. LANE-PETTER

CHANGE AND DECAY

Aging

Proceedings of the Symposium held at the University of Oregon Medical School, 1964. Edited by William Montagna. (Advances in Biology of Skin, Vol. 6.) Pp. xv + 273. (London and New York: Pergamon Press, 1965.) 100*s*. net.

Behavior, Aging and the Nervous System

Biological Determinants of Speed of Behavior and Its Changes with Age. Edited by A. T. Welford and James E. Birren. (A Monograph in The Bannerstone Division of American Lectures in Geriatrics and Gerontology.) Pp. xvi + 637. (Springfield, Ill.: Charles C. Thomas, 1965.) 22.50 dollars.

THESE volumes record the contributed papers—but not the discussions—of two symposia on ageing. At first sight they have little in common except in so far as they demonstrate how widely disparate subjects and interests can be included under the general heading of ageing research. Yet while the topics were obviously very different there is one common feature. The detailed semantic arguments about how to define ageing, which often seem to distract gerontologists and to divert them from their main business, were avoided and both meetings clearly got down straight away to the description of fresh experimental findings and the interpretation of results.

Advances in the Biology of Skin is the sixth of a series of reports of meetings devoted to the skin and is concerned solely with its ageing. The skin is, indeed, an organ that is very vulnerable to the accumulation of environmentally induced injuries during life and it is also much more complex and variable from site to site, both in structure and function, than is often imagined. In general, it shows an increasing disarray with increasing age together with a decreasing number of functioning units the individual activity and efficiency of which are declining. Oberste-Lehn, in a very detailed paper, however, suggests that there may be new growth of follicles, and while some receptor organs, for example touch corpuscles, get fewer, pacinian corpuscles are being constantly remodelled and others, like free nerve endings, seem to be little altered.

For many people, perhaps, ageing of the skin means primarily loss of or greying of hair and a wish for some way of rejuvenating the cosmetic appearance. Little information and less comfort are provided on the first topic, but the capacity of hormones to restore the character and the biochemical nature of the ageing skin is entered into very thoroughly.

In his paper about the changes in nerve endings in skin Winkelman begins by saying that "it is accepted that ageing dulls the senses but how this occurs has scarcely interested anyone but philosophers". The second book, *Behavior, Aging and the Nervous System*, shows that the psychologists at least are aware of the force of the criticism and accept the truth of the first statement. By and large the whole volume is devoted to providing psychological and physiological data from clinic and laboratory that are appropriate bases for discussions about the slowing of behavioural patterns with increasing age. Almost all the work mentioned has been carried out on human subjects and the occasional papers describing animal experimentation are not of particularly high quality.

There are many possible places in the nervous system where slowing of reaction patterns and of skilled performance or memory storage may be initiated—in the sensory organs, the conductor mechanisms, the central areas or in the effector systems, and both autonomic and voluntary nervous systems may be involved. All are considered in various degrees of detail and completeness. In addition, it is noteworthy and commendable how vigorously the participants faced up to the constant difficulty of deciding whether a change in function in old people is a "normal ageing change" or is a consequence of superimposed disease. They do this by considering the slowing of performance brought about by physical disease, whether local, as in brain injury, or general, as in cardiovascular disease, or by psychological disease, as in schizophrenia. Nor are practical problems forgotten. For example, how best to use and train older workers in industry and the ability of older airline pilots to make correct decisions when under load are both considered in some detail.

Neither of the books is cheap and it is unlikely that any one person will want to buy both. But without doubt, there is much more justification than usual for

publishing the proceedings of what were evidently, within their respective frameworks, interesting and illuminating meetings. P. L. KROHN

MUSIC AND PHYSICS

The Physics of Musical Sounds

By C. A. Taylor. (Applied Physics Guides.) Pp. xii+196+record. (London: The English Universities Press, Ltd., 1965.) 42*s*. 6*d*.

MUSIC is important to many of us, and sound is important to all but the deaf. Yet, as Prof. Taylor writes concerning his experience with budding physicists, "sound had been missed out and the problem of teaching it at a higher level became more challenging".

This book can serve for a useful undergraduate course in physics with an emphasis on sound and music. It contains neat, self-contained treatments of the wave equation, the vibration of strings, square membranes, and cylindrical and conical pipes, harmonic analysis, Fourier transforms, convolution, resonance, coupled systems and starting transients. Mathematical sections are indicated by an asterisk at the heading, so that the musician can skip them in reading the book. The purpose and general nature of the mathematics is clearly explained in the rest of the text.

On the musical side there are wise, if necessarily incomplete discussions of the nature of musical sound, hearing, sum and difference tones, scales, consonance and dissonance, and architectural acoustics. The theme of "the possibility of designing a completely new musical instrument" unifies the musical and the physical content of the book.

The book seems quite reliable. On page 39 it says "It is thus clear that the ear is insensitive to phase . . ." which contradicts some experimental results for steady tones[1] but conforms to traditional experiments. As Prof. Taylor notes in many places, our knowledge of acoustics, and especially of psychoacoustics and physiological acoustics, is small.

We may hope that the publication of *The Physics of Musical Sounds* will help to overcome the present neglect of acoustics. Other influences are at work. Advances in electronic instrumentation, and especially in digital computers, have given to-day's acousticians much more flexible and powerful tools than the ingenious optical and mechanical techniques on which Prof. Taylor dwells. Concerning experiment, the book is a good summing-up of what has gone before, and a useful source of references for musicians and physicists alike. But to-day techniques which are merely mentioned are pushing our knowledge of acoustics forward. J. R. PIERCE

[1] *J. Acoustical Soc Amer.*, 31, 1579 (1959).

TRAFFIC CONTROL

Traffic Control

Theory and Instrumentation. Edited by Thomas R. Horton. (Based on papers presented at the Interdisciplinary Clinic on Instrumentation Requirements for Traffic Control Systems, held December 16–17, 1963, at New York City.) Pp. xi+218. (New York: Plenum Press, 1965.) 12.50 dollars.

THIS book contains ten papers divided into two sections, "Traffic Control Instrumentation, Theory and Practice" and "Case Studies of Large-scale Integrated Traffic Control Systems", and provides a useful summary of American work on road traffic control, from both the theoretical and the practical point of view. It contains a very readable introduction and a useful short list of publications for the reader who wishes to go further. Most of the information has been published elsewhere, but it is very useful to have it collected in a single volume. The information on instruments is not too detailed, but is sufficient to show the principles of operation of devices for measuring the basic quantities required for control.

The fundamental relations between the three variables, flow, concentration and speed, are discussed in one paper, although no reference is made to the basic work of Lighthill and Whitham[1] in this field. Several theoretical models give relations between speed and concentration which are fairly close to observed results over most of the range, although they all tend to be unrealistic to a greater or lesser extent at low concentrations.

In order to design a traffic control scheme, one needs a judicious blend of theoretical analysis and practical engineering ability. On the whole the book brings out the need for both, although the relationship between them may not always seem clear. A theoretical approach which includes a large empirical element appears to have been the most successful one. The paper by D. L. Gerlough on simulation of traffic situations, using digital electronic computers, rightly points out that comparatively rough models can be adequate for making the required calculations. In most cases what is required is the effect on the overall travel time of a change in the control system. Travel time can be very susceptible to small changes in the flow of traffic, the types of vehicles, the presence of parked vehicles and a large number of other factors, not all of which can be fully controlled. It may seem to be desirable to allow precisely for all these effects, averaging out over the whole spectrum, but in practice it is sufficient to assume that conditions are uniform, at any rate during normal working hours. Conditions during peak periods are special and the traffic demand may be changing rapidly. Intersections in particular are frequently overloaded during peak hours and methods of minimizing delay under these conditions are discussed in an interesting paper by D. C. Gazis.

There is at present a great deal of interest in the central control of traffic signals by computer—two major schemes are being put in hand in West London and Glasgow. Methods of tackling such situations are given, and the experiment in Toronto, which was begun several years ago, is described with useful details of costs.

Some recent work at the Road Research Laboratory, using a computer programme which approximately minimizes total travel time in a network of signals, has been described by Hillier[2].

Another topic discussed is the control system for the Congress Expressway (an urban motorway) in Chicago. In this system congestion on the expressway at critical times is avoided by restricting the flow of traffic entering on a particular ramp. Assessment of the scheme has included measurements of the effects on traffic which finds an alternative route, and showed that during the whole peak period there was an average small reduction (2·5 per cent) in travel time.

[1] Lighthill, M. J., and Whitham, G. B., *Proc. Roy. Soc.*, A, 229, 317 (1955).
[2] Hillier, J. A., *Traffic Engineering and Control*, 7, (8, 9), 502, 569 (1965).

SUNDIALS REVIVED

Les Cadrans Solaires

By R. R. J. Rohr. Pp. 208+4 colour plates. (Paris: Gauthier-Villars et Cie., 1965.) 58 F.

IN the preface to this book, Dr. Henri Michel, of the Academie Internationale de l'Histoire des Sciences, cites three essentials of a good book: to have something to say; to say it; and to say nothing else. These are well realized in Captain Rohr's treatment of his subject, the sundial in all its forms. Beyond this, he achieves a remarkable blend of the philosophical, historical, artistic and practical, thus appealing to a wide range of readers.

In his introduction, the author transmits his enthusiasm for all these aspects (and notes that he makes allowance for the non-mathematically minded by printing passages relating to calculations in smaller type) and acknowledges the help received from other specialists in his field.

The first of ten chapters traces the evolution of the sundial from Egypt in the fifteenth century B.C. to the heliochronometers used as late as the turn of this century by French railways to regulate the clocks at rural stations, and the modern elaborate ornamental dial. Chapter 2 covers the movement of the Earth, co-ordinates, equation of time and other factors to help the constructor of dials find the meridian and determine the orientation of walls.

Classical dials, in which the gnomon is fixed parallel to the Earth's axis of rotation, and inclined plane dials, such as those carved on the faces of polyhedrons, are dealt with in their major variations in two detailed chapters. The reader is then acquainted with the effects of changes in declination, the various reckonings of the day from sunrise to sunset, and the delineation of the tables of the dials. Chapter 6 deals with analemmatic dials, that is, those in which the gnomon is movable.

Although one may be surprised by the ground so far covered, these six chapters have only been concerned with what may be considered normal dials. Rohr now embarks on the rarer dials, including lunar dials, those by which the Sun's elevation above the horizon is measured and azimuthal dials which indicate time by means of the variation in the Sun's azimuth.

The penultimate chapter, complementary to the first, illustrates the evolution of the sundial by existing examples intended for public use. It is interesting to note that the earliest dial showing equal hours dates from 1493, all previously indicating temporal hours (that is, based on the ancient division of the day from sunrise to sunset).

Outstanding examples of the work of modern artists are noted, such as the armillary sphere at Phillips Academy, Massachusetts, and the most recent Swiss instruments which have an error of about 1 min.

For completeness the author concludes with some fifty inscriptions found on sundials (mainly offering advice of an improving nature) ranging from the plainly depressing to the distinctly hopeful. A neat anecdote rounds off the book.

Les Cadrans Solaires is a most informative and readable work, clearly printed on good paper, extremely well illustrated with a frontispiece in colour, 51 photographs and 105 figures. Appended, before a bibliography and index, are tables giving the conversion of time to arc, refraction, equation of time, the Sun's declination, hour angle and magnetic variation.

These are an obvious hint to move courageous readers to revive the ancient art of dialling. P. S. LAURIE

PHYSICS FOR SOPHOMORES

The Feynman Lectures on Physics

By Richard P. Feynman, Robert B. Leighton and Matthew Sands. Vol. 1: Pp. 530. 66*s*. Vol. 2: Pp. 540. 66*s*. Vol. 3: Pp. 375. 51*s*. (Reading, Mass., and London: Addison-Wesley Publishing Company, Inc., 1964 and 1965.)

WHAT to include in the undergraduate course, and how best to teach and stimulate the student, whether by lectures, tutorials, seminars or practical classes, are questions which face every university physics teacher. There are no ready answers. A lecturer may succeed because of his personality, the novelty of his subject, his impressive demonstrations or showmanship, or merely because of his adaptability to his audience. Advances and discoveries made in the research laboratories are very rapidly publicized these days through the medium of the Press, radio or television, and often they are presented in a most elegant and convincing manner. The student becomes aware of these advances long before they can be incorporated into the material of his course. The enthusiastic and bright school-leaver complains that the course is dull and old-fashioned; he expresses boredom and frustration. Nevertheless, it is important to direct his attention to fundamentals and to the avoidance of elaborate and sometimes involved mathematical treatments of physical phenomena, in the introductory stages of his course. He must prepare himself to be receptive of new ideas and concepts and future developments and to judge their applicability and soundness on the basis of previous knowledge and fundamentally established principles. He must try to avoid cluttering up his mind with the rapidly growing and vast number of present-day achievements and applications in physics and technology, however exciting they may seem to be when first met. There is no doubt that undergraduate physics courses need to be altered to meet the challenge of modern requirements. Physics teaching and syllabuses at the "O" and "A" levels are now being remodelled, but the link has yet to be forged between the new school physics and the university introductory course.

At the California Institute of Technology the development of such an introductory course began some five years ago under the supervision of Profs. R. B. Leighton, H. V. Neher and M. Sands. Prof. R. P. Feynman, who had been giving the two-year sophomore lectures, agreed at Sands's suggestion to revise his course and with the help of his colleagues to deliver a set of lectures specially devised and addressed mainly to the more intelligent and actively minded of the students. The needs of the less able students were not to be overlooked; they would be provided in the lectures with a backbone of material. And what Feynman calls the "extra fireworks", included in the lectures, would be presented in such a way that no student would be made nervous. The sequence would not be traditional, and topics not usually found in such courses would be included.

The lectures Feynman gave were tape-recorded, edited carefully and to some extent re-arranged. The three volumes of *The Feynman Lectures on Physics* are the final result. They are not text-books, though in some respects they come very close to being such. The text retains the easy flow of language used by the lecturer; illustrations are brought in unhesitatingly from other parts of physics not yet discussed to clarify some new step or application; and always the emphasis is on the simple underlying physical principles.

Volume 1 contains the lectures given in the first year. Its fifty-two chapters cover mechanics, relativity, geometrical and physical optics, the kinetic theory of gases, the principles of statistical mechanics, thermodynamics, sound and waves, and symmetry in physical laws. It was to be expected that Feynman would want to include as early as possible the concepts of quantum physics, and Chapters 37 and 38 on quantum behaviour and the relationship between waves and particles represent the talks on these subjects which were introduced before dealing with the kinetic theory of gases. The more formal treatment of quantum mechanics was postponed until later in the course.

Volume 2 is based on the lectures given in the second year. These consisted mainly of electricity and magnetism, and the account printed in Volume 2 is a strongly revised, expanded and re-arranged treatment of the lecture material. The differential calculus of vector fields was discussed first, enabling the subsequent electromagnetic field theory to be dealt with in rigorous mathematical form, using vectors and tensors. In addition to electricity and magnetism, the geometry of crystals and crystal growth, elasticity and elastic materials, and fluid flow, are also discussed in Volume 2. Feynman, in his preface, mentions that he was satisfied with his first-year lectures, but was

unhappy about those on electricity and magnetism. His complaint is that he failed to discover a unique or different way of presenting the subject from that usually followed. The lengthy mathematical discussions needed to deal with electromagnetic phenomena may indeed worry the student as he looks through Volume 2 and considers that he must absorb the contents together with many other topics during his all-too-short academic course.

Twelve lectures at the end of the second year were devoted to quantum mechanics, but these were later supplemented by seven additional lectures in May 1964. By reprinting Chapters 37 and 38 of Volume 1 as the first two chapters of the third volume and adding the edited version of the quantum mechanics lectures, Volume 3 is a self-contained unit relatively independent of the other two volumes. Following the initial statement of quantum behaviour, probability amplitudes, spin and one-state systems are discussed. The ammonia maser is used to introduce the consideration of two-state systems, and then the propagation of electrons in crystals, semiconductors, symmetry and conservation laws, and the quantum mechanics of angular momentum are dealt with. The volume concludes its introduction to quantum mechanics by a treatment of the Schrödinger wave equation and its solution in the case of the hydrogen atom.

Feynman was well aware that the best teaching can be done only when there is a direct individual relationship between student and teacher. His lectures suffered because there was little or no feedback from the audience to the lecturer, but he puts forward the excuse that when to-day there are so many students to teach there must be some substitute for the ideal, and he expresses the hope that his lectures and subsequent text will meet the present need. Lecturers in universities and technical colleges will be grateful for the experiment he made, and for the record that they can study in detail. The *Lectures* provide a first-rate background for the further study of physics, and an excellent guide to the preparation of lectures which will capture and maintain the interest of students with the obvious success and enjoyment that Feynman's achieved.

S. WEINTROUB

MODERN ELECTROCHEMISTRY

Treatise on Electrochemistry

By G. Kortüm. Second, completely revised Englih edition. Pp. xxii+637. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 170*s*.

THE previous English edition of Prof. Kortüm's *Treatise on Electrochemistry* (1951) was a two-volume work, based on the first German edition (1948), but with a great deal of additional material added by J. O'M. Bockris, including chapters on experimental techniques in electrochemistry, numerical examples for the student and tables of electrochemical data. The volume under review is a new translation of the third German edition (1962), with some modifications to bring it up to date. It does not include the additional material of the first English edition, and thereby regains the unity of the German text. The translation is adequate but inelegant, and the literal translation of German words often produces terms which will be unfamiliar to the English-speaking reader, for example 'fertility' and 'influence constant' (in electrostatics), and 'first and last heats of solution'. Occasionally this leads to mis-translations such as 'prüfen' as 'prove' and 'eventuell' as 'eventually'.

The first three chapters are introductory in character, and could well have been shortened considerably, or even omitted altogether, in a book of this nature. Chapter 1 contains much elementary electricity and magnetism, such as Ohm's law, Gauss's theorem, the Poisson equation, etc., all worked through in detail. Chapter 2 works through thermodynamics from scratch in a straightforward but somewhat old-fashioned way, based on the Carnot cycle. Chapter 3 is entitled "The Solution of Ions", but includes discussions of polar and non-polar bonding, and detailed accounts of different types of molecular interaction, of lattice energies, and of the theory of liquid dielectrics, all with a good deal of algebra. These three chapters occupy nearly one-quarter of the book, and contain little which could not be found in general treatises on physical chemistry.

Chapters 4–9 deal with the usual topics of classical electrochemistry, such as strong and weak electrolytes, ionic interaction, and the theory and applications of conductance and e.m.f. measurements. The exposition and choice of material are excellent, and (as throughout the book) there are copious references to general articles and original papers. A few sections have not been brought fully up to date: thus three pages are devoted to the quinhydrone and antimony electrodes but only one to the glass electrode; the section on dissociation of acids in deuterium oxide gives no references later than 1938; and the kinetic salt effect is not related to transition state theory.

The final three chapters, comprising more than one-third of the book, represent its most valuable contribution. Topics treated include potential differences at phase boundaries, over-potential, electrode kinetics, corrosion and passivity, and polarography and other electrochemical analytical techniques. These chapters are truly up to date, and the author provides a welcome guide to the confusing variety of facts and theories in controversial fields such as electrode polarization. There is much interesting information about practical applications in fields such as electrometallurgy, fuel cells and ion-exchange resins, and attention is drawn to many important theoretical and practical problems which remain unsolved.

To sum up, this book contains much of value to research workers in the field of electrolytes and to advanced students of physical chemistry, though it could have been made more digestible (and cheaper) by abbreviating the earlier sections. It should certainly be in any well-stocked chemical library.

R. P. BELL

HOT GLASS

Structural Transformations in Glasses at High Temperatures

Edited by N. A. Toropov and E. A. Porai-Koshits. Authorized translation from the Russian by E. B. Uvarov. (The Structure of Glass, Vol. 5.) Pp. ix+223. (New York: Consultants Bureau, 1965.) 25 dollars.

STUDIES in the field of glass technology and the chemistry and physics of the silicates have been very active in the U.S.S.R. for some considerable time. In recent years four All-Union conferences on the glassy state have been held and the Consultants Bureau has made available translations of the proceedings of these conferences. This is a very useful service, particularly as some of the volumes have been published in translation almost simultaneously with their appearance in Russian.

Volume 3, which formed the proceedings of the fourth All-Union conference on the glassy state, was sub-titled "Catalysed Crystallisation of Glass". This showed that a very considerable effort was being placed in the U.S.S.R. on the development of the glass-ceramics, introduced in the late 1950's as "pyrocerams" by Corning Glass Works. *Structural Transformations in Glasses at High Temperatures*, published only one year later, shows that this effort is not being relaxed at all. Although the title is different, with one exception the papers in the present volume are also concerned with nucleation and crystal growth, an understanding of which is a necessary background for the development of successful glass ceramics.

The problem of nucleation is considered by V. N. Filipovich by discussing a very simple model of a mixture of two particles. As he says, " . . . success depends on the extent to which the model reflects the real system. The development of a detailed theory of crystallization of complex glasses and melts directly depends on the state of the theory of liquid and glass structure, which is still far from perfection".

The editor of this volume, E. A. Porai-Koshits, has made important contributions to the study of glasses by X-ray diffraction and his laboratory has for some years now concentrated on the use of small-angle X-ray diffraction, light scattering and electron microscopy to study the development of immiscibility in simple glasses and the initiation of crystallization. This experimental work is of a high standard and very useful data on microphase separation in lithium oxide and sodium oxide silica glasses are included.

In the third volume most of the work was concentrated on glasses in the Li_2O–Al_2O_3–SiO_2 system and some papers on crystallization in this system are included in the present volume. In addition glasses containing sodium oxide in place of lithium and/or gallium oxide in place of alumina have been examined. In the alkali aluminosilicate glasses, the devitrification products retain the alumina in the anionic framework, but the gallium glasses give only crystals in which the anionic framework appears entirely of silica.

Work by Toropov and his co-workers on the MgO–FeO–SiO_2 system shows that thought is still being given to the production of useful ceramic materials from blast furnace slags.

There is a little overlap with the previous volume particularly in the theoretical section and space could have been saved by editorial action; for example, the same argument and the same diagram are repeated on pages 35 and 40. The appearance of this volume so soon after the previous one shows very definitely that these new materials are considered to be of great importance in the U.S.S.R.

R. W. DOUGLAS

UNCERTAIN MATERIAL

Radiation Damage in Graphite

By J. H. W. Simmons. (International Series of Monographs in Nuclear Energy, Vol. 102.) Pp. xii+242+8 plates. (London and New York: Pergamon Press, 1965.) 80*s*. net.

Radiation Damage in Graphite will be of great value to those who are concerned with the effects of reactor irradiation on the properties of graphite and particularly to those whose interest arises from practical reactor problems. The introductory chapter is followed by a clear account of the physical processes in which the atoms are involved during radiation and a description of the defects which are produced. This is followed by four chapters which describe, in turn, the behaviour of electrical and mechanical properties, thermal and structural properties, the dimensional changes and the mechanical properties. Finally, a full account is given of stored energy effects and the way in which this can be measured and of the phenomena of radiation annealing. Particularly impressive is the series of excellent photographic plates which provide visual evidence of the radiation damage, notably as seen in the electron microscope.

To one who was concerned with the early investigations of this subject it comes as rather a shock to see how ill-defined many of the concepts still are. A reader approaching the subject for the first time might well be confused by the mass of data which is available but which, because of the heterogeneity of the radiation and the complications of the damage processes, is very incompletely digested. In assessing this, one is left with much doubt, and certainly no information, about the reliability of some of the experimental results, in a field in which reproducibility is not to be taken for granted. Figs. 45, 56 and 81 provide typical examples of justifiable doubts concerning the data. The theoretical calculations seem to be similarly fraught with difficulties, and it is no surprise to find a note added in proof which, concerning the displacement energy T_d, says "It now appears that 60 eV is a better value than 25 eV, and if this is accepted, the number of displaced atoms given in this book should be reduced by the ratio 25 : 60".

The author has achieved a very creditable performance with his cast of eccentrics and has provided a valuable, up-to-date story of his field.

G. E. BACON

EXPERIMENT SIZE IN INDUSTRY

Sample-Size Determination

By Arthur E. Mace. Pp. x+226. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1964.) 96*s*.

THE title of *Sample-Size Determination* is extremely misleading, suggesting as it does a treatment of an important problem in the design of surveys. The book is, in fact, "a compilation of mathematical procedures for determining the optimum size of a research experiment" and is concerned exclusively with industrial experimentation.

The heart of the book is contained in Chapters 5–7. The first of these is devoted to the estimation of confidence limits for parameters of normal, binomial, and exponential distributions. Each problem is discussed in a separate section, which begins with an introduction, in which the relevant statistic is described, its distributional properties are stated, and a formula is given for determining the degree of replication required to achieve a confidence interval of specified width; this is followed by an example, and a postscript (a good feature) discussing the robustness of the sampling distributions involved. The examples are presented, in monotonously stereotyped form, as little stories, the hero of which is an engineer ("An engineer wishes to estimate the mean change in resistance of a certain type of 3-Kohm resistor during the first 1,000 hours of a life test"), who usually has to deal with some rather well-informed manufacturers ("The manufacturer . . claims that knowing the initial resistance value of an individual specimen he would be able to predict the tolerance range for resistance of this specimen after 1,000 hours to within 120 ohms"), and who after a certain amount of arithmetic, laid out in considerable (at times unnecessary) detail, arrives at a happy ending in the form of a conclusion ("Therefore, the engineer concluded that a sample of 46 specimens of the resistor type should be placed on test").

Chapter 6 is similar in character to Chapter 5, except that it presents its problems in terms of hypothesis testing, with control of errors of the second kind, and Chapter 7 deals with problems of selection.

The examples in these chapters could provide quite a useful 'cook-book' for a reader prepared to tolerate the 'child's guide' flavour of the presentation (though I hope no experimenter would be perverse enough to do an experiment consisting of 160 identical half-replicates of a 2^3 factorial design, as suggested in one example!). The introductions to the examples are unfortunately much less satisfactory. The author's attempt to make each section self-contained has involved him in much unnecessary repetition, while many of these sections are far too condensed to mean much to the sort of reader who needs to have examples spelled out in the detail given here; the writing also is not always as clear as it might be. These criticisms, and especially the last, apply also to the first four (short) chapters, intended to provide a logical founda-

tion for Chapters 5–7. These are written in terms of the utmost generality, with no examples, and contain a number of sentences at the exact meaning of which I can only guess.

I noticed errors in two formulae. In the equation at the foot of page 69 the term $p_0^n/(n-1)$ should be p_0^n/n, and (more seriously) in the formula for f on page 82 the complete numerator of the fraction should be, but is not, squared.

The book is completed by four chapters on sequential sampling, lot acceptance sampling plans (illustrated by discussion of two U.S. Defense Department Military Standards), sample-size precision schedules, and decision functions, a three-page bibliography, and a useful 28-page appendix of tables. The tables are photographically reproduced, with results that though always legible are sometimes unattractive: this detracts from the otherwise high standard of production.

Perhaps this book can best be summed up as a cook-book with pretensions: as such it is expensive at the price.

M. R. SAMPFORD

General Protozoology

By V. A. Dogiel. Revised by J. I. Poljanskij and E. M. Chejsin. Second edition. Pp. xiv+747. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 147*s*. net.

A NEW text-book devoted to the general aspects of protozoology has been greatly needed, as the classical books of the past (Doflein, Wenyon, Calkins, etc.) have become much out of date. Included in the latter was the *General Protozoology* of Dogiel, published in 1951 in Russian. It has now been practically rewritten by two of his students, J. I. Poljanskij and E. M. Chejsin, from the Institute of Cytology, Leningrad, and well translated into English by C. A. Hoare. It will prove invaluable to protozoologists and biologists in general; on account of the lucid exposition of the main features of the protozoa, with particular reference to their morphology, cell-division and evolution. Chapters are accordingly devoted to the cytoplasm, nucleus, skeletal and fibrillar structures, organelles of locomotion, methods of reproduction and regeneration, and evolution. The physiology of the organisms, including metabolism and irritability, are considered separately, while there are two special chapters on life-cycles and ecology. Examples are taken from the free-living and parasitic protozoa, but there is, of course, no detailed treatment of individual species, and medical and veterinary protozoology, as such, is entirely omitted from consideration. This book provides the fundamental background to a subject which is advancing so rapidly, particularly in the elucidation of fine structure, genetics and metabolism, that still another edition will be required in a few years' time. Readers will find it stimulating and challenging; in many places, it is emphasized that the "problem leaves much to be investigated" and this recommendation applies as much to fundamental phenomena of biology as to host–parasite relationships, systematics and other questions of more specialized interest. The book is illustrated with 326 figures of exceptional clarity and its references are thoroughly international.

P. C. C. GARNHAM

Biophysics and Cybernetic Systems

Edited by Myles Maxfield, Arthur Callahan and Lawrence J. Fogel. (Proceedings of the Second Cybernetic Sciences Symposium, October 13, 1964.) Pp. vii+184. (Washington, D.C.: Spartan Books, Inc.; London: Macmillan and Co., Ltd., 1965.) 45*s*. net.

INTENDING readers would be well advised to begin with this book at the Appendix. The conference of Californian scientists which it reports is one of the many cross-disciplinary explorations that seem nowadays to find eager publishers, and the Appendix contains a candid and not altogether flattering debate as to its value. The time must surely come soon when "quality control" will be applied to papers for symposium volumes as for those in reputable journals, and publishers who neglect to do so will become known accordingly.

It is not suggested that all or even most of the papers here presented fall short by journal standards. The subjects range from the biophysics of natural and synthetic membranes to the modelling of complex biological processes and the theory of artificial intelligence, and almost all the material will be of some interest to specialists in the respective fields. Most original and stimulating, perhaps, is L. J. Fogel's discussion of "Artificial Intelligence through a Simulation of Evolution". He proposes a method of allowing an automaton to evolve its own logic by a search process of "natural selection" which he considers to have reasonable prospects of convergence. In a different direction R. L. Binggeli's finding, that pigeons improve in left–right discrimination when they lose one optic tectum, throws intriguing light on the functional significance of brain symmetry.

But a good many of the reports here presented—especially in the field of network-modelling—are acknowledged to be preliminary or inconclusive. In a research meeting of specialists this may be positively beneficial to the freedom and vigour of discussion. Whether it justifies their addition in that form to world literature is another question.

D. M. MACKAY

Vistas in Astronomy

Edited by Arthur Beer. Vol. 6: Celestial Mechanics, Instrumentation, Meridian Astronomy, Solar Research, Astrophysics. Pp. vii+214. (London and New York: Pergamon Press, 1965.) 84*s*. net.

THIS is the sixth volume in a series containing sets of review papers on subjects covering all the fields of astronomy and astrophysics, written by internationally recognized specialists. The volume includes seven such papers by eight scientists, working in six different countries.

Of particular interest is the paper on relativistic theories of gravitation by G. J. Whitrow and G. E. Morduch, in which one finds an extensive comparison of the effects predicted by the general relativity and eight Lorentz-invariant theories of gravitation for the three astronomical tests of relativity: the deflexion of a light-ray near a mass; the gravitational red-shift; and the advance of perihelion in the two-body problem. Additional advances of perihelion and node due to the rotation of the central body are also considered. None of the theories are strictly equivalent to the general relativity in their predicted effects. The final conclusions are particularly well presented and can be read fruitfully without going through all the mathematics.

Another interesting article is that on the diffuse emission nebulae by S. R. Pottasch. The problems of structure, brightness and interpretation of their spectra are very clearly presented in a broad review of all data concerning these important members of our Galaxy.

The other papers are: one on catalogues of meridian observations of stars (F. Schmeidler); two on optical and seeing problems of long-focus refractors (P. A. Ianna and S. L. Lippincott); and two on active regions of the Sun (S. B. Pikelner and J. Kleczek). All, without doubt, will be quoted frequently in future works and constitute an important contribution in their respective fields.

J. KOVALEVSKY

MEETINGS

THE MEASURE OF THE MOON

AFTER many decades spent in astronomical semi-obscurity, the Moon has emerged to claim renewed interest. The reasons for this are of historical significance. From time immemorial, astronomy has been debarred from the status of a genuine experimental science by the remoteness of its objects of study. With the exceptions of the meteors intercepted by the Earth, the properties of all celestial bodies could be studied only at a distance—from the effects of attraction exerted by their mass, and from the ciphered message of the light brought by nimble-footed photons across the intervening gaps of space. The dramatic emergence of long-range spacecraft in recent years promises to change this picture.

It was against this background that an international conference on selenodesy met at the University of Manchester between May 30 and June 4, under the joint sponsorship of the Department of Astronomy and of the Terrestrial Sciences Laboratory, U.S. Air Force Cambridge Research Center. Mahlon S. Hunt of the Terrestrial Science Laboratory and I were joint chairmen. The aim of the conference was to bring into focus our knowledge of the motion of the Moon about its centre of gravity, its shape and gravitational field, and the large-scale topography of its surface. More than forty scientists from thirteen countries (Algeria, Austria, Egypt, France, Greece, Holland, India, Italy, Japan, Poland, South Africa, the United Kingdom and the United States of America) congregated at Manchester.

The first day of the conference was devoted to a discussion of the problems of lunar librations (underlying all determinations of the shape of the Moon by stereoscopic methods). Prof. Karol Koziel of Krakow University surveyed present knowledge, based very largely on visual heliometric observations of the past hundred years—a necessary time baseline for studies of long-period terms. Such measurements represented the pinnacle of precision astrometry: their masters all belong to the past, for surprisingly few contributions to these studies have been made by photography even if it has been necessary to await the development of electronic computers to make full use of them. Prof. Koziel, together with Drs. Maslowski and Mietelski of Krakow Observatory, recently performed such computations at the University of Manchester, and their results were presented at the conference for the first time. The most significant establish a free libration of the Moon in longitude, suggesting that (to this extent) the Moon behaves like a rigid body. The elastic parameters of the lunar globe have not yet been introduced explicitly into the equations of the problem, and a determination of their values represents important scope for future work.

Further contributions to the general problem of libration of the Moon and other planetary satellites were made by Prof. Colombo of the University of Padua, and Dr. D. H. Eckhardt of the Cambridge Air Force Research Laboratories in the United States, who succeeded to a remarkable extent in making automatic the solution of the entire problem.

The second day of the conference was devoted to a survey of observational methods and techniques. Prof. Jean Rosch of the University of Paris reviewed the principal methods of observation from the days of Bessel to the present and emphasized the effects of atmospheric disturbances on the accuracy of the measurements. The highlights of this session were the contributions by Drs. R. Julian, Aerospace Division of Hughes Aircraft Company, Los Angeles, and M. Orszag, Centre Nationale des Recherches Spatiales, Paris, on the proposed methods for exploration of large-scale lunar topography by means of nano-second (ruby) laser flashes whose optical echoes can be timed so precisely as to measure the instantaneous slant range between the transmitter and the reflecting segment of the lunar surface with an accuracy of the order of 100 metres.

The third day of the conference opened with introductory addresses by Prof. J. Hopmann of the University of Vienna and Dr. Constantine L. Goudas, Boeing Scientific Research Laboratories, Seattle, Washington, who summarized present knowledge of the form of the lunar globe, based on observations made from the Earth. Extensive investigations have revealed that, within an error of a few kilometres in altitude, the Moon is essentially a sphere of mean radius 1,738 km. However, the departures of the actual lunar surface from such a sphere appear to be quite complex and can only be adequately represented by several tesseral harmonic terms of increasing order, of which numerically the most important appear to be those of fourth order. A mere ellipsoid certainly does not seem to represent a better approximation to the actual shape of the Moon than a mean sphere.

Determining the positions and altitudes of the underlying selenodetic control points from the Earth is one of the most exacting tasks of photographic astrometry, which can be successfully approached only by sequential photography with the aid of long-focus telescopes; these alone can enable us to eliminate the effects of imperfect quality of "seeing". Their use in turn calls for a massive effort which can be mounted only with well-equipped and large groups of investigators. The two principal groups of recent contributors to this field—the Aeronautical Chart and Information Center of the U.S. Air Force in St. Louis, Missouri, and the U.S. Army Map Service in Washington, D.C.—were represented at the conference by R. W. Carder and D. Meyer for the Air Force, and A. Nowicki for the Army, with additional contributions by Dr. G. A. Mills and M. D. Davidson of the University of Manchester.

The gravitational field of the Moon is not uniquely determined by the shape of its surface (which represents only a "boundary condition" of the dynamical problem). Nevertheless, it is probable that all conspicuous harmonic terms in the description of the geometry of the lunar surface will have similar counterparts in the exterior potential of the lunar globe. Important papers on the motion of the artificial satellites in the gravitational field of the Moon and on the converse problem of its specification from an analysis of satellite orbit perturbations were presented at the conference by Prof. W. M. Kaula of the University of California in Los Angeles (*in absentia*) and Dr. J. Lorell of the Jet Propulsion Laboratory of the California Institute of Technology.

The fourth and last day of the conference was devoted to the problems of local topography of the lunar surface. Prof. M. J. G. Minnaert of the University of Utrecht summarized in his introductory address the photometric methods which can be used for this purpose and emphasized the reduction of photographic data secured by the *Ranger* or *Orbiter* spacecraft. Following Minnaert's masterly exposition of his subject, J. Sasser of the NASA Manned Space Flight Center in Houston outlined the plans of the National Aeronautics and Space Administration for mapping the lunar surface in quest of *Apollo* landing sites; the afternoon session was largely devoted to an exposition (by Z. Kopal, T. W. Rackham, M. T. Jones, A. Rifaat and P. V. Sudbury) of the photographic methods for lunar mapping which have been developed at the University of Manchester in the course of the past seven years.

What have been the principal features of the present state of selenodesy which have emerged from the recent Manchester meetings? First, that it is inexpedient to undertake new extensive measurements of lunar librations, for there would scarcely be time to reap their harvest in the pre-*Apollo* era; once men have installed zenith telescopes on the lunar surface all Earth-bound observations of lunar librations will become obsolescent. On the other

hand, a clear need exists to reduce again existing observations on the basis of Eulerian equations, taking account of the finite elasticity of the lunar globe, and to develop their solutions to a greater accuracy than has been done so far, for much smaller librations than those perceptible from the Earth should soon become observable from the lunar orbiting satellites.

The observations to determine the lunar figure from the distance of the Earth can be tackled successfully only by experienced groups of investigators utilizing extensive series of sequential photography. The law of diminishing returns is clearly operative here, yet the current effort cannot be relaxed, because in this particular field a closer proximity to the object of the study is not necessarily an asset. In the future, optical echoes of laser pulses should represent the royal road to the problem, but at present these are not even around the corner.

There are many ways in which lunar shadow techniques to determine local topography of the Moon can be pushed further (for example, by a resort to over-exposed terminator photography) to yield resolutions of only a few metres difference in altitude, but there is no doubt that massive contributions of data from spacecraft will have to be handled largely by the automated photometric methods.

ZDENĚK KOPAL

COMPUTERS IN LIBRARIES

By Our Own Correspondent

THE view that the large libraries of the world should now move forward to use computers in managing their affairs is one of the general conclusions of the Anglo-American Conference on Mechanization of Library Services held at Oxford during June 30–July 3. A parallel theme running through the proceedings of the conference was that the large libraries should put their heads together to work out common standards and to pool their individual solutions to common problems. Neither librarians nor computer engineers at any time suggested that readers should be in any way restricted in the use made of libraries for the sake of easier mechanization. Indeed, it would appear that librarians feel as strongly about the principle that readers should not be told what to read as do doctors about the Hippocratic Oath.

The conference, which was attended by some sixty librarians and computer engineers from the United Kingdom and the United States, was a natural sequel to a number of exploratory discussions held in the past year or so between the principal libraries in the two countries. The chairman was Sir Frank Francis, director and principal librarian of the British Museum. Most of the meetings were held in the Curator's Room at the Bodleian, across the quadrangle from the dramatically restored seventeenth century façade above the entrance to the Divinity School.

The problems of British libraries, apparently shared by the Library of Congress and the University Libraries of Harvard and Yale, were described at the beginning of the conference by representatives from the British Museum, the Bodleian and Cambridge. At each place there is a large central library with a number of dependent institutions. At each of the British libraries the collection is ancient and the maintenance of the catalogue is a continuing problem. The rate of acquisition is high, not only because of the demands of scholars, but also because of the shower of books and papers descending on all copyright depository libraries.

The university libraries in particular are anxious to create union catalogues that will enable readers to know what books are stored at dependent libraries, in university departments for example, as well as centrally. This implies that it would be desirable to acquire and catalogue books and papers centrally. Steps in this direction are being taken by the Radcliffe Science Library in Oxford and by the Science Library at Cambridge. The Oxford project will yield a catalogue of scientific periodicals on magnetic tape and eventually in print as well, but this is counted as a pilot experiment. At all three British libraries, thought is being given to the mechanization of the catalogue in its entirety—a task which is made less formidable at the Bodleian by the fact that the existing catalogue of books acquired before 1920 has in any case to be redone.

Some of the advantages, and some of the accompanying problems, of mechanizing an existing catalogue were described at the conference by Mr. Foster M. Palmer, associate University librarian for reference and circulation at Harvard. A project to put on tape the shelf list at the Widener Library was begun some years ago. One product is a printed catalogue of parts of the library. Another is the facility which now exists for searching through masses of information about the books at Harvard for statistical analyses. Mr. Palmer thought that costs at Harvard so far worked out at 25 cents for a catalogue entry, but other librarians offered estimates both lower and higher than this. In producing a mechanized catalogue, the cost of computer time appears to be small compared with that of manual labour involved.

The British Museum is perhaps more aware than any other library of the difficulties of devising mechanized catalogues. Mr. A. M. Cain and Mr. J. W. Jolliffe, of the Department of Printed Books at the Museum, described some of the alphabetical problems which impede the use of mechanization. How best, for example, to deal with books whose titles are in Punjabi or Tamil. Transliteration is not a practical proposition, chiefly because of the difficulty of making a one-to-one correspondence between an outlandish language and its equivalent. This view was given powerful support at the conference by Bodley's librarian, Mr. R. Shackleton, who said that scholars would not be satisfied with anything but the best. The systems team at the British Museum under Mr. Cain has apparently recommended that in future the general catalogue at the Museum should use only Roman, Greek, Cyrillic, Slavonic and Hebrew, with Irish as well for output, and that transliteration into one or other of these should be attempted. But everybody seemed to agree that there are great and growing problems in dealing with the proliferation of individual languages and alphabets in south-east Asia. For computer engineers this means that the task of cataloguing a large library has to be conducted with an alphabet which may for practical purposes have as many as a thousand characters, when provision is made for numbers and for capital letters and punctuation marks.

How is all this to be done? Mrs. Barbara Evans Markuson of the Information Systems Office of the Library of Congress described the work being done in Washington to standardize the production of catalogues. American practice has for several years been simplified by the way in which the Library of Congress classifies and catalogues all books published in the United States in such a way that local librarians anywhere in the United States can obtain a standard catalogue card to go with each new acquisition. The importance of this service can be estimated by the statement of one delegate at the conference that the seventy-odd libraries affiliated to the Association of Research Libraries in the United States are reconciled to spending 18 million dollars a year on the maintenance of catalogues. What is now being done at the Library of Congress is to carry out this cataloguing operation by computer. Work on a small system called 'Project *MAR'C* has been running for some months which will produce a catalogue record of books currently acquired by one part of the Library. Magnetic tapes carrying this information will then be distributed to libraries elsewhere in the United States which will be chosen for their diversity. Librarians will be able to use the tapes for constructing their own

catalogues and for keeping themselves informed of the developing pattern of acquisitions at Washington.

Mrs. Markuson appeared to have been overwhelmed and even surprised to find that even more ambitious plans for mechanizing the Library of Congress have recently been welcomed by those who hold the pursestrings in Washington. Evidently the prospect is not unreal of a complete automation of cataloguing at the Library of Congress and, by extension, of other American libraries as well. Yet it is plain that much remains to be done before it will be known for certain how much information about a book should be included in the bibliographic record. This, it would seem, is one of the most fruitful fields for international collaboration and agreement, and Mrs. Markuson was eloquent about its urgency. Some librarians present were so mistrustful of the necessarily subjective process of constructing a description of a book that they were inclined to think that nothing but the title page would be a sufficient record.

The computer engineers are clearly aware of all these subtleties. Indeed, the undertone of the conference was that it would necessarily be a long time before enough work had been done to know how quickly and how completely libraries would be mechanized. Yet there is more than cataloguing in prospect. Some engineers talk of how computers will help with keeping records of borrowings from a library, how they could help with finding books scattered through several libraries dependent on a central institution, and how eventually they might also allow librarians to keep track of the frequency with which books are used so as to be able to arrange more adequately their stocks for the convenience of the readers. All this is also linked with the possibility that systems for transmitting information from one library to another may help enormously with the sharing of stocks, or at least with the sharing of experience. And nobody knows, of course, what will not happen when the books are on tape as well as the catalogues.

OBITUARIES

William Henry Eccles

W. H. ECCLES, who died on April 29, 1966, at the age of ninety, was a physicist who tackled with notable success some of the early scientific problems associated with radio communication. He was an adviser to both the Army and the Navy during the First World War, and later was directly associated with the development of world-wide wireless telegraphy and telephony, including the beginning of broadcasting.

William Henry Eccles was born at Ulverston, Lancashire. He went from a secondary school to the Royal College of Science at South Kensington, from which he graduated in 1898. The following year he joined Marconi in the laboratory of the Wireless Telegraphy and Signal Company at Chelmsford, where he investigated electrical oscillations in coupled circuits. His study of coherers led to results which were presented in his D.Sc. thesis in 1901.

Shortly afterwards he was appointed head of the Department of Mathematics and Physics at the South-western Polytechnic, Chelsea, and 10 years later he was reader in graphics at University College, London. In 1916 he became professor of applied physics and electrical engineering at the City and Guilds of London Technical College, Finsbury. It was during these early years that Dr. Eccles made his principal contributions to radio research. His work on coherers was continued in a comprehensive study of crystal detectors for radio reception, and in 1912 he presented a paper to the Royal Society expanding the theory put forward some years earlier by Oliver Heaviside to explain the propagation of radio-waves around the Earth by reflexion in the ionized conducting regions of the upper atmosphere. He suggested that the ionizing influence of the Sun's radiation would account for the observed differences in wave propagation by day and by night. During 1912–13 Eccles played a leading part in the British Association's investigations into the characteristics of atmospheric disturbances in radio reception. His book on *Wireless Telegraphy and Telephony*, of which the second and enlarged edition was published in 1918, was for many years one of the very few comprehensive text-books on the subject. *Continuous Wave Wireless Telegraphy*, published in 1921, was a pioneer work of reference in this subject.

Eccles's interest in electrical science and engineering was wide. He was president of the Institution of Electrical Engineers in the 1926–27 session, president of the Physical Society in 1928–30, and of the Institute of Physics from 1929 until 1931. He was keenly interested in the activities of the radio amateur, and was a founder member of the Radio Society of Great Britain and its president for 1923–24. R. L. SMITH-ROSE

Prof. F. Y. Henderson

THE death occurred on April 9 of Prof. F. Y. Henderson only 6 years after his retirement as director of the Forest Products Research Laboratory. Born in 1894, he was educated at the High School, Glasgow, and at the University of Glasgow, where he gained the Gold Medal for Botany and a D.Sc. In the First World War he enlisted in the Royal Engineers but was recalled while on the way to France in order to become superintendent of the Detonator Department of Nobel's Explosive Co. In 1919 he joined the Geological Survey in Nigeria and returned in 1921, as a plant physiologist, to the Imperial College of Science and Technology, where eventually he became professor of timber technology. He investigated the relationship of the food reserves of hardwoods to *Lyctus* powder-post beetle attack, and his book *Timber, Its Properties, Pests and Preservation* is still widely used. He was an outstanding lecturer and was extremely popular with his students.

During this period, Henderson also became director of biological studies at the Sir John Cass Institute (1932–39) and superintendent of examinations in biology for the Pharmaceutical Society from 1928 until 1945. With T. A. Oxley, now director of the Forest Products Research Laboratory, but then at the Pest Infestation Laboratory, Henderson investigated the physiology of grain storage.

In 1945, Prof. Henderson was appointed director of the Forest Products Research Laboratory when post-war timber shortages were a severely limiting factor in the reconstruction of Britain's economy. Investigations into the properties of tropical timbers helped to introduce many new species to the trade.

The programme of the Laboratory was concentrated on the utilization of home-grown timbers when the afforestation schemes, begun after the First World War, yielded mature timber. Henderson co-ordinated programmes of work by serving on the research committees of the Timber Development Association and Furniture Development Council. An excellent chairman of committees, he formed panels with industrial representation to plan work on adhesives and plywood which later became the Composite Wood Panel.

An exceptionally modest man with a dry Scots humour, Henderson was keenly interested in his staff and ever ready to help them in personal problems. His high sense of public duty was evidenced by his war-time service in the Home Guard, in which he commanded a platoon. He was a skilled craftsman in woodwork, a lover of music and a keen gardener. He married Elizabeth Douglas Pringle in 1932 and is survived by her and two daughters.

J. D. BLETCHLY

FOSSIL HOMINID REMAINS FROM UBEIDIYA, ISRAEL

By PROF. PHILLIP V. TOBIAS
Department of Anatomy, University of the Witwatersrand, Johannesburg

PROMINENT among the faunal and cultural discoveries in the Central Jordan Valley[1–4] has been a small series of hominid fragments found at Ubeidiya near the Jordan outlet of Lake Tiberias. The fauna with which they were found has been recognized as Cromerian, while the cultural remains have been classified by Stekelis as

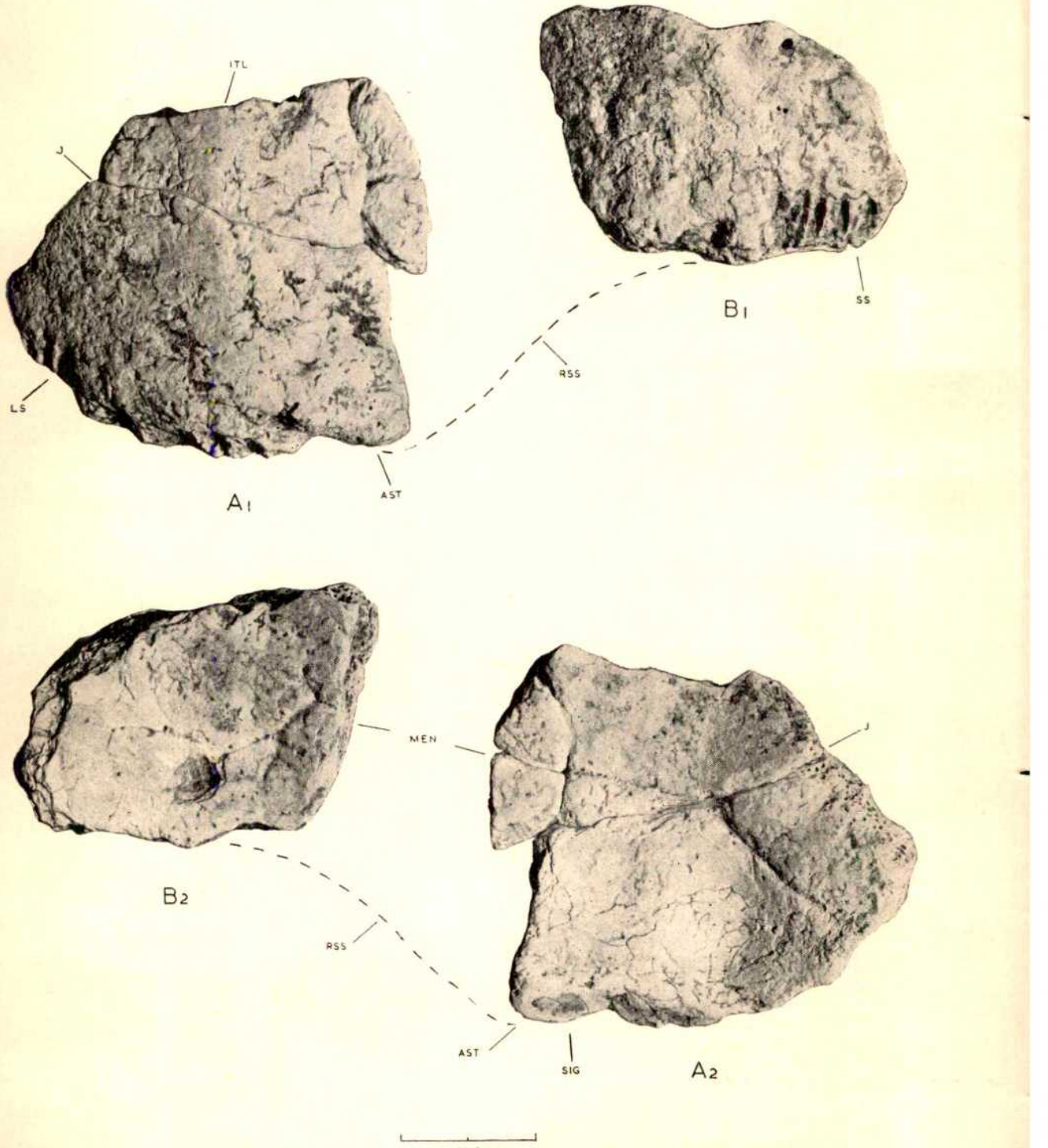


Fig. 1. Ubeidiya hominid right parietal fragment *A* (A_1, ectocranial; A_2, endocranial) and right parietal fragment *B* (B_1, ectocranial; B_2, endocranial). The two fragments are in approximately the correct alignment with each other, and the dotted line represents the probable position of the intervening portion of the squamosal suture. *AST*, Asterion; *ITL*, inferior temporal line; *J*, junction of *in situ* and bulldozed fragments; *LS*, lambdoid suture; *MEN*, meningeal vascular grooves; *RSS*, reconstructed squamosal suture; *SIG*, sigmoid sinus groove; *SS*, squamosal suture

Israeli Variant of Oldowan I and II, and Israeli Variant of Abbevillian.

Five pieces (two fragments of parietal bone, one of temporal bone, a molar and an incisor) can definitely be identified as of human origin, while a sixth morsel (possibly part of an occipital bone) is probably, though not definitely, hominid. Slight differences in the colour and texture of the fragments do not rule out the possibility that the pieces belonged to the same skull. Samples of all six remains were submitted to Dr. K. P. Oakley for relative dating.

Description of Remains

Hominid parietal fragment A (Fig. 1). The larger parietal fragment comprises four pieces, which fit well together and had been glued before I received them. Two of the fragments are very small and fit on to a third, bigger piece: this tripartite portion was exposed by a bulldozer. The fourth and largest portion, effecting perfect contact with the third, was later found *in situ* in layer 23 of the sequence. The hominid teeth were also found in this layer.

The combined fragment is from the asterionic region of a right parietal bone. On the endocranial surface is a sulcus occupied by branches of the middle meningeal vessels. Near the mastoid angle is a well-marked sigmoid sulcus, indicating that the sigmoid venous sinus rose on to the parietal bone. In *Homo erectus pekinensis* it does not extend so far upwards[5]. The ectocranial surface is marked by a robust inferior temporal line, which would be more in keeping with maleness than femaleness. Traces of the lambdoid suture are evident near the mastoid angle.

Towards the mastoid angle, an ectocranial heaping up of bone suggests a torus angularis, but the surface is eroded and damaged and the maximum development of the area would seem to be missing. Nevertheless, the heaping up at this point is sufficient to give the bone its large maximum thickness of 12 mm. Thicknesses elsewhere on this fragment vary from 7 to 11 mm.

Hominid parietal fragment B (Fig. 1). This smaller parietal fragment was uncovered by the same bulldozer. Two features help to place this fragment: an externally bevelled sutural surface, and a pattern of meningeal vascular grooves. The grooves are fine and shallow, in contrast with the broad, deeply etched grooves on fragment *A*. The general direction of the grooves is parallel to the sutural margin. The most likely area with these morphological features is the right parietal abutting on the squamosal suture in front of the midpoint between the mastoid and sphenoid angles, and therefore a short distance in front of parietal fragment *A*. Thus, fragments *A* and *B* could have belonged to the same parietal bone.

This fragment is also thick and its thickness varies from 9 to 11·5 mm.

The temporal fragment (Fig. 2*A*). This specimen comprises the lower part of the right squamous temporal, including a well-developed supramastoid crest, a distinct suprameatal spine and triangle, and the lateral part of the roof of the bony external acoustic meatus. A small portion of pars mastoidea is present and mastoid air-cells are exposed. A very small fragment of tympanic plate in the antero-superior part of the meatus abuts on to a process of bone, which is either a large postglenoid tubercle or perhaps the down-turned edge of the tegmen tympani: a squamotympanic fissure separates the process from the tympanic fragment. The structure is unmistakably human and the robust supramastoid crest suggests maleness rather than femaleness.

Indeterminate cranial fragment (Fig. 2*B*). This fragment cannot positively be identified as human. It may have belonged to the nuchal area of an occipital bone.

The molar tooth (Fig. 3). The entire crown and about 7 mm of the root of a right third upper molar was found *in situ* in layer 23. Unfortunately, much of the surface is eroded and morphological detail is partly obscured. In addition, the lingual half of the occlusal surface shows moderate attrition but no dentine is apparently exposed.

Despite the wear and erosion, the main cusps of the trigon are apparent. The worn mesiolingual cusp or protocone is ill-defined. Only a trace of the longitudinal occluso-mesial fissure is suggested, and this is so situated that the transverse (buccolingual) diameter of the protobone exceeds that of the paracone. No trace can be found of the occluso-lingual fissure. The moderate attrition is unlikely to have obliterated the lingual fissure if one had been present. It seems more likely that the fissure was poorly developed or absent: in other words, that the hypocone was nearly, or even entirely, absent, as is not uncommon in third molars. Poor development or absence of the hypocone occurs in some Neandertal teeth, such as those of Monsempron[6], Petit-Puymoyen, La Quina, and Krapina (the unworn third molar)[7]; in one or two of the seven third molars of Choukoutien[8]; and in modern man (see, for example, the Javanese material of Korenhof[9]).

The metacone is small but bulges buccally and distally. Its mesial limit cannot be located precisely. A mesiolingual prolongation of the metacone appears to make contact with the protocone, but no oblique or trigon crest connects the two cusps; instead, their line of contact is marked by a deep fissure running distad and lingualwards from the central fovea.

No posterior fovea is evident, but traces of the anterior fovea are present, connected by a slight mesial fissure to the central fovea. An anterior trigon crest connects protocone to paracone, mesial to the anterior fovea.

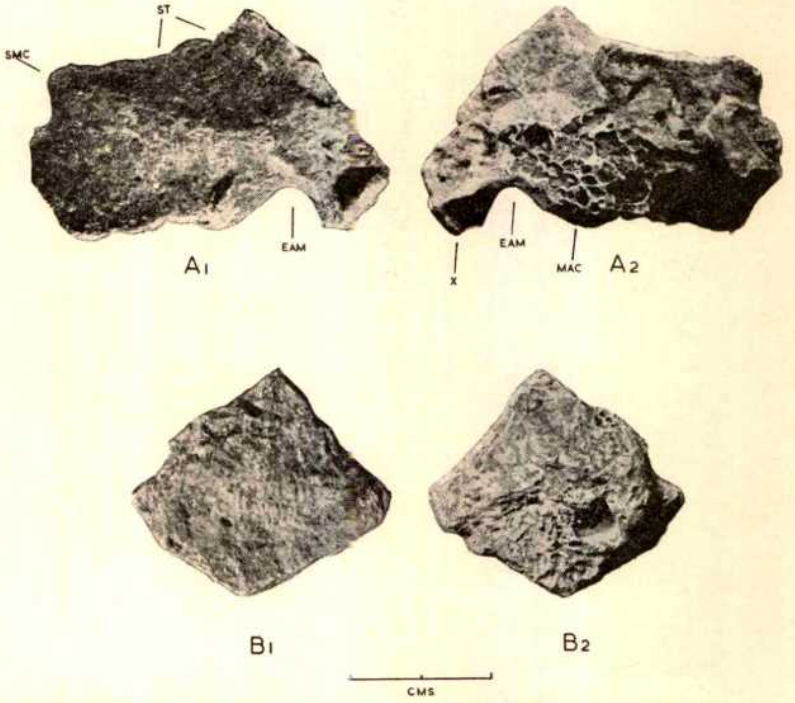


Fig. 2. Ubeidiya hominid right temporal fragment (A_1, ectocranial; A_2, endocranial) and possibly hominid cranial fragment, perhaps of occipital (B_1, ectocranial; B_2, endocranial). *EAM*, external acoustic meatus; *MAC*, mastoid air-cells; *SMC*, supramastoid crest; *ST* squamous part of temporal; *X*, down-turned process of bone

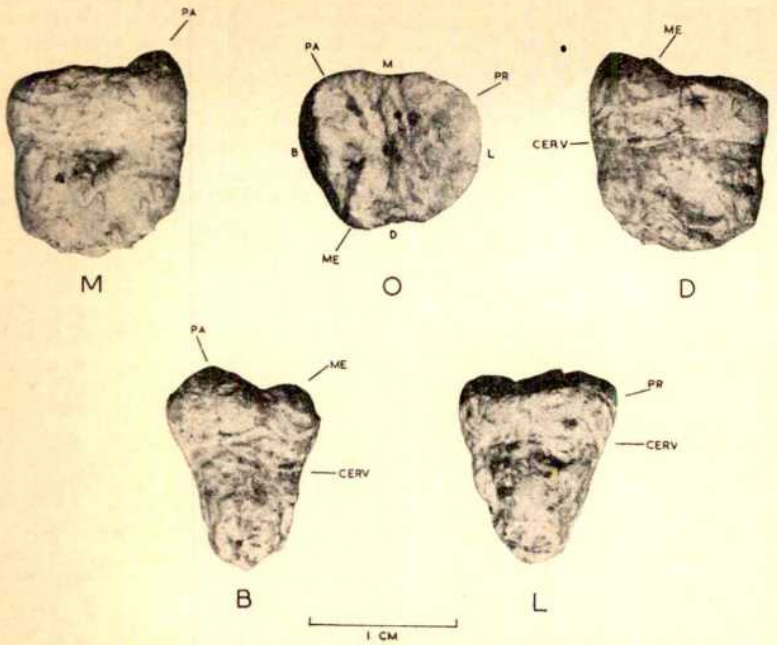


Fig. 3. Right third upper molar of Ubeidiya hominid. *M*, Mesial; *D*, distal; *B*, buccal; *L*, lingual (palatal); *O*, occlusal. *CERV*, Cervical enamel line; *ME*, metacone; *PA*, paracone; *PR*, protocone

The roots are fused so far as they are preserved, though separate root-canals are manifest. A longitudinal groove is apparent on the mesial face of the fused roots, between the larger buccal and smaller lingual components.

The diameters of the crown of the tooth are 9·6 mm (mesiodistal) by 11·4 mm (buccolingual). These measurements may be fractionally too small on account of the abraded surface. The metrical characters are compared with those for various other hominids in Table 1.

Table 1. METRICAL CHARACTERS OF MAXILLARY THIRD MOLARS OF UBEIDIYA AND OTHER HOMINIDS*

		Crown diameters Mesiodistal (mm)	Buccolingual (mm)	Module (mm)	Mesiodistal/ buccolingual shape index (per cent)
Ubeidiya		9·6	11·5	10·55	83·5
Homo erectus ($n = 10$)		8·7–10·8	10·4–14·0	9·55–12·4	77·1– 89·5
Australopithecus africanus ($n = 11$–12)		11·6–15·2	14·6–17·9	13·2 –16·5	77·7– 93·7
Australopithecus robustus ($n = 16$)		12·8–17·0	15·9–18·2	15·0 –17·4	70·3– 95·5
Neandertal man ($n = 13$)		9 –13	11 –14·5	10·0 –13·75	71·1–101·7
Modern man					
Aleuts	27♂	7·1–11·2	7·3–12·5	—	76·9–102·0
	15♀	7·8–10·2	10·2–11·0	—	70·9– 91·7
Bantu	265♂	6·3–11·0	9·1–14·3	—	61·5–104·7
	81♀	4·4–10·3	4·7–12·7	—	64·3– 93·6

* The figures for *H. erectus* and the australopithecines are from Tobias (ref. 10), Neandertal man after Patté (ref. 7) and Stewart (ref. 17), the Aleuts from Moorrees (ref. 11), and the Bantu from Jacobson (ref. 12).

The dimensions of the Ubeidiya third molar fall well below those of the australopithecines, but comfortably within the ranges for *H. erectus*, *H. sapiens neanderthalensis* and *H. sapiens sapiens*. The same might be said for its morphology: it is undoubtedly a hominine tooth, but nothing in its dimensions or morphology indicates to which species of *Homo* its owner belonged.

The incisor tooth (Fig. 4). A second tooth was found *in situ* in layer 23 and has been identified as a probable left lateral mandibular incisor. It is represented by the crown and the upper few millimetres of the root. The tooth is fairly well preserved, and even the perikymata are clearly detectable. The incisal edge is moderately worn and two small linear areas of exposed dentine are present; the mesial corner of this edge is right-angled, the distal corner rounded, exactly as described by Weidenreich for Pekin man[8]. The labial face is moderately curved from above downwards and strongly curved mesiodistally. On the lingual face is a definite though slight lingual swelling. The cervical enamel line on the mesial face extends upwards as a smooth curve, whereas on the distal face it ends in a point, higher up than on the mesial surface. The crown is narrow mesiodistally and shows little tendency to broaden from cervical line to incisal edge, the distal face bulging slightly more than the mesial.

The crown diameters are 5·6 mm (mesiodistal) and 5·6 mm (labiolingual), giving a module of 5·6 mm, a crown area of 31·36 mm² and a crown shape index of 100 per cent. These dimensions fall below those of australopithecine second incisors. The mesiodistal diameter falls within the sample range for *H. erectus*, just below the sample range for Neandertal man, and within the range for modern man, as judged by Aleut, Bantu and other series. The labiolingual diameter of 5·6 mm falls below the lower limits of all available fossil hominine incisors and can be accommodated only in the range for modern man. Even if we compare the labiolingual diameter of the Ubeidiya incisor with those of first incisors, its value remains well below the lower limits for available samples of *H. erectus* and Neandertal man. The differences are, however, not significant. In sum, the Ubeidiya incisor is very small and it can be accommodated somewhat more comfortably within series of modern human incisors than in samples of fossil hominine incisors. Notwithstanding, these comparisons cannot rule out the possibility that the owner of the tooth belonged to an extinct species or sub-species of *Homo*.

All the morphological features indicate hominine rather than australopithecine status for the individual(s) represented by this group of remains. The only decidedly archaic feature seems to be the great thickness of the parietal bone. The Ubeidiya thickness measurements are compared with other hominid data in Table 2.

The Ubeidiya parietal is thick, in comparison not only with the thin-skulled australopithecines and modern man, but also with the thick-skulled Neandertal group. On the other hand, it does not attain the thickness of the very thick-skulled *Homo erectus* group. An occasional normal modern cranium might attain a thickness of 12·0 mm in the asterionic region, but if the Ubeidiya parietal represents a normal and fairly typical member of its population, and not an extreme variant, we should have to regard its massive thickness as a truly archaic feature.

Nevertheless, on the fragmentary evidence before us, the Ubeidiya hominid cannot specifically be identified.



Fig. 4. Left lower incisor of Ubeidiya hominid. *LA*, Labial; *LI*, lingual; *D*, distal

Table 2. MAXIMUM THICKNESS (in mm) OF PARIETAL BONE NEAR MASTOID ANGLE

Ubeidiya	12·0	This investigation
Neandertal group	4·0–9·0 (mean 7·25)	Weidenreich[5]
Swanscombe	7·0–9·0	Campbell[13]
Spy I	7·1 (mean)	Twiesselmann[14]
Spy II	6·5 (mean)	Twiesselmann[14]
Fontéchevade	8	Vallois[15]
La Ferrassie	4	Vallois[15]
La Chapelle-aux-Saints	5	Vallois[15]
Piltdown	11·0 (mean)	Twiesselmann[14]
H. erectus pekinensis	13·5–17·4 (mean 14·8)	Weidenreich[5]
H. erectus erectus	14·0	Weidenreich[5]
Zinjanthropus	6·5–7·0	Tobias[10]
S. African australopithecines ($n = 6$)	4·5–12·0* (mean 6·4)	Tobias[10]
Modern man		
Unspecified	4·5–5·2	Martin–Saller[16]
Australians ($n = 16$)	7·75 (mean)	Twiesselmann[14]
Europeans ($n = 200$)	2–7	Twiesselmann[14]

* In five australopithecine crania, the thickness ranges from 4·5 to 6·5 mm (mean 5·6 mm), but in the Kromdraai cranium the thickness appears to be 12·0 mm (ref. 10).

The evidence presented by Miss Molleson and Dr. Oakley[18] that the hominid fragments are younger than the Cromerian fauna does not resolve the taxonomic problem: the Ubeidiya hominid could belong to an extinct or an extant taxon of *Homo*. It is hoped that the painstaking excavations of Profs. Stekelis and Haas will be rewarded by the discovery of further remains which it will be possible to identify. Meantime, the available evidence allows the assignment of the remains to the genus *Homo*, but does not permit recognition of the species. The present group of Ubeidiya hominid fossils should therefore be classified as *Homo* sp.

I thank Profs. G. Haas and M. Stekelis for their generous invitation to investigate these remains. I also thank Mr. A. R. Hughes for the photographs, and Miss J. Soussi for her assistance.

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IMMUNOLOGICAL TOLERANCE OF THE FEMALE TO HOMOLOGOUS SEMINAL PLASMA PROTEIN

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As a result of their sexual activity, women may repeatedly receive protein from another individual into their reproductive tracts. Although the females of most other mammalian species engage in coitus fewer times during their lives than do most humans, immunization of females against the components of semen, though rarely observed[1], might be expected to occur with repeated coitus. Seminal plasma proteins have served as antigens[2–4], and sensitization to foreign proteins has been shown to occur through the female reproductive tract[5].

The male ejaculate contains proteins in solution in the seminal plasma, and proteins which are part of the spermatozoa and other cells (mostly leucocytes) of the ejaculate. The leucocytes, though relatively small in number when compared with the spermatozoa, would be expected to be highly antigenic. Kiddy, Stone, Tyler and Casida[6] reported specific antibodies in the blood of heifers which received intrauterine injections of bull blood, though the sensitized animals were fertile when mated with the same bull from which the blood was taken. It has been demonstrated that antispermatozoal antibodies are formed if heterologous spermatozoa are injected[7–9] or introduced into the vagina[10,11]. Homologous spermatozoa have been shown to produce antispermatozoal antibodies and lowered fertility[12]. Franklin and Dukes[13,14] have recently reported the presence of antispermatozoal antibodies in the blood of some women. However, there does not appear to be any evidence that the spermatozoa, seminal fluid, or leucocytes entering the female reproductive tract during coitus have any detrimental effect on health or fertility.

Because ample evidence exists that the introduction of soluble antigens into the female reproductive tract results in specific antibody formation, we have undertaken a comparison of the antigenicity of homologous and heterologous seminal plasma proteins. Previous workers have found that the injection of heterologous seminal plasma proteins elicits specific antibody formation (see ref. 7). Because seminal plasma proteins do function as antigens, and because the female reproductive tract is a route by which immunization may occur, we formulated the working hypothesis that the female does not become sensitized to seminal plasma because she is immunologically tolerant to seminal plasma proteins of her own species. We reasoned that if the foregoing hypothesis were true, heterologous seminal plasma proteins would be antigenic if introduced into the female reproductive tract, while homologous seminal plasma proteins would not be antigenic even when injected intravenously or subcutaneously.

Adult virgin female rabbits were administered the antigen twice weekly. We utilized 'Bovine Albumin Fraction V Powder' (Nutritional Biochemical Corporation) as a control antigen, and bull and rabbit seminal plasma. Bovine albumin was dissolved in 0·85 per cent sodium chloride to attain a concentration of 200 mg/ml. Bull semen was obtained from a local supplier on the morning it was ejaculated, and rabbit semen was collected in our laboratory by means of an artificial vagina. The bull and rabbit semen were diluted three times with phosphate buffered saline and centrifuged three times at about 1,600*g* for 5 min to remove the spermatozoa. The diluted seminal plasma was kept frozen in small portions until used. Antigen was placed in the vagina of the test rabbit by means of a glass tube with a polished end which was inserted deep into the vagina. Great care was take to avoid trauma to the vaginal lining. No attempt was made to prevent leakage of the antigen from the vagina,

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though some leakage was observed with all three antigens used. Antigens were administered twice weekly. No attempt was made to keep the dose of antigen the same for each injection, but rather to build up an antibody response as quickly as possible by giving as much antigen at each injection as the rabbit seemed able to tolerate. Blood samples were drawn from each rabbit at intervals during the course of the experiment for testing.

Double-diffusion agar plates were prepared according to the method of Ouchterlony[15], using a 1 per cent solution of 'Special Noble Agar' (Difco). After antigen and antisera were placed in the appropriate wells, the plates were placed in a moist chamber at room temperature for 24 h and then observed for signs of precipitation. Heavy precipitation was given a rating of + + + +, and very light precipitation was given a rating of +; + + and + + + reactions were intermediates. Reactions were designated as negative only when no evidence of precipitation could be discerned.

The results of these experiments are summarized in Table 1. Rabbits which received a total of 800 mg of serum albumin intravenously or 950 mg of bovine serum albumin subcutaneously were observed to be + + + + shortly after the final injection. Intravaginal doses of 3,750–4,550 mg of the same preparation of antigen were required to obtain + or + + reactions. These results provide an approximate assessment of the relative antigenicity of the same antigen administered by way of the intravaginal route as compared with the intravenous and subcutaneous routes. In order to determine whether or not the antigen was being absorbed through the peritoneum, tubal ligations were performed on two of the rabbits 2 weeks preceding the initiation of antigen treatments. No difference in antibody titre was observed between rabbits with and without tubal ligations.

Table 1. A COMPARISON OF ANTIBODY RESPONSE TO BOVINE SERUM ALBUMIN, BOVINE SEMINAL PLASMA AND RABBIT SEMINAL PLASMA

Antigen	Route	Notal No. of injections	Total amount of antigen injected	Response
Bovine serum albumin	Intravenous	4	800·0 mg	+ + + +
	Subcutaneous	4	950·0	+ + + +
	Intravaginal	10	4,550·0	+
	Intravaginal	10	4,550·0	+
	Intravaginal*	8	3,750·0	+ +
	Intravaginal*	10	4,550·0	+
Bovine seminal plasma	Intravenous	3	2·5 ml.	+ + + +
	Intravenous	3	1·5	+
	Intravenous	3	1·5	+
	Intravenous	3	1·5	(anaphylactic death)
	Subcutaneous	6	9·0	+ + +
	Subcutaneous	5	5·0	+ + +
	Subcutaneous	5	5·0	+ + + +
	Subcutaneous	5	5·0	+ + +
	Intravaginal	9	15·0	+
	Intravaginal	9	15·0	+
	Intravaginal*	9	15·0	+
	Intravaginal*	9	15·0	+
Rabbit seminal plasma	Intravenous	12	21·0	– –
	Intravenous	6	20·0	– –
	Intravenous	6	20·0	– –
	Subcutaneous	12	21·0	– –
	Subcutaneous	6	20·0	– –
	Subcutaneous	6	20·0	– –
	Intravaginal	9	16·0	– –
	Intravaginal	9	16·0	– –
	Intravaginal*	9	16·0	– –
	Intravaginal*	9	16·0	– –

* Rabbits with tubal ligations.

Bovine seminal plasma injected intravenously caused a + + + + reaction after 2·5 ml. in one rabbit, and a + reaction after 1·5 ml. in two others. Another rabbit died of an anaphylactic reaction following the third injection. Subcutaneous injections of bovine seminal plasma resulted in + + + or + + + + reactions after 5–9 ml. of antigen. Four rabbits (two of which had tubal ligations) were observed to have a + reaction after receiving 15 ml. of bovine seminal plasma intravaginally.

Rabbit seminal plasma was ineffective as an antigen in rabbits. No discernible precipitates were seen in the blood after 16 ml. of antigen was placed intravaginally, nor after 21 ml. of antigen was injected intravenously or 20 ml. subcutaneously.

The finding that the female rabbit is apparently immunologically tolerant to the seminal plasma proteins of the male cannot be explained by the presence of blood plasma proteins in the seminal plasma, for some of the seminal plasma proteins are different from those that occur in the blood plasma[16]. One possible explanation for the immunological tolerance of the female to homologous seminal plasma proteins lies in the common embryonic origins of the male and female reproductive tracts and, more specifically, in the accessory sex glands of the female which are homologous to those of the male. The following consideration of accessory sex glands which occur in the female is quoted from Price and Williams-Ashman[17]. "In many female mammals, homologues of the male prostate and bulbo-urethral glands develop in the foetus. These glands may retrogress prenatally, remain vestigial, or develop postnatally and become functionally active. These homologues are the female prostate glands (paraurethral glands of Skene) and the bulbovestibular (major vestibular or Bartholin's glands). In addition, there are urethral glands (minor vestibular) which are homologous with the male urethral glands of Littre, and female preputial or clitordal glands corresponding to the male preputials. The major vestibular, when present, and the minor vestibular and clitordal glands are *functional* in many mature females. In a few cases, well-developed prostate glands which are actively secretory have been found in females of four mammalian orders."

We reasoned that the accessory sex glands of the female might be producing the same proteins as those in the seminal plasma of the male and, if this were so, might be present in the female reproductive tract. We therefore obtained five reproductive tracts from cows and five reproductive tracts from heifers. 150 ml. of saline was inserted into the fimbrium of a Fallopian tube and flushed out through the vagina. This was repeated with the same 150 ml. of saline for each Fallopian tube of each cow reproductive tract. The process was repeated with another 150 ml. of saline for the heifer reproductive tracts. A highly reactive rabbit antiserum obtained by injecting bull seminal plasma intravenously was then adsorbed against cow blood plasma until no further precipitate was obtained. The adsorbed rabbit anti-bull seminal plasma serum was then tested against the washings from the cow reproductive tract and from the heifer reproductive tract. In both cases, significant precipitates were formed.

The possibility existed that the rabbit antiserum against bull seminal plasma contained antibodies against bull protein that were not limited to the seminal plasma. If that were true, the washings which we obtained from the reproductive tracts of cows and heifers could have contained such proteins. Therefore, the rabbit antiserum against bull seminal plasma protein was adsorbed with cow blood, saline extract of homogenate of beef liver, and saline extract of homogenate of beef kidney. When adsorbed antiserum was then reacted with washings from cow and heifer reproductive tracts, a precipitate formed.

It therefore seems that the immunological tolerance of the cow to seminal plasma protein can be explained by the presence of the same protein in the female reproductive tract. Some of the proteins common to the cow and the bull reproductive tract are not found in blood, liver or kidney. The present evidence does not indicate the source of these proteins in the female. While it is evident that other possibilities exist, a reasonable expectation would be that the accessory sex glands of the female reproductive tract produce the same proteins as do their homologous glands in the male reproductive tract.

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NUCLEIC ACID SYNTHETIC PATTERNS IN SYNCHRONIZED MAMMALIAN CELLS

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As a result of a number of elegant autoradiographic analyses of cytological systems, it is recognized that chromosomal DNA replication occurs in definite sequential patterns[1]. Not only is the sequence well ordered but the length of the synthetic (*S*) phase appears constant in relation to other phases of the cell cycle[2]. An excellent review of the cell cycle is given by Baserga[3]. Some insight into the operation of the genetic control mechanism responsible for this stability requires analyses of biosynthetic DNA patterns during the cell cycle. It is equally important to delimit the replication period with respect to time of other macromolecular components as well, so as to understand their modes of interaction with DNA. Randomly growing cell populations obviously present difficulties because of the exact nature of the DNA synthetic process. The use of synchronized cultures obviates this difficulty and allows precise autoradiographic analyses of synthetic patterns of nucleic acids and proteins. The present article is concerned with observations of the patterns of incorporation of tritiated thymidine into DNA and tritiated uridine into RNA.

CMP human epithelial adenocarcinoma cells were synchronized in tube cultures by double-blockage with excess thymidine, as described previously[4]. After removal into normal medium at the end of the second blockage, the cell population emerged as a synchronized unit into the *S* phase. Tritiated thymidine was added for a 1-h pulse at 12·5 μc./ml. (New England Nuclear Corporation, Lot 108–55–114, 6·7 c./mM) and then fixed in Carnoy's acetic–alcohol (1 : 3). At the beginning of the second hour of synchrony, another tube was labelled for 1 h. This pattern was followed for 14 h in all, a period long enough to include *S*, *G*2, *M*, and the onset of *G*1. Randomly growing cells were likewise exposed to labelled thymidine for 1 h. An identical sequential pulse procedure was followed using tritiated uridine at 20 μc./ml. (Schwarz, Lot 6501, 4·42 c./mM) to evaluate the incorporation patterns into RNA. Autoradiographic and staining procedures were carried out as described previously[4]. It was not possible to make quantitative grain counts because of the intense labelling at different times in the cell cycle, but the intracellular labelling patterns were evident.

There was no sign of DNA synthesis during the first hour of synchrony, as was shown by the complete lack of labelling with tritiated thymidine. This was undoubtedly due to the presence of an endogenous store of unlabelled thymidine from the previous blockage medium which was not yet fully metabolized. The typical pattern of RNA synthesis was also delayed in some cells during this first hour. For these reasons, the *S* phase was considered to begin at the end of the first hour in normal medium. All time periods mentioned in the subsequent discussion are corrected for the 1-day delay. Fig. 1 illustrates the dramatic increase in *S*-phase cells which took place in the first hour. By the third hour, almost 98 per cent of the population was labelled with tritiated thymidine. The fact that 95–98 per cent of the population exhibited DNA synthesis during a 3-h period (1–4 h), compared with 30 per cent in a randomly growing culture, demonstrates the remarkable degree of synchrony achieved in the *S* phase. The maximum percentage labelling at 3 h was followed by a gradual decrease to 10 per cent labelling after 11 h which remained constant during the next 2 h. This 10 per cent group represents the fraction of the population which was out of synchrony and was still synthesizing DNA when the main synchronized population was passing through during the *G*2 and *M* (mitotic) phases. The mitotic rate in this particular experiment attained a peak of 17 per cent at a single sampling time (11 h) following the initiation of DNA synthesis, a rate which was almost six times that measured in random cultures and sixty times higher than in blocked cells. In other synchrony experiments, we observed mitotic bursts as high as 50 per cent. Presynchrony treatment with 2·5 mM thymidine did not produce

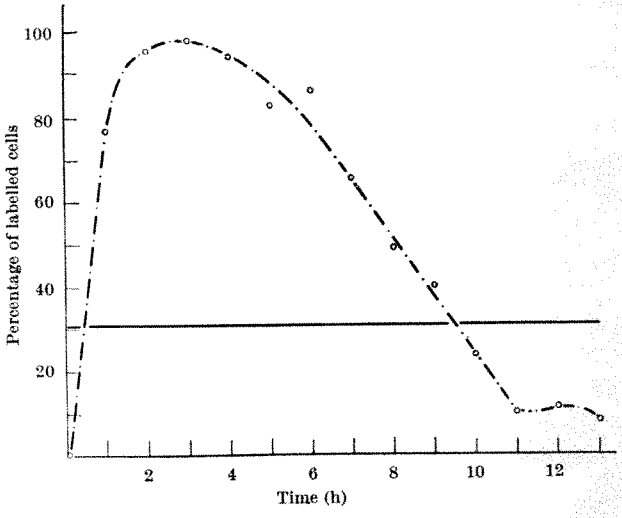


Fig. 1. Percentage of synchronized *CMP* tumour cells labelled with tritiated thymidine for 1 h in normal medium after final blockage in 2·5 mM unlabelled thymidine. Time 0 h occurs 1 h after blocked cells are placed in normal medium. A minimum of 1,000 cells were counted for each point indicated. A cell was considered to be labelled if its nucleus had any detectable silver grains over background. The unusually high level of synchrony is especially well demonstrated in the early- and mid-*S* phase (0–5 h) by the rapid and almost 100 per cent labelling rate. During the *G*2 (5–11 h) and *M* (11–12·5 h) phases, synchrony drops to 85–90 per cent. ———, Synchronized culture; ———, untreated control culture

morphologically abnormal cells in the synchronized cycle which followed. Chromosomal anomalies due to the chemical mode of synchrony also were not observed at any time during the mitotic burst. Although we cannot entirely exclude the possibility at this time that the synchronized population was an abnormal one in terms of macromolecular synthetic patterns, this possibility seems highly unlikely in view of the actual cell cycle parameters we obtained. The breakdown of this particular synchronized population revealed an *S* phase of approximately 9 h, a *G*2 of 2 h, and an *M* phase of 1·5 h. Less than 1 h of the next *G*1 phase was sampled before the end of this experiment. The generation time of untreated *CMP* cells is about 28·5 h according to our unpublished ciné film analyses. Since 30 per cent of such a population is labelled after being pulsed with tritiated thymidine, the *S* phase is close to 8·6 h—a figure which corresponds well with the 9-h *S* phase observed in synchronized cultures. It was reported[5] that excess thymidine in a single treatment of 26 or 45 h produces an apparent stimulation of mitosis of cultured cells which results from a prolonged metaphase time. The conditions of treatment and results are entirely different from our experiments since we use two successive exposures to thymidine which produce an almost complete premitotic block.

The pattern of incorporation of tritiated thymidine showed unusual differences during the *S* phase. A graphical summary of the relative incorporation pattern based on visual analysis of all slides is presented in Fig. 2. Untreated control cells displayed a relatively uniform labelling pattern in nuclei undergoing DNA synthesis during exposure to tritiated thymidine. In some nuclei, there was also incorporation into nucleoli (Fig. 3). In early *S* (0–2 h), labelling was moderate and uniform over the entire nucleus, except for nucleoli which were almost devoid of synthetic activity, and became progressively more intense with time (Fig. 4). Beginning at 2 h, DNA synthesis was especially prominent in regions corresponding to nucleoli in size and appearance. Further support for intranucleolar DNA in *CMP* cells is based on cytochemical staining and ultrastructural evidence of chromatin[6]. Thymidine incorporation into nucleoli progressively increased and reached a high level 3–4 h in the *S* phase. During the same period of preferential nucleolar labelling, the remainder of the nucleus was still undergoing DNA synthesis, but at a lower rate than the nucleolus (Fig. 5). During the next hour (4–5) there was an abrupt stoppage of nucleolar DNA replication and a marked reduction of extranucleolar DNA synthesis. Thymidine incorporation picked up again between 5 and 6 h in both regions, leading

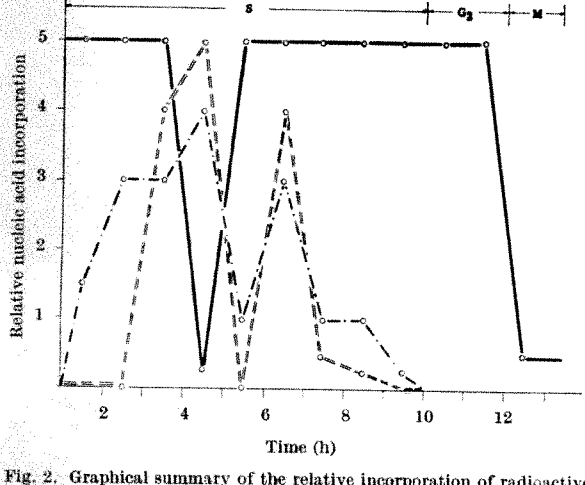
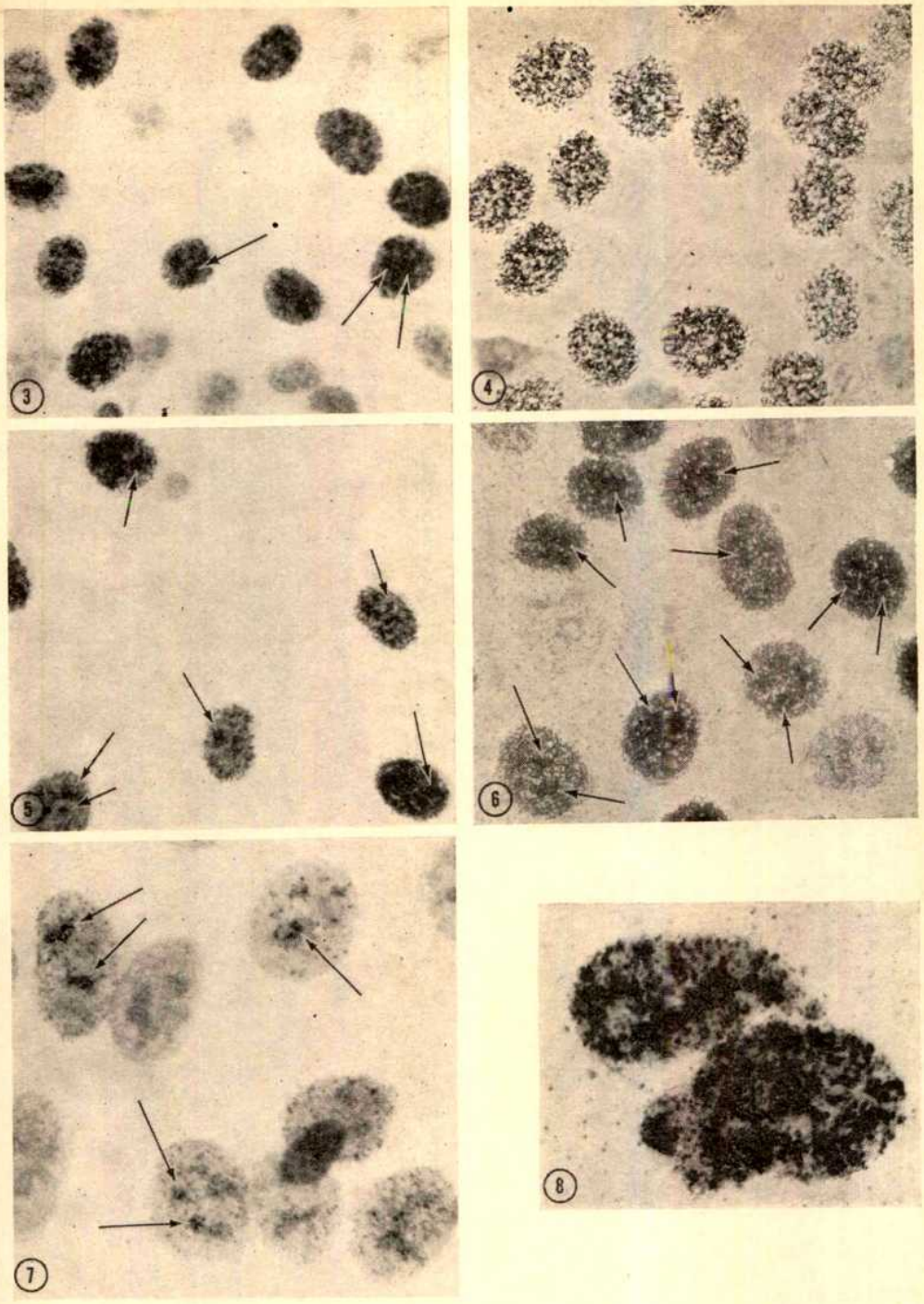


Fig. 2. Graphical summary of the relative incorporation of radioactive specific precursors into RNA and DNA of synchronized cells. Data are based on visual estimations of developed silver grains in autoradiographic preparations and are presented to illustrate dramatic changes in nucleic acid patterns. See text for discussion. ———, RNA; —·—·—·—, chromatin DNA; = = =, nucleolar DNA

to the synthesis of a second distinct nucleolar DNA fraction (Fig. 6). This fraction was still being synthesized between 6 and 7 h in some nucleoli (Fig. 7), but there was a general decline in nucleolar DNA synthesis. In the terminal end of the *S* phase, labelling was especially marked over heterochromatin, as judged by the concordance of grains with heteropyknotic blocks of chromatin (Fig. 8). The rate of DNA synthesis in nuclear chromatin was generally much greater in the mid-*S* phase (except for a sudden drop between 4–5 h) than in the early or late *S* replicating periods, as judged by the relative density of silver grains. The onset of the *S* phase was accompanied by a relatively high rate of incorporation of tritiated thymidine, whereas the termination of the *S* phase was attended by a slow decline in DNA synthesis. The small proportion of cells out of synchrony (10 per cent or less of the population) lagged behind the others and were detected during the mitotic burst and in the main *G*1 phase population (11–13 h). Some of the lagging group incorporated thymidine (*S* phase) or were fixed in cell division (*M* phase). Cells delayed in the *G*2 phase were unlabelled and contained larger nuclei than *G*1 phase cells.

With the exception of one brief period, there was a continuously high level of RNA turnover and probable net synthesis in nucleoli and chromatin of all cells throughout the DNA synthetic and the premitotic *G*2 phases (Fig. 2). The density of labelling over nucleoli, nuclei, and cytoplasm was in the approximate ratio of 6 : 3 : 1 respectively; the same was true for untreated control cells (Fig. 9). The exceptional pattern of RNA incorporation was first noticed at 2–3 h (Fig. 10) and became fully developed between hours 3 and 4 in the *S* phase. At this time, there was a complete absence of nucleolar RNA synthesis, a slight decrease in chromatin RNA synthesis, and a lack of cytoplasmic labelling of RNA (Fig. 11). Nucleoli were frequently outlined by grains in the perinucleolar regions, which suggested an incorporation into chromatin localized here. The sudden cessation of intranucleolar RNA synthesis coincided with the time of peak intranucleolar DNA synthesis. After the natural inhibition of nucleolar and extranucleolar RNA synthesis, there was an immediate return to the usual incorporation pattern (Fig. 12) which persisted into the *G*2 phase (Fig. 13). After exposure to tritiated uridine for 1 h, cells undergoing division frequently showed very low but clear-cut levels of radioactive RNA on the chromosomes and in the cytoplasm. The pattern of response was by no means uniform as many cells in mitosis showed no labelling at all, either on the chromosomes or in the extrachromosomal regions. These observations refer to cells labelled just before and during the mitotic burst (Fig. 13) as well as to randomly dividing populations (Fig. 9). We did not attempt to make a detailed analysis of the labelling pattern of different mitotic stages. Our results indicate that chromosomal synthesis of RNA during mitosis makes a negligible contribution to the total RNA produced during the cell cycle. Cells in the early *G*1 phase were small and demonstrated the typical RNA incorporation pattern described earlier for most of the *S* and *G*2 phases.

Nucleolar DNA has been reported in some cells on the basis of light microscopy of stained preparations[7]. Its presence is often regarded as exceptional and without significance since the nucleolus itself appears to be of chromosomal origin[8,9]. Following the work of McClintock[10] intranucleolar DNA was assumed by some workers to represent the attached nucleolar-organizing region of a chromosome which was genetically inactive and without special biochemical significance—a point of view which necessarily biases the interpretation of macromolecular control mechanisms[11]. A contrary and somewhat prophetic view was stated almost 20 years ago by Schultz[11]. Recent biochemical data[12], in addition to cytochemical[13] and electron microscope observations[14] of the nucleolus,



Figs. 3–8. Untreated or synchronized *CMP* cells exposed to tritiated thymidine at 12·5 μc./ml. for 1 h fixed in acetic–alcohol (1 : 3), and washed in 5 per cent trichloroacetic acid at 5° C for 15 min. Developed '*AR* 10' autoradiographs stained lightly with haematoxylin and eosin and photographed by bright-field except for Figs. 4 and 5. (Figs. 3–6, × *c.* 560; Figs. 7 and 8, × *c.* 965)

Fig. 3. Randomly growing control cells demonstrate intense tritiated thymidine incorporation into nuclear DNA. A few nucleoli also appear labelled (arrows) in addition to nucleolar-associated chromatin

Fig. 4. Synchronized cells in the very early *S* phase (1–2 h) demonstrate uniform DNA synthesis in the nucleoplasm. There is no apparent DNA synthesis in nucleoli. Phase-contrast photomicrograph

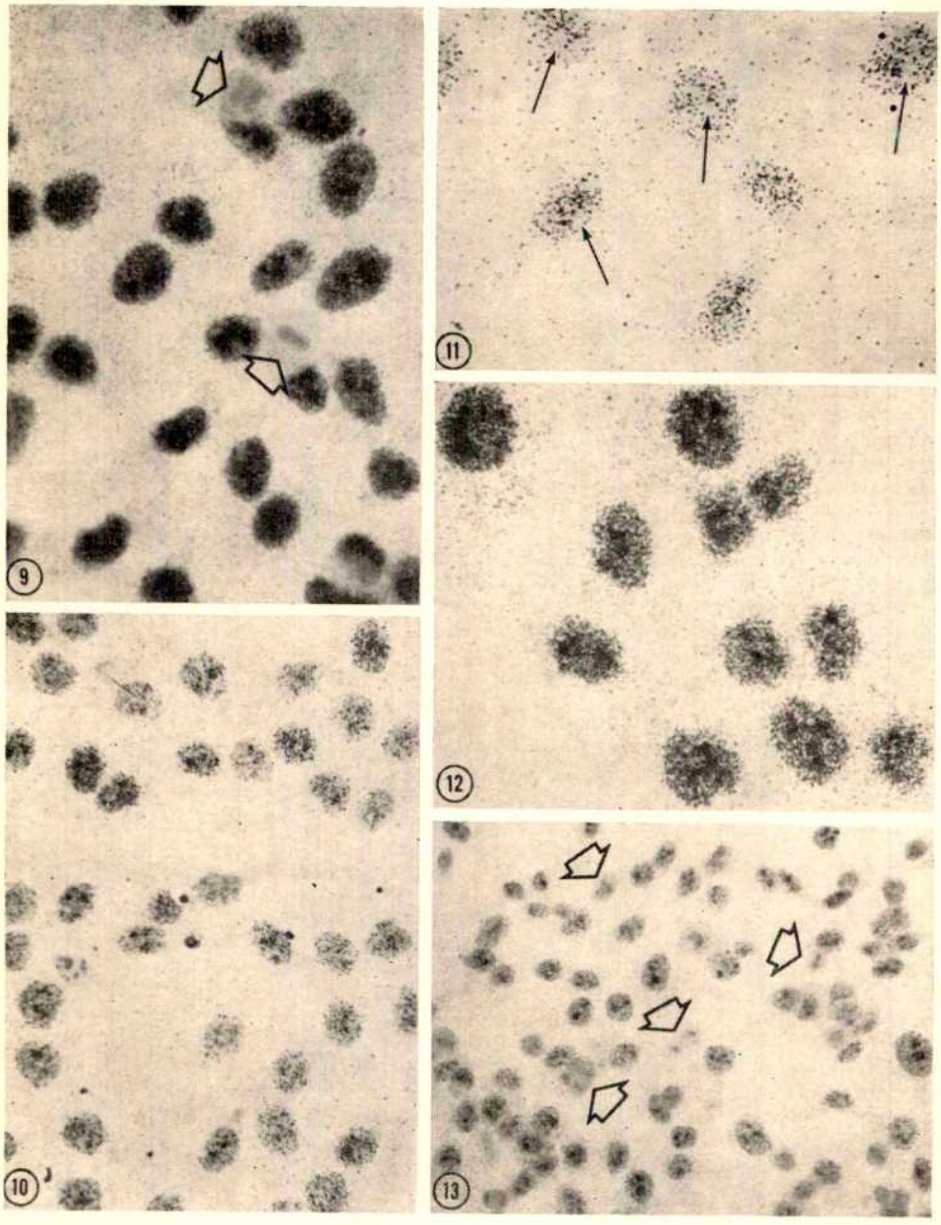
Fig. 5. During early *S* phase (3–4 h) the first intranucleolar DNA fraction is synthesized (arrows). Synthesis of this fraction is superimposed on extranucleolar DNA replication

Fig. 6. Preferential tritiated thymidine incorporation is demonstrated into a second intranucleolar DNA fraction (arrows) between 5–6 h of the *S* phase. Phase-contrast photomicrograph

Fig. 7. Specific and exclusive replication of the second intranucleolar DNA fraction and its associated chromatin is revealed in several nuclei of a culture labelled between 6–7 h of the *S* phase

Fig. 8. Synthesis of heterochromatin is indicated in these nuclei where prominent heteropyknotic blocks are heavily labelled in the late *S* phase (7–8 h)

revealed distinctive RNA and protein fractions to be present. One of the first biochemical reports of nucleolar DNA was made by Monty *et al.*[12]. Since then, actively synthesizing DNA has been demonstrated by autoradiography at the light[15] and electron microscopic[16] levels. Untreated *CMP* cells contain large quantities of nuclear DNA, substantial amounts of which are concentrated in the perinucleolar and intranucleolar regions[6]. At the fine structural level, we observed that perinucleolar chromatin often intrudes directly into the nucleolar mass. In other cases, distinct isolated patches of chromatin lie within partly vacuolated areas of the nucleolus. The appearance of intranucleolar chromatin stained with metallic salts is identical with that found in the perinucleolar region and the marginal nuclear areas. This morphological similarity may not extend to chemical base composition since it is known that a DNA template with a high adenine and thymine content gives rise to an RNA of high guanine and cytosine content in the region of nucleoli[17]. In cultured cells infected with DNA viruses such as *SV40*, substantial quantities of Feulgen-positive material are observed within the nucleolus in very early stages of infection[18]. At the ultrastructural level, unusual dense particles, which appear to be DNA viral cores, are



Figs. 9–13. Untreated or synchronized *CMP* cells exposed to tritiated uridine at 20 μc./ml. for 1 h. Subsequent handling is the same as described for preceding plate. Photomicrographs obtained by bright-field. (Figs. 9, 11 and 12, × *c.* 560; Figs. 10 and 13, × *c.* 220)

Fig. 9. Randomly growing control cells. Radioactive RNA is heaviest in nucleoli, followed by nucleoplasm and then cytoplasm. Cells in metaphase and anaphase (arrows) are unlabelled.

Fig. 10. During the very early *S* phase (2–3 h), nucleolar RNA synthesis begins to be turned off. Incorporation into chromosomal RNA also is diminished

Fig. 11. Complete repression of nucleolar RNA synthesis is demonstrated in these synchronized cells labelled between 3–4 h in the *S* phase. Arrows mark several unlabelled nucleolar areas

Fig. 12. The normal intracellular pattern of RNA synthesis is resumed immediately following the natural RNA repression illustrated in Fig. 11. The photomicrograph demonstrates cells labelled between 5–6 h of the *S* phase. The incorporation pattern is typical of most of the *S* phase and of untreated control cells

Fig. 13. A low-power autoradiograph of synchronized cells exposed to the radioactive RNA precursor during the *G2* phase. Unabated RNA synthesis is demonstrated with the usual intracellular distribution of label found in control cells. A few cells are already in mitosis and do not appear labelled (arrows)

likewise observed within nucleoli of *SV40*- (ref. 19) and adenovirus-infected cells[6]. It is reasonable to assume that the mechanism responsible for intranucleolar viral DNA synthesis also operates at a lower level of activity in uninfected cells.

As detected and defined in the present report by its first appearance in the early *S* phase (2·5–3·5 h) and again in a second burst of synthesis soon afterward (4·5–5·5 h), this intranucleolar DNA undergoes its own distinctive pattern of renewal as does the DNA found elsewhere in the nucleus. The extent to which either of these fractions is a part of the nucleolar organizing chromosome region is not known with certainty. However, we are inclined to regard the second one as genetically inactive or repressed (hoterochromatin) and to be derived from the nucleolar organizer since there are no significant RNA renewal changes in the nucleolus during its period of replication. It also is synthesized later in interphase when extranucleolar heterochromatin is duplicated. The first nucleolar DNA fraction may likewise represent part of the normal chromosomal complement, but in view of the concomitant RNA changes which accompany its replication we attach greater significance to it and regard it as a reservoir of genetic information and a possible site of the DNA control mechanism (see later discussion). Taken collectively, these observations and others on nucleolar function[20] would suggest that this organelle is more than a repository or transient stop for nucleoproteins but serves important synthesizing functions, one of which is the production of at least one biochemically significant DNA fraction.

There seems to be general acceptance of the concept, DNA⟶RNA⟶protein, in which DNA serves as a template for the formation of messenger RNA which, in turn, is essential for cytoplasmic protein synthesis in ribosomes[21]. With low doses of actinomycin *D*, it was shown that binding of the DNA template[22] prevents the action of DNA-dependent RNA polymerase[23] and selectively blocks nucleolar RNA synthesis[24]. Since RNA polymerase is a nucleolar constituent[25] and is normally associated with DNA[26], we emphasized recently[4] that nucleolar DNA is the logical site of the trigger for genetically coded RNA synthesis in the nucleolus. In this connexion, it was demonstrated by Granboulan and Granboulan[27], using electron autoradiography, that some nucleolar RNA is synthesized at the sites of nucleolar-associated chromatin. Blockage of DNA with 5-fluorodeoxyuridine prevents the formation of ribonucleoprotein in granular components of the nucleolus[28]. Our present results substantiate this relationship, as we detected a complete block in nucleolar RNA synthesis in the early *S* phase during the time when the first nucleolar DNA fraction was being replicated. The normal presence in the nucleus of messenger RNA[29] and transfer RNA[30] would suggest that these fractions are all responsive to nucleolar DNA. The fact that chromatin RNA production was also depressed at the same time as nucleolar RNA in our experiments suggests that this RNA fraction also is nucleolar DNA-dependent. The second nucleolar DNA fraction, which is synthesized as a block in the mid-*S* phase, apparently does not code for nucleolar RNA as does the first fraction since RNA synthesis continues unabated in this region.

Proceeding from this point, our working hypothesis is that in a multiplying cell population continuous renewal of DNA must occur, including template DNA of the nucleolus and chromatin. While template DNA is in the process of having both its strands replicated, primarily during the early *S* phase, it is impossible for either of these strands to serve as a matrix for RNA synthesis. As an obligatory consequence, RNA synthesis is temporarily switched off during this period. This may be considered as one of the periods of crisis during macromolecular replication in the cell cycle. In support of this hypothesis is a recent finding that a particularly sensitive DNA fraction is synthesized in the early *S* phase[31]. In addition, Prescott and Kimball's results on the ciliated protozoan *Euplotes* are in accord since they found that nuclear regions engaged in DNA synthesis were incapable of incorporating RNA precursors[32]. We should emphasize in this connexion that after this critical period of major template DNA renewal, when DNA replication occurs in various nuclear sites, continuous RNA synthesis is carried on in the chromatin and nucleolus. Presumably the synthesis of messenger RNA and coded ribosomal RNA precursors account for much of this main stream of RNA activity during interphase, which is in harmony with the suggestion of Hurwitz *et al.*[33] that the information for protein synthesis has to be continually renewed by the synthesis of new messengers. This stream of RNA is derived primarily from the nucleolus, which has already completed its template replication. However, the observation that there is continuous chromosomal RNA synthesis going on (except for the early crisis period) at the same time as DNA replication is under way suggests that additional multiple DNA template sites are continuously being inactivated and activated on the chromosomes, depending in part on whether or not they are undergoing replication. We are aware that part of the labelled RNA may not be DNA-dependent and may have undergone some translocation during the labelling period, but do not propose in this article to enter into the question of the origin(s) of cellular RNA[34]. Continuous RNA synthesis throughout interphase has been reported[35] and is generally confirmed in our investigations. The fact that this pattern is interrupted briefly in both chromatin and nucleoli of early *S* phase cells is a new finding to our knowledge, but is not unexpected in view of other evidence that RNA synthesis may be interrupted during the cell cycle[36].

On a molecular level, the model requires template DNA to be in the double-stranded state while coding the synthesis of RNA and in a partially unwound but replicating state at other times during the *S* phase. In this connexion, it is known that RNA polymerase is more active when primed by non-replicating DNA than by single-stranded DNA[37]. It may be premature to speculate further about the molecular nature of a DNA-protein model which allows multiple regions to prime the synthesis of RNA while other portions undergo self-replication. It has been suggested that chromosomal DNA exists in at least two different molecular associations, an early labelling and a late labelling variety[38]. Our observations support the idea that RNA polymerase can initiate RNA synthesis from the middle of the DNA chain and can copy selected regions of the molecule[26]. We have not dealt here with the role of histones in the regulation of RNA synthesis[39]. Another suggestion, derived from our data, is that RNA polymerase is likely to be found associated with a template DNA fraction in the chromatin as well as with a nucleolar DNA template as already reported[25]. As a consequence, it is predicted that DNA-coded RNAs will be produced in both these regions. The fact that actinomycin *D* in low doses affects the nucleolus and in higher doses acts on chromatin as well[24] is in harmony with this concept. Additional support comes from the finding that ribosomal RNA may be coded by chromosomal DNA[40], in addition to being associated with the nucleolus[24,41].

The specific location of the genetic control system, which triggers and regulates the intranuclear DNA replication pattern, is a matter of conjecture at present. Although we consider nucleolar DNA to be a reasonable candidate for this role, this is only a working hypothesis at the moment which is supported by the findings of Harris[42]. There is other experimental evidence, as detailed in the present article, for stating that RNA synthesis is primed for the most part by intranucleolar DNA and to a lesser extent by chromosomal DNA. These principal template DNA fractions are themselves replicated during the early *S* phase of the cell cycle. Shortly afterwards, in the

mid-*S* phase, a second nucleolar DNA fraction is synthesized which is probably derived from the nucleolar organizer. It differs from the first fraction in its time relations and in the fact that nucleolar RNA synthesis is not simultaneously repressed.

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Note added in proof: Relevant studies on amino-acid incorporation patterns during the cell cycle of synchronized mammalian cells were presented by the senior author at a symposium on "The Nucleolus, its Structure and Function", at Montevideo, Uruguay, during December 5–10, 1965, and will appear in a forthcoming monograph of *J. Nat. Cancer Inst.*

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SECONDARY CLUMPING EFFECT IN HUMAN CITRATED PLATELET-RICH PLASMA PRODUCED BY ADENOSINE DIPHOSPHATE AND ADRENALINE

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THE addition of thrombin to platelet-rich plasma (PRP) induces platelets to release rapidly adenine nucleotides and 5-hydroxytryptamine[1], and the platelets undergo 'viscous metamorphosis'. As only a small amount of protein is released at the same time, it is probable that a generalized increase in membrane permeability does not occur. These changes may depend on adenosine diphosphate (ADP), which is released from the platelets[2]. The release of ADP and 'viscous metamorphosis' are also produced by collagen[3,4]. This article reports a secondary clumping phenomenon of human platelets which occurs at an interval after the addition of ADP and results in changes of the same magnitude as those produced by the addition of collagen or thrombin. Its presence or absence affects the response of any particular PRP to ADP and also to adrenaline.

Human citrated venous blood (0·38 per cent sodium citrate) was withdrawn by a two-syringe technique and centrifuged at 160*g* for 10 min. All containers were either plastic or siliconized glass. PRP was removed and stored at 37° C. 1-ml. samples of PRP were examined by the nephelometric method of Born[5] and the transmission of light was recorded continuously: the details of this technique have been described previously[6]. ADP (Sigma Chemicals, Ltd.) in saline was added by micrometer syringe in 0·5 to 2 per cent dilution of PRP.

The morphology of samples of PRP fixed in 4 per cent formalin[7] taken before and after clumping has been examined by phase-contrast microscopy. Single platelets were examined in wet films. This allowed platelets to turn over and aided identification of their shape. Three categories have been recorded: thin discs of normal size[8], larger degenerating shapes, and smaller irregular ovals plus spheres. Detail of clumps could only be seen after they had been firmly squashed between a coverslip and a slide[9]. The compression separated the platelets in

the clumps and so the clumps have been classified according to the resistance of the platelets to separation. 'Loose adhesion' indicates that most of the platelets in a clump have been separated; 'moderate adhesion' that individual platelets cannot be seen at the centre of the clump but are still visible at the edges; 'marked adhesion' that single platelets have not been separated even at the edges of the clump. Some plasma contained more than one type of clump and some clumps had the features of two grades of adhesion in different portions. The specimens were classified on the dominant features. The grades of adhesion could still be identified when the degree of compression was varied.

Platelets have been counted by the method of Dacie[10]. In the post-ADP specimens platelet debris and granules were present and were not included in the counts. The term 'maximal clumping' has been applied to specimens in which most clumps showed marked adhesion, and in which there were few clumps of less than fifty platelets and there were less than 25,000 single whole platelets/mm^3.

Supernatants were prepared from clumped specimens of PRP which had been stirred for 2–3 min after the addition of ADP or adrenaline by cooling the specimens to 4° C and centrifuging for 5 min at 1,250g. The supernatant was stored in ice. It was warmed to 37° C immediately before the addition of 0·25 ml. by plastic Mantoux syringe to 1 ml. of PRP. Platelet-poor plasma (PPP) was prepared from the same blood specimen used in each experiment. ADP was added to aliquots of this PPP which were then added to the PRP used for testing the supernatants. A calibration curve was thus obtained. The ADP-like activity in a specimen is given by multiplying the activity in the 0·25 ml. of its supernatant by five.

It has been shown previously that the stirring of disk-shaped platelets causes a swirling effect in the plasma, resulting in variable light transmission which when recorded gives an oscillating tracing[6]. Following the addition of ADP, there is rapid conversion of disks to irregular ovals and spheres. This reduces the resistance of the platelets to stirring and so there is a loss of oscillations and, due to the more uniform distribution of the platelets in the PRP, an initial decrease in light transmission. Clumping of these changed platelets results in a subsequent increase in light transmission. This sequence of changes produces a 'hump' tracing[6].

Fig. 1 shows the changes in platelet shape and clumping produced by the addition of different clumping agents to stirred PRP. 0·25 ml. of PPP or saline produced an increase in light transmission due to the dilution of the PRP and did not alter the oscillations or cause clumping. Adrenaline caused a gradual loss of oscillations and a slow increase in light transmission. Many clumps of platelets which had retained their disk shape were present. 5-Hydroxytryptamine (5-HT) and ADP produced an immediate hump tracing. Only a small subsequent increase in light transmission occurred following the addition of 5-HT. Thrombin produced a hump tracing but after a delay of 3–10 sec. The delay decreased as the thrombin concentration was increased. The clumping activity of adrenaline was inhibited by phentolamine and partially by adenosine (see later), and of all other clumping agents by adenosine. In addition, the action of 5-HT was also inhibited by phentolamine and of thrombin by heparin. These results confirm the observations of Born[12] and O'Brien[11,13,14]. Thus, the nature of a clumping agent present in a supernatant can be detected from the change it produces in platelet shape plus the effect of inhibitors on this change. The concentration of the agent can be determined from the increase in light transmission produced. When more than one clumping agent is present in a supernatant, the initial changes in platelet shape are those of the agent with the most rapid action. Quantitative estimation of one clumping agent in a supernatant containing more than one must be made in test PRP to which inhibitors of the other agents have been added.

The dose-response curve of PRP to the addition of ADP was examined. The PRP was stirred for 30 sec and then the ADP was added. The changes in light transmission were followed for 10 min (for 2 min at the same chart speed and 8 min at a slower chart speed (Figs. 2 left, 3 left)). Then the platelet count, the frequency of different sizes of platelet clumps and the morphology of the larger clumps were noted (see Table 1).

The response of a PRP to increasing concentrations of ADP appears as an increase in light transmission (clumping phase) followed by a decrease in light transmission (reversal phase) (Fig. 2 left). A small reversal phase was still present after the addition of 10^{-5} M ADP but not after 10^{-4} M ADP. The samples removed after stirring for 10 min (Table 1) demonstrated that increasing concentrations of ADP caused a reduction in the number of single platelets and small platelet clumps and an increase in the number of larger platelet clumps. The degree of platelet adhesion also increased with increasing ADP concentrations and 10^{-4} M ADP produced 'maximal clumping'. The addition of supernatant from a similar PRP to test PRPs (Fig. 2 right) demonstrated a loss of ADP-like activity between the time of adding the ADP and of testing the supernatant.

Fig. 3 left shows the response of another PRP. The addition of 5 × 10^{-7} M ADP (0·5) led to clumping and reversal phases similar to those illustrated in Fig. 2)

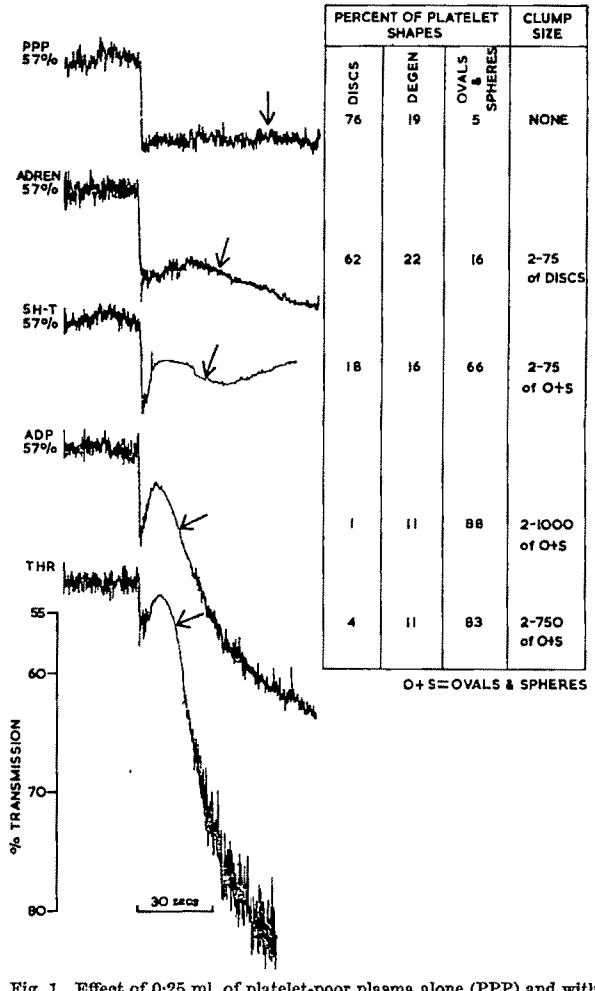


Fig. 1. Effect of 0·25 ml. of platelet-poor plasma alone (PPP) and with adrenaline (adren), 5-hydroxytryptamine (5-HT), and ADP and of 0·25 ml. saline with thrombin (THR) on platelet-rich plasma. The final concentrations were adren 4 × 10^{-6} M, 5-HT 5 × 10^{-6} M, ADP 2 × 10^{-6} M, Thr 0·2 u./ml. The percentages of single platelets which were disk-shaped, degenerating larger shapes (degen), and ovals plus spheres (O + S) have been averaged from six experiments

Table 1. EFFECT OF SECONDARY CLUMPING ON THE PLATELET COUNT, FREQUENCY OF DIFFERENT SIZES OF PLATELETS CLUMPS, AND MORPHOLOGY OF LARGER CLUMPS IN SPECIMENS OF PRP STIRRED FOR 10 MIN AFTER THE ADDITION OF ADP

	ADP initial concentration 1 unit = 1×10^{-6} M	Platelet count Th/mm³	Clumps 2–10 platelets	Clumps 10–50 platelets	Clumps 50–1,000 +	Morphology of clumps of 50–1,000 +
No secondary clumping (Fig 2)	PRP	385	+	+	–	–
	1·5	290	+ + +	+ + +	–	–
	4	167	+ + +	+ + +	+ +	*l*
	10	73	+ + +	+ + +	+ + +	*l* and *m*
	20	32	+ +	+ +	+ + +	*m* and *M*
	100	8	*r*	+	+ + +	*M*
Secondary clumping at 8×10^{-7} M ADP (Fig. 3)	PRP	360	+	+	–	–
	0·5	280	+ + +	+ +	–	–
	0·75	250	+ + +	+ +	–	–
	0·8	8	*r*	–	+ + +	*M*
	1·0	12	*r*	–	+ + +	*M*
	100	5	*r*	–	+ + +	*M*

The dose-response curves of the PRPs are shown in Figs. 2 and 3. 1 unit ADP = 10^{-6} M ADP, that is, 10 = 10^{-5} M ADP. + + +, Many; + +, several; +, few; *r*, rare; *l*, loose adhesion; *m*, moderate adhesion; *M*, marked adhesion.

The addition of 8×10^{-7} M ADP produced the expected clumping phase, but the reversal phase was interrupted by a second clumping phase which continued until the increase in light transmission equalled the change produced by 10^{-4} M ADP (100). There was no reversal of this secondary phase during the 10 min of observation. A specimen taken after stirring for 10 min showed that 'maximal clumping' had occurred. In this PRP an increase in the added ADP concentration of only 2×10^{-7} M caused fusion of the initial and second clumping phases. The addition of supernatant from a similar PRP to a test PRP demonstrated a marked release of clumping activity during the second phase (Fig. 3 right). This increase in clumping activity was inhibited by pre-incubation of the test PRP with adenosine. Heparin and phentolamine did not inhibit this activity. Thus it produced the same changes in this test system as ADP. This activity was only released by ADP when platelets were present in the plasma and the plasma was also stirred.

In some PRPs, therefore, a second clumping phase occurred which resulted in 'maximal clumping' and also the release of ADP. This phenomenon will be called 'secondary clumping'. It may occur at any ADP concentration from 10^{-7} M to 10^{-5} M. The change from the curve showing secondary clumping interrupting the reversal phase to the curve showing fusion of the two clumping phases was produced by a small increase in ADP concentration. These three phases cannot be shown on one tracing in most PRPs which require more than 4×10^{-6} M ADP to produce secondary clumping. It was possible to produce 'maximal clumping' in all PRPs (Fig. 2 in Table 1), but when secondary clumping had not occurred no increase in clumping activity could be demonstrated in the test system used (Fig. 2 right). Secondarily clumped specimens stirred for 1 h at 37° C showed some break-up of the smooth edge of the clumps. Increase in platelet debris gave a decrease in light transmission. There was no increase in recognizable single platelets.

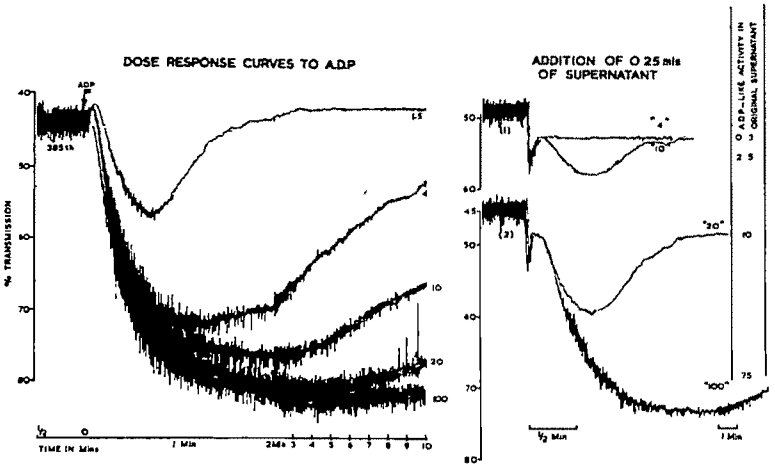


Fig 2. *Left*, Dose-response curve to ADP of PRP which did not give a secondary clumping phenomenon after the addition of ADP. 1 unit = 1×10^{-6} M ADP. *Right*, 0·25 ml. of supernatant from specimens of a similar PRP, stirred for only 2 min after the addition of ADP, has been added to two test plasmas (1) and (2) which had been previously calibrated with PPP containing known amounts of ADP. The parent specimen from which the supernatant was prepared is shown in quotation marks. The right column gives the ADP-like activity in the parent specimen

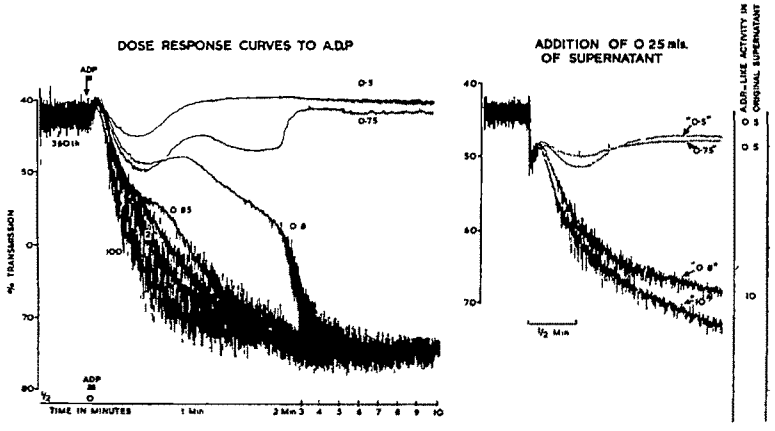


Fig. 3. *Left*, Dose-response curve to ADP of PRP which gave a secondary clumping phenomenon at 8×10^{-7} M ADP (0·8). 1 unit = 1×10^{-6} M ADP *Right*, 0·25 ml of supernatant from specimens of a similar PRP, stirred for only 2–3 min after the addition of ADP, has been added to PRP which showed secondary clumping at $1{\cdot}5 \times 10^{-6}$ M ADP

Secondary clumping resulted in the release of up to 10^{-5} M ADP-like activity (Fig. 3). One-eighth of the supernatant of a specimen which had been secondarily clumped by $7{\cdot}5 \times 10^{-7}$ M ADP contained sufficient activity to clump secondarily another specimen of the same PRP. Thus a self-propagating platelet clumping reaction can be produced *in vitro* despite dilution of the clumping activity released by the platelets.

Adrenaline induces one phase of clumping in all PRPs and two phases of clumping in most platelet-rich plasmas[15]. Secondary clumping to ADP and a second phase of adrenaline-induced clumping either were both present or were both absent in the same specimen of PRP when it was tested separately with both substances (Table 2). The concentration of adrenaline required to produce a second phase of clumping was in most instances less than the concentration of ADP required to produce secondary clumping (Table 2).

Adrenaline was added to PRP which was incapable of secondary clumping following the addition of ADP (Fig. 4). The initial changes shown in Fig. 1 occurred and many clumps of up to 1,000 disk-shaped platelets were present. Their formation was also associated with a variable loss of oscillations of the tracing. Phentolamine added after the formation of the clumps of disks rapidly reversed the increase in light transmission and oscillations reappeared. Microscopy showed only very few clumps of more than ten platelets.

In those PRPs which showed secondary clumping to ADP, a second phase of adrenaline clumping interrupted the first phase at a time after the addition of adrenaline which was directly proportional to both the adrenaline concentration and the ADP concentration necessary to induce secondary clumping. The second phase started with a reduction in oscillations which was associated with the presence of many oval and spherical platelets. This was followed by a rapid fall of the tracing and then the appearance of marked oscillations (Fig. 5*A*, *a*). At this point 'maximal clumping' was present. Equimolar phentolamine added during the first phase again caused complete reversal, but during the early part of the second phase reversal was only partial. No oscillations reappeared and some further clumping occurred after the addition of the phentolamine (Fig. 5*A*, *b*). The clumping at this point was not due, therefore, to adrenaline. However, the adrenaline stimulus was still required at this point for the second phase to proceed to maximal clumping. Phentolamine added later in the second phase did not influence the development of maximal clumping.

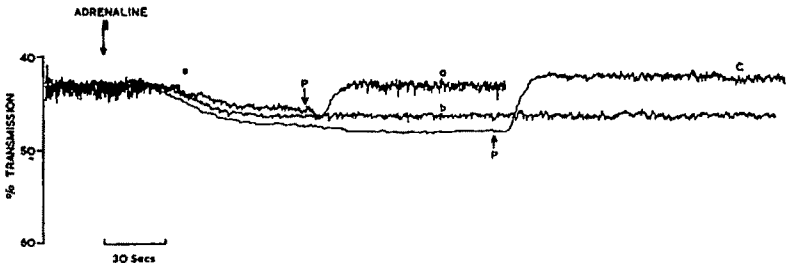


Fig. 4 Reversal of the first phase of adrenaline clumping by phentolamine. Phentolamine 2×10^{-5} M was added at the point *P* to PRP (*a* and *c*) after it had been clumped by adrenaline 2×10^{-6} M (*a* and *b*) and 1×10^{-4} M (*c*)

The supernatant from PRP maximally clumped by adrenaline (Fig. 5, *B*) produced an immediate hump curve in the test PRP. The test specimen containing phentolamine (which inhibits adrenaline and 5-HT) was clumped by the supernatant. This showed that ADP-like activity had been released during the maximal clumping and the fall in LT indicated that 'secondary clumping' had occurred. Both adenosine and phentolamine were required to inhibit completely the clumping activity in the supernatant. The clumping which occurred in the specimen which contained only adenosine (Fig. 5 *B*, *b*) was reversed by phentolamine. Thus adrenaline-induced clumping is not completely inhibited by equimolar adenosine.

In summary and conclusion, the addition of ADP or adrenaline induced one phase of clumping in all, and two phases of clumping in many, citrated plasmas when stirred maximally. The first phase was the direct result of the addition of the clumping agent. The adrenaline-induced first phase consisted of clumping of disk-shaped platelets and was rapidly and completely reversed by phentolamine. This may be unique to the catecholamines. The second phases produced both by ADP and adrenaline are a property of the individual plasma and resulted in clumping which did not reverse itself *in vitro*, and in the release of ADP-like activity. In those plasmas in which 'secondary clumping' occurs at low ADP or adrenaline concentration, a self-propagating reaction was present *in vitro* despite dilution of the ADP-like activity released by the platelets. The relationship of these phenomena to haemostasis and thrombosis requires investigation.

I thank Dr. M. F. Oliver for his advice and help and for providing research facilities, and Mrs. N. S. Davidson for technical assistance. This work was under-

Table 2. Relationship in Representative Specimens of Platelet-rich Plasma (PRP) between the Presence of Secondary Clumping to ADP and a Second Phase of Clumping to Adrenaline

Subject	PRP Platelets/mm³	Secondary clumping (ADP)	Second phase (Adrenaline)
AT	785	$2{\cdot}5 \times 10^{-7}$ M	5×10^{-9} M
KD	210	$7{\cdot}5 \times 10^{-7}$ M	5×10^{-8} M
BM	410	$7{\cdot}5 \times 10^{-7}$ M	2×10^{-7} M
JT	455	$1{\cdot}5 \times 10^{-6}$ M	1×10^{-6} M
CC	305	3×10^{-6} M	5×10^{-7} M
TH	307	4×10^{-6} M	$7{\cdot}5 \times 10^{-6}$ M
AA	360	5×10^{-6} M	5×10^{-6} M
JH	309	8×10^{-6} M	3×10^{-6} M
RWD	530	Absent	Absent
MH	260	Absent	Absent
NM	480	Absent	Absent

The lowest concentration producing secondary clumping has been recorded. Following the addition of ADP the PRP was stirred for 3 min and following adrenaline for 5 min.

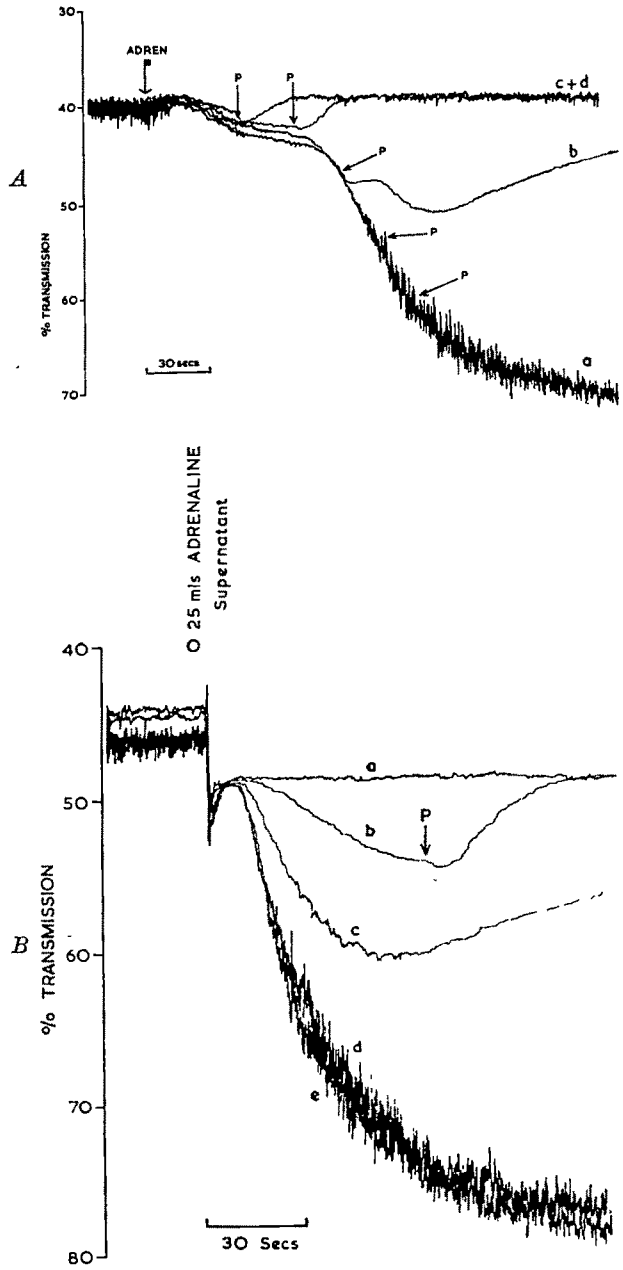


Fig. 5. *A*, The effect on the two phases of adrenaline-induced clumping (2×10^{-6} M) of phentolamine 2×10^{-5} M (*P*) added at increasing intervals after the addition of the adrenaline. During the first phase (*c* and *d*) complete reversal occurred; during the early part of the second phase (*b*) partial reversal occurred, and later in the second phase there was no reversal (*a*). *B*, The effect of inhibitors on the clumping activity present in the supernatant (0·25 ml.) from PRP maximally clumped by adrenaline. The initial increase in transmission is a dilution effect. The inhibitors present were: (*a*) incubation in advance for 5 min with adenosine 5×10^{-3} M and phentolamine 2×10^{-5} M; (*b*) adenosine only—phentolamine was added at *P*; (*c*) phentolamine only; (*d*) heparin, 25 u./ml.; (*e*) saline

taken during the tenure of a British Heart Foundation research fellowship.

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THYROTROPHIN RELEASING FACTOR IN HYPOPHYSEAL PORTAL BLOOD

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SINCE the formulation of the hypothesis that the secretion of anterior pituitary hormones is controlled at least in part by neurohumoral agents passing to the pituitary by way of the hypophyseal portal vessels[1,2], evidence for the existence of a specific thyrotrophin releasing substance (TRF) has been forthcoming (for reviews see refs. 3 and 4). Proof that such releasing substances are concerned in regulation of pituitary activity requires their identification in the hypophyseal portal blood in concentrations which vary as appropriate pituitary activity varies[2,3]. We believed that rat portal blood, if it contained a substance(s) which could release rat thyroid stimulating hormone (TSH), might, when infused into the pituitaries of rabbits, increase the peripheral level of TSH in the recipient rabbits. The evidence presented in this article suggests the presence of a factor in the portal blood of rats which influences TSH release by the anterior pituitary.

Lister female rats of 160–200 g and males of 180–280 g from the colony of the Department of Human Anatomy, University of Oxford, were used. In the females, bipolar electrodes, and in the males, unipolar electrodes of platinum wire (s.w.g. 34) coated with glass except for 0·5 mm at the tips were placed stereotaxically in the anterior hypothalamus. These rats with implanted electrodes were screened (under urethane anaesthesia, 1,000 mg/kg, 10 per cent in 0·9 per cent saline) to determine whether electrical stimulation (50 c/s sine wave) for 30 min could elevate blood radioactivity levels while the rats were under a thyroxine (T4) dosage (5–10 μg T4/100 g/day) sufficient to prevent release from their thyroids of previously incorporated iodine-131. Of fifty rats screened in this way, sixteen responded with an increase in blood radioactivity, suggesting release of TSH as a result of stimulation. Stimulation was performed at voltages of 1·4–2·5 V and current readings ranging from 80–120 μamp (peak to peak).

7–10 days later, rats which had given a positive screening response were anaesthetized with urethane as before and prepared for the collection of portal blood from the severed pituitary stalk by a procedure described elsewhere[5]. After collection of an initial sample of 0·7–1·5 ml., stimulation was begun as before. Further portal blood samples were collected during stimulation. The volume of blood at several time intervals during collection was recorded, making possible an estimate of the overall rate of collection. The samples (in graduated tubes) were held in an ice bath, centrifuged, the haematocrit measured and the plasma stored at −10° C. The portal blood plasma was afterwards used for infusion into rabbits. Some samples were also assayed for TSH content.

Rabbit pituitaries were cannulated with platinum cannulae by the technique described by Campbell *et al.*[6]. 2–4 weeks later, rat portal samples of 0·135–0·160 ml. were infused at the rate of 0·065 ml./h into the conscious rabbits and peripheral blood samples of 2·5 ml. were withdrawn from the rabbit ear veins before and after infusion.

TSH was assayed in the rabbit peripheral blood samples and in four of the rat portal blood samples by an *in vitro* method described previously[7,8], and modified to allow expansion of the assay to include eight or nine unknowns. Rabbit TSH values were expressed as mU./100 ml. plasma. The values of λ obtained in these assays fell between 0·19 and 0·48, except in one assay (0·64) the results of which were discarded.

The electrodes of four of the sixteen screened rats became detached from the skulls before portal blood collection. Portal blood plasma was collected from the remaining twelve rats both before and during electrical stimulation. Assays of TSH in four of these samples showed that the levels before stimulation ranged from 68 to 208 mU./100 ml. while those collected during subsequent stimulation ranged from 5·3 to 39 mU./100 ml. plasma. These high levels are probably due to ischaemia of the pituitary after section of the portal vessels causing a passive leakage of TSH into the blood flowing backwards through the gland. Paired samples of stimulated and unstimulated plasma from the rat portal blood collections were infused into a total of fifteen rabbits through cannulae in the pituitaries. A 24–48 h period was allowed for recovery between the first and second infusions in those rabbits which received two. Half the rabbits were given the unstimulated plasma and half were given stimulated plasma at their first infusions. One rabbit died immediately after its infusion. The plasmas of two other rabbits were included in an assay of doubtful validity (λ = 0·64) and were discarded.

Nine portal plasma samples collected before electrical stimulation of eight rats were infused into the pituitaries of recipient rabbits. Peripheral TSH levels fell in five rabbits, but showed no significant change in the other four. The samples of portal blood collected from the same eight rats during electrical stimulation induced the following changes in the levels of TSH in the peripheral blood of recipient rabbits: in two the TSH levels rose, in two they fell, and in five they showed no significant change (see Table 1).

When the data from individual rats are compared across Table 1, it can be seen that while the pre-stimulation portal blood of rats Nos. 5, 13, 14, *M*9 and *N*6 caused falls in recipient TSH levels, plasma collected from the same rats during stimulation caused the level of TSH in the recipient to rise or to remain unaltered. In rat *M*7 (both plasmas from which induced TSH falls) and rat *M*9 the electrode tips were in the mamillary and premamillary areas respectively. In all other rats shown in Table 1, the electrodes were in the anterior hypothalamus. The exposed tips extended downward to lie above and in the ventromedial nuclei (Fig. 1). Sample 14 (ii) may indicate a decline in TRF activity after a short initial response to stimulation. For each rabbit in Table 1, the plasma TSH level after infusion is expressed as a

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Table 1. CHANGES IN BLOOD TSH LEVELS IN RABBIT AFTER INFUSION OF HYPOPHYSEAL PORTAL BLOOD FROM RATS BEFORE OR DURING ELECTRICAL STIMULATION IN THE HYPOTHALAMUS

Rat No.	Rabbit No.	Peripheral TSH levels in recipient rabbits (mU./100 ml. plasma) Pre-stimulation rat plasma Initial	Post-infusion†	Percentage of initial (*A*)	Stimulated rat plasma Initial	Post-infusion†	Percentage of initial (*B*)	Net response (*B*/*A*)
5	1*C*	4·6	2·2	48	6·1	17·3 − +	286	5·95
13	3*C*	40·8	14·6 +	36	84·3	101·1	120	3·35
14*	4*C*	13·0	4·8 +	37	(i) 34·7	54·2	155	4·20
	7*C*	19·2	6·9 +	36	(ii) 8·4	3·7 −	44	1·23
16	6*C*	25·4	27·0	106	34·3	22·9	67	0·63
*M*7	15*C*	10·7	4·2 + + +	39	28·8	7·4 − + +	26	0·66
*M*9	16*C*	19·3	9·0 +	47	8·3	8·2	99	2·12
*N*6	14*C*	11·7	5·6	48	3·3	7·0 −	215	4·46
	17*C*							
*N*10	13*C*	4·4	6·6	150	7·8	5·7	73	0·49
	18*C*							
Mean ± *S.E.*				60·8 ± 13·3			120·6 ± 28·3	2·57 ± 0·67
P (by *t* test)								< 0·05

* During stimulation of rat No. 14, plasma (i) was collected in the first 12 min and plasma (ii) in the following 15 min.
† Significance of change from initial value: +, $P < 0.05$; + +, $P < 0.01$; + + +, $P < 0.001$ (by variance ratio test).

percentage of the same animal's initial level (*A* and *B*) taken immediately before infusion. The percentage responses for each paired infusion are combined to give an index of the net response by the rabbit pituitaries (*B*/*A*) to hypothalamic stimulation in the donor rat. The mean index of response for all paired portal samples was significantly greater than unity (2·57 ± 0·67, $P < 0.05$).

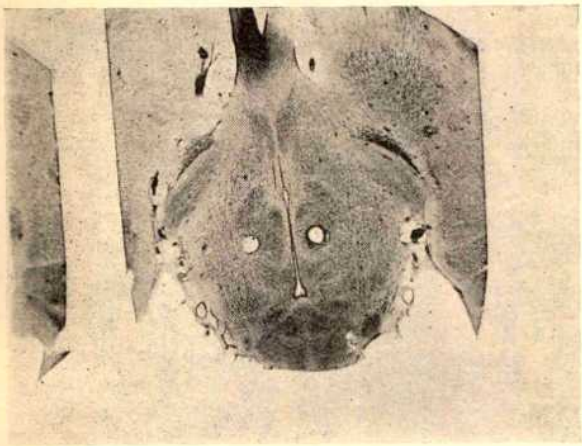


Fig. 1. Horizontal section through rat hypothalamus showing electrode tracks as holes in ventromedial nuclei. (× 17)

Although the onset of stimulation was always followed by an increased rate of flow of portal blood (mean before stimulation, 1·35 ml./h; mean during stimulation, 2·22 ml./h) the rate of flow was not correlated with the ability of the sample to raise or lower the blood TSH level in the infused rabbit. Thus flow in rat No. 5 before stimulation was at 1·2 ml./h and during stimulation was only 1·4 ml./h.

Plasma from blood samples obtained during the surgical approach to the pituitary stalk but before the stalk was cut, and plasma from the pituitary stalk of rats without electrodes, were infused into rabbit pituitaries as a control procedure. These samples did not raise the peripheral TSH levels in the recipient rabbits. In two out of four cases, a significant fall in TSH levels was induced, comparable to those shown in Table 1 after infusion of plasma collected before stimulation.

This series of experiments demonstrates that blood collected from the hypophyseal portal vessels after stalk section in the rat, when infused into the pituitaries of recipient rabbits, was able either to raise or to lower peripheral TSH levels, the direction of the change depending on whether or not the hypothalamus was stimulated electrically. It has been shown that under stressful conditions of surgery and anaesthesia there is a marked inhibition of TSH secretion in the rat[9,10]. Under the equally or more stressful procedures required for portal blood collection from the rat, the portal blood appeared to contain substances which depressed TSH levels in the recipient rabbit. When electrical stimulation was applied in the hypothalamus, however, the rat portal blood appeared to contain substances which either prevented this fall in the TSH levels of the recipient rabbits or in some cases caused them to rise. This was not a result of the TSH content of the infused plasma samples, as these were assayed directly for TSH in four cases and in each case the TSH level of the pre-stimulation sample was higher than that of the sample collected under stimulation. In an infusion volume of approximately 0·16 ml. there would be less than 0·3 mU. TSH, insufficient to be detected after its infusion into a 2·5–3·5 kg rabbit.

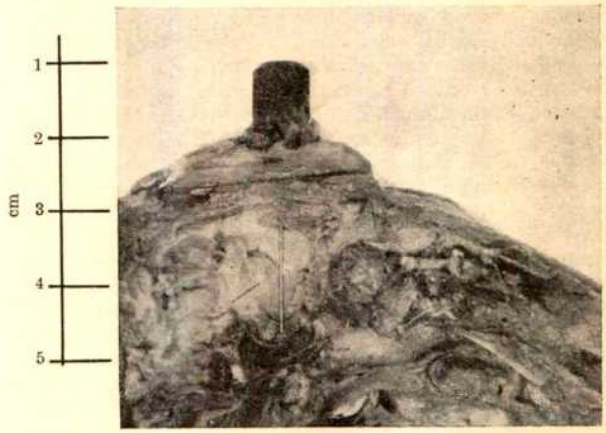


Fig. 2. Cutaway section of rabbit skull showing tip of cannula lying in the anterior pituitary. (× 3·1)

These preliminary findings, although by no means conclusive, are consistent with the hypothesis that following electrical stimulation in the hypothalamus TSH may be released from the pituitary due to the liberation of a thyrotrophin releasing factor (TRF) which passes by way of the hypophyseal portal vessels to the adenohypophysis. Further investigation into this problem is under way.

We thank Prof. G. W. Harris in whose laboratories this work was done, and Miss Linda Palfry for valuable technical assistance. The work was financed by a grant from the U.S. Air Force and by the U.S. National Institutes of Health and the Medical Research Council.

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TOXICITY OF HEXAMETHYLPHOSPHORAMIDE IN RATS

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CHANG *et al.*[1] reported that hexamethylphosphoramide (HMPA), an analogue of tepa, is an effective chemosterilant for houseflies. They emphasized that it has a low toxicity to mammals, and also that it has a wide margin of safety for flies between a lethal dose and a dose effectively sterilizing the males. Another advantage claimed for HMPA is that it is not an alkylating agent as, for example, are the ethyleneimine compounds which are also now being considered as chemosterilants. In view of the reported low acute toxicity of HMPA to mammals and its value as an insect chemosterilant, we undertook investigations of its toxicity to rats.

Male and female Sherman strain rats were used, and technical grade HMPA was either dissolved in water and given by stomach tube (while the controls were dosed with tap water) or mixed into ground Purina chow used as food. LD_{50} values for HMPA were determined according to the procedure developed by Gaines[2] for other pesticides; calculations were made according to the method of Litchfield and Wilcoxon[3]. All rats had free access to Purina chow and water. For white blood cell counts, blood was obtained by tail clipping. Complete autopsies were undertaken with most animals. Tissue for microscopic study was fixed in 10 per cent neutral formalin, and routine staining procedures were performed with haematoxylin and eosin. The left testis and epididymis were removed from a number of experimental male rats, which had been repeatedly dosed, and from a corresponding group of controls. This procedure was carried out with the animals under sodium pentobarbital anaesthesia 38–45 days after onset of exposure; microscopical examinations were then performed. These animals were then allowed to live for an additional 52 days.

The LD_{50} values for HMPA given by oral and dermal routes to adult rats are shown in Table 1. The compound when given by the oral route was more toxic to male rats than to females. The acute toxicity of HMPA by the oral route in male rats was less than that of the alkylating agents, apholate, metepa and tepa, by a factor of about 19 or greater. Gaines and Kimbrough[4] reported oral LD_{50} values of 98, 136 and 37 mg/kg, respectively, for the latter three compounds. Owing to considerable variation in the mortality among rats given single doses of HMPA by the dermal route, the LD_{50} could only be estimated. The main clinical symptoms observed in acutely poisoned rats were involuntary urination, mild muscle fasciculation, convulsions and bloody urine. (Large numbers of intact red blood cells were observed in the urine when it was investigated under the microscope.) Because HMPA is an organic phosphorus compound and because muscle fasciculation was observed, the blood cholinesterase level was determined titrimetrically in six adult male rats. When each animal was used as its own control, the change in plasma enzyme activity varied little (−3 to −42 per cent) at the time of most severe illness—about 3 h after an oral dosage of 2,000 mg/kg. Five hours after this dosage, there was some return to normal (+8 to −19 per cent). There was no significant change in red cell cholinesterase at any time.

Table 1. ACUTE TOXICITY OF HMPA IN ADULT SHERMAN STRAIN RATS

Route	Sex	LD_{50} (mg/kg)	19/20 conf. limits (mg/kg)	Lowest dose (mg/kg) Tested	Lethal
Oral	M	2,650	2,325–3,021	500	2,000
Oral	F	3,360	2,754–4,099	2,000	2,500
Dermal	M	3,500–4,500*	–	3,000	3,500
Dermal	F	3,500–4,500*	–	3,000	3,500

* Estimated.

A dose of 2,200 mg/kg was given to ten adult male rats. The total white blood cell and differential counts were determined before, 3 days after, and 1 week after treatment. The white blood cells did not appear to be affected. The only animals that showed a drop in total white blood cell count were 6- to 7-week-old male rats which had been treated daily with 400 mg/kg HMPA by stomach tube for 30 days. Their average count was 8,820 white blood cells/mm³ blood (range: 5,500–13,050); the total white blood cell count of ten controls of the same age averaged 16,670/mm³ blood (range: 10,600–23,050). The lowest single dose which resulted in a loss in weight in males was 1000 mg/kg; the lowest repeated dose with this effect was 100 mg/kg/day.

In order to determine acute tissue changes, six adult male rats that had been given a single dose of 2,500 mg/kg HMPA were killed when moribund (3 days after poisoning) and their tissues examined. Two instances of necrotizing cystitis and four instances in which the tubules of the kidneys contained red blood cells and hyaline casts were recorded; these findings are consistent with the haematuria mentioned earlier. Intra-alveolar haemorrhage and bronchiectasis were observed in three rats. The spleen was smaller than normal in all animals. Even among the six rats killed only three days after a lethal dose, the testes of two of them showed a reduced number of sperms and spermatids, and occasionally multinucleated cells were observed. As will be described later, the testis proved to be the organ most consistently affected by single or repeated doses of HMPA, but other organs were also affected in some animals. For example, repeated dosing produced bronchial pneumonia with the formation of abscesses in the poisoned animals. The pneumonia was not related to the stomach tubing (Table 2), as it also occurred in the animals which received HMPA in their diet but not in the animals which received only water by stomach tube. It is possible that HMPA affects the reticuloendothelial system and thus lowers the resistance to infection, as many poisoned animals showed very small atrophic spleens. The lymph nodes of these animals, on the other hand, were normal or enlarged.

In a further investigation of the effect of HMPA on the testes, additional groups of adult male rats were killed about 40 days after receiving single doses of varying amounts of HMPA by stomach tube. The lowest single dosage damaging the testes was 1,000 mg/kg; a single dosage of 500 mg/kg produced no effect. The lowest dosage given repeatedly by stomach tube to rats in which the testes were examined microscopically was 100 mg/kg/day, and this dosage caused testicular atrophy (see Table 2). When given in the diet at a level of 750 p.p.m., the daily intake, ranging from 80 to 40 mg/kg, produced partial or complete atrophy (Table 2). The testes were obviously smaller in living rats even when microscopic study revealed only partial atrophy. However, in a preliminary breeding experiment, it was found that when HMPA was administered by stomach tube at a rate of 25 mg/kg/day for 56 days it did not affect the fertility of male rats during that period, and the testes of the living animals appeared to be normal in size.

Microscopic examination of the testes, 36–41 days after a single dosage of 1,000 mg/kg or more, indicated that at

Table 2. PNEUMONIA AND TESTICULAR ATROPHY IN MALE SHERMAN STRAIN RATS CAUSED BY HMPA

Dosage level (mg/kg)	No. of doses Stomach tube	Diet	Days between last dose and pathological examination	Number of animals: Total examined	Killed	Died	With pneumonia	With partial or complete testicular atrophy
2,000	1		36–41	9	9	0	2	9
1,000	1		38	5	5	0	3	2
500	1		38	6	6	0	1	0
0	1		38	6	6	0	0	0
400	36–99		0	8	2	6	2	8
200	26–99		0	5	3	2	3	5
100	47		0	4	surgery*	0	–	4
	60–99		0	10	9	1	5	10
	48		0	4	surgery*	0	–	0
0	98		0	10	10	0	0	0
		45	0	5	surgery*	0	–	5
80–40†		61–103		11	8	3	8	11
0		45	0	3	surgery*	0	–	0
		103	0	8	8	0	0	0

* Left testis was removed under sodium pentobarbital anaesthesia. The same animals as well as others in the group that did not undergo surgery are included in the autopsy material.

† The dietary level of HMPA was 750 p.p.m. In the first few weeks the animals ate about 110 g/kg/day so that the intake of HMPA was about 80 mg/kg/day; later as the rats matured the average food intake dropped to 55 g/kg/day and the HMPA intake to about 40 mg/kg/day.

this time the spermatids are the cells of the seminiferous epithelium most affected by HMPA, but the effect is not uniform throughout the testes. Particularly after repeated dosing at or above the rate of 80–40 mg/kg/day, some tubules of some of the testes still contained normal or almost normal seminiferous epithelium while others were devoid of it or showed only a few atypical cells. We have called this condition 'partial atrophy', as in complete testicular atrophy (Table 2) all tubules showed loss of the seminiferous epithelium except for a few remaining spermatogonia. Similar pictures of partial or complete atrophy were also observed with metepa in an experiment conducted earlier[4].

A single dose of 2,500 mg/kg was given to six females. No effect on the female reproductive organs was observed when the animals were killed 36 days later.

HMPA was given to ten female rats by stomach tube at a rate of 200 mg/kg/day. The dosing began 7 days before mating to undosed males and continued until the twentieth day of pregnancy. The females were then killed and their uteri opened. The offsprings and placentae were weighed and examined grossly; the foetuses were fixed in Bouin's solution, and cross-sections were examined later under the dissecting microscope. Abnormalities were not detected in the offsprings of either the rats dosed with HMPA or eight female control rats that had been dosed daily for the same period with tap water. The weight of each foetus, the weight of each placenta, the number of animals per litter, and the number of resorption sites in the treated animals did not differ statistically from the controls.

A dosage of 100 mg/kg/day of HMPA is about 4 per cent of the acute oral LD_{50}. In earlier investigations in which metepa was administered by stomach tube, the lowest dosage level that caused partial testicular atrophy in some rats after 55 days was 10 mg/kg/day; this represents about 7 per cent of the acute oral LD_{50} dose. Metepa was found to be more toxic to rats when given daily by stomach tube in water than when given as a component in the diet—10 mg/kg/day by stomach tube had about the same effect as 76 mg/kg/day in the diet[4]. On the other hand, HMPA had about the same effect whether it was given in the diet or by stomach tube (see Table 2)—probably owing to its greater stability. Because of its stability, HMPA might present a greater problem as a residue than the ethyleneimines, which deteriorate rapidly, although both kinds of compound have a cumulative effect on the testes.

These data show that HMPA has a very specific effect on the testes of rats when fed at a dietary level of 750 p.p.m. which may be compared with a level of 2,500 p.p.m. in food necessary to cause 100 per cent sterility in male flies[1]. This comparison shows that the safety of any future use of HMPA must be based on its moderate toxicity to mammals and on limitation of their contact with it rather than on any important difference in the inherent susceptibility of mammals and insects to the compound.

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[3] Litchfield, jun., J. T., and Wilcoxon, F., *J. Pharmacol. Exp. Therap.*, **96**, 99 (1949).
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CONVERSION OF PHENOTHIAZINE DERIVATIVES TO THE CORRESPONDING SULPHOXIDES ON THIN-LAYER PLATES

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PREVIOUS experimental work[1] reported from this laboratory on the phenothiazine derivatives has dealt mainly with colour, crystal and spectrophotometric tests as a means of identification. In a preliminary report[2] at a meeting of the American Academy of Forensic Science, attention was directed to the value of the sulphoxides as a further means of identification of these drugs, and a method for preparing them quickly and accurately was described. The sulphoxides have been used only in a limited manner up to now, because only a few of them have been available commercially and their preparation in the laboratory was very tedious and time-consuming. The present article extends the data of the preliminary report and deals with a complete investigation of forty phenothiazine derivatives and their respective sulphoxides using ultra-violet spectrophotometry, thin-layer and gas chromatography. It also makes it possible now to investigate these phenothiazine derivatives and their sulphoxides using infra-red spectrophotometry.

Reference Compounds

A few sulphoxides were obtained from the various manufacturers. Others were prepared according to the

method of Schmalz and Burger[3]. 20 mg of the salt of the phenothiazine derivative were dissolved in 20 ml. of a solution prepared by mixing 20 ml. of 3 per cent hydrogen peroxide with 70 ml. of 85 per cent ethanol. The solution was heated in a water bath at 90° C for 3·5 h, after which time the *p*H was adjusted to 12 with ammonia. The sulphoxide formed was extracted with ether and the solvent evaporated. The identity of the finished product was established by means of its ultra-violet and infra-red spectra, colour tests, thin-layer and, where applicable, gas chromatography.

The ultra-violet spectrophotometric data were obtained on a Beckman '*DK-2A*' ratio recording spectrophotometer, the infra-red data on a Beckman '*IR-4*' infra-red spectrophotometer and the gas chromatographic work was done on a 'Microtek *GC-2500R*' gas chromatograph. A short-wave ultra-violet lamp, model '*SL2537*' (Ultra-violet Products, Inc., South Pasadena, California), was used to locate the spots on the thin-layer chromatography plates.

Each of the following drugs in the form of their salts was used in the experiments described here: chlorpromazine, thioridazine, trifluoperazine, thioproperazine, levopromazine, perphenazine, fluphenazine, triflupromazine, promazine and promethazine.

During routine work on the identification of phenothiazines it was observed that when a developed thin-layer plate was left in the open air for some days, the ultra-violet spectrum of an eluted spot no longer resembled that of the original product, but was close to that of its sulphoxide.

Gas–liquid and thin-layer chromatography confirmed that the sulphoxide had indeed been formed. Because of this certain conditions influencing the oxidation of phenothiazines on thin-layer plates were further investigated.

Plates were prepared for ten different compounds. Each plate contained eight spots of the drug assigned to it. The plates were developed in the solvent and were allowed to stand in open air in daylight at approximately 20° C. Each day one spot was eluted and the ultra-violet spectrum recorded. After an interval of 48–72 h, depending on the derivative under investigation, marked changes in the absorption spectrum had taken place; the spectrum began to resemble that of the corresponding sulphoxide. At the end of 8 days the spectrum was that of the pure sulphoxide (see Fig. 1). While a change in colour of the spot was noted a few minutes after the dry plate was exposed to the daylight, it was not accompanied by a change in the ultra-violet spectra. Gas–liquid and thin-layer chromatography confirmed that only the sulphoxide was formed.

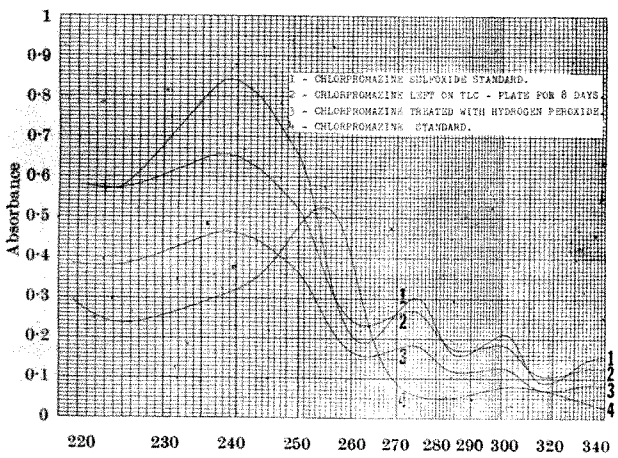


Fig. 1. Ultra-violet spectra of oxidized and unoxidized chlorpromazine. (1) Chlorpromazine sulphoxide standard; (2) chlorpromazine left on thin-layer chromatography plate for 8 days; (3) chlorpromazine treated with hydrogen peroxide; (4) chlorpromazine standard

Table 1. SPECTROPHOTOMETRIC DATA AT THE END OF 8 DAYS IN DAYLIGHT AND OPEN AIR (ABOUT 20° C) OBTAINED FROM THE ELUTED SPOTS

Generic names	Maxima of the standard drugs	Maxima of the sulphoxides
Chlorpromazine	254–305	238–274–298–340
Thioridazine	262–313	235–260–276–304
Trifluoperazine	257–308	244–275–304–336
Thioproperazine	234–264–315	249–275–304–342
Levopromazine	251–303	240–275–296–329
Perphenazine	255–307	233–275–300–340
Fluphenazine	258–308	233–255–274–302–340
Triflupromazine	257–308	233–255–274–300–340
Promazine	252–303	233–272–295–340
Promethazine	249–299	234–260–295–330

The oxidation was complete at the end of 8 days and was dependent mainly on the availability of oxygen. When the ten compounds, spotted and developed on plates, were placed in total darkness in an oven at 20° C over circulating air for 8 days, oxidation was complete. If similar experiments were carried out without circulating air in darkness or in daylight no oxidation occurred. The same was observed when the plate, spotted in the usual manner, was kept in a tightly closed vessel in an atmosphere of nitrogen for 2 weeks. This was confirmed by gas–liquid and thin-layer chromatography.

These experiments emphasize the importance of having sufficient air (oxygen) in order to obtain conversion of the phenothiazines to their respective sulphoxides. It was also evident that light was not essential in the oxidation process.

Numerous adsorbent gels are available on the market for thin-layer chromatography. Our experience with 'Silica Gel *G*', 'Adsorbosil *P*-2', 'Aluminium Oxide *G*' and 'Chromsorb-*G*' is that they do not by themselves have any effect on the formation of the sulphoxides in these experiments. It was further established that the thickness of the gels was not a decisive factor in the oxidation reaction since the ultra-violet spectra obtained from phenothiazine derivatives on plates of varying gel thickness were identical under standard conditions. The gels were not affected by the presence of hydrogen peroxide used to oxidize the phenothiazine derivatives to their sulphoxides. After spotting with 10, 20 and 30 per cent solutions of hydrogen peroxide, plates with silica gel were chromatographed, dried, eluted and examined in ultra-violet light. No absorption was detected.

The oxidation reaction appears to be associated with a relatively large increase in the surface area of the phenothiazine drugs on the plate. Such increase is brought about by the development of the plate after spotting with the drug.

Effect of Ultra-violet Irradiation

No change in the ultra-violet spectra was observed on the spotted plate up to and after 4 days of irradiation with ultra-violet light in a dark room without circulating air. It was observed, however, that deep brownish colours had developed in the spots. A control plate placed in the same room but excluded from ultra-violet irradiation had acquired a faint brown colour only, but no change in the ultra-violet spectra was noted. It is our opinion that ultra-violet irradiation does not have any appreciable effect on the oxidation of the phenothiazines by the method used. Further experiments may shed some light on the changes occurring under these conditions.

Effect of Temperature

When a series of plates, spotted with these drugs, is placed in an oven at 60° C with circulating air, conversion to the sulphoxides becomes measurable after about 2 h and is complete after 3–4 days. A peculiar phenomenon was encountered when a plate spotted with phenothiazine derivatives and developed in the usual manner was placed in an oven with circulating air at 120° C. Contrary to our expectations, only traces of oxidized material were detectable by ultra-violet measurements at the end of 3 days. Further experiments are in

progress to elucidate this phenomenon. Oxidative activity was practically absent at low temperature; no oxidation took place on a plate left at 0° C for 5 weeks.

Formation of Colour

Our experiments were carried out on plates, some of which were exposed to daylight while others were kept in darkness or exposed to ultra-violet irradiation. Other plates, in addition, were maintained in ovens where air was not circulated, in jars where there was no circulation of air, or in an atmosphere of nitrogen. The temperature of the atmosphere in which the plates were kept was also varied. In each case a colour developed in the spotted areas and varied as described later.

When plates were spotted with these drugs and maintained in daylight at approximately 20° C with or without oxygen, colour developed in the spots within 15 min. In darkness, the development of colour was delayed. Under these conditions the colours varied widely, depending on the concentration of drugs used. Also, the colour of any individual spot was not uniform, but varied from light shades in the central areas to deeper and darker shades in the periphery, or vice versa. When ultra-violet irradiation was applied to spots at 20° C in a dark room without circulating air, colour developed about as rapidly as it did in daylight. The shades were pastel at first and resembled those on the plates exposed to daylight, but with longer irradiation they changed colour. In those developed in daylight, on the other hand, the shade of the colour increased. The temperature at which the plates were stored was important in the development of the colour. After 6 weeks at 0° C only faint spots were evident, whereas with temperatures well above 20° C the spots appeared very rapidly and were more intense.

Hydrogen Peroxide as Oxidizing Agent

In our preliminary report[2] it was shown that spots of phenothiazine developed on thin-layer plates could be oxidized rapidly to the sulphoxide, using 3 per cent solutions of hydrogen peroxide (see Fig. 1). At that time the optimum concentration of peroxide had not been determined. Further investigations have shown that the concentration of hydrogen peroxide is not critical, but that best results are obtained when the concentration is between 10 and 20 per cent. The oxidation product obtained in this manner on the plate was confined to one spot only, as can be seen in Fig. 1 above the spot treated with 10–20 per cent hydrogen peroxide. The R_F value corresponds to that of the pure sulphoxide. The optimum of hydrogen peroxide concentration was determined as follows.

In Fig. 2 the original compound chlorpromazine HCl was spotted in such a manner as to obtain a large spot without tailing and the chromatogram was allowed to develop in direction 1. The spot was outlined under ultra-violet light and one strength of peroxide was applied to the upper part and another concentration to the lower part of the spot. Two reference spots were applied on line with and next to the spot, one with chlorpromazine and one with chlorpromazine sulphoxide. The plate was

Table 2. Spectrophotometric Data on Phenothiazine Derivatives kept in Circulating and Non-circulating Air (about 20° C) under Oxidizing and Non-oxidizing Conditions

Generic names	Maxima standard drugs	Maxima non-circulating air or nitrogen atmosphere	Maxima circulating air (sulphoxide)
Chlorpromazine	254–305	253–302	240–251*S*–274–300–340
Thioridazine	262–313	262–312	260–276*S*–304–338
Trifluoperazine	257–308	257–308	235–260–276–306–344
Thioproperazine	234–264–315	234–265–314	238–264–310–340
Levopromazine	251–303	250–302	250–275–298–332
Perphenazine	255–307	255–307	239–251*S*–275–306–344
Fluphenazine	258–308	256–307	234–260–276–306–348
Trifupromazine	257–308	255–307	238–258–281–310–342
Promazine	252–303	251–301	233–254*S*–270–300–340
Promethazine	249–229	249–300	232–250*S*–270–298–334

S, shoulder.

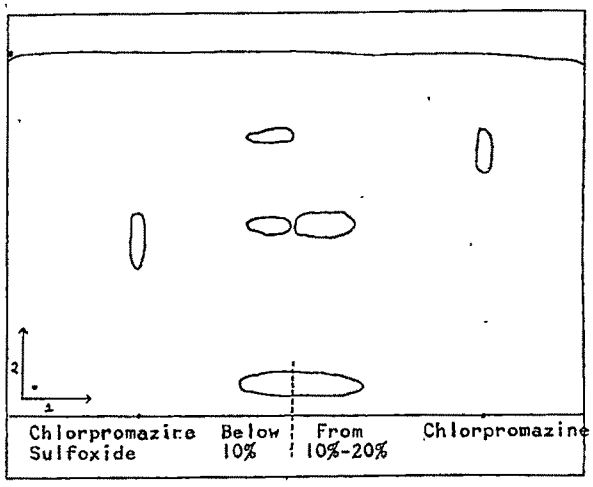


Fig. 2. Effect of concentration of hydrogen peroxide on complete oxidation of chlorpromazine

now allowed to develop in direction 2 perpendicular to the first one. This experiment indicated that in order to obtain a complete conversion to the sulphoxides for the quantity used, a concentration of at least 10–20 per cent hydrogen peroxide was essential. Below that concentration, only portions of the drug were oxidized and two spots were obtained on re-chromatographing. There was some indication that a second compound was formed when 30 per cent hydrogen peroxide was used together with hot air to dry the spot. This compound travelled behind the sulphoxide on the plate and might be the corresponding sulphone. The sulphoxides were identified by their ultra-violet spectra, colour tests, thin-layer chromatography and, where applicable, by gas–liquid chromatography.

Gas Chromatography

Gas chromatography is at present the most satisfactory method of separating and identifying the phenothiazines and their respective sulphoxides. Excellent separations were achieved using a 2-ft. 3 per cent '*SE*-30' column at different temperatures (Tables 3 and 4). It should be noted, however, that only very broad peaks could be obtained for the sulphoxides of the four phenothiazines with the highest molecular weights.

The retention times for these four compounds varied from 40 to 60 min. All compounds were injected in the free state as well as in salt form without any differences appearing in the chromatogram.

Table 3. Retention Times for Phenothiazine Derivatives

Compound	Temperature: Flow rate (ml./min)	Retention time 200° C 80	210° C 90	250° C 120
Trifupromazine		6·8	2·4	—
Promethazine		7·8	2 8	—
Promazine		9 1	3·1	—
Chlorpromazine		—	4·5	—
Levopromazine		—	5·7	—
Trifluoperazine		—	8·6	—
Fluphenazine		—	28·7	4·3
Thioridazine		—	29 8	5·8
Perphenazine		—	—	9 6
Thioproperazine		—	—	17 6

Table 4. Retention Times for the Sulphoxides

Sulphoxide of	Temperature: Flow rate (ml./min)	Retention time 210° C 90	250° C 120
Trifupromazine		4·2	—
Promethazine		6·6	—
Promazine		7·8	—
Chlorpromazine		10·2	2·5
Levopromazine		11·8	3 1
Trifluoperazine		16 8	3 6
Fluphenazine		—	—
Thioridazine		—	—
Perphenazine		—	—
Thioproperazine		—	—

Table 5

No.	Generic name	R_F of original drug	R_F of sulphoxide	Spectrophotometric data of sulphoxides obtained in the manner described
1	Acetophenazine	0·69	0·16	251–274*S*–310
2	Acetopromazine or acetylpromazine	0·58	0·39	251–272*S*–310–343
3	Aminopromazine or proquamazine	0·60	0·28	232–266–295–333
4	Carphenazine	0·71	0·16	246–277*S*–310
5	Chlorpromazine	0·62	0·48	238–273–298–340
6	Chlorprothazine	0·69	0·49	238–250*S*–273–298–340
7	Chlorprothixene	0·66	0·45	255–302
8	Cyamepromazine	0·61	0·39	243–274*S*–304–340
9	Diethazine	0·69	0·44	233–268–293–338
10	Dimethoxanate	0·56	0·41	240–274–295
11	Ethopropazine or prophenamine	0·70	0·47	233–267–292–336
12	Fluphenazine	0·75	0·59	232–273–304–343
13	Isopromethazine	0·63	0·39	233–267–291–336
14	Isothipendyl	0·64	0·41	238–273–336
15	Levopromazine	0·87	0·59	250–276*S*–296–333
16	Mepazine	0·58	0·39	231–272–299–342
17	Methdilazine	0·64	0·43	232–272–298–342
18	Methopromazine or methoxypromazine	0·67	0·35	244–274*S*–294–330
19	Methylpromazine	0·62	0·40	238–272–299–340
20	Perphenazine or chlorpiprozine	0·65	0·45	240–250*S*–274–342
21	Phenothiazine or penethazine	0·69	0·37	232–266–294–334
22	Pipamazine	0·83	0·53	239–274–300–342
23	Prochlorperazine	0·55	0·15	238–274–300–340
24	Promazine	0·51	0·37	231–271–299–342
25	Promethazine	0·66	0·38	232–270–297–340
26	Propiomazine	0·77	0·54	246–265*S*–304–360
27	Prothipendyl	0·69	0·48	238–276–340
28	Pyrathiazine or pyrrolazate	0·64	0·43	232–269–295–336
29	Thiazinamium	0·53	0·33	232–269–294–336
30	Thiethylperazine	0·47	0·28	238–272–301–350
31	Thiopropazate	0·77	0·25	238–274–300–340
32	Thioproperazine	0·43	0·26	245–262*S*–275–304–342
33	Thioridazine	0·71	0·46	237–273–302–340
34	Transergan	0·53	0·33	225–266–291–330
35	Trifluoperazine	0·63	0·41	233–273–302–343
36	Triflupromazine	0·69	0·50	233–274–301–343
37	Trimeprazine or alimemazine	0·71	0·50	232–297–340
38	No 6710 Rhône-Poulenc	0·63	0·46	251–273–298–332
39	No 9269 ,,	0·85	0·59	240–274–305
40	No. 7261 Smith Kline and French	0·78	0·27	233–272–302–340

Proposed Method of Identifying Phenothiazine Derivatives

The phenothiazine derivative is spotted in the lower two corners of a thin-layer plate (20 cm × 20 cm) approximately 3 cm from the edges. The plate is placed in the developing tank, with the spots at the bottom, until the solvent has travelled to about 3–4 cm from the top, after which time it is taken out and allowed to dry. The spots are now outlined under the short-wave ultra-violet light (cited previously) and the right-hand one is just wetted with small drops of 10–20 per cent hydrogen peroxide solution added drop by drop. The spot is dried in a hot air stream (60° C), after which the plate is placed in the tank again with the peroxide-treated spot at the bottom and allowed to develop in a direction perpendicular to the initial one. In this way, two R_F values can be obtained, one for phenothiazine derivative and one for the sulphoxide. The spots can now be removed from the plate and eluted with distilled water. After the ultra-violet spectra are obtained from the supernatant, the original phenothiazine and its sulphoxide can be compared with reference compounds with the use of thin-layer and gas–liquid chromatography. It should be noted that even after the most careful elution the gel remaining in the centrifuge tube still contains some sulphoxide which can be demonstrated by colour tests.

Table 5 shows the ultra-violet spectrophotometric data on the forty phenothiazine sulphoxides investigated, together with the R_F values and those of the parent compound.

We thank Smith Kline and French Laboratories and Dr. I. S. Forrest, Biochemical Research Laboratory, *T*-47 Veterans Administration Hospital, Palo Alto, California, for supplying samples of sulphoxides.

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MECHANISM OF THE CYTOTOXIC ACTION OF ALKYLATING AGENTS IN MAMMALIAN CELLS AND EVIDENCE FOR THE REMOVAL OF ALKYLATED GROUPS FROM DEOXYRIBONUCLEIC ACID

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THE reaction of alkylating agents of the mustard type with cellular deoxyribonucleic acid has been proposed as the basis for their cytotoxic action[1,2]. In an earlier report[3] results were presented which supported this view in the case of the reaction of mustard gas di-(2-chloroethyl)sulphide with mouse lymphoma cells (Fisher *L*5178*Y*) growing in culture. In this work two cell lines differing by a factor of about 2·5 in their sensitivity to the agents were investigated. With the more sensitive line, it was found that a dose which killed 90 per cent of the cells resulted in a depression of about 50 per cent in the rate of DNA synthesis within the first 2 h. This was the only rapid biochemical effect which was observed, protein and ribonucleic acid synthesis not being affected. It was also observed, however, that administration of a similar dose of sulphur-35-labelled mustard gas to the resistant line, which had very little biological effect, resulted in a similar extent of reaction of alkylating agent with the DNA of the cells. Walker has also recently reported[4] that the DNA of cells in the *S* or G_2 phases of

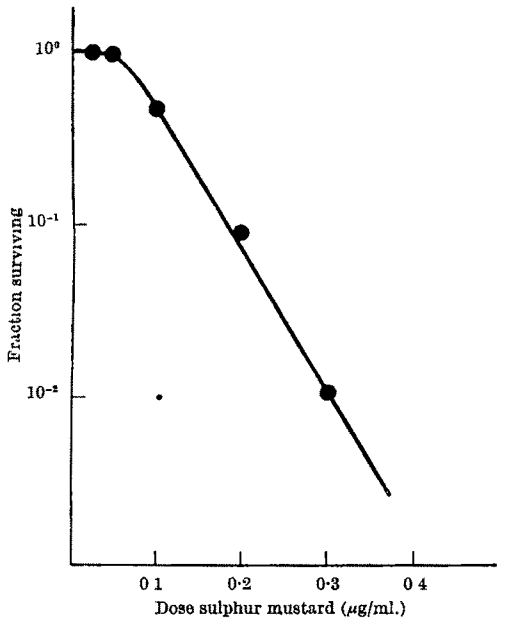


Fig. 1 Dose response of HeLa cell survival to sulphur mustard treatment. Method of derivation of the curve is described in the text

the cell cycle, which exhibit a difference in their sensitivity towards mustard gas, nevertheless reacted with [^{35}S]mustard gas to the same extent.

A logical explanation for these effects would be the existence of mechanisms capable of repairing the damage to DNA in cells less sensitive to the action of the mustard. Repair of damage to DNA could proceed by a sequence of steps which involves removal of the alkylated groups from alkylated DNA followed by subsequent degradation and resynthesis of a portion of the DNA. Evidence for an enzyme-mediated serial repair mechanism of this kind has been found in bacterial systems for the repair of damage caused by ultra-violet radiation[5,6], while it has been found that alkylated bases are excised from the DNA of *Escherichia coli B/r* following alkylation with mustard gas[7]. The purpose of this article is to present evidence that a mechanism exists in mammalian cells for the removal of alkylated bases from alkylated DNA.

Under conditions where extensive killing of cells occurs, the extent of reaction of the alkylating agent with DNA is very small ($\sim$ 1 alkylation per 10^5 nucleotides, see later) and it was considered that the only method having sufficient sensitivity to demonstrate a loss of alkyl groups from DNA of this kind would involve measurement of the change in specific radioactivity of DNA after alkylation with a labelled reagent. Despite the inhibition in the rate of DNA synthesis caused by mustard gas, DNA synthesis does proceed for many hours after administration of mustard and in cells which will not divide, so that the normal G_2 amount of DNA is synthesized[3,8]. Levis *et al.*[9] have reported that after human amnion cells (*AUP* strain) are treated with HN2 the DNA content per cell can reach much more than the G_2 level. Any loss in the specific radioactivity of DNA alkylated with labelled reagent could therefore be the consequence of an increase in the amount of DNA in the culture. In order to correct for any reduction in the specific radioactivity of the alkylated DNA due to continued DNA synthesis, cells were labelled with tritiated thymidine before they were treated with [^{35}S]mustard gas. Not only did this provide a method whereby one could allow for the dilution of the sulphur-35 label by subsequent DNA synthesis, but it also enabled the rate of DNA synthesis to be compared with that of a non-alkylated culture—this comparison is independent of the need for quantitative isolation of DNA.

For the present investigation, HeLa cells growing in suspension cultures were used. These cells had been found to have a sensitivity to mustard gas similar to that of the resistant line of mouse lymphoma cells previously investigated[3]. Their use presents the practical advantages that it is easier to grow large volumes of culture to high cell density ($\sim 10^6$/ml.) and that the DNA content per cell is greater ($1{\cdot}7 \times 10^{-11}$ g compared with $1{\cdot}2 \times 10^{-11}$ g). A dose-survival curve (Fig. 1) was obtained by normal plating procedures. Petri dishes (Falcon *TC*) were inoculated with an appropriate number of cells to give about 200 colonies per dish 30 min after treatment of the cells in suspension culture with various doses of mustard gas. Colonies were counted after 10 days' growth in a gassed incubator (95 per cent air, 5 per cent carbon dioxide). One factor which was observed, but not taken into account in the derivation of the curve, was the apparently slower rate of division of colonies derived from treated cells. For the purpose of constructing this curve, however, it was considered legitimate to count all visible colonies irrespective of apparent growth rate[10]. The dose for 37 per cent survival of the cells (D_0) was 0·05 μg/ml.—calculated from the straight portion of the survival curve (Fig. 1). As was the case with the resistant lymphoma cell line, the dose-response curve for the action of mustard gas on HeLa cells exhibits a shoulder which has been thought to be indicative of the operation of repair mechanisms[11].

In a typical experiment DNA was labelled by incubating HeLa cells (at a concentration of 4×10^5 cells/ml. growing in suspension culture in Eagle's medium) with tritiated thymidine at 5 mμc. (2 mμmole)/ml. After 24 h, the cells were transferred to the same volume of fresh medium for 6 h before half the culture was treated with [^{35}S]mustard gas at 0·32 μc. (0·5 μg)/ml. DNA, RNA and protein were then isolated after 45 min and at various times thereafter by the Kirby phenol method[12,13] from suitable measured portions of the mustard treated and control cultures (about 5×10^7 cells). The DNA was freed from any contaminating RNA by treatment with ribonuclease, then centrifuged in a mixture of 2 per cent sodium acetate and 1·5 per cent sodium chloride for 1 h at 64,000*g*. The following determinations were made at various times: the yields of DNA, RNA and protein in equal portions of control and treated cultures, the specific radioactivity of sulphur-35-labelled DNA, RNA and protein from mustard-treated cultures and the specific radioactivity of tritium-labelled DNA in treated and control cultures.

The results showed that the rate of synthesis of RNA and protein after mustard treatment, as indicated either by the increases in weights of material isolated (Fig. 2*b*) or by the decreases in their specific radioactivities (Fig. 2*a*), was the same as in the control culture. Furthermore, these syntheses proceeded at the same exponential rate as the increase in cell number of the control culture. This occurred despite the lack of increase in cell number of the mustard-treated culture which became, after 48 h, a uniform population of enlarged cells. The increase in the amount of DNA isolated from control cultures and the decrease in its specific radioactivity also proceeded at the same rate as the increase in cell number (Figs. 2*a* and *b*), thus providing evidence that in this system the thymidine was not being lost from the DNA owing to its turnover, or being re-utilized after such a loss. In mustard-treated cultures, however, there was a decrease in the rate of DNA synthesis as reflected by the slower rate of drop in the specific radioactivity of tritium-labelled DNA. This drop in rate of DNA synthesis has been confirmed by pulse-labelling experiments with [^{3}H]thymidine of high specific radioactivity for both HeLa and mouse lymphoma cells[3]. As this decrease occurred within 2 h of treatment with mustard, it could not have arisen from a mustard-induced mitotic block resulting in fewer cells entering the next synthetic period, contrary to the findings of Layde and Baserga for HN2-treated Ehrlich

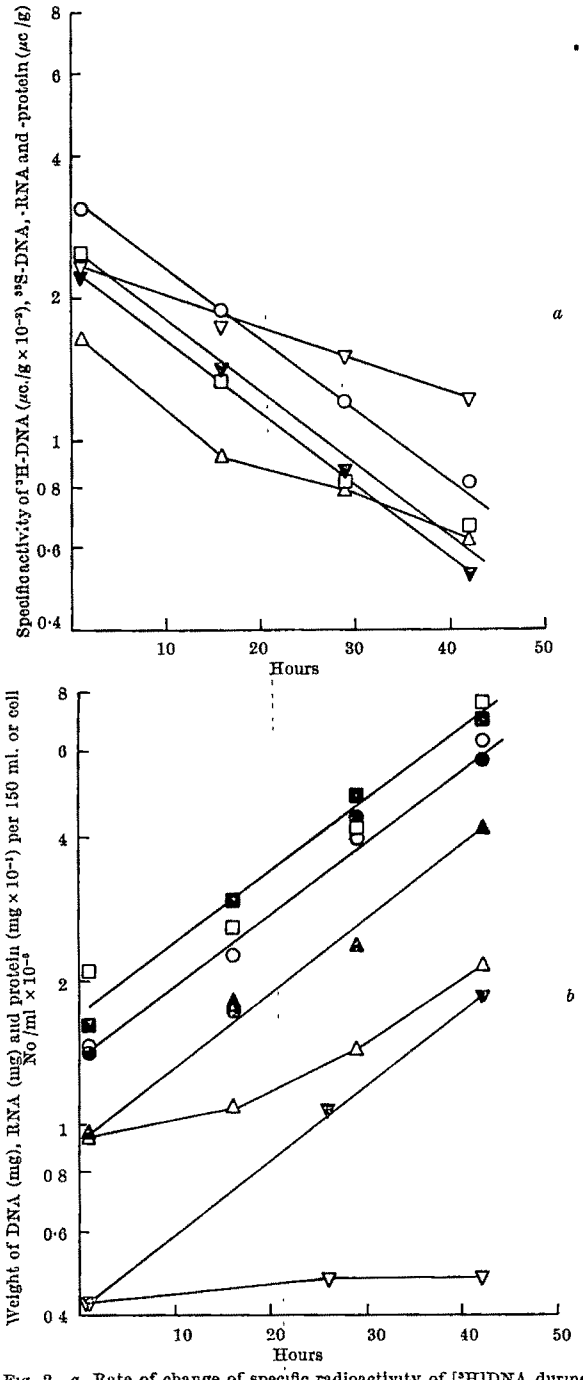


Fig 2. *a*, Rate of change of specific radioactivity of [³H]DNA during growth of HeLa cell culture and rate of change of specific radioactivity of [³⁵S] DNA, RNA and protein and [³H]DNA isolated from a similar HeLa cell culture after reaction with [³⁵S]mustard gas (0 5 μg (0 32 μc.)/ml.) A Packard 'Tricarb 3003' liquid scintillation spectrometer was used for all radioactive assays For the double-labelling experiments, settings were used such that in one channel tritium was counted at 18 5 per cent and sulphur-35 at 15 per cent while in a second channel tritium was 10^{-3} per cent and sulphur-35 29 per cent efficiencies. The specific radioactivities of DNA and RNA were corrected to an E_1^1 200 ▼, Tritiated DNA control; ▽, tritiated DNA mustard treated; △, sulphur-35 DNA mustard treated, □, sulphur-35 RNA mustard treated; ○, sulphur-35 protein mustard treated

Fig. 2. *b*, Rate of increase of protein, RNA and DNA and cell number in HeLa cell culture and in a similar culture after treatment with [³⁵S]mustard gas (0 5 μg (0 32 μc)/ml.). ●, Protein control; ○, protein mustard treated; ■, RNA control; □, RNA mustard treated, ▲, DNA control, △, DNA mustard treated, ▼, cell number control, ▽, cell number mustard treated

ascites tumour cells[14]. The fact that the increase in total amount of DNA isolated paralleled this drop in specific radioactivity showed that either method of estimation was a true indication of net DNA synthesis and eliminated the possibility that any gross additional turnover of DNA occurred following alkylation. The loss of sulphur-35 label from DNA proceeded initially at a greater rate than the loss of tritium label from DNA, which indicated that a loss of the alkylated groups from DNA had occurred and had been accompanied by, or was a prerequisite for, continued DNA synthesis. The increase in yield of DNA could be calculated from the decrease in the specific radioactivity of the tritium in DNA, and from this and from the drop in specific radioactivity of the sulphur-35 label in the same DNA a value for the absolute loss of alkylating agent residues from DNA was derived. Three such experiments were carried out under these conditions of mustard treatment and gave essentially the same result of an overall loss of sulphur-35 label from DNA of about 50 per cent (Fig. 3, II, III and V). A fourth experiment (Fig. 3, IV) was also performed in which the pre-labelled HeLa cells were treated with unlabelled mustard gas (0·25 μg/ml.) and unlabelled cells were treated with the same concentration of [³⁵S]mustard gas. The changes in radioactivity due to tritium and sulphur-35 in the DNAs isolated after various times were combined, as with the double-labelling experiments, to give the percentage loss of sulphur-35 radioactivity (the sulphur-35 labelled DNA was used for the chromatography discussed later (Fig. 4)). It is evident that the major portion of this loss had occurred within the first cell generation time after treatment, and that the rate of loss decreased with time. At present there is insufficient evidence to indicate either the precise time-course for the reaction or its dependence on dose.

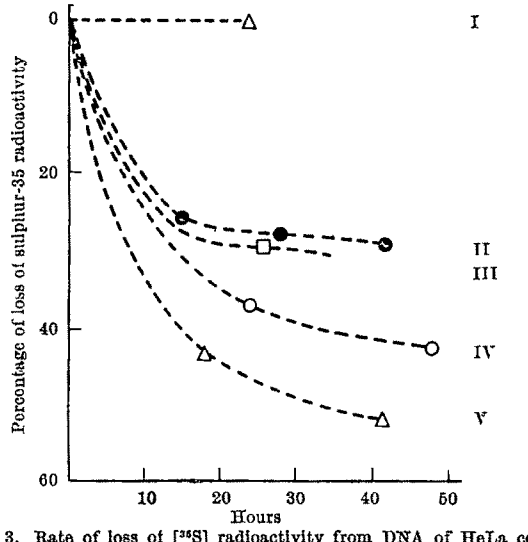


Fig. 3. Rate of loss of [³⁵S] radioactivity from DNA of HeLa cells following their reaction with various doses of [³⁵S]mustard gas. Loss of radioactivity was calculated from changes in [³H] and [³⁵S] specific radioactivity of DNA as illustrated in Fig. 2*a* and described in text. Curve II corresponds to data in Fig. 2*a* and *b*. I, 0·25 μg/ml. + IAA; II, 0·5 μg/ml.; III, 0 5 μg/ml ; IV, 0·25 μg/ml ; V, 0 4 μg/ml.

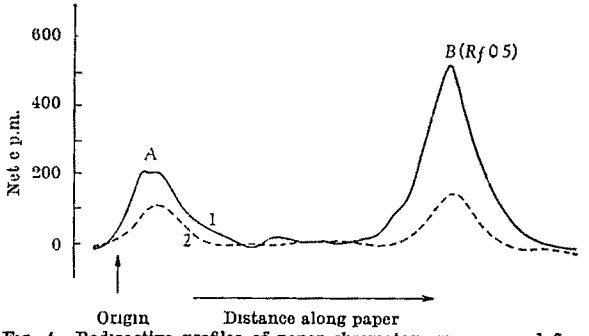


Fig 4. Radioactive profiles of paper chromatograms prepared from radioactive DNA (2 mg and not less than 4 mμc) isolated from a HeLa cell culture 0 5 h (1) and 48 h (2) after treatment with [³⁵S]mustard gas (0·25 μg (0 4 μc.)/ml.), and heated in N hydrochloric acid (1 mg/0 05 ml.) at 100° C for 0·25 h Papers were developed by downflow chromatography with isopropanol–conc. hydrochloric acid–water (78 : 16·4 : 15·6) for 18 h

That the loss of mustard from DNA was enzymatically mediated and did not arise from spontaneous hydrolysis of alkylated DNA[15] was indicated by the fact that it could be inhibited by iodoacetamide under conditions where DNA synthesis did not occur. Cells (2×10^8/500 ml.) which had been treated 0·5 h previously with [^{35}S]-mustard gas (0·25 μg/ml.) were disrupted by the method of Rosenberg and Cavalieri[16]. DNA was isolated from half the cell lysate (2·5 ml.) while the remainder was placed in a dialysis bag with 0·5 mM iodoacetamide and dialysed at 37° C overnight against 500 ml. of Eagle's medium. The specific radioactivity of the sulphur-35-labelled DNA which was later isolated was the same as that isolated initially (Fig. 3, I).

It has been established[1] that, at low levels of reaction, mustard gas reacts preferentially with guanine moieties of DNA. A proportion of the mustard reacts with two guanine moieties in adjacent base pairs, and it is thought that these lie in opposite strands of the macromolecular structure: it is this latter reaction which is supposedly responsible for the cytotoxic action of mustard gas. The preferential removal of these cross-links in the form of di(guanin-7-yl)ethyl sulphide has been shown by Lawley and Brookes[7] to be associated with resistance to mustard gas in *E. coli* strain *B/r*. Clearly it was of interest to determine whether the loss of mustard from the DNA of mammalian cells occurred in an analogous preferential manner. Accordingly, DNA isolated from cultures 45 min and 48 h after mustard treatment was hydrolysed with N hydrochloric acid and chromatographed on Whatman No. 1 paper.

The distribution of radioactivity on the paper was determined using a Nuclear Chicago 'Actigraph II' chromatogram-scanner and revealed the usual pattern of two radioactive products for 2-hydroxyethylthioethyl-guanine (*B* in Fig. 4) and at the origin the cross-linked product, di-(guanin-7-yl)ethyl sulphide (*A* in Fig. 4)—the latter present initially to the extent of 26 per cent of the total radioactivity on the paper. In DNA isolated after 48 h, the proportion of this product on chromatograms had increased to 34 per cent of the total. While the quantitative aspects of this phenomenon need further investigation, nevertheless some preliminary conclusions can be drawn from the result.

If it is assumed that all product *A* results from inter-strand cross-linking, then three simple modes of action for a possible excision mechanism can be considered:

(1) Exclusive excision of cross-linked material. This would lead to a maximum loss of activity in the experiments described of 26 per cent with a complete elimination of spot *A*.

(2) Excision occurring on only one strand of DNA. This would lead, if all alkylated bases were removed, to a loss of activity of 37 per cent and an increase in the percentage of compound *A* to 40 per cent.

(3) Completely random excision from both chains of the DNA, with the additional assumption that removal of an alkylated base from one chain does not affect the probability of the subsequent removal of the cross-linked base on the other chain. From these assumptions, if half compound *B* is removed, then a quarter of compound *A* is also removed, and a loss of 44 per cent of the total activity results in an increase in the proportion of *A* to 33 per cent.

The experimental value obtained of a reduction of 43 per cent in total sulphur-35 label in DNA accompanied by an increase in the proportion of *A* from 26 to 34 per cent agrees best with mechanism (3), although the errors inherent in deriving values from the chromatograms would not preclude mechanism (2). It is apparent, however, that the mechanism of excision of alkylated groups from DNA in mammalian cells as described here differs from that reported for the removal of alkylated bases from *E. coli B/r* (ref. 7) in which the cross-linked material is preferentially removed.

These investigations with a radioactive alkylating agent permit a calculation of the amount of reaction occurring with HeLa cell DNA in relation to the dose of mustard gas given and a comparison can be made with other published values[4]. The lethal dose of mustard gas can further be compared with that of X-rays. At a dose of 0·5 μg/ml. the results show that one molecule of mustard gas reacts per $1·3 \times 10^5$ molecules of DNA-P, and at the D_0 value obtained from Fig. 1 of 0·05 μg/ml. the amount of reaction would therefore be 1 molecule per $1·3 \times 10^6$ molecules DNA-P. As there are 3×10^{10} DNA-P atoms in the HeLa nucleus this is equivalent to $2·3 \times 10^4$ alkylations in the total cellular DNA. The corresponding value for either mouse *L* cells[4] or mouse lymphoma cells is 4×10^4 (ref. 17). Assuming that, as already discussed, 25 per cent of the reaction of mustard gas with HeLa cell DNA leads to cross-links which are the assumed lesions, then the overall reaction is equivalent to $5·7 \times 10^3$ cross-links in the total cellular DNA. It has been calculated[18,19] that for ionizing radiation the D_0 value is equivalent to one ionization per DNA of molecular weight 10^9–10^{10}, or 10^3–10^4 total hits per cell. This value is therefore of the same order as the number of mustard cross-links per cell, and this comparison thus lends support to the view that DNA is the target for both agents. The extent of reaction with RNA or protein molecules would in both cases be very small, because the molecular weight of these is much less than that of DNA and there is no evidence for anything other than random attack on all cellular constituents. If the existence of the shoulder in the survival curve (Fig. 1) is indicative of a repair mechanism[11], then this repair mechanism is saturated at a dose of 0·07 μg/ml. which corresponds to 8×10^3 DNA cross-links per cell.

Furthermore, the sole biochemical action of mustard gas at a dose about equal to the D_0 value detected in these experiments on HeLa cells was a depression in the rate of DNA synthesis, and this depression was a prelude to the premitotic block which ensued and was not a consequence of it. Similar findings have been reported for the action of similarly low doses of X-rays[18,19]. For both cytotoxic agents it is therefore indicated that the lethal damage is the result of direct effect on the DNA which interferes with its replicative ability, but not with its function as a template for RNA synthesis at the low doses capable of preventing cell division.

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RIBONUCLEIC ACID SYNTHESIS IN MACROPHAGES IN RELATION TO THE SECONDARY IMMUNE RESPONSE *IN VITRO*

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RECENT investigations have suggested that macrophages take an intermediary role in the mechanism of antibody formation, and most investigations of the fate of antigens have indicated that they are taken up by phagocytic cells in spleen and lymphoid tissues, and in various other organs including the liver[1–3], where they have been reported as complexed with nucleic acids[4].

Fishman and Adler[5] reported that phenol extracts of ribonucleic acid (RNA) from macrophages after contact with bacteriophage induced phage-neutralizing antibodies when added to lymphoid cells in tissue culture or transplantation chambers. They concluded that the material was most likely to be a low-molecular-weight RNA, and further investigation indicated that such an RNA from macrophages was incorporated by lymph node cells[6]. In all these experiments, the RNA was extracted from cell preparations consisting largely of macrophages, but cell-transfer studies[7] with similar preparations showed that potential antibody-forming cells were also present.

Haemocyanin was used as an antigen in mice[8] to find whether macrophages actively process antigens as a necessary step in the induction of the immune response. It was concluded that RNA preparations from mouse peritoneal cells after uptake of haemocyanin labelled with iodine-131 *in vivo* were immunogenic in primed mice, but the RNA contained radioactivity with properties compatible with the antigen or fragments thereof, and the preparations were found to be more immunogenic than original antigen.

A recent short report[9] has indicated that preparations of bovine serum albumin (BSA) after "biological filtering", by injecting into intact, non-immunized rabbits and taking the serum after an interval to inject into fresh animals, had lost its ability to stimulate an antibody response and some of the rabbits injected with filtered BSA became tolerant to it. The authors suggested that macrophages played a part in the immune response and that processing of antigens by these cells was necessary for the synthesis of antibody. A recent investigation[10] using autoradiography combined with electron microscopy has shown that iodine-125-labelled *Salmonella* flagellar protein was present on the surface of the dendritic macrophages of the lymphoid follicles, perhaps bringing the antigen into close contact with the responsive cells.

It has been found that peritoneal exudate cells induced in normal rabbits by intraperitoneal injections of oyster glycogen and triolein can take up heterologous proteins and that these primed cells can stimulate the rate of DNA synthesis in spleen cells from rabbits immunized to the specific antigen[11]. This response was antigen-specific and was considered to be an *in vitro* manifestation of the secondary immune response. Using this system, investigations of the role of RNA synthesis in peritoneal cells in relation to this transfer mechanism were carried out.

Methods for obtaining spleen cell suspensions[12] and macrophages[11] have been described in detail, DNA synthesis in spleen cells being measured by the incorporation of carbon-14-labelled thymidine during 24–48 h of culture[12]. RNA synthesis in macrophages was measured by the incorporation of tritiated uridine. Uptake of protein by macrophages was measured by the incorporation of iodine-131-labelled human serum albumin (HSA). All radioactive materials were obtained from the Radiochemical Centre, Amersham.

When 1×10^6 peritoneal exudate cells were incubated in tritiated uridine for 8-h periods and the rate of incorporation was measured (Fig. 1), the peak of uridine incorporation was maximal at about 20 h of culture and then fell to zero by 44 h. No effect, due to the presence of heterologous protein, was noted in these experiments.

Actinomycin-*D* (Act-*D*) obtained from Merck, Sharp and Dohme, Ltd., Rahway, New Jersey, was used to inhibit uridine incorporation, and typical results are shown in Fig. 2. No effect was seen during the first 24 h of culture until the concentration of Act-*D* was more than 10^{-5} μg/ml. in the medium, while lower concentrations produced some inhibition when uptake of tritiated uridine was measured over 48 h of culture

Table 1 illustrates that inhibition by Act-*D* was time-dependent and that the effect was not reversed by washing the cells and incubating in the absence of Act-*D* for up to 48 h.

The results in Table 2 show that the presence of Act-*D* in the medium in concentrations sufficient to inhibit significantly the incorporation of tritiated uridine into macrophages did not affect their ability to transfer an antigen-specific response to spleen cells from an immunized rabbit. Pre-incubation of macrophages in Act-*D*

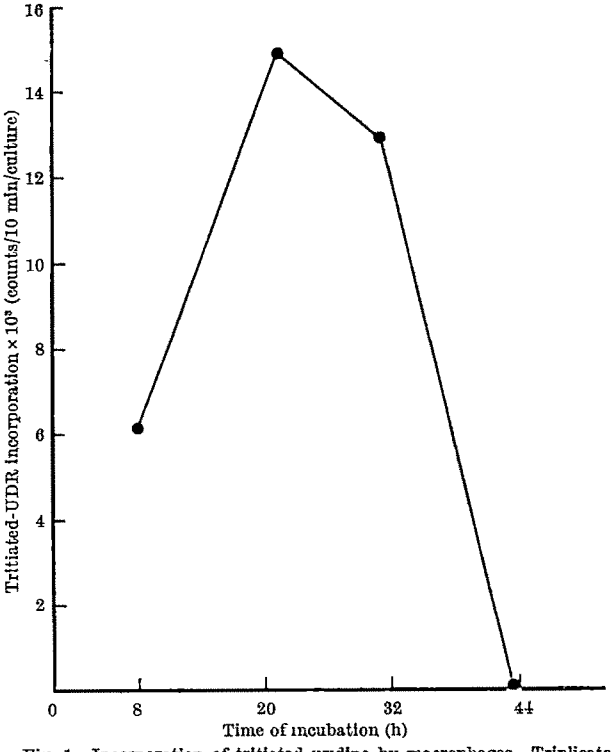


Fig. 1. Incorporation of tritiated uridine by macrophages. Triplicate cultures of 1×10^6 cells were incubated with 5 μc./ml. of tritiated uridine (200 mc./mM) for periods of 8 h. After washing in warm medium, the cells were removed from the culture beaker with cold 0·02 per cent sodium versenate, washed in ice-cold 5 per cent TCA, the precipitates washed in cold methanol, and dissolved in hyamine for measurement of radioactivity by scintillation counting

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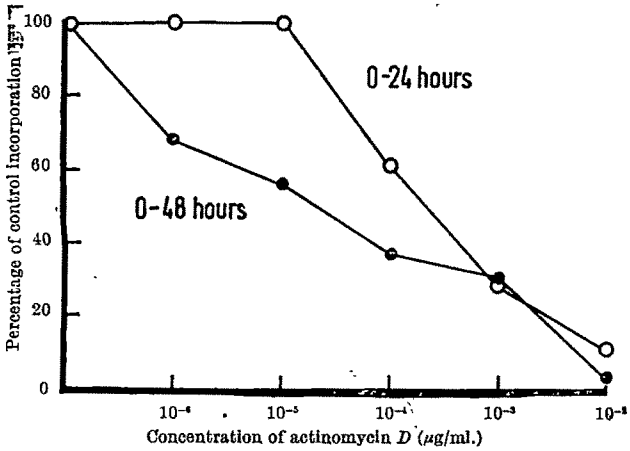


Fig. 2. Inhibition of tritiated uridine incorporation into macrophages by actinomycin-*D*. Act-*D* and tritiated uridine were added initially and uridine incorporation measured as in Fig. 1

for 6 h before exposing them to antigen also failed to prevent this transfer, and, as shown in Table 3, Act-*D* does not influence the uptake of iodine-131-labelled HSA by macrophages. Similar experiments using RNA preparations extracted with phenol[13] from macrophages incubated with antigen have all been negative. Intact macrophages were necessary for positive results although cells, heat-killed or irradiated with a dose of 2,000 rads after incubation in antigen, could still stimulate responsive cells.

The results presented here indicate that macrophages from peritoneal exudates took up antigen and transferred its specific stimulation of the rate of DNA synthesis in spleen cells from an immunized rabbit. If RNA played a part in this process it was unlikely to be that of a specific template produced by macrophages after contact with antigen. Other investigations[11] have shown that antigen was present on the surfaces of primed cells and that antibody to antigen could block transfer. It is therefore tentatively concluded that the role of macrophages in the stimulation of cell proliferation in the immune response is to take up antigen on to their cell surfaces, where it plays some part in the regulation of lymphoid cell proliferation. The mechanism of this is obscure. Previous investigations[11] have not been able to establish any evidence of direct contact of the dividing cells with macrophages. Of course, it is still possible that macrophages produce an informational RNA molecule which can direct the specificity of antibody formation in cells synthesizing immunoglobulins. This possibility is being further explored. The experiments presented here were only concerned with the mechanism of stimulation of cell division in response to antigen.

Table 1. Inhibition of Tritiated Uridine Incorporation by Macrophages in Relation to Time of Contact with Act-*D*

Time of contact with Act-*D*	RNA synthesis (counts/10 min/culture)					
	Time of wash–24 h		24–48 h		Time of wash–48 h	
1×10^{-2} μg/ml.	Act-*D*	Control	Act-*D*	Control	Act-*D*	Control
1 h	2,464	4,006	15,565	23,626	2,408	3,768
3 h	1,070	7,663	6,172	23,968	1,251	11,124
6 h	1,024	12,635	2,033	26,141	1,308	10,854
24 h	429	8,615	29	24,548	29	24,548

Macrophages were incubated in Act-*D*, then washed in warm medium and the incorporation of uridine was measured as described in Fig. 1.

Table 2. Effect of Act-*D* on the Ability of Macrophages primed with Antigen to stimulate Spleen Cells

Act-*D* (μg/ml.)	DNA synthesis rate (counts/min/culture)			
	C	*C* + *M*(*C*)	HGG	*C* + *M*(HGG)
0	560	547	15,220	5,076
1×10^{-6}	—	433	—	3,925
1×10^{-5}	—	600	—	4,465
1×10^{-4}	—	503	—	4,360
1×10^{-3}	—	543	—	4,786
1×10^{-2}	—	560	—	4,790

Cultures of 1×10^5 macrophages were incubated in Act-*D* (*M*(*C*)) or Act-*D* and HGG (*M*(HGG)) for 18 h, washed three times in warm medium, and 1×10^7 spleen cells from a rabbit immunized to HGG were added. The rate of DNA synthesis in the spleen cells was measured by the incorporation of carbon-14-labelled thymidine during the 24–48 h after adding the spleen cells to the macrophages.

Table 3. Uptake of Iodine-131-labelled HSA by Macrophages in the Presence of Act-*D*

Act-*D* (μg/ml.)	Uptake of HSA (counts/min/culture)
0	3,990
1×10^{-4}	4,883
1×10^{-3}	3,630
1×10^{-2}	3,170
1×10^{-1}	3,775

Triplicate cultures of 1×10^6 macrophages were incubated in the presence of iodine-131-labelled HSA and Act-*D* for 24 h, washed three times in ice-cold saline and dissolved in 6 *N* hydrochloric acid for gas-flow counting.

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AN EARLIER PHASE OF THE LIGHT-EVOKED ELECTRICAL RESPONSE FROM THE PIGMENT EPITHELIUM-CHOROID COMPLEX OF THE EYE OF THE TOAD

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An extremely rapid biphasic response has been found which is evoked by light from the retinal photo-receptors of the monkey, and which has been designated the early receptor potential[1–3]. This type of response has also been found in a number of other vertebrate species[4,5]. Its second phase may be selectively abolished by either lowered temperature[6] or light adaptation[5], so the two phases of the early receptor potential are separately generated. The early receptor potential is followed by a much slower response called the late receptor potential[1,3], which may be selectively abolished by anoxia[1]. Thus the response of the retinal photoreceptors consists of three separate phases. More recently, it has been shown that the pigment epithelium-choroid complex of the eye of the toad contains photosensitive cells which generate an electrical response to light stimuli[5]. For convenience, this type of response will be referred to in the present article as the 'non-retinal response'. It seems evident that the cells which generate the non-retinal response are not as specialized for light detection as the retinal rods and cones. This provides an opportunity to compare the responses from retinal photoreceptors with those from

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a more primitive type of photosensitive cell. The non-retinal response was first described as biphasic in form, and its latency was about 25–30 μsec[5]. We have now found an earlier phase of the non-retinal response which has no detectable latency. Thus the non-retinal response likewise contains three phases, which we have separated by appropriate procedures, and evidence is presented which indicates that the three phases of the non-retinal response are comparable with those generated by retinal photoreceptors. These findings greatly increase the probability that a fundamental sequence of electrical responses has been found which occurs in many or all types of photosensitive cells within the animal kingdom.

The general methods for this work have been described previously in detail[5]. Briefly, the eye was enucleated from the toad (*Bufo marinus*). The front of the eye was then cut away, and the retina was removed from the eye cup with a piece of dry cotton. The remaining eye cup consisted only of the pigment epithelium-choroid complex, which was supported by the sclera. This eye cup was set on a cotton wick soaked with frog Ringer's solution, which acted as the reference electrode. A second cotton-wick electrode made contact with Ringer's solution inside the eye cup. The polarity of all records was such that an upward deflexion indicated positivity of the inner side of the eye cup. The frequency of the recording system was flat over the range 6–60,000 c/s, which was sufficient to give undistorted responses. Light stimuli were provided by a condenser-discharge lamp (Honeywell 'Strobonar 65C'), which gave an intense flash having an effective duration of 0·7 msec. This lamp was mounted outside the electrically shielded recording room, and the light was led to the preparation by a fibre optics bundle, one end of which was placed close to the flash tube, and the other mounted directly over the eye cup. Both electric and photoelectric artefacts were completely absent from the records, except when introduced deliberately for timing purposes. The preparation was mounted in a small flat-bottomed glass dish, which was partially submerged in a water bath, the temperature of which could be held constant or varied. The temperature of the preparation was monitored by a small thermistor probe in the Ringer's solution inside the eye cup.

Fig. 1 shows the major effects of lowered temperature on the non-retinal response, and these effects have all been observed consistently in many experiments. The response at 24·5° C consisted of a rapid negative phase, followed by a slower positive phase. These are the two phases reported previously at room temperatures[5], and they comprise the second and third phases of the total response. When the temperature was lowered to 16·5° C, the positive third phase was decreased in amplitude; also its peak occurred at a later time, and the response was broadened. By comparison, the negative second phase showed a distinct increase in amplitude. The different sensitivities of the second and third phases to lowered temperature strongly suggest that they are generated by different mechanisms, and the apparent increase of the negative second phase is probably due to the greater effect of lowered temperature on the positive third phase. When the temperature was lowered to 7° C, the effects on the third phase became more marked. The second phase showed a slight further increase in amplitude, while its peak was delayed and its time course broadened. Also at this temperature a positive first phase became apparent. When the temperature was further lowered to 2·5° C, the negative second phase became smaller and markedly slower in its time course, and the positive first phase became even more prominent. Thus the total response contains an initial positive phase, which normally is masked by the negative second phase. Because this masking occurs, and results in a normal response with a clearly measurable latency, the second phase must normally begin at the same time as the first phase or very shortly thereafter. Lowered temperature affects the second phase more than the first; thus as the negative second phase is slowed in its time course, and reduced in amplitude, the initial positive phase is progressively revealed. Because the second phase is more temperature-dependent than the first phase, these two phases also appear to be generated separately. The effects of lowered temperature on the first and second phases of the non-retinal response are closely analogous to those found by Pak and Cone for the early receptor potential of the whole eye of the rat[6]. In their work on the rat an initial positive phase of the early receptor potential was not seen clearly at room temperatures, but was revealed by lowered temperature as the negative second phase was decreased in amplitude and slowed in

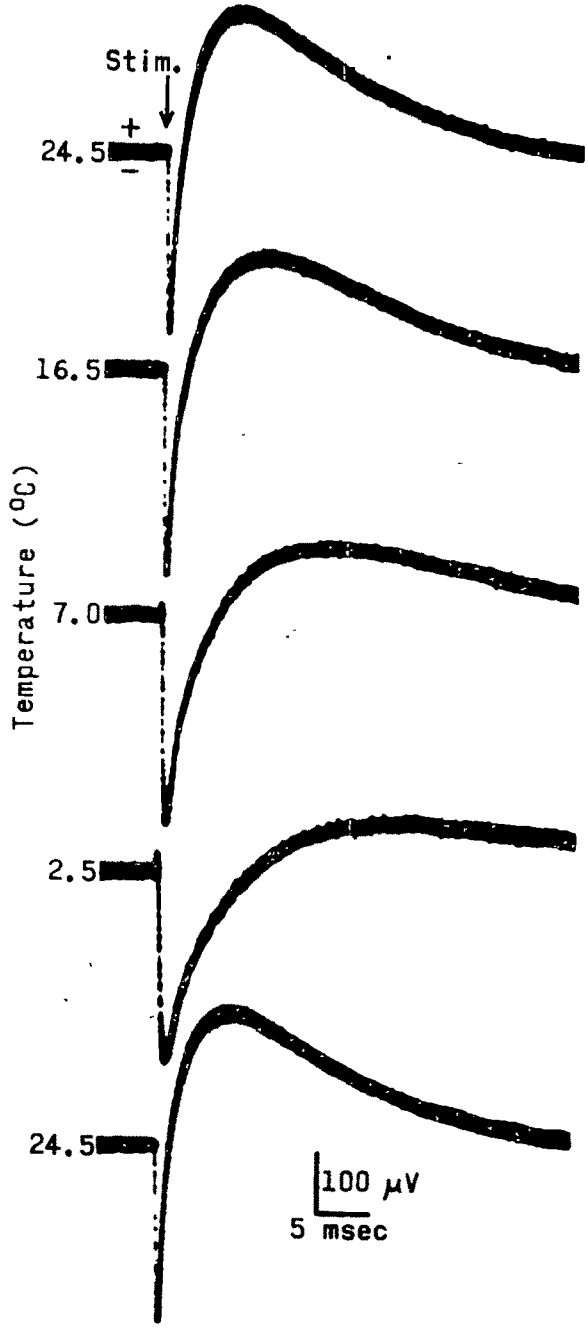


Fig. 1. The effect of lowered temperature on the non-retinal response. The temperature was first lowered, and representative records are shown reading from the top downward; the temperature was then returned to the control value, as shown by the bottom record. Stimuli were delivered at 1-min intervals for 5 min before this series of records, and throughout the series of records, in order to maintain constant light adaptation. The time of delivery of the stimulus is shown by an arrow over the top record

its time course. The first phase was essentially isolated at about 0° C, and Pak and Ebrey[7] found that the first phase could be detected at temperatures as low as −35° C.

In certain experiments we lowered the temperature of the preparation as far as −8° C. Glycerol was used in the wicks to prevent freezing, but was not added to the solution in the eye cup because it shrinks cells. Within this sub-zero range, progressive effects of lowered temperature were noted which were qualitatively similar to those shown in Fig. 1. At −8° C the third phase was extremely slowed and had almost disappeared, while the second phase was much smaller and broader; the first phase was larger, but at this temperature it was still not so large as the second phase. Thus lowered temperature might isolate the first phase of the non-retinal response, but in this case the temperatures required are inconveniently far below 0° C.

While investigating the non-retinal response at sub-zero temperatures, the introduction of Ringer's solution and glycerol (1 : 1) into the eye cup caused a reduction in amplitude of both the second and third phases of the response. This occurred within a few seconds, revealing an initial positive phase. The second and third phases sometimes recovered gradually, but incompletely, over a period of several minutes. It seemed likely that these effects were due to the high osmotic pressure of the glycerol solution, which initially dehydrated the photosensitive cells, with gradual penetration of the glycerol into the cells resulting in re-hydration and partial recovery of the response in some cases. If this interpretation were correct, the initial positive phase should be isolated stably by increasing the extracellular osmotic pressure with biologically inert substances which do not penetrate cell membranes. For this purpose we tried Ringer's solutions containing polyethylene glycol ('Carbowax, 1500') and having osmotic pressures above 1,000 mosmols. Fig. 2 shows the sequence of effects when 'Carbowax' Ringer's was introduced into the eye cup. The third phase was reduced more rapidly than the second phase, as shown in record 2. Then the third phase disappeared, as shown in record 3, at which time the second phase was greatly reduced and the initial positive phase was revealed. In record 4 the second phase has also disappeared, and the initial positive phase appears to be isolated. The different sensitivities of the three phases of the response to this procedure provide another type of evidence that the three phases are generated by somewhat different mechanisms. As shown by record 5, the isolated first phase of the response has no detectable latency. It would seem that the latency of this first phase could not exceed a few μsec, which is comparable with that of the first phase of the early receptor potential of the toad retina[5].

When Ringer's solution was saturated with sucrose, similar results were obtained. Also if the preparation was allowed to dry by standing in air for several hours, and if a wick electrode was then inserted into the eye cup without adding fluid, the response consisted only of a small initial positive potential. Thus any method of removing the intracellular fluid seems to isolate the first phase. With 'Carbowax' Ringer's the form and amplitude of the isolated first phase have been stably maintained for at least 15 min. This was sufficient for our experiments, so longer periods were not attempted. Thus, 'Carbowax' provides a simple and effective method for isolating the initial positive phase, and for maintaining it stably so that some systematic experiments may be performed on it.

When the pigment epithelium-choroid complex was removed from the preparation, and the sclera was partially filled with 'Carbowax' Ringer's, no response was detected. Also no response was recorded when the wick electrodes were in direct contact. Similar controls have been performed for the second and third phases of the response[5]. Thus all three phases of the non-retinal response are generated bioelectrically within the pigment epithelium-choroid complex. The non-retinal response of the frog has been recorded recently by micropipette electrodes in the pigment epithelial cells, which lie immediately at the surface of the eye cup preparation[8]. Both the first and second phases of the response are inverted in these intracellular records, so the non-retinal response appears to be generated by the cells of the pigment epithelium.

The non-retinal response is so resistant to light adaptation that true effects of light adaptation were not clearly demonstrated in the initial report[5]. We have now found that during a sequence of maximum intensity flashes at 15-sec intervals, both the second and third phases decrease slightly in amplitude; initial amplitude is then recovered after dark adaptation. Following isolation of the first phase by 'Carbowax' Ringer's, a similar schedule of stimulation and dark adaptation produces similar effects on the amplitude of the first phase. Thus all three phases of the response show quite small effects of light adaptation. This is in contrast with responses of the toad's retinal photoreceptors, which show marked effects of light adaptation[5]. An important question is whether all three phases of the non-retinal response result from the absorption of light by a single type of photopigment. Because resistance to light adaptation is a unique property of this response, the similar small effects of light adaptation

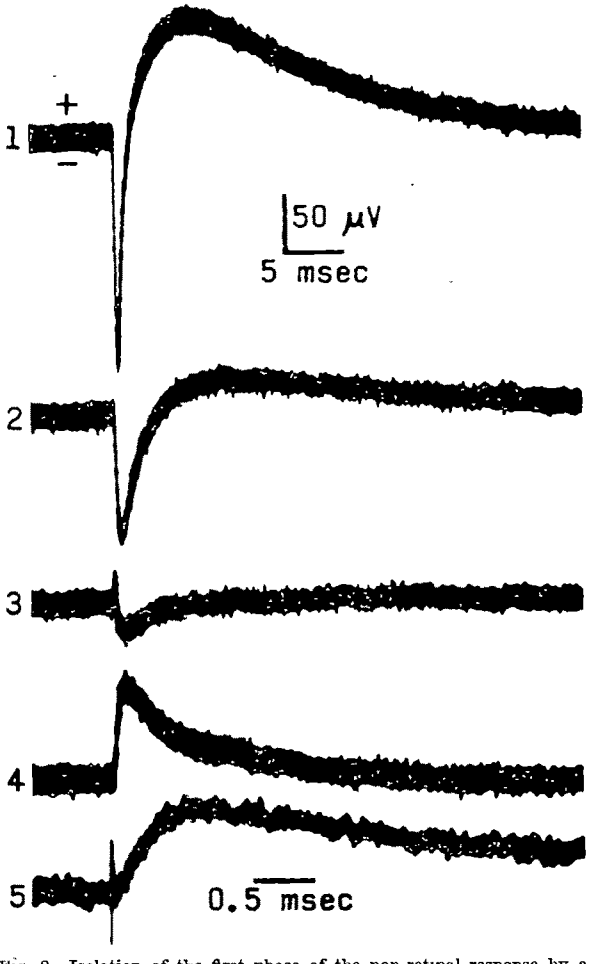


Fig. 2. Isolation of the first phase of the non-retinal response by a hypertonic extracellular medium. Record 1 is a control response with Ringer's solution in the eye cup. Then the Ringer's solution was sucked out and a Ringer's solution containing 'Carbowax' was introduced (see text). Record 2 was taken about 20 sec after this procedure, and record 3 was taken after 170 sec. Record 4 shows the final effect in a different preparation, in which 'Carbowax' Ringer's gave an isolated initial phase which was particularly large, and record 5 shows this isolated first phase at higher sweep speed. In record 5 a stimulus artefact, which resulted from discharge of the condenser in the light source, was introduced deliberately for timing purposes, it was recorded as a vertical line, and the response rises immediately after the artefact. During these records the stimulus was repeated at 15-sec intervals, for control of light adaptation, and the temperature was about 25° C

on the three phases are strong evidence that the three phases result from the absorption of light by a single type of photopigment. Recent results indicate that this pigment is melanin. The response which resists light adaptation is large and readily demonstrated in the eyes of pigmented rats, while a superficially similar but smaller response from the eyes of albino rats[5] now seems to be due to the early receptor potential. These findings were first made by Arden[9], and have been confirmed by Brown and Crawford[8].

We also observed the effects of lowering the temperature on receptor responses in the isolated retina of the toad, using previously described methods of isolating the retina and recording from it[5]. This was done in order to compare the effects of lowered temperature on the retinal responses with effects on the non-retinal responses of the same animal. The *a*-wave of the electroretinogram, which results from the rise of the late receptor potential[3], disappeared when the temperature was reduced to the range of 5°–9° C. Further reduction of temperature eliminated the second phase of the early receptor potential and isolated the first phase; this occurred at about 0° C. Thus the three phases of the response from retinal photoreceptors show a similar order of temperature-dependence. But the second and third phases of the retinal response do not survive to such low temperatures as analogous phases of the non-retinal response in the same species.

In both the retinal and non-retinal responses, the first and second phases are quite rapid by comparison with the slower third phase. But at room temperature the second phase of the non-retinal response attains its peak after about 0·3 msec, while the second phase of the early receptor potential of the toad occurs after about 1·75 msec[5]. Also the third phase of the non-retinal response must begin after less than 0·3 msec, because its amplitude can affect the measured amplitude of the second phase, while the late receptor potential of the toad's retina does not seem to begin until after at least 5 msec. Thus the second and third phases of the retinal response appear to be slower than analogous phases of the non-retinal response. Of several possible explanations for this, one is the greater length of the retinal photoreceptors. The first two phases of the non-retinal response have the same polarity as the two phases of the early receptor potential. But the third phase of the non-retinal response is positive, while the late receptor potential is negative, when recorded under comparable conditions, This is an intriguing point which requires further investigation.

The entire non-retinal response was well supported by placing either 0·9 per cent sodium chloride or frog Ringer's in the eye cup. When the sodium chloride in the Ringer's solution was replaced by sodium nitrate, there was no distinct effect. But when the sodium chloride was replaced osmotically by sucrose or lithium chloride, the third phase was reduced in amplitude within 1 or 2 min and then abolished. Thus the third phase is dependent on sodium ions in the eye-cup solution. Also ouabain (5×10^{-4} M), which depresses active transport of sodium across cell membranes[10], slowly and selectively reduced the amplitude of the third phase. This suggests that the third phase is dependent on an active transport of sodium ions. But the second phase seems insensitive to the ionic composibion of the eye-cup solution, and the first phase can even withstand dehydration of the cells. In these respects, also, the non-retinal response seems comparable with responses of retinal receptors. The *a*-wave of the electroretinogram, which serves as an index of the late receptor potential, is critically dependent on sodium ions[11,12]. It is also abolished by high potassium concentrations, which presumably depolarize the retinal receptors[13]. On the other hand, the early receptor potential is not affected by the ionic composition of the bathing medium[13,14].

Thus the findings reported in this article greatly increase the likelihood that the sequence of electrical responses in retinal photoreceptors is a fundamental one which occurs also in a more primitive type of photosensitive cell in the pigment epithelium-choroid complex. It seems possible to explain readily the detailed differences which have been noted on the basis of differences in the absorbing photopigments or in the generating cells. Thus the two response sequences seem to represent variations on a basic theme, as expected if a fundamental response sequence is investigated in photoreceptors at different levels of specialization. In this sequence it seems very likely that the first phase is generated by a solid-state potential. It survives dehydration of the cells, is not affected by the ionic composition of the bathing medium, and has a very low temperature-dependence which permits its survival well into the sub-zero range. The general possibility of solid-state potentials in excitable cells has already been suggested[15–17], and this type of potential would now seem to have been found in photoreceptors. The characteristics of the second phase are rather transitional between those of the first and third phases, and the third phase appears to result from a transmembrane ion flux.

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BIREFRINGENCE OF DIAMOND

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IT is generally considered, both from its morphology and etching figures, that diamond belongs to the holohedral cubic system and that it should not show birefringence unless strained. Yet it has been common knowledge since 1815, when Brewster described double refraction in some diamonds, that many diamonds are, in fact, birefringent. It has generally been assumed that these were strained either during growth or later. There have been conflicting views as to how widespread is this diamond birefringence. The most significant contribution to this subject is that of G. Friedel[1], who described a polariscope investigation of 200 diamonds and of 150 thin cleavage sheets. He reported that all the crystals showed some degree of birefringence and that so also did many of the cleavages.

In the extensive literature dealing with the diamond, very little has been published concerning terrestrial micro-diamonds, if we call as such (quite arbitrarily) diamonds smaller than 0·2 mm across. With the advent of some millions of carats of synthetic diamond of just this size, it is now of interest to compare the properties of synthetic diamond with natural diamond of comparable size. In the standard grease-table recovery technique used at diamond mines, quantities of small-mesh tailings are collected, but it is not economic or useful to sort this material. I have acquired 350 carats of small-mesh South African mine tailings and a search through this has yielded 3,000 clear transparent well-formed micro-diamonds. They are present in the tailings to the extent of a fraction of 1 per cent, the rest being breakage fragments from much larger crystals.

These small whole crystals are about equally divided between octahedra, dodecahedra and triangular twins. There are no cubes, which is in conformity with usual South African experience. Some 10 per cent of the octahedra show a small black inclusion. Inclusions are quite rare in both the dodecahedra and the triangular twins. These crystals are now being investigated from the point of view of determining their morphology, surface micro-topography, fluorescence and birefringence. This report will deal only with their birefringence.

Surprisingly enough, without exception, all 3,000 exhibited birefringence. Despite the small thickness, in most, the double refraction can be fairly large, and frequently several bands appeared under the polarizing microscope. Observations were deliberately made with a light source of only moderate intensity, so that any near approach to extinction would not be over-illuminated. The occurrence of birefringence in all these small, clear, well-formed crystals was so unexpected that arrangements were made with the central diamond distributing organization (Industrial Distributors) to examine a considerable number of selected gem-quality diamonds for birefringence. Two thousand such diamonds were specially selected by their expert sorters, as follows: (*a*) 1,000 crystals (1 carat each), mixed octahedra and dodecahedra; (*b*) 400 flat triangular twins (1 carat each); (*c*) 200 half-carat selected "Cape dies" (dodecahedra), traditionally for drilling, for wire dies, and reputed to be long-lasting in use; (*d*) 400 1 carat fragments (chips).

All 2,000 diamonds were examined, singly, with a simple pocket polariscope consisting of two crossed 'Polaroid' disks and a ×10 eyepiece, the combination fitting over a small hand torch. Without exception, all these gem-quality selected crystals showed some degree of birefringence. There was a wide range in response, but complete extinction was not obtained with any of the diamonds. Twenty crystals (that is, 1 per cent) exhibited a strong localized birefringent region which was clearly linked with a slight local defect or inclusion. About 100 crystals (that is, 5 per cent) were only weakly birefringent, and in most of these the transmitted light appeared in rectilinear patches lying in crystallographic directions. On average, the 200 Cape dies showed less birefringence than did the rest of the diamonds, thus confirming the traditional belief in their strength. Yet many did show several bands of polarization. Notable were the 400 chips, for nearly all showed considerable double refraction, some long chips being the most birefringent of all those examined.

On the basis of these observations made on 3,000 fine-quality micro-diamonds and on 2,000 good-quality gem-stones, it is certainly not rash to conclude that the chances of finding a non-birefringent diamond appear to be remote. There is clearly now a very strong case for believing that almost all diamonds are birefringent. My observations on 5,000 good-quality diamonds confirm the tentative conjecture made by Friedel (in 1924 with his 200 diamonds) that probably all diamonds are birefringent; however, I am quite unable to accept the explanation which he proposed, and I shall offer here an alternative one.

Finding a transition temperature at 1,885° C (ref. 2), where diamond tends to shatter catastrophically, Friedel argued that all diamonds, initially isotropic at formation, pass through a plastic state, sufficient to accept permanent deformation the cause of which is associated with this transition temperature. He went on to argue that this creates the strain leading to the ubiquitous birefringence.

I shall offer quite a different explanation. Harrison and I[3] have sectioned and etched numerous diamonds and have always been able to demonstrate the existence of a stratigraphical etch pattern, often strictly linear, which clearly indicates that diamond must have grown by successive layer formation. Fig. 1 is a typical stratigraphical etch pattern on the surface of a 1-mm slice cut from the mid (girdle) region of a fine-quality transparent octahedron observed by oblique illumination. Bright regions are areas which have accepted heavier etching.

By photographing this slab in light of wave-length just shorter than 3000 Å (which can distinguish type 1 from type 2 diamond) I found that the darker regions of Fig. 1, which are those free from etch (or relatively less etched), are type 2 diamond, while the brighter (heavily etched regions) are of type 1.

Now this diamond like the rest is birefringent; a polariscope picture of the slab is shown in Fig. 2. The slab is highly birefringent and a remarkable correlation is obvious between the etch pattern and the birefringence. To account for this, it is only necessary to postulate that there exists some small difference in either the thermal expansion property or the elastic strength or the lattice dimension of the two distinct types of diamond. Such an assumption would adequately explain the close correlation between the birefringence pattern and the stratigraphy.

To account for the birefringence it is then necessary to propose that all diamonds are layer mixtures of type 1 and type 2 material. Such a conjecture not only explains the universal occurrence of birefringence, but it also accounts for the catastrophic shatter described by Friedel and Ribaud. It explains also the microcharacter of diamond cleavage surfaces. In most diamonds despite the intermixing, one type usually dominates the other, and it is this dominant type which determines the ultimate classification. I have already demonstrated[5]

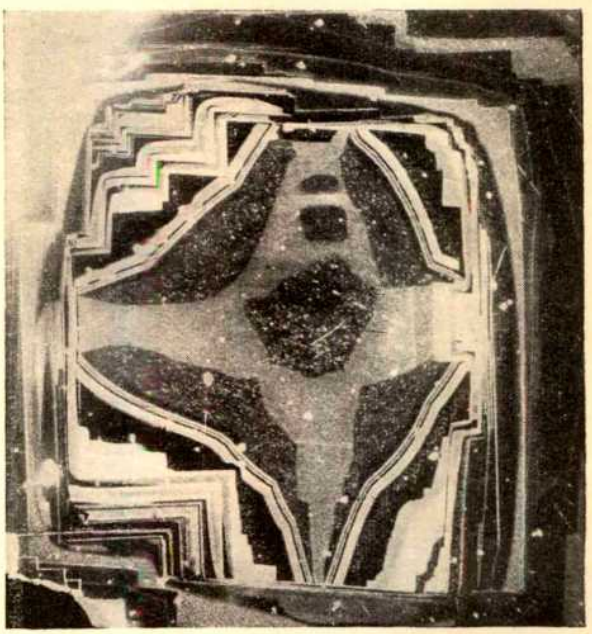


Fig. 1

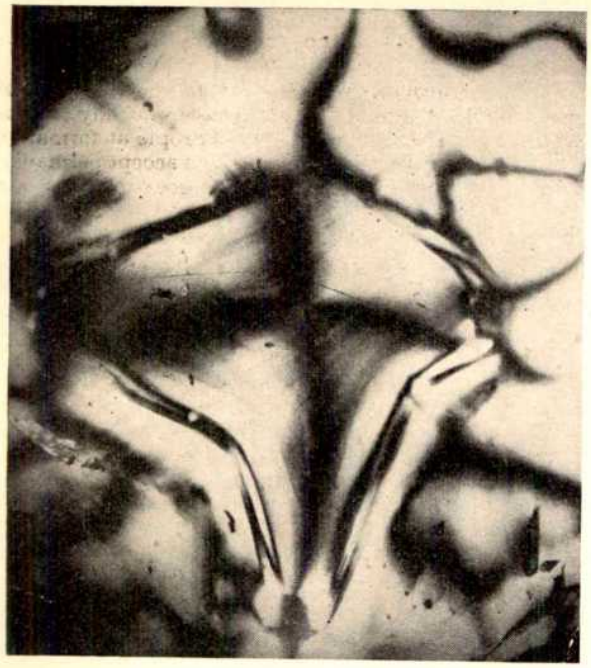


Fig. 2

by interferometric studies on cleavage planes that diamonds which are predominantly type 2 cleave much flatter than those which are predominantly type 1. Yet any cleavage usually shows distinct regions, some of which cleave well, mixed with regions which cleave poorly. The conjecture that I make here as to an intermix of types would also explain this aspect of cleavage.

Friedel's idea of a mere mechanical strain is not able to explain why so many of our observed strain patterns follow linear crystallographic directions. On the contrary, because in my view the crystal has grown by way of oriented sheet layers, it is clear that the interfaces between type 1 and type 2 can, in fact, be expected often to orient parallel to crystallographic directions; thus the appearance can be predicted of strain pattern fringes lying along crystallographically oriented directions—and this is found.

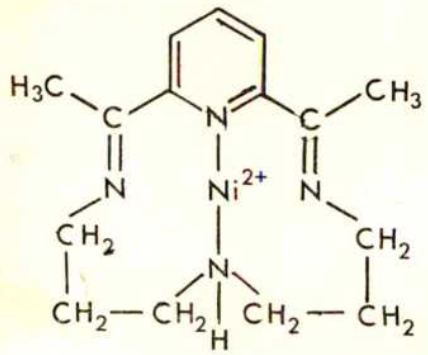
Friedel's theory also fails to explain the appearance of strong birefringence in the many chips or fragments. If mere direct mechanical strain were the cause of birefringence in diamond, it could reasonably be anticipated that when such a strained crystal fractured it would tend to do so in such a manner as to tend to relieve the mechanical strain. Thus it would be expected that such strain-relieved fragments would, on the whole, show less birefringence than complete crystals. Yet the contrary is true, which implies that the birefringence is built into the fragments and cannot be released by the act of fracture. In our view, a complete mixing of types would automatically lead to high strain and, if such a strained crystal fractured, the individual pieces, being also complex, would still remain highly strained, as in fact is observed. Thus I noted considerable strain in all the 400 chips which I studied. The strain cannot be relieved by the act of fracture if an intermixing of type 1 and type 2 is responsible for the birefringence as I suggest here.

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NICKEL (II) COMPLEXES OF THE TETRADENTATE MACROCYCLE 2,12-DIMETHYL-3,7,11,17-TETRAAZABI*CYCLO* (11.3.1) HEPTADECA-1 (17),2,11,13,15-PENTAENE

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As part of the investigation[1,2] of the reaction of linear polyamines with 2,6-diacetylpyridine in the presence of metal ions, Curry prepared the macrocyclic complex cation, 2,12-dimethyl-3,7,11,17-tetraazabi*cyclo* (11.3.1) heptadeca-1 (17),2,11,13,15 pentaene-nickel (II), hereafter referred to as NiL^{2+} (Structure I).



(I)

The perchlorate salt was characterized by its infra-red spectrum, elemental analyses (carbon, hydrogen, nitrogen and chlorine), and determination of the formula weight in water using a 'Mechrolab' Osmometer (calculated for monomer, 516; found, 518). Rich and Stucky[3] claimed to have isolated the tetrachlorozincate salt of this cation but have offered spectral data for a product that has not been analysed. They have identified the copper complex as the tetrachlorozincate.

The complex ion of structure I, NiL^{2+}, is planar with unhindered axial sites, so that salts containing anions or other donors, such as water, provide a series of tetragonal complexes in which the nickel ion is subjected to a relatively constant in-plane ligand field but varying axial perturbation. Some of the chemical and physical properties of a number of these complexes have been examined in the course of a general investigation of tetragonal macrocyclic nickel (II) complexes.

Syntheses

$NiL(ClO_4)_2$ was prepared as described by Curry[1] by the reaction of stoichiometric quantities of $NiCl_2 \cdot 6H_2O$, diacetylpyridine, and diaminodipropylamine in 50 : 50 water-ethanol solution for several hours at 60° C. After

the removal of the bulk of the ethanol by evaporation, the crude product was precipitated by the addition of concentrated aqueous sodium perchlorate. The product was recrystallized from warm water, yielding grey-brown needles which turn brick red on removal of co-ordinated water *in vacuo*. The dihydrate of the perchlorate salt was prepared by letting the anhydrous salt stand in a water-saturated atmosphere.

The chloride and thiocyanate salts were prepared by the addition of lithium chloride or sodium thiocyanate to an acetone solution of the perchlorate salt, the chloride or thiocyanate immediately precipitating. These products were recrystallized from chloroform. Both these products precipitate as anhydrous salts, but the chloride readily picks up water to form the hydrate, turning from olive green to grey. The brown thiocyanate shows no tendency to pick up water.

Ni*L*Br$_2$·H$_2$O was precipitated by the addition of a concentrated solution of sodium bromide to aqueous Ni*L*Cl$_2$. The product was recrystallized from warm water. The addition of concentrated sodium bromide solution to aqueous solutions of Ni*L*(ClO$_4$)$_2$ or Ni*L*(BF$_4$)$_2$ results in the precipitation of the mixed salts Ni*L*Br(BF$_4$)·H$_2$O or Ni*L*Br(ClO$_4$)·H$_2$O.

The analytical data are reported in Table 1.

Magnetic and Spectral Properties

The magnetic susceptibilities of the samples were determined from room temperature to liquid nitrogen temperatures by the Gouy method. Ni*L*(ClO$_4$)$_2$ and Ni*L*ZnCl$_4$ (ref. 3) are diamagnetic, and are presumed to be square planar complexes. The infra-red spectrum of the perchlorate salt shows no evidence of co-ordination by the perchlorate anions. Ni*L*Cl$_2$, Ni*L*(SCN)$_2$, and Ni*L*(H$_2$O)$_2$(ClO$_4$)$_2$ are paramagnetic species which exhibit normal Curie-Weiss temperature dependence. The Curie-Weiss plots for these complexes are shown in Fig. 1, where the curves are constructed from a least squares fitting of the depicted data points.

Ni*L*Br$_2$·H$_2$O is diamagnetic. This is interesting in view of the fact that similar macrocyclic complexes of nickel, such as the bromide salt of the hexamethyltetraaza*cyclo*-tetradecane complex reported by Curtis[4], are paramagnetic. Since stereomodels indicate that the axial sites in Ni*L*$^{2+}$ are considerably less hindered than in the Curtis macrocycle, the failure of bromide to produce a paramagnetic complex cannot be attributed to steric factors. It may be that, as a result of the stronger field produced by the azo-methine groups in Ni*L*$^{2+}$, the weak axial perturbation produced by the bromide ion is insufficient to unpair the electrons of the nickel(II) ion. It seems quite unlikely, however, that Ni*L*Br$_2$·H$_2$O is a 6-co-ordinate species with both bromides co-ordinated, since the complex shows no solubility in chloroform in which Ni*L*Cl$_2$, Ni*L*(SCN)$_2$, and the tetragonal chloride and bromide salts

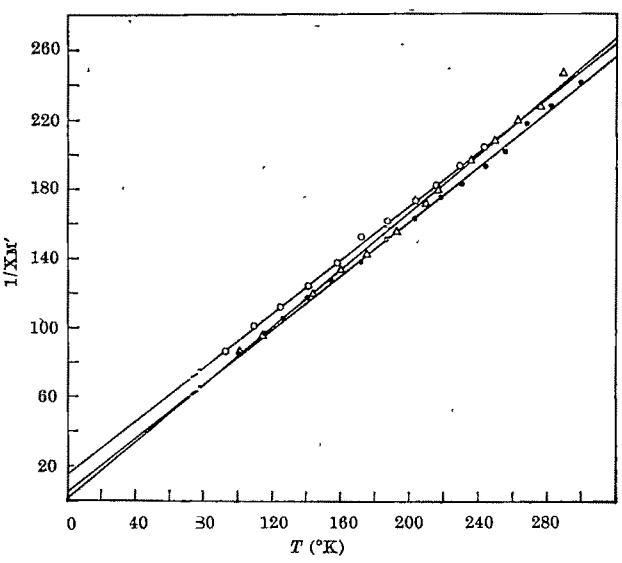


Fig. 1. Temperature dependence of susceptibility of tetragonal complexes of 2,12-dimethyl-3,7,11,17-tetraazabi*cyclo* (11 3.1) heptadeca-1 (17),2,11,13,15-pentaene ○, Ni*L*(H$_2$O)$_2$(ClO$_4$)$_2$, μeff = 3·22 B M., θ = 19·1°; △, Ni*L*(SCN)$_2$, μeff = 3·11 B.M., θ = 0·3°, ●, Ni*L*Cl$_2$, μeff = 3·22 B M., θ = 6·9°

of the Curtis macrocycle are soluble. It is also difficult to explain the intense colour of this complex if 6-co-ordination is assumed.

A particularly striking observation is that the visible absorption spectrum of Ni*L*Br$_2$·H$_2$O is virtually identical to that of the mixed salts Ni*L*Br(BF$_4$)·H$_2$O and Ni*L*Br(ClO$_4$)H$_2$O and markedly different from that of the planar Ni*L*(ClO$_4$)$_2$ complex or the tetragonal Ni*LX*$_2$ (where *X* is chloride, thiocyanide or water) complexes. Examples of these spectra, which were run on Nujol mulls using a 'Cary 14' spectrophotometer, are given in Fig. 2, and the band maxima are tabulated in Table 2.

The possibility that water is co-ordinated at one axial site in these complexes with bromide at the opposite position is rather remote, since the average field thus produced should be at least as strong as that resulting from the axial co-ordination of chloride, which produces a paramagnetic complex. Although the possibility of 6-co-ordinate bridged bromide complexes cannot definitely be ruled out for Ni*L*Br$_2$·H$_2$O, Ni*L*Br(BF$_4$)·H$_2$O, and Ni*L*Br(ClO$_4$)·H$_2$O, the objection to such structures on the basis of the deep colour of these complexes can again be raised. The co-ordination number of five seems highly likely for these substances.

The part played by water in these complexes is almost certainly a significant one. Ni*L*Br$_2$·H$_2$O and the two mixed salts contain 1 mole of water which is not removed by heating to 109° C *in vacuo*. The infra-red spectra of

Table 1. Analytical Data for the Complexes

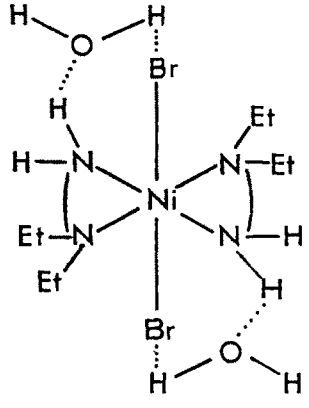
Compound		Carbon	Hydrogen	Nitrogen	Chlorine	Sulphur	Bromine	Fluoride
Ni*L*(ClO$_4$)$_2$	*T*	34·91	4·26	10 86	13·74			
	F:	35·36	4·41	10 89	14 06			
Ni*L*Cl$_2$	*T*·	46 43	5·72	14·44	18·28			
	F	46 32	5 80	14·35	18 34			
Ni*L*(SCN)$_2$	*T*.	47 13	5 12	19·40		14 80		
	F.	47 30	5 64	19·15		14 82		
Ni*L*Br$_2$ H$_2$O	*T*	36 40	4 88	11 32			32·29	
	F	36 48	5 02	11·20			32 15	
Ni*L*Br(BF$_4$)·H$_2$O	*T*·	35 90	4 82	11 17				15 14
	F:	36 21	4 82	11·02				14 64
Percentage water loss on drying Ni*L*(H$_2$O)$_2$(ClO$_4$)$_2$		*T*. 6 9						
		F: 7·1						

Table 2

Compound	ν_1	ν_2	ν_3	ν_4	ν_5	ν_6	ν_7
Ni*L*Cl$_2$	9,350 (wk)	14,300	19,200				
Ni*L*(SCN)$_2$	10,800 (sh)	13,900	18,900 (sh)	25,400 (sh)			
Ni*L*(H$_2$O)$_2$(ClO$_4$)$_2$		13,900	21,800 (sh)	25,200			
Ni*L*(ClO$_4$)$_2$					17,900 (sh)	24,200	28,600
Ni*L*Br$_2$ H$_2$O					16,500	18,200	21,800
Ni*L*Br(ClO$_4$) H$_2$O					16,500	18,200	21,800
Ni*L*Br(BF$_4$) H$_2$O					16,500	18,200	21,800

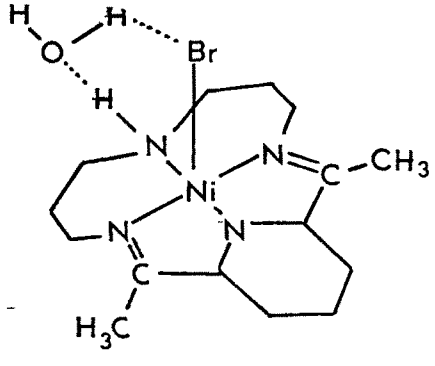
these complexes show sharply split bands in the region of the O—H stretching frequency, and it seems likely that the molecule of water exerts a profound effect on the structure of the complex. A number of examples of the interaction of water with co-ordinating anions have been observed previously. The anhydrous chloride and bromide salts of the Curtis macrocycle are paramagnetic, while their dihydrates are diamagnetic[4]. Since the spectrochemical series would lead one to expect, if anything, the reverse behaviour, it must be assumed that water is interacting with the halides in some manner which either prevents their co-ordinating or weakens their donor strength. Similarly, Melson and Busch have found that the bromide and chloride salts of the complex ion tetrabenzo-[*b*, *f*, *j*, *n*] [1, 5, 9, 13] tetraaza*cyclo*hexadecine-nickel(II) contain one mole of water, which can only be removed with difficulty, and have postulated that hydrogen bonding between this water molecule and the halides is responsible for lowering the ligand field of the halides and producing the abnormally low magnetic moments observed for these complexes[5].

A particularly interesting hydrate structure has been proposed by Goodgame and Venanzi to explain the fact that the dihydrate of *bis*(*N*,*N*-diethylethylenediamine) nickel(II) bromide is paramagnetic whereas the anhydrous compound is diamagnetic[6]. In this case Goodgame and Venanzi suggest that hydrogen bonding as shown in Structure II is responsible for holding the bromide ions in the axial sites.



(II)

A similar interaction of water with the bromide in Ni*L*Br$_2$ and the mixed salts, which have only one N—H bond, would produce essentially 5-co-ordinate, diamagnetic complexes. The choice of this structure (III) is a compelling one, since it accounts for the tendency of the complex to bind only one molecule of water and for the splitting of the O—H band in the infra-red spectrum.



(III)

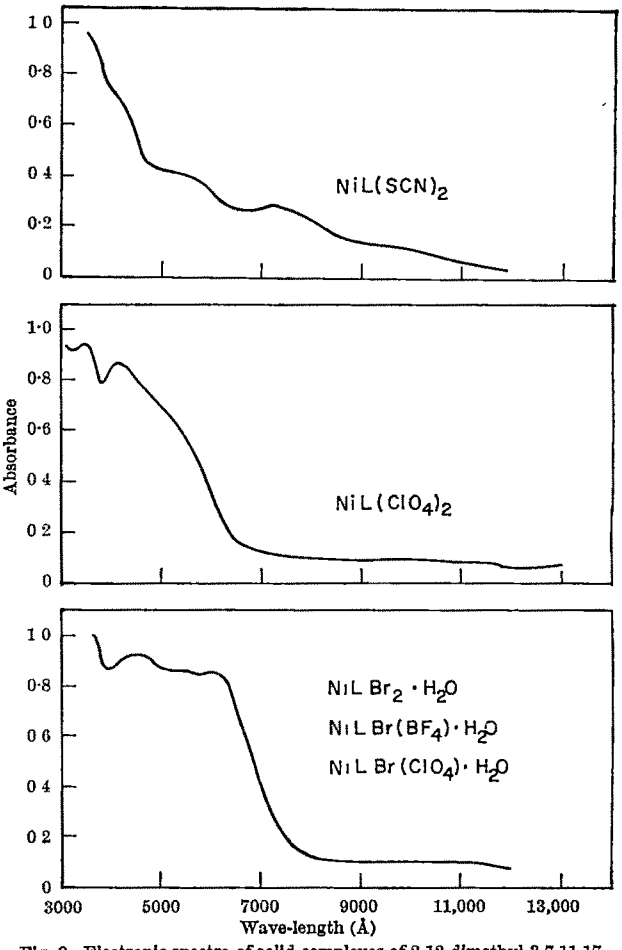


Fig. 2. Electronic spectra of solid complexes of 2,12-dimethyl-3,7,11,17-tetraazabi*cyclo* (11.3.1) heptadeca-1 (17),2,11,13,15-pentaene

The intense colour of the complexes, which exist as fine black needles, is consistent with this low degree of symmetry. The molar conductance of Ni*L*BrBF$_4$·H$_2$O in CH_3NO_2 is 84·1 ohm^{-1} at 21°, as expected for a uni-univalent electrolyte[7].

Reduction of the Azo-methine Linkages

The catalytic reduction of the azo-methine linkages in this macrocycle yields two isomeric products which are red and yellow in colour. with the red form predominating. The infra-red spectra of the products show enhancement of the N—H band at 3,180 cm^{-1} and diminution of a band at 1,570 cm^{-1}, presumably the C=N band in the starting material. The complete disappearance of this band is not observed because it coincides with a band originating from the diacetyl-pyridine moiety. Hydrogen uptake is consistent with that expected for the reduction of both azo-methine linkages. It can be concluded that these two isomeric forms are geometric isomers with the two methyl groups extending on either the same or opposite sides of the plane of the nickel ion (meso and racemic, respectively). Further results on the reduced complexes and its salts will be reported elsewhere.

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EVIDENCE OF TWO SOUND CHANNELS IN THE POLAR ATMOSPHERE FROM INFRASONIC OBSERVATIONS OF THE ERUPTION OF AN ALASKAN VOLCANO

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AND
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AT daybreak on January 24, 1966, ash-charged vapour columns were seen over Mt. Redoubt from Kenai and Anchorage, Alaska. Reports soon confirmed that the column was being erupted through a new fissure vent on the north ridge of the volcano below the old crater. Mt. Redoubt (3,110 m) is located at 152° 50′ W. long., 60° 30′ N. lat., 550 km from College, Alaska, at an azimuth of 210°. The volcano has been active during at least five previous occasions in historical time as summarized in Table 1.

Table 1. MT. REDOUBT VOLCANO

Year	Type of activity
1778	Active (type unknown)
1819	Smoke (steam and/or ash clouds)
1902	Active (type unknown)
1933	Smoke (steam and/or ash clouds)
1965	Sporadic emission of vapour plumes (from a new fissure on north flank)

The chronology of the recent eruptive cycle is:

January 24, 1966. A turbulent ash-charged cloud which reached an altitude of 6,100 m was reported over the volcano; the presence of a new vent at the 2,300-m level on Redoubt was reported by aircraft in the area.

January 25–27, 1966. Heavy cloud cover obscured the volcano; however, on January 25 an eruptive cloud appeared above the cloud deck. Its height was estimated at 12,000 m by a jet pilot flying near Redoubt.

January 28, 1966. Helicopter reconnaissance by one of us revealed that activity had decreased to the rather steady emission of a turbulent but ash-free steam cloud.

February 5, 1966. A second eruptive cycle of ash-charged gas columns began after a quiescent period of 9 days.

February 6–8, 1966. Visual observations were hampered by bad weather conditions, but reports on February 8 indicate the emission of a persistent 4,570–6,100 m ash-charged vapour column throughout the day.

February 9–10, 1966. Another day of marginal visibility; two columns were erupted which were closely monitored by U.S. Air Force radar facilities and aircraft at Kenai, Alaska. Shortly after 1900 h U.T., a U.S. Air Force plane reported the emergence of a brown-coloured turbulent cloud which mushroomed at 13,720 m from a closed deck below. This elevation was confirmed by U.S. Air Force radar control at Kenai. On February 10, at 0050 h U.T., the radar station at Kenai reported another eruption cloud which mushroomed at 9,700 m.

February 20, 1966. Activity was markedly reduced from February 10 until February 20 when a Geophysical Institute seismic research team on the south ridge of Mt. Redoubt reported a steam and ash cloud at 1938 h U.T. which rose to 5,000 m. There has been no further activity other than the emission of a continuous column of steam similar to the activity which characterized the two interim quiescent periods.

So far, lava has not been erupted from the new vent and no crater development has been observed. The ejecta deposited by the eruptive clouds are composed of andesite pumice, scoria and lithic fragments, and a sand-sized fraction consisting of discrete and fractured grains of quartz, feldspar and hornblende, which has apparently been derived from granitic sub-volcanic basement rocks. Glass shards are rare or absent in ejecta deposited up to 1 km from the vent and the lithic and erupted fragments show no evidence of magmatic transport.

The explosive clouds erupted by Mt. Redoubt were apparently derived from the rapid degassing of an andesitic magma which was initiated at considerable depth. The lithic fragments were probably derived from the attrition of andesite flows within the volcanic pile, but the pumice and scoria are believed to represent the vesiculated and degassed equivalent of the juvenile magma.

During the period of January 24–February 20, 1966, infrasonic pressure waves travelling at acoustic velocities were observed at the Geophysical Institute at College, Alaska, following the eruptions of Mt. Redoubt. The infrasonic signals were detected by a quadrilateral array of microphone stations using equipment and techniques developed by the National Bureau of Standards[1]. Assuming a plane wave solution, one can determine the azimuth of the arriving signal as well as the horizontal trace velocity V_h of the waves from the arrival times of the pressure waves at the four microphones. If the angle between the wave normal and the horizontal plane is α, then α is given by $V_h = V_\varphi \sec \alpha$, where V_φ is the local velocity of sound. From the analogue recording of the pressure variations as a function of time one can obtain the absolute amplitudes, the periods and dispersion of the infrasonic waves. The acoustic waves from the Mt. Redoubt eruptions were recorded without filtering on magnetic tape and also on charts with two different bandpass filters, namely, an $N7$ channel filter with half-amplitude points at 9 sec and 70 sec, and an $N6$ channel bandpass from 0·1 sec to 35 sec.

Six separate infrasonic events were associated with the various eruptions of Mt. Redoubt. Each of these events can be related to the eruption of a specific turbulent cloud. The strength of the infrasonic signals was generally proportional to the height of the erupted cloud. From the observed character of the eruptions the infrasonic pressure waves were probably generated by the sudden ejection of large volumes of high-pressure gas by the volcano rather than by explosions of the type reported by Gorshkov[2] and Goerke *et al.*[3] for Bezymianny and Agung volcanoes.

The characteristics of these six infrasonic events are given in Table 2 together with the height of the erupted cloud in metres and the component of the wind in m/sec at the 30-km level from an azimuth of 210°. The average azimuth of all the infrasonic signals from Redoubt was 210·8° with seldom more than ±5° variation from this direction. The various waveforms of the infrasonic pressure waves are shown for events numbers 1, 2, 4 and 6 in Fig. 1 in which the waves have been superimposed to give the best correlation of phase of the pressure waves among the four microphone sites.

Table 2 shows that for all events, with the exception of number 3 in which the signal was only a single pressure

Table 2. MT. REDOUBT INFRASONIC SIGNALS

No.	1966 Date	U.T.	N6 channel φ	N6 channel V_h (m/sec)	N7 channel φ	N7 channel V_h (m/sec)	Amplitude (dynes/cm²)	Duration (min)	Period (sec)	Cloud height (m)	Wind (m/sec)
1	1–24	1000		—	202°	339	1·5	6	75–60	6,100	20·6
		1012		—	220	430	2·0	8	70–50		
2	1–25	2130	209°	363	206	331	>100	25	65–25	13,000?	14·9
		2139		—	211	404	>100	15	15–10		
3	2–5	0230	210	336	211	389	2 5	spike	10 sec		−9·8
4	2–9	1944	210	316	211	360	7	20	30–20	13,720	−9 8
		1956	213	407	214	408	16	20	30–20		
5	2–10	0102	207	346	215	353	2·5	8	40–30	9,700	−13·9
		0112		—	207	450	2·0	3	40–30		
6	2–20	2003	206	319	207	366	1·8	3	10	5,000	−34
		2010	208	455	209	429	1·0	1	10		

spike, there were two arrivals of infrasonic energy associated with each volcanic eruption. The two successive arrivals of infrasonic energy can best be seen in Fig. 1 for the events on January 24 and February 20. The first arrivals are at 1000 h and 2003 h, while the second are at 1012 h and 2010 h on January 24 and February 20, respectively. A difference in the alignment of the superimposed pressure records between the first and second signals is indicated by the break in the time line. The sequences of numbers 1, 2, 4, 3 on the time line in Fig. 1 give the time of arrival sequence of the waves at the four microphone sites. The difference in the character of the incoherent noise and the presence of an infrasonic signal can be clearly seen in Fig. 1 for the January 25 event. The four pressure traces exhibit random fluctuations from the beginning of this record until 2130 h when the coherent signal arrived and all four traces became superimposed.

The average delay time between the two infrasonic signals is 10 min for the five events. The trace velocity is greater for the second signal than for the first in all these events. The averaged velocities are $V_h = 343$ m/sec for the first wave and $V_h = 426$ m/sec for the second. If the local acoustic velocity is given by $C = 20{\cdot}60\sqrt{T}$ m/sec, where T is the absolute temperature, then for the temperature measured by balloons at the 7-km level of 230° K, we find that $C = 304$ m/sec. Using this velocity as an approximation of the group velocity of the infrasonic waves, the travel time to cover the 550 km distance by a level path would be about 30 min. Thus, a 10-min delay time for the second signal indicates that the path for the second signal was about 30 per cent longer than that for the first signal. If V_φ is assumed to be 304 m/sec, then the angle of incidence of the first wave is about 28° while that of the second is about 45°. From these considerations we conclude that the infrasonic pressure waves are travelling from Mt. Redoubt to the observing site by two different acoustic channels in the atmosphere. The signal which arrives first travels in the lower sound channel, described by Diamond[4], between the ground and the 50-km level. The second signal which arrives from a larger angle of incidence travels in the higher channel, described by Pfeffer and Zarichny[5], from the 50-km to the 110-km level.

The normal dispersion (that is, group velocity increasing with increasing period) of the wave trains of the first signal in events numbers 2 and 4, which can clearly be seen in Fig. 1, is consistent with the dispersion expected for acoustic propagation by the fundamental mode in the first sound channel[5]. Further evidence of the normal dispersion of the ducted infrasonic waves can be found by averaging the trace velocities in Table 2 for the high-frequency $N6$ and lower-frequency $N7$ bandpass filters. The average velocity for the "first signal" which propagated in the lower sound channel for the $N6$ bandpass is 342 m/sec while that for the $N7$ bandpass is 359 m/sec. Thus a higher group velocity, characteristic of normal dispersion for the longer period waves in the lower sound channel, is indicated by the higher average trace velocity for the signals observed on the $N7$ relative to the $N6$ bandpass filters. Superposition of the second signal on the tail of the first makes it impossible to determine the dispersion characteristics of the second signal.

No evidence was found of infrasonic waves that had propagated around the Earth by a great circle path through the Antipodes from any of the Mt. Redoubt eruptions as were observed in the case of the eruptions of Mt. Agung[3]. The lack of such east-to-west propagation of acoustic signals is consistent with the inhibiting effect of the westerly winds on the lower sound channel in winter[4].

The effect of the wind on the strength of the received signals may account in part for the difference in the amplitude and duration of the infrasonic waves. The amplitude of event number 2 was much greater than that of number 4. Although the heights of the erupted clouds were about the same for these two events, the wind at the 30-km level, as shown in Table 2, would favour the development of a strongly ducting sound channel[4] for event 2 while inhibiting it in event 4.

The difference in the observed periods of the pressure waves for these two events can be used to show that event number 2 was intrinsically more energetic than number 4

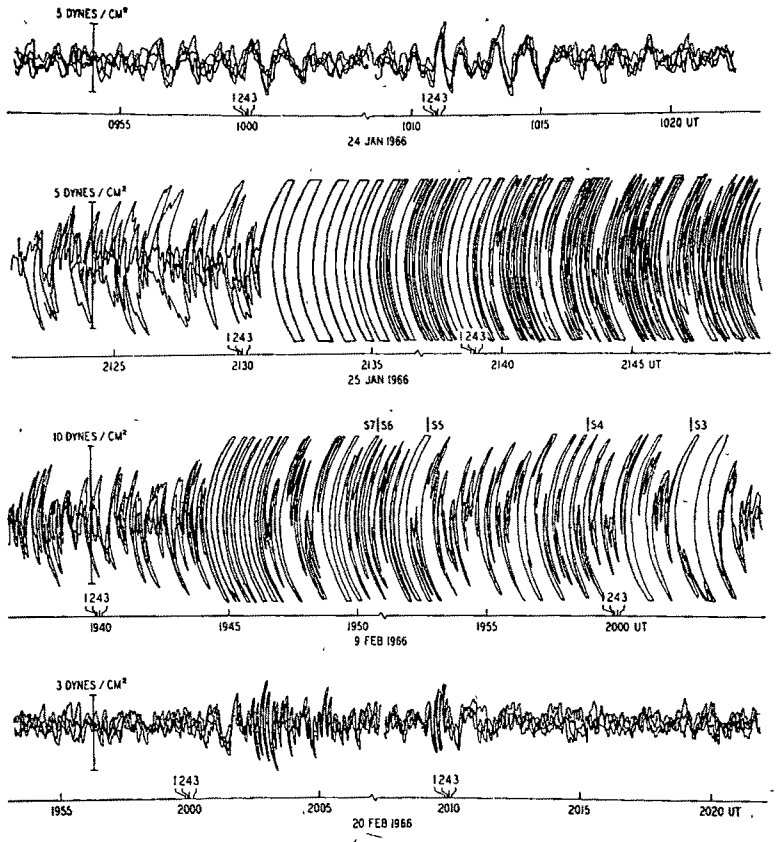


Fig 1. Infrasonic waves from Mt. Redoubt

even though some of the differences in their amplitudes can be explained by wind effects. Hunt *et al.*[6] have shown that the average period of the largest wave due to an explosion in the atmosphere is roughly proportional to the cube root of the energy released by the explosion. The mean periods of the waves in events 2 and 4 averaged over the first four cycles are 55 sec and 21 sec, respectively. The ratio of the infrasonic energy released in event 2 to that in 4 would then be $(T_2/T_4)^3$ or about 18 to 1. Unfortunately the bad weather conditions on January 25 prevented this being verified by visual observations.

It is possible to estimate from the amplitude of the pressure waves the volume of gas ejected by the volcano by use of a model developed by Hunt *et al.*[6]. They calculate the pressure variations as a function of time and distance from a source which injects 1 km³ of gas into the atmosphere. By extrapolation of their curves in Fig. 7*a* for the pressure variations at a distance of 1,000 km from the explosion site to the closer distance of 550 km (the Redoubt to College distance) it was found that the injection of 1 km³ of gas at the explosion site would cause a 30-dynes/cm² pressure wave at a distance of 550 km from the explosion. The volume of gas ejected by the Redoubt eruptions which generated the infrasonic pressure waves can be found by calculating the ratio of the observed pressure variation at College to the extrapolated theoretical variation of 30 dynes/cm² and multiplying this by 1 km³.

The maximum amplitude of the February 9 infrasonic pressure wave from the magnetic tape record was 16 dynes/cm². This gives a volume of gas injected into the atmosphere by the eruption of about 0·5 km³. Because the signal from the January 25 eruption was off-scale on the chart recorders and had saturated the magnetic tape record, we can only estimate the volume of gas ejected by Mt. Redoubt for this event as eighteen times that of the event of February 9, or 9 km³. This follows from our previous discussion of the relative energy of the events of February 9 and January 25 which we derived from the ratio of the periods of the two infrasonic waves.

The energy of the eruptions which ejected the gas clouds from the fissure vent on Mt. Redoubt can be found by calculating the work done by the ejected gas in expanding against the ambient atmospheric pressure at the level of the vent. The energy is thus given by $\int_0^V P\mathrm{d}v$ where V is the volume of the ejected gas and P is the constant pressure at the 2300-m level or $0{\cdot}8 \times 10^6$ dynes/cm². The energy necessary to inject 0·5 km³ of gas in the February 9 eruption is thus $(0{\cdot}8 \times 10^6$ dynes/cm²) $(0{\cdot}5 \times 10^{15}$ cm³) or 4×10^{20} ergs. For the January 25 eruption the energy would be about 7×10^{21} ergs.

Our findings can be compared with those of G. S. Gorshkov[2] in his study of the explosion of Bezymianny volcano, Kamachatka, U.S.S.R., on March 30, 1959. Using microbarograph data from eight meteorological stations at distances from 45 km to 760 km from the volcano, Gorshkov calculated an average value of 3×10^{22} ergs for the air wave generated by the explosion. Gorshkov stated that about 10 per cent of the total energy of the volcanic explosion was transformed into resultant air wave energy. Thus Gorshkov estimated the total energy of the Bezymianny eruption to be approximately 3×10^{23} ergs.

In the case of Mt. Redoubt, because of the non-explosive character of the eruptions, the fraction of the total energy of the eruption which was consumed as work necessary to eject the gas clouds against atmospheric pressure is thought to represent the majority of the energy of the eruption. Some energy would be used in imparting kinetic energy to the solid ejecta; perhaps one-third of the total energy. Thus we can establish lower limits for the energies of the Mt. Redoubt eruptions as 6×10^{20} ergs for the event of February 9 and $1{\cdot}05 \times 10^{22}$ ergs for that of January 25.

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PRODUCTION OF MESONS IN THE NUCLEI OF GALAXIES

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THE emission of radiation by electrons from the decay of mesons produced in high-energy astrophysical processes has frequently been discussed. Some years ago, Burbidge[1] and Ginsburg[2] considered π meson production in the interaction of fast cosmic ray protons with galactic material; while Burbidge and Hoyle[3] considered π meson production in the annihilation of anti-nucleons. The electrons from the meson decay reactions:

$$\pi \rightarrow \mu + \bar{\nu} \text{ and } \mu \rightarrow e + \nu + \bar{\nu} \qquad (1)$$

could then, under favourable conditions of electron energy and galactic magnetic field, produce synchrotron radiation in the optical and radiofrequency regions of the spectrum. Burbidge and Hoyle found that it was possible, on the basis of their proposed model, to meet the luminosity requirements of the most energetic radio sources, provided that the number of anti-nucleons in the galaxy could be maintained at a constant level—a level which would, moreover, be consistent with the pair production of nucleons at a rate given by the requirements of steady-state cosmology.

In these models, no assumptions had to be made regarding any possible localization of the meson production process within the galaxy.

Later, Burbidge, Burbidge and Sandage[4] presented detailed evidence for the occurrence of violent events in the nuclei of galaxies, including the Seyfert galaxies and a number of the strong radio sources, and suggested that the nuclei may have many effects on the structure and evolution of these galaxies and may be the ultimate sources of their energy. Hoyle and Fowler[5,6] at the same

time proposed a model on which an energy sufficiently large to account for the strong radio sources could be produced by a galactic nucleus, in the form of a massive star, as a result of its gravitational contraction.

Following the discovery of the quasi-stellar radio sources[7,8], several further models were advanced to account for the existence of highly localized sources of great energy: Fowler[9,10] developed the massive star model, and a similar system was discussed by Greenstein and Schmidt[11]; while Field[12], Woltjer[13] and Gold *et al.*[14] considered various processes which could occur if the galactic nucleus were to consist of a stellar cluster rather than a single star.

I shall propose here a model which has features in common with several of those already suggested. We consider a process whereby new material is accreted from intergalactic space by a galactic nucleus consisting of hydrogen already at an advanced stage of condensation; and we shall now investigate the situation for which this material, before its entry into the galactic nucleus, has acquired an energy exceeding the threshold for the production of π mesons and anti-nucleons. Adopting a phrase originally applied by Salpeter[15] to the accretion of interstellar matter by a massive star, this process can be described as one of "catastrophic accretion". I suggest that such a process, centred on a galactic nucleus of radius somewhat greater than its Schwarzschild limit, could lead to the production of π mesons and anti-nucleons: decay of the π mesons would give rise to fast electrons as already discussed, while the anti-nucleons would give the same ultimate decay products, but by way of the production of π mesons in the process of annihilation

For a proton or hydrogen atom, henceforth referred to simply as a proton, entering the gravitational field of the galaxy from a distance which is large by comparison with the radius (R) of the nucleus, the appropriate equation of motion gives the final velocity (βc):

$$(\beta c)^2 = \frac{2MG}{R} = \frac{8\pi}{3} G (R^2 \rho) \qquad (2)$$

where M and ρ are the mass and density of the galactic nucleus, and G the constant of gravitation. (R is the Schwarzschild radius for the mass M at $\beta = 1$.)

The production of π mesons and anti-nucleons may occur in initial interactions of the types:

$$p + p \rightarrow N + N + n\pi \qquad (3a)$$

and

$$p + p \rightarrow N + N + N + \overline{N}: \qquad (3b)$$

In the first the proton energy threshold for the production of a single π meson ($n = 1$) is 300 MeV, while in the second the threshold for the production of an anti-nucleon is 5·6 GeV. We calculate from equation (2) the values of $R^2\rho$ required for the attainment of the corresponding values of β : $R^2\rho$ equals $0{\cdot}685 \times 10^{27}$ g cm^{-1} for equation (3*a*), and $1{\cdot}575 \times 10^{27}$ g cm^{-1} for equation (3*b*).

We consider proton–proton interactions at an arbitrary energy of 7 GeV, for which $R^2\rho$ has a value very similar to the second of these figures, and for which many data have been collected from experiments with the *Nimrod* and *Bevatron* proton synchrotrons. From this required value of $R^2\rho$ we calculate ρ and also M, expressed in solar mass units, for different values of R. Some of the results are given in Table 1.

A galactic nucleus specified by one of the sets of figures given in Table 1 could act as a 7-GeV cosmic proton accelerator, and give rise to the production of π mesons

Table 1. PARAMETERS DESCRIBING GALACTIC NUCLEUS

R (cm)	ρ (g cm^{-3})	$\frac{M}{M\odot}$
10^{14}	$1{\cdot}6 \times 10^{-1}$	$3{\cdot}3 \times 10^{6}$
10^{15}	$1{\cdot}6 \times 10^{-3}$	$3{\cdot}3 \times 10^{9}$
10^{16}	$1{\cdot}6 \times 10^{-5}$	$3{\cdot}3 \times 10^{12}$

and anti-nucleons in the process of catastrophic accretion occurring at this energy.

It is essential, however, that the charged π mesons should have a large probability of free decay, eventually to electrons, and only a small probability of secondary interaction. This condition immediately sets an upper limit to the density of the hydrogen of the galactic nucleus. We estimate from the results of calculations by Morgan[16] that an approximate mean momentum for π mesons produced by 7 GeV protons is 1 GeV/c. In general, the mean free path for meson decay at a momentum of p is given by:

$$\lambda\,(\text{decay}) = \tau c\beta\gamma = \tau\left(\frac{p}{m_0 c^2}\right)$$

where τ is the proper lifetime of the meson. Now it is the lifetime of the π meson which is relevant here, because only the strong interactions of the π mesons offer competition to the production of decay electrons, even though the μ meson has the longer lifetime. Taking, therefore, a value of τ equal to $2{\cdot}55 \times 10^{-8}$ sec, we find that λ (π decay) $= 5{\cdot}45 \times 10^{3}$ cm. For the interaction of charged π mesons in hydrogen we estimate, from data summarized by Kallén[17], that the total cross-section at 1 GeV/c is 38 millibarns: the mean free path for interaction is then given by $\lambda(\pi$ interaction$) = (N\rho\sigma)^{-1}$ $= (0{\cdot}023\ \rho)^{-1}$. In order that, for the π mesons, decay should be more probable than interaction, $(0{\cdot}023\,\rho)^{-1} \geqslant 5{\cdot}45 \times 10^{3}$, which sets an upper limit to ρ equal to $0{\cdot}8 \times 10^{-2}$ g cm^{-3}. This corresponds to a lower limit for R of $4{\cdot}45 \times 10^{14}$ cm.

The range of possible values of R is also limited by the mass of the galactic nucleus. If we take as a guide the figures for the dimensions and mean density of our own Galaxy[18], we find that unless $R \lesssim 3 \times 10^{16}$ cm the whole of the galaxy mass is concentrated in the nucleus; and if we consider only galactic systems similar in total mass to our own, this value of R may be taken as an upper limit to the radius of the galactic nucleus on our model.

With regard to these limits, we conclude that an acceptable value for R would be 10^{15} cm, with values for ρ and M as given by Table 1, although it could be as high as 10^{16} cm if an appreciable fraction of the galaxy mass were concentrated in the nucleus. The former figure is taken in most of the subsequent calculations.

For a catastrophic accretion process centred on a galactic nucleus described by these figures, the interaction mean free path for the incident protons, initially in the tenuous material of the envelope and then in the denser material of the nucleus, may be estimated approximately in terms of the proton–proton cross-sections and the appropriate densities of the material. A survey of the values taken by the cross-section over the energy range corresponding to the difference in radii of the galactic envelope and the galactic nucleus suggests that at no stage is the proton acceleration process inhibited appreciably as a result of proton interaction with the material of the envelope: for a mean density of galactic material[18] of 7×10^{-24} g cm^{-3}, a proton can easily derive enough energy from the gravitational field over a distance of an interaction mean free path to more than compensate for any energy loss by collision. For the galactic nucleus, we may take for the inelastic cross-section the particular value of 27 mbarns[19] which, for the value of ρ already estimated, corresponds to a mean free path of $3{\cdot}9 \times 10^{4}$ cm. It can therefore be concluded that the protons incident on a galaxy described by our model reach the centre without an appreciable loss of energy, and that their interactions are localized in the extreme periphery of the nucleus.

We now estimate the rate of production of energy in the form of fast electrons inside the galactic nucleus. First, we calculate the flux of protons, dN/dt, gravitating from the region of intergalactic space, at a distance $\mathfrak{R}$

from the galactic centre, where the density of protons is ρ_0 cm^{-3}:

$$\frac{dN}{dt} = \left(\frac{64}{3\pi}\right) MG\rho_0 T \text{ protons sec}^{-1} \quad (4)$$

where T is the accretion time for protons from this region, and which is related to $\mathfrak{R}$ by the expression:

$$T = \pi\left(\frac{\mathfrak{R}^3}{8MG}\right)^{\frac{1}{2}} \text{ sec} \quad (5)$$

If we take for $\mathfrak{R}$ a distance equal to half the mean separation between galaxies in a cluster of typical size, and set the maximum energy attained by the protons equal to our arbitrary energy of 7 GeV, so that M has the value already estimated, we find that:

$$\frac{dN}{dt} = 3{\cdot}92 \times 10^{54}\rho_0 \text{ protons sec}^{-1} \quad (6)$$

The corresponding value of T is $1{\cdot}31 \times 10^{18}$ sec, which is of the order of the Hubble time. Only a comparatively brief phase towards the end of this total accretion time is, however, relevant to the occurrence of the high-energy processes at present being considered on our model.

Secondly, we estimate the mean energy produced, in the form of fast electrons, per incident proton. We assume that the whole of the inelastic cross-section of 27 mbarns[19] represents direct π meson production in proton interactions of the type (3*a*). The meson multiplicity n measured at this energy, the π meson charge ratio deduced from charge independence, and a small correction for the finite probability of π meson secondary interaction together give the result that the mean number of decaying charged π mesons is 1·2 per incident proton. We estimate that for a π meson momentum of 1 GeV/*c* about 4 per cent of the meson decay energy appears in the form of fast electrons, and we finally obtain the figure of $7{\cdot}6 \times 10^{-5}$ ergs for the mean electron energy produced per incident proton.

To see if the catastrophic accretion process could give a rate of energy production comparable with that observed for the quasi-stellar sources, we shall leave aside for the moment any consideration of the other products of the incident proton interaction, and try to explain the energy production in terms of electron energy alone. Combining the figures obtained for incident proton flux and electron energy produced per proton, and leaving ρ_0, the density of protons in intergalactic space, as an undetermined parameter, we put the product equal to the total luminosity of a quasi-stellar source, taking for example the figure of 10^{46} ergs sec^{-1} for the source 3*C* 273 derived from the estimate made by Greenstein and Schmidt[11]:

$$(3{\cdot}9 \times 10^{54}) \times (7{\cdot}6 \times 10^{-5})\ \rho_0 = 10^{46} \quad (7)$$

which has the solution $\rho_0 = 3{\cdot}4 \times 10^{-5}$ cm^{-3}. If we had taken R as 10^{16} cm, the right-hand side of equation (6) would have been $(1{\cdot}24 \times 10^{55})\ \rho_0$ protons sec^{-1}, which would have given $\rho_0 = 1{\cdot}1 \times 10^{-5}$ cm^{-3}. These values of ρ_0 correspond to hydrogen densities in intergalactic space of $5{\cdot}7 \times 10^{-29}$ g cm^{-3} and $1{\cdot}8 \times 10^{-29}$ g cm^{-3} for $R = 10^{15}$ cm and $R = 10^{16}$ cm, respectively.

These figures are very similar to the theoretical values discussed by various authors: Sciama[20], for example, suggests that matter exists in intergalactic space with a density of 2×10^{-29} g cm^{-3}. An upper limit to the density of neutral hydrogen in intergalactic space, derived from the results of observations at a frequency of 21 cm discussed by Davies[21], is $4{\cdot}3 \times 10^{-29}$ g cm^{-3}. On our catastrophic accretion model, the density is the total density of intergalactic hydrogen, both neutral and ionized, so that our figures are consistent with this upper limit for a wide range of levels of ionization, each level corresponding to a different temperature for the intergalactic hydrogen. Sciama[20] has, however, pointed out that if the temperature of the intergalactic gas exceeds 10^5 °K, the gas is thermally stable against collapse. As we may expect a similar condition to apply to our model, it is clear that the model may be feasible only if the temperature is, in fact, less than this limiting value.

Recently reported[20] observations by Gunn of the Lyman-α radiation from the quasi-stellar source 3*C* 9 have suggested that the density of intergalactic neutral hydrogen may be as low as 5×10^{-35} g cm^{-3}. If this result is confirmed, then we may have to revise our conclusions in one of two ways: either we may take this figure to be an indication of a very high level of ionization, in the event of which our model in its present form may no longer be feasible for the correspondingly high gas temperature; or we may take the figure to be an indication of a very low total gas density, in which case our model is still feasible but gives a lower rate of energy production. For the second alternative, an improved situation would result if the "local" density of the intergalactic gas within a galactic cluster were several orders of magnitude higher than that outside: the rate of energy production could then be as large as previously estimated.

Whether a given intergalactic hydrogen atom is neutral or ionized would seem to matter most when it enters the envelope of the galaxy. At this stage of the accretion process, the kinetic energy is already greater than the ionization energy for hydrogen, so that ionization of a neutral atom occurs when it interacts with the material of the outer envelope. Any free electrons produced in this process can be expected to diffuse inwards, but to an extent which is significantly affected by local values of the galactic magnetic field.

In considering energy production in the nucleus of the galaxy we have only, so far, dealt with the contribution made by fast electrons from charged meson decay, and have assumed that these electrons when injected into the magnetic field of the nucleus can produce observable synchrotron radiation. Our conclusions would be modified, in favour of our obtaining a more powerful energy source for a given intergalactic gas density, if other products of high-energy proton interactions of the types indicated in equation (3) were also taken into consideration. We estimate that whereas less than 1 per cent of the incident proton energy is converted into the energy of fast electrons via charged π meson decay, about 10 per cent appears in the form of gamma-ray photons from π° meson decay, and 15 per cent in the form of neutrinos. Some of the gamma-ray photons, which are of mean energy 50 MeV, should undergo secondary interactions in the nucleus, also producing fast electrons.

Electrons which escape into the envelope, where the strength of the galactic magnetic field is probably much less than inside the nucleus, should radiate at a lower energy level than they would if retained in the nucleus: perhaps the energy of most of these electrons is emitted in the radiofrequency continuum, while that of electrons retained in the nucleus is emitted in the optical continuum.

Whatever the mechanism of its production may be, radiation emitted from a galactic nucleus such as we have described is strongly affected by the gravitational field of the nucleus. For a nucleus which is capable of accelerating particles to a velocity of βc, the gravitational red-shift $(\delta\lambda/\lambda)$ of radiation emitted from its surface is $\frac{1}{2}\beta^2$. In order for this effect to be consistent with the Doppler ("cosmological") interpretation of the observed red-shifts associated with the quasi-stellar sources, we must assume that the emission lines of the optical spectrum are produced in material outside the nucleus, in regions where the gravitational potential of the nucleus is much lower than at its surface. Any gravitational red-shift which these lines may have is inextricably combined with the Doppler red-shift due to any radial component of velocity within the galactic material. We estimate that for a galactic nucleus having mass and radius as calculated, the gravitational red-shift for material at a distance of

only 10^{-1} parsec (pc) from the galactic centre is equal to the Doppler red-shift for velocities of the material of 10^3 km sec^{-1}.

Now the occurrence of velocities of 2×10^3 and 3×10^3 km sec^{-1} in the quasi-stellar sources 3*C* 48 and 3*C* 273 has been deduced by Greenstein and Schmidt[11] from the widths of emission lines from regions of diameter 10 pc and 1 pc, respectively. If the events inside and in close proximity to the galactic nuclei we have described are indeed related to the production of quasi-stellar sources, then the small gravitational red-shifts predicted may well be completely obscured by this additional observational feature of high velocities in the source material.

These emission lines, originating in the innermost region of the galactic envelope, are presumably excited by the electrons which escape from the nucleus. (An electron at a distance of 10 pc from the galactic centre has an energy of about 10 eV, and so is capable of inducing optical transitions in hydrogen and other light elements of the galactic material.) In general, it would seem that electrons which give up their energy at a large radius ($\gtrsim 10^{-1}$ pc) from a galactic centre provide a means whereby energy produced as the result of a gravitational process can be radiated without appreciable modification by gravitational red-shift.

The picture we now have representing high-energy events occurring in the galactic nucleus and its immediate environment is related by several common features to the model of quasi-stellar sources constructed by Greenstein and Schmidt[11] from detailed considerations of all the available observational data. On this model, a quasi-stellar source consists of three concentric regions: the first, of radius $\lesssim 1$ pc, gives rise to the optical continuum and may contain a central object of mass $\sim 10^9 M_\odot$; the second, of radius 1 pc–10 pc, is the source of the emission lines; and the third is an outer region from which the radiofrequency radiation is emitted. Observations by Smith and Hoffleit[22] of the variations in optical intensity of 3*C* 273 over a number of years have indeed indicated that the size of the optical component of the source is $\lesssim 1$ pc, at a conservative estimate, and could be much smaller, $\sim 10^{-2}$ pc (3×10^{16} cm).

Greenstein and Schmidt found that, in order to satisfy total energy requirements, for an estimated lifetime of 10^6 years for the quasi-stellar source and for a synchrotron emission model with equipartition between magnetic field and total particle energies, it was necessary to have a source of energy, possibly associated with the central massive object, which provided a steady supply of high-energy electrons. Now the process of catastrophic accretion which we have discussed is a mechanism by which this central massive object, if identified with the galactic nucleus of our model, could act as the required high-energy electron source. (Regarding the continuous generation of relativistic particles in, for example, the nucleus of 3*C* 273, we note the possible similarity of the nucleus in this respect to that of the Crab nebula, pointed out by Shklovsky[23], who suggested that if the two phenomena are similar in nature and differ only in scale, then there would be difficulties in explaining them on an accretion model[24].)

The length of time over which a galactic nucleus acts as a source of high-energy electrons cannot be estimated from our model alone. Electron production begins when the accretion process has produced an energy above the threshold for π meson production, and ends when the "stellar" evolution of the accreted material of the galactic nucleus has attained a stage beyond which the density of the material exceeds the limit corresponding to the meson decay condition. The lifetime of the source is, therefore, defined in principle; although it has not been estimated, because our own knowledge of the probable course of evolution of such a massive accretion is not at present sufficient.

After I had completed the work described here, I realized that Zel'dovich and Novikov[25] had considered a similar gravitational process—the accretion of interstellar material by a neutron star with the production of soft X-radiation. While the two gravitational processes are quite distinct, they are clearly of the same general type and form an interesting comparison.

The calculations made here have been based throughout on a model involving a number of simplifying assumptions, and may best be regarded as numerical illustrations of the working of our model. They do indicate, however, that in the process of catastrophic accretion of intergalactic hydrogen by the nucleus of a galaxy, highly localized sources of great energy may be produced; and they suggest that the nucleus, taken together with the innermost region (radius $\lesssim$ 10 pc) of the galactic envelope, could perhaps be identified observationally with the optical component of a quasi-stellar source.

The calculations also indicate that, with favourable conditions, such a source may occur at a phase during the lifetime of a galactic system having fairly typical properties. (Although I have taken a galaxy in a cluster as an example, I do not mean to suggest that quasi-stellar sources should necessarily be associated with clusters.)

It would be interesting to correlate stages of evolution of a galaxy with different stages of energy production on the accretion model. Both the total luminosity and the spectral distribution of the emitted radiation would be expected to change as the galactic material is continuously redistributed. I propose to investigate the consequences of the model when it is extended to cover such time variations of the system.

For the present I suggest that catastrophic accretion may be one possible mechanism whereby some of the gravitational energy of a galactic system is converted into electromagnetic energy, and that this conversion may operate through the intermediary processes of π meson production and decay.

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LETTERS TO THE EDITOR

PHYSICS

High Elastic Modulus Carbon Fibre

PRESENT-DAY interest in fibre reinforced composite materials has been centred on the difficulty of preparing suitable filamentary materials in large quantities. The attractive physical properties of whisker crystals have not, as yet, been exploited, because of the difficulties of bulk production of uniform whiskers.

Of all filamentary materials, their physical properties give graphite whiskers greatest potential for composite development. Small quantities have been prepared and they are reported[1] to possess the highest tensile strengths (3×10^6 lb./in.^{2}) and Young's moduli (about 130×10^6 lb./in.^{2}) yet recorded for any material. In addition to this, the specific gravity is low (2·27) and the high strength and high modulus can be expected to be maintained to high temperatures. But, again, these whiskers are not amenable to bulk production by present techniques.

Carbon fibres are available commercially in relatively large quantities, but their tensile strengths and Young's moduli are too low (about 10^5 lb./in.^{2} and $5–10 \times 10^6$ lb./in.^{2}, respectively) for them to be of any significant use as structural elements in composite materials.

We have prepared carbon fibres of high elastic modulus in continuous lengths and in relatively large quantities, by precisely controlled heating of commercially available textile fibres to 3,000° C in both reactive and inert atmospheres. Average values of Young's moduli greater than 60×10^6 lb./in.^{2} with peak values more than 100×10^6 lb./in.^{2} have been attained. Average tensile strength values of 250,000 lb./in.^{2} are typical. Fig. 1 shows typical modulus and strength distributions. The specific gravity of such fibres is usually in the range 1·8–2·0. The tensile strength and modulus values reported for graphite whiskers indicate that these carbon fibre values are potentially capable of great improvement, but the present values are sufficiently high to make these fibres outstanding.

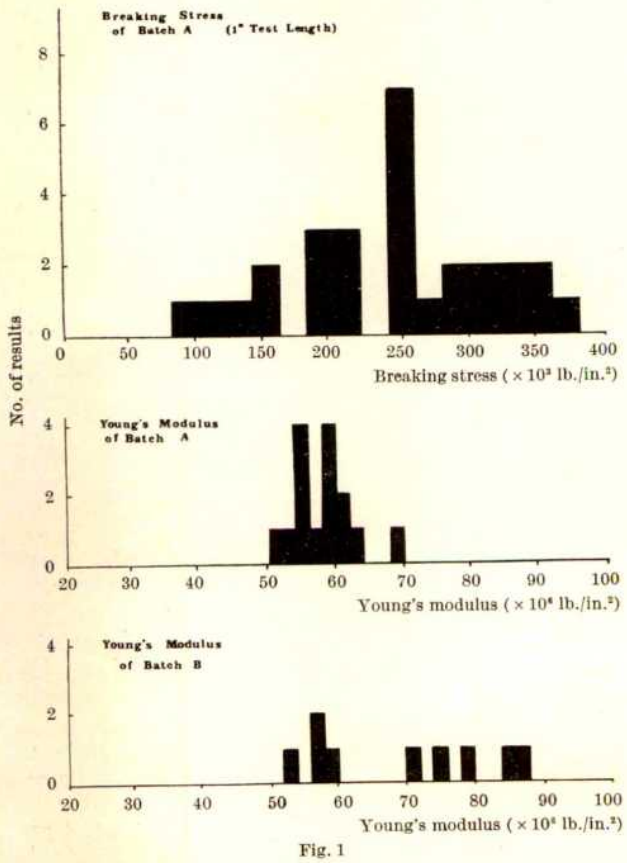


Fig. 1

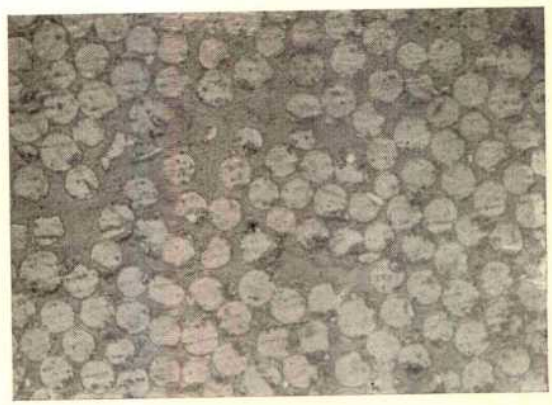


Fig. 2. Cross-section of carbon fibre reinforced polyimide resin (× 400)

Table 1. COMPARISON OF THE MECHANICAL PROPERTIES OF A NUMBER OF ALLOYS WITH THOSE OF A CARBON FIBRE EPOXY RESIN COMPOSITE

Material	Density (lb./in.^{3})	Tensile strength (lb./in.^{2})	Young's modulus (lb./in.^{2})	Specific strength (in.$^{-1}$)	Specific modulus (in.$^{-1}$)
Aluminium alloy—*A/FLS*	0·100	45,000	$10·5 \times 10^6$	450×10^3	105×10^6
Titanium alloy *T/AM*	0·164	65,000	17×10^6	400×10^3	105×10^6
Fibre glass epoxy *XP219*	0·073	155,000	7×10^6	$2,000 \times 10^3$	92×10^6
Carbon fibre epoxy	0·051	90,000	40×10^6	$1,500 \times 10^3$	660×10^6

Composites produced from this material include carbon fibre reinforced epoxy resin and carbon fibre reinforced polyimide resin. A comparison of the properties of the carbon fibre epoxy composite with those of other materials is shown in Table 1.

Fig. 2 shows a typical cross-section of carbon fibre reinforced polyimide resin.

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Effects of Long-chain Molecule Additives in Water on Vortex Streets

THE spectacular reductions of turbulent friction which can occur as a result of dissolving certain long-chain molecule substances in water[1] have still not been adequately explained. An explanation might be sought in terms of a continuum theory relating the stresses in the fluid to its motion. Various logically possible sets of such constitutive equations have been proposed, and both the second-order fluid of Coleman and Markovitz[2] and the anisotropic liquid of Ericksen[3] seem attractive as possible models of dilute polymer solutions. It is desirable, therefore, to try to devise simple experiments which will help to decide which model fits the real fluids best and determine the disposable constants in the theoretical model.

One of these constants in either model corresponds to the Newtonian viscosity as measured in conventional steady-flow viscometers. This must be equal to the viscosity of water for zero additive concentration, and in fact it varies comparatively little for concentrations of additive up to about 100 weight parts per million (w.p.p.m.). The other disposable constants would be expected to be linearly proportional to concentration for small concentrations.

As turbulence is a process dependent on time, it might be conjectured that the time-dependent terms in the theoretical models would be of particular significance with regard to turbulent drag reduction, and additions of different drag-reducing substances, in concentrations of roughly equal effectiveness for turbulent drag reduction, might all have been expected to produce similar effects on simple time-dependent flows. Further, these effects would seem likely to vary linearly with concentration. However, these reasonable expectations do not seem to be borne out, at first sight in any event, by the following experiments.

A suitable simple time dependent flow can be generated by a circular cylinder of diameter d placed transversely to a flow of speed v. As is well known, alternate eddies are regularly shed from the cylinder at a frequency n, forming a 'vortex street' wake, provided the Reynolds number vd/ν (where ν is kinematic viscosity) is in the appropriate range. In the present experiments the flow was a jet of circular cross-section and diameter 2·0 mm falling under gravity from a header tank, and the cylinder was a wire of diameter 0·15 mm placed transversely to the axis of the jet at a point where the gravity head was 15·7 cm, so that the speed was 1·76 m/s. Thus $R \simeq 240$ and the Strouhal number $S = nd/v$ ought to be about 0·17, according to Relf's results[4]. The eddy-shedding frequency was measured by a strain gauge element which sensed the small oscillations of a stylus 1·8 mm below the wire and in its wake. The results of measurements of the Strouhal number in water and various concentrations C (w.p.p.m.) of various drag-reducing additives are shown in Table 1.

Table 1. MEASUREMENTS OF THE STROUHAL NUMBER (S) IN WATER AND WITH VARIOUS CONCENTRATIONS (C IN W.P.P.M.) OF VARIOUS DRAG-REDUCING ADDITIVES

Water	S	0·15 – 0·17					
Polyethylene oxide (Union Carbide 'Polyox *WSR* 301')	C	5	10	15	20	30	40
	S	0·16	0·16	0·13	0·08–0·11	0·08–0·09	0·08–0·09
Polyacrylamide ('Dow Separan *NP* 30')	C	18	36	72			
	S	0·16	0·16	0·16			
Guar gum	C	80	120				
	S	0·15–0·16	0·16				

Small concentrations of all the additives made the eddy-shedding more regular than for water, where doubtless some breakdown of the vortex street into turbulence was beginning to occur. Presumably the additives inhibited this breakdown. However, as can be seen, larger concentrations of polyethylene oxide produced a large reduction in eddy-shedding frequency, quite unlike the behaviour of the other two additives, and far from linear in concentration. The large reductions in frequency were accompanied by proportionate increases in amplitude.

The threshold concentration of about 10 w.p.p.m. for polyethylene oxide, below which no reduction of the Strouhal number occurs, has no real counterpart in turbulent drag reduction. There—although there is a threshold shear stress which must be exceeded for drag reduction to occur[5]—the drag reductions are linear in concentration for small concentrations. It is conceivable that in the present eddy-shedding experiments there is some mechanism which tends to lock the Strouhal number to about 0·16 or some sub-multiple of this, as for concentrations greater than 20 w.p.p.m. the Strouhal number tends to about 0·08—half its initial value. Thus the present results do not entirely rule out a constitutive equation in which the coefficients are linearly proportional to concentration. Furthermore, the tendency for the lower frequencies to be favoured at the expense of higher ones is not inconsistent with the behaviour of turbulent flows, where the smaller eddies are suppressed[1]. However, polyacrylamide solution of 30 w.p.p.m. concentration or guar gum solution of 100 w.p.p.m. concentration would typically produce the same order of turbulent drag reduction as 15 w.p.p.m. polyethylene oxide solution, yet these fluids clearly do not behave similarly in the present eddy-shedding experiment.

All this suggests, perhaps, that various different mechanisms may be responsible for turbulent drag reduction. This conjecture is supported by normal-stress difference measurements in progress at this Division, which show that here again polyethylene oxide behaves differently from solutions of polyacrylamide or guar gum of equal turbulent drag reducing potency.

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Turbulent-flow Properties of Deoxyribonucleic Acid Solutions

MANY natural and synthetic high polymer solutions have lower friction in turbulent flow than the pure solvent[1]. In an effort to devise an experiment to explain the mechanism involved, flow studies were made using deoxyribonucleic acid (DNA) together with various carcinogens, steroids, acridine dyes, an antibiotic, and other materials which modify the effective molecular size, shape, and weight of the dissolved DNA. While the mechanism of friction reduction is still as elusive after these experiments as before, the interesting differences in turbulent flow properties of the various DNA-additive complexes may point to a simple way of estimating the effects of additives on the configuration of the DNA molecule.

An apparatus for measuring the turbulent-flow properties of fluid samples, based on the change in pressure loss due to pipe friction compared with that of distilled water, was used for the DNA tests[2,3]. This instrument gives an indication of the friction-reducing properties of high polymer solutions. The test consists of measuring the pressure drop in a section of small pipe at constant flow rate corresponding to a Reynolds number of 14,000, first with distilled water, then with the test solution. Then the friction reduction can be calculated using the relation:

$$\text{Percentage of friction reduction} = 100\,(1 - \Delta P_t/\Delta P_w)$$

where ΔP_t is the pressure drop using test solution, and ΔP_w is the pressure drop using distilled water.

All chemicals were commercially obtained, as well as the calf thymus DNA (Sigma Chemical Co.), and were used without further purification. The tests were made at 23° C, with a 0·15 M sodium chloride, 0·015 M sodium citrate, *p*H 7·0 solution as the solvent. This solution shows no difference from distilled water in flow characteristics in the test apparatus. Additives which dissolved in this solvent (no DNA present) did not affect the friction properties.

Fig. 1 shows the results obtained for DNA and for DNA mixed with steroids, a purine, and a mustard. In each case a weight of chemical equal to half the weight of DNA in solution was added and stirred at 23° C for at least 1 h before testing. While DNA itself seems to be a very effective material in terms of reducing the turbulent

friction, mixtures of DNA plus these chemicals showed little consistent change in effect. From these data it would seem that the binding[4,5] of 17β-oestradiol, testosterone, caffeine, and *tris*(2-chloroethyl) amine has little effect on the flexibility or other structural characteristics of DNA.

The dyes 9-amino acridine and proflavine showed large increases in friction reduction (Fig. 2) when mixed with DNA. These materials are believed to intercolate strongly in the DNA helix[6], extending the length of the molecule. Repeated frictional tests show that these materials also greatly strengthen the DNA to shear degradation.

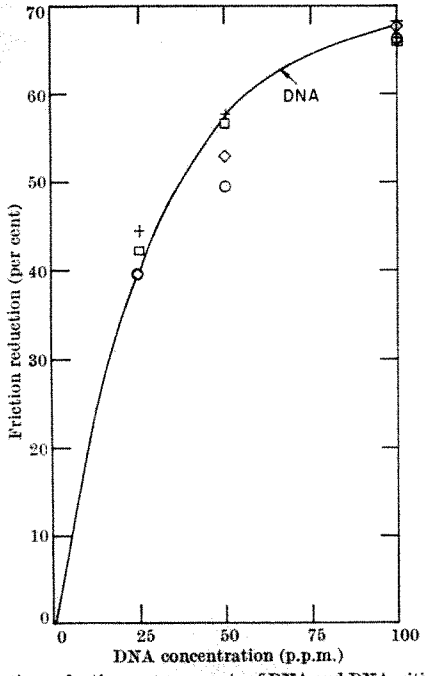


Fig. 1. Friction reduction measurements of DNA and DNA with steroids, a purine, and a mustard, as a function of DNA concentration. ○, 17β-Oestradiol; □, testosterone; +, caffeine; ◇, *tris*(2-chloroethyl)amine; —, DNA alone

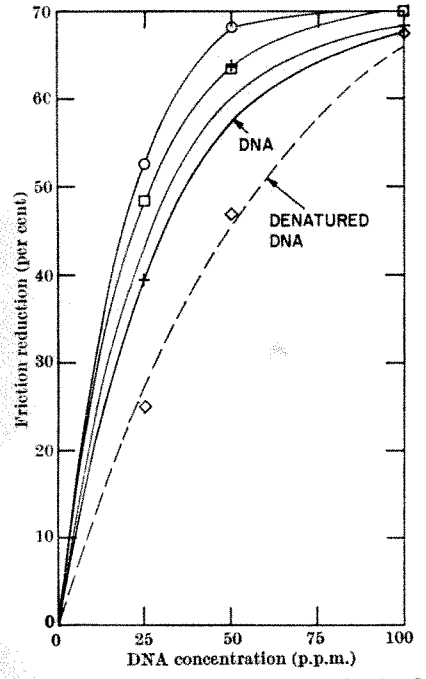


Fig. 2. Friction reduction measurements of DNA, denatured DNA and DNA with acridine dyes and an antibiotic as a function of DNA concentration. ○, 9-Amino acridine; □, proflavine; +, actinomycin-*D*; ◇, acridine orange; ——, DNA alone; — —, denatured DNA

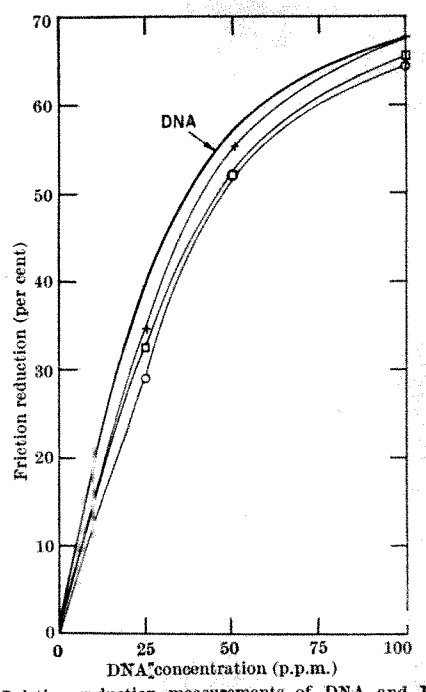


Fig. 3. Friction reduction measurements of DNA and DNA with carcinogens and a hydrocarbon as a function of DNA concentration. ○, 3,4-Benzpyrene; □, 1,2,5,6-dibenzanthracene; +, phenanthrene; —, DNA alone

Actinomycin-*D* also exhibits the same characteristics with DNA although the binding may not involve intercolation in this case[7]. In contrast, acridine orange seems to intercolate[8] to such an extent as to separate the DNA helix components so that the friction-reduction of this mixture was practically identical to that of a DNA preparation which had been denatured by bringing a water solution to 90° C and rapidly cooling.

Tests with the carcinogens 3,4-benzpyrene and 1,2,5,6-dibenzanthracene (Fig. 3) indicated substantial lowering of the friction reduction effect. I surmise this is due to a partial unwinding of the helix or at least a weakening which allows mechanical shear partially to separate the helix pairs. These materials seem to act by intercolation[9] and the test data show that phenanthrene which is a near carcinogen by several measures[10] may act in almost the same fashion.

In summary, the method of measuring the turbulent friction resistance of DNA-complexes may offer some insight into the changed structural characteristics which occur. Since the method requires such simple apparatus, it seems that refinement of the technique may be of value in further investigations of the physical effects of additives on the DNA molecule.

I thank Lt. M. F. Hayes, of the Naval Postgraduate School, Monterey, California, who performed these experiments during a summer assignment at this Station, and Dr. H. D. Brown, of Merck, Sharp and Dohme Research Laboratories, for a gift of 'Dactinomycin'.

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Growth of Ice Tubes

IT has been known for many years that when supercooled water suddenly freezes, whisker-like growths may occasionally form on the upper side of the layer of ice covering the water. It would seem that this was first reported by H. G. Dorsey in 1921 (ref. 1), but it has since been mentioned by several other observers[2–6].

Two theories have been put forward to account for the whisker-like growths. The earlier observers thought they were produced by reverse sublimation, that is, the capture of vapour molecules by the ice, and it was suggested that they might even be of single-crystal form. Bally[4], however, argued that the needles might, in fact, be tubes of ice with a core of water. Unfortunately, their small size (diameters a few tenths of a millimetre and lengths up to about a centimetre are typical), and the fact that neither the time nor the place of their sudden appearance can be predicted, have hitherto prevented detailed observation of their growth so that direct evidence of the growth mechanism has been absent.

A new method of cultivating these growths was discovered in the course of other work, which results in specimens up to an order of magnitude larger than those grown by the conventional method. These large growths have been filmed, and this has provided conclusive evidence that Bally's 'tube' theory of growth is correct.

The growths are formed in a vacuum from frozen deaerated water. About 50 ml. of distilled water contained in a beaker is vigorously agitated with a vibrating stirrer, inside a vacuum chamber where the air pressure is reduced to about 0·1 mm mercury absolute. Under these conditions the water boils vigorously at room temperature, and the air previously dissolved in the water is extracted. Then the stirrer is switched off, and the vacuum is momentarily released. This sudden increase in pressure forces any traces of undissolved air into solution in the deaerated water, thus removing any nuclei that might later have initiated boiling.

When the vacuum is restored the water does not boil but enters the metastable region, where the pressure is below the saturated vapour pressure, that is, it becomes superheated. When the stirrer is in action it promotes vigorous agitation in the water, which still does not boil but remains in a superheated state. This may seem surprising in view of the widely held belief that metastable conditions in water are disrupted by vigorous agitation. Numerous experiments in this laboratory have shown, however, that agitation has no effect on a moderately superheated liquid, or even on a liquid under moderate negative pressure—provided that gas nuclei have been completely removed and that the agitation is not such as to introduce fresh nuclei.

In this condition of agitated superheat, rapid evaporation occurs. This cools the water progressively, and after a few minutes its temperature falls well below freezing

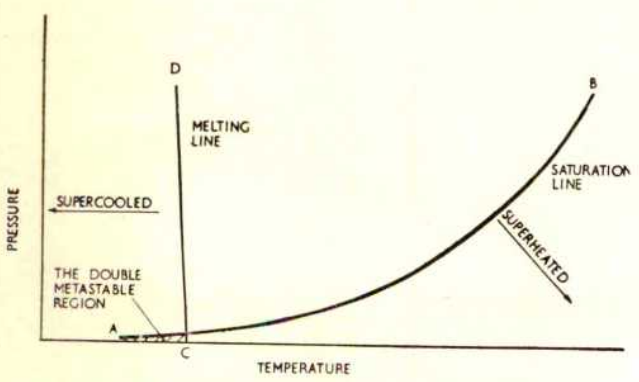


Fig. 1. Graphical representation of the double metastable state in liquid water (supercooling-cum-superheating)

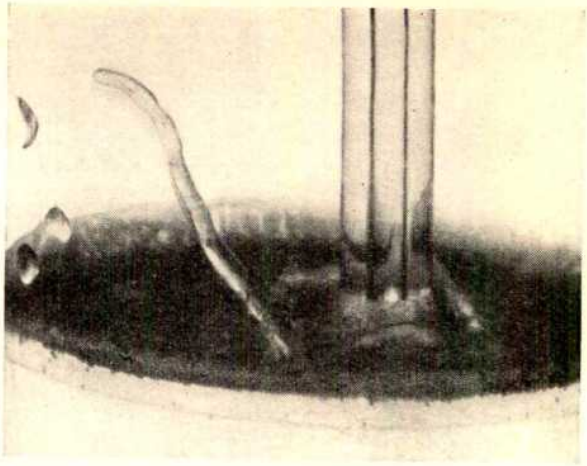


Fig. 2. Typical ice-tube formation. (The stirrer rod is 0·25 in. in diameter)

point. The water is then doubly metastable: it is both superheated and supercooled, simultaneously.

This condition is illustrated in Fig. 1. The region below the saturation line AB corresponds to the superheated state, while the region to the left of the melting curve CD corresponds to the supercooled state. Consequently, the shaded area, which is below AB and to the left of CD, corresponds to the double metastable state, where the liquid is both superheated and supercooled. If seeded with an ice crystal it will freeze, but if seeded with a gas bubble it will boil.

When the temperature of the water falls to about $-10°$ C, spontaneous freezing occurs. A solid disk of ice forms on the surface, and a network of ice crystals interspersed with water forms beneath. After a period which may be anything from a few seconds to a few minutes, an ice needle may grow rapidly from the upper surface of the ice.

The needles may take a wide variety of forms. A needle may be straight or gently curved as in Fig. 2. It may bend very sharply, and even bend through 180° until it ends by returning to the ice surface from which it emanated. Needles grown by this method are usually between 2 mm and 3 mm in diameter, and up to 5 cm long; their rate of growth is of the order of 1 cm per sec.

The growth of a number of these large specimens has been filmed at 50 frames per second, and when the film is projected at 24 frames per second it can be seen that the 'needles' are, in fact, hollow tubes of ice with a core of water, as was conjectured by Bally[4]. This is most clearly apparent at the moment of cessation of growth, when the core of water can be seen to freeze. Sometimes the wall of the tube can be seen to burst, under the pressure generated by the freezing of the water inside it.

While a tube is growing its tip is hemispherical in shape, and the surface of the hemisphere can be seen to pulsate. It is evident that the tip consists of an unfrozen drop of water. The outer edges of this drop of water rapidly freeze, thus progressively lengthening the walls of the tube, while the drop is continuously replenished by fresh water passing up the inside of the tube.

Pressure is obviously required to force the water up the tube. This pressure is evidently generated by the expansion of water as it freezes slowly in the enclosed space between the upper disk of ice and the walls of the beaker. That this is so may be seen by using a slightly contaminated beaker for the experiment; in this case the disk of ice does not adhere perfectly to the wall of the beaker, no pressure is generated, and an ice tube does not form.

A film of these experiments, complete with a sound commentary, has been prepared and may be borrowed

on application to the Director, National Engineering Laboratory, East Kilbride, Glasgow.

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Technique for the Measurement of the Transit Time of an Optical Signal

DURING the past several decades numerous arrangements have been developed for measuring the time of transit of a beam of light propagated over a tropospheric path. In these arrangements the transit time is determined by measuring the phase shift of modulation on the beam[1–6]. Most of this work has been carried out in connexion with measurements of (1) the velocity of electromagnetic waves, (2) geodetic distances, and (3) atmospheric turbulence.

This communication presents still another configuration for such phase measurements by combining elements found in several of the approaches already referred to. The combination described here appears to possess several characteristics which might, in certain applications, provide improved performance compared with those suggested in the literature to date.

The suggested scheme is similar to the techniques described by Bergstrand[1] and that used by the Central Radio Propagation Laboratory at microwave frequencies since 1955[7,8]. In fact, it may be considered to be the latter technique modified to propagate the microwave measuring signal as polarization modulation on an optical frequency carrier rather than by direct radiation and reception of the microwave signal. The principal differences between this technique and the one used by Bergstrand are the heterodyning process for reducing the measuring signal to lower frequencies and the use of direct linear phase comparison.

The basic system is shown in Fig. 1. At terminal 1 a light beam is polarization modulated by modulator 1 and transmitted along the path to terminal 2, where it is modulated again by modulator 2. The modulating signal at terminal 1 is obtained from a stable reference oscillator operating at frequency f_1 of, say, 10 GHz. The modulating signal at terminal 2 is obtained from a voltage-controlled oscillator the frequency (f_2) of which is chosen such that $f_2 - f_1 = f_t$, where f_t is, say, 50 kHz. The polarization modulation is then converted to intensity modulation by polarizer 2.

The intensity-modulated light emerging from polarizer 2 has a component at frequency f_t which is detected and converted to an electrical signal by photodetector 2. This intermediate frequency (IF), f_t, is compared in the phase detector with a 50 kHz signal generated at terminal 2 by the IF reference oscillator. The output from the phase detector is applied as an error signal to the voltage-controlled oscillator. This phase locks the voltage-controlled oscillator to the microwave modulation on the incoming light beam and ensures that $f_2 - f_1 = f_t$.

Simultaneously, a light beam is transmitted from terminal 2 to terminal 1. This beam is first modulated by modulator 2, transmitted along the path, then passed through modulator 1 and polarizer 1. When this beam emerges from polarizer 1 it also has a component of intensity fluctuation at frequency f_t, which is converted by photodetector 1 to an IF signal of frequency f_t. This IF signal is returned to terminal 2 for phase comparison with the IF signal generated by photodetector 2.

Except for a constant term, the phase difference between the IF signals generated at terminals 1 and 2 is equal to the phase difference of the modulation signals travelling along the path, and thus provides a measure of the transit time. Since the same analysis applies here as applies to the Bergstrand and Central Radio Propagation Laboratory systems referred to above, this aspect will not be repeated.

The polarization modulation of the light beams is accomplished by the common technique in which the original beam from the source is passed through a polarizer and an element the birefringent properties of which depend on an electrical potential (Kerr cell, Pockel cell, etc). Aside from focusing and intensity considerations, the coherence properties of the beam itself have no particular significance for this application.

By the use of a single transit of the beam over the path, more light power and less scintillation are achieved than with systems in which the beam is reflected back over the path to the point of origin[9]. For an order of magnitude calculation, assume a collecting aperture diameter of 20 cm, divergence of 0·1 milliradian for the beam (representative of a laser beam: the relative improvement realized in the case of a wider beam is correspondingly greater), and the atmospheric attenuation of 0·5 dB/km. There results an increase of light intensity given by:

$$(P_1/P_2) = (0{\cdot}5L + 20 \log 0{\cdot}5L) \text{ dB}$$

where L is the path length in kilometres, P_1 is the light intensity available at the photodetector for a one-way

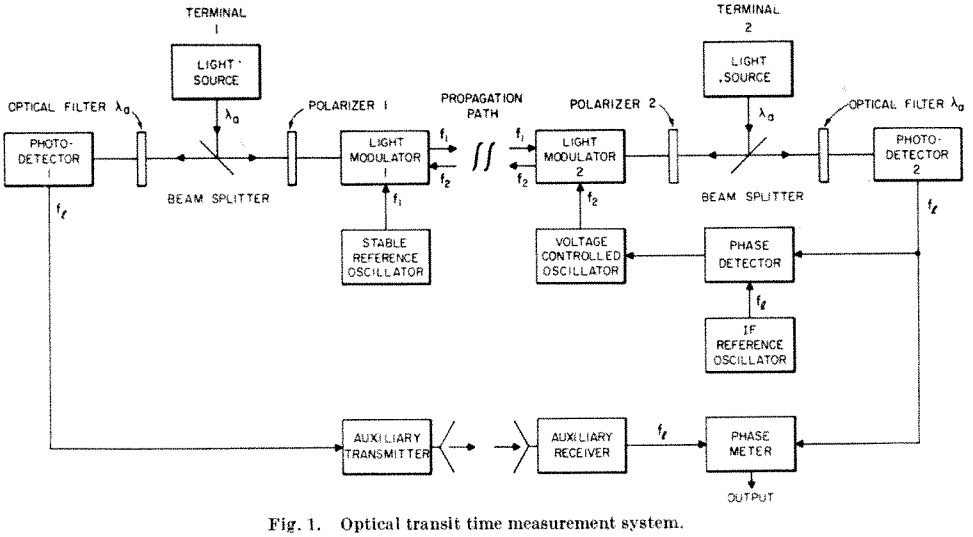


Fig. 1. Optical transit time measurement system.

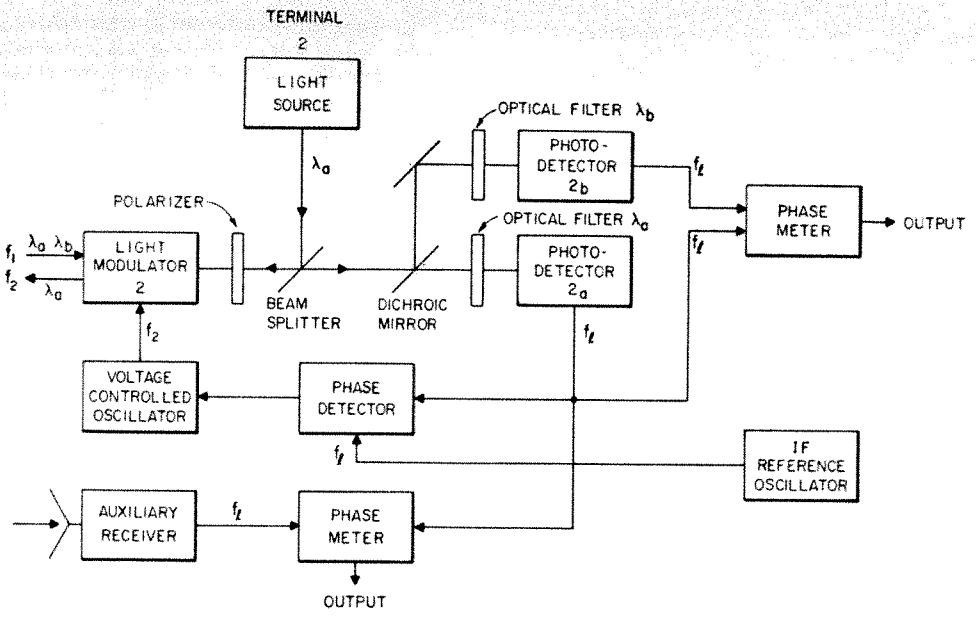


Fig. 2. Modification to permit determination of atmospheric dispersion

transmission, and P_2 is the light intensity available at the photodetector after reflexion back over the path. The first term on the right-hand side arises from the attenuation of the light beam in the atmosphere. The second term expresses the fraction of the beam area intercepted by the collector at the terminal where it is received and assumes that the latter lies completely within the former. For a 10-km path (P_1/P_2) is about 19 dB, for 20 km about 30 dB, and so on. This does not take into account reflexion losses.

This gain in received light intensity is purchased at the cost of providing the auxiliary link, but the essential problems involved in obtaining a suitable link have been solved already[7]. Other advantages include the use of linear phase measuring with its attendant simplicity, accuracy, and high-frequency response and the absence of variable light paths, obviating problems of depolarization by additional optical elements.

The upper value to the modulation frequency may be limited by the state of available modulators to frequencies no higher than a few GHz for continuous operation. Pulse operation may be required for higher frequencies.

The use of a second telescope and modulator might appear to be a disadvantage compared with some systems[4,10,11]. However, these systems have a back-scatter problem which may be quite serious. In this arrangement, because of the increased received light intensity compared with a system using a reflector at one terminal, back-scatter should be accordingly less important. Furthermore, back-scatter can be further reduced in this system by the use of nearly monochromatic light sources at slightly different wave-lengths and the appropriate narrow-band optical filters before each photodetector.

In the measurement of geodetic distance through the troposphere one of the more serious difficulties is the determination of the average refractive index along the path. It has been suggested[9,12] that the atmospheric dispersion in or near the visible portion of the spectrum is large enough to be useful in determining this average refractive index.

The extension of this system to include the measurement of atmospheric dispersion is relatively straightforward as shown in Fig. 2. The source at terminal 1 supplies light at the two wave-lengths desired. This may be achieved by using a source which generates spectral lines and optical filters to select those lines desired. It might also be accomplished by combining, optically, light from separate sources. At terminal 2, the incoming beam containing both light wave-lengths is passed through modulator 2, where the modulation on each is heterodyned to the IF frequency. The voltage-controlled oscillator is phase-locked as before to one of the incoming signals. By means of the narrow-band optical filters each of the photodetectors responds to the modulation on the corresponding component of the incoming beam. The phase shift between these photodetector output signals will be proportional to the dispersion of the propagation path. As discussed in the references, this information can then be used to correct the path length indicated by the first part of the system.

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ASTROPHYSICS

Radio Astronomical Detection of Helium

WE report the detection of three radio lines arising from transitions between highly excited states of neutral helium (^{4}He). The observations were made with the 60-ft. telescope of Harvard University during the months February–April 1966. The observed helium lines result from transitions of the $n + 1 \rightarrow n$ type between states 160 and 159, 159 and 158, and 157 and 156. Conforming to the notation suggested in our recent work[1] on excited hydrogen, we shall call these transitions 'He 156α',

'He 158α' and 'He 159α', respectively. Their respective rest frequencies are 1716·372 Mc/s, 1652·214 Mc/s and 1621·332 Mc/s. (Adopting a more recent value of the Rydberg constant R_∞ (ref. 2), the rest frequencies of the lines reported in our previous reports (refs. 1, 3) are slightly modified. The frequencies for H 156α, H 158α and H 166α are 1715·673 Mc/s, 1651·541 Mc/s and 1424·734 Mc/s, respectively.)

The Harvard 60-ft. telescope is equipped with a cavity maser ten-channel radiometer having a frequency resolution of 80 kc/s. The antenna beam-width is 52 ± 2 min of arc and the aperture efficiency is 0·38 ± 0·02 over the range of frequencies utilized. The total noise temperature of the system is about 100°K.

Fig. 1 shows the H 159α and He 159α profile obtained in 14 h of integration time. Also included is a profile of the He 158α line with 21 h of integration time. The zero level of the He 158α data is arbitrarily elevated for visual comparison. Fig. 2 is the profile of a comparison source, Taurus *A*, obtained over the same frequency range as the 159α profile shown. The theoretical separation of the H 159α and He 159α lines is 0·660 Mc/s and this value has been indicated on Fig. 1 by the vertical lines through the hydrogen and helium features.

We have considered the possibility that the helium signals are produced by another atomic or molecular species. Because we have observed the helium line at three different frequencies we can exclude the possibility that these are anything other than $n\alpha$ type lines. The total abundance of all the elements other than hydrogen or helium has been estimated by a number of methods to be of the order of 1 per cent of the total mass of the galaxy and, if so, they will not produce $n\alpha$ type signals of sufficient intensity to be measured by our telescope. We have calculated the frequencies of the H $n\beta$ and H $n\gamma$ transitions which lie near the frequencies of the three helium lines and find that they do not coincide with our observed signals. Furthermore, the normal theory of differential galactic rotation would exclude the possibility of an HII region having a radial velocity of ~ -100 km/s in the direction of *M*17. While we cannot rule out the possibility that an HII region with an extremely large peculiar velocity lies along the line of sight to *M*17, the Doppler shift must be exactly the separation of the He $n\alpha$ and H $n\alpha$ lines in this frequency range, a coincidence we regard as unlikely.

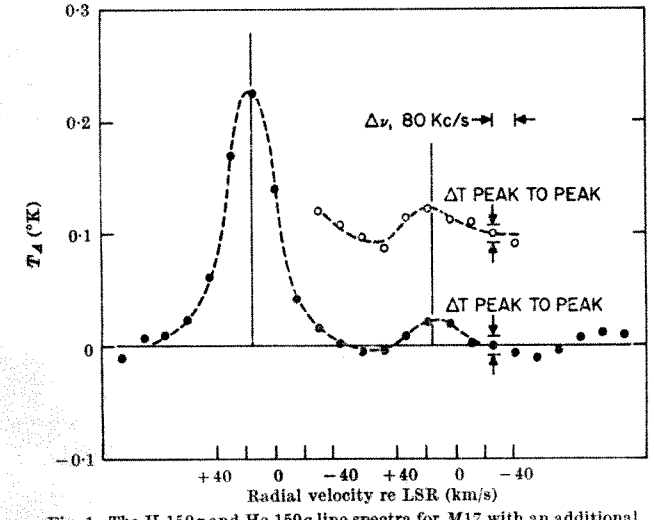


Fig. 1. The H 159α and He 159α line spectra for *M*17 with an additional set of ten-channel data covering the He 158α line. The ordinate is antenna temperature and the abscissa is velocity with respect to the local standard of rest. The continuum antenna temperature of *M*17 is 20° K. ●, 159α, 14-h integration; ○, 158α, 21-h integration

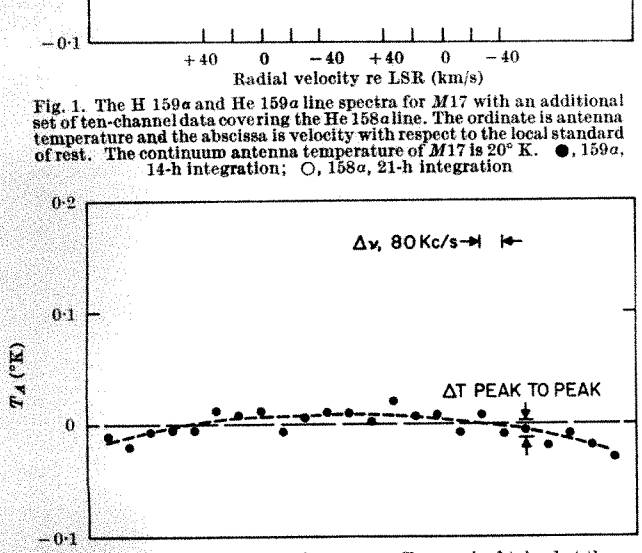


Fig. 2. The spectrum of a comparison source, Taurus *A*, obtained at the same frequency range used in the 159α measurements on *M*17. The continuum antenna temperature of Taurus *A* is 29° K. 18-h integration

For a system capable of achieving larger signal-to-noise ratios for the helium lines (preferably at much higher frequencies where the line strengths are greater), simultaneous measurements of hydrogen and helium should yield several important quantities. First, a comparison of helium and hydrogen line widths should make it possible to separate the thermal and turbulent velocities of the nebulae. Secondly, the data in most cases provide a measure of the helium to hydrogen abundance ratio in HII regions, a quantity of cosmological interest.

If the hydrogen and helium share the same thermal and turbulent motions, and if these are the only broadening mechanisms of importance for $\nu > 1{,}420$ Mc/s (as we believe to be the case[1,3]), then with sufficiently large signal-to-noise ratios, we have a possible method for determining the electron temperature and turbulence in the nebula. However, the accuracy of the method depends on the relative magnitude of the turbulent and thermal contributions to the observed line widths. This method would be important because of the apparent disagreement between optical temperature determinations (see, for example, O'Dell[4]) and recent radio determinations of nebular electron temperatures[1,3,5] following the method suggested by Kardashev[6]. Recent work by Goldberg[7] has shown that determinations of electron temperatures by the method suggested by Kardashev may be inaccurate in some cases. Thus, the method utilizing the hydrogen and helium line widths may be the only way to determine electron temperatures from the $n\alpha$ line results.

We have planimetered our *M*17 profiles and obtained a mean ratio of the area under the helium line to that under the hydrogen line equal to 0·10 ± 0·05. Two principal effects must be evaluated before this quantity can be related to the abundance ratio of helium to hydrogen. Because the degree of ionization of hydrogen and of helium is controlled by the radiation field of the exciting stars in the nebula and not by the electron temperature, one should examine the radio lines of both neutral and ionized helium to determine the total helium abundance. If the exciting stars are relatively cool[8], the fact that the Stromgren spheres for helium and for hydrogen are different must also be taken into consideration. With these factors accounted for, it will be possible to obtain the helium : hydrogen ratio from the radio profiles.

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Radio Emission of Venus at 3 cm and the Solar Activity

BECAUSE of the favourable observing conditions, the planet Venus has frequently been observed in the radio spectrum for some years now. Recently, precise observations at the wave-length 3·3 cm were made by Bibikova *et al.*[1]. These observations were made after the inferior conjunction in Summer 1961. The authors found that the temperature of the planet was distinctly dependent on its phase. The mean temperature observed was about 544° K for the mean phase 0·47, and the value derived from the phase dependence for the inferior conjunction was found to be about 372° K. This large day–night contrast in temperature gives some support to Sagan's[2] greenhouse model over Opik's[3] aerospheric model of the Venusian atmosphere.

Bibikova *et al.* have also compared their results with those obtained by Mayer *et al.*[4] for the 1958 conjunction. For the temperature difference of about 100° K between the two inferior conjunctions, they admitted (in one of their two suggested hypotheses) the possibility that the difference found may be caused by solar activity, because the observations were made at different phases of the solar cycle.

As can be seen from the data of Bibikova *et al.*, the differences between the individual temperature values are in some cases greater than their mean error. Thus the variability of the observed temperature must be real and an adequate explanation of this phenomenon must be sought.

We have therefore investigated the possible dependence of the Venusian temperature as given by Bibikova *et al.* on the instantaneous solar activity, represented by the daily Wolf's numbers R. When making correlations the longitude differences of the Earth and Venus must be respected. Because of the rotation of the Sun, this difference gives a shift in phase in the scales of the time of observation of the solar activity on the Earth and the influence of the activity on Venus. We therefore corrected all the dates of observations of Venus (t_φ) for this geometric effect and obtained fictitious dates (t'_φ) suitable for correlating the Venusian temperature T_φ with R. To obtain a good correlation between the curves of T_φ and R the T_φ curve must be shifted to the left by about 8–9 days. The result using a supplementary shift, Δt, of 8·5 days is given in Fig. 1. In this case the ratio $\Delta T/\Delta R$ is about unity, and for $R = 0$ the dependence of T_φ on the phase of the planet disappears. The resulting value for the temperature, reduced to the conditions without solar activity, is $T_\varphi = 475°$ K.

In order to confirm this finding we have made a short analysis of the observations made by Mayer *et al.*[5] at λ3·15 cm during the 1956 inferior conjunction. The dispersion of the temperature values was greater in this case, and the correlation was not as detailed, but the results correspond in general to those for the 1961 conjunction. Only the reduced temperature for zero activity was lower and was estimated to be about 440° K. A more complete analysis of the dependence of Venusian temperature on solar activity, using data published by different authors for other frequencies, will be published elsewhere[6].

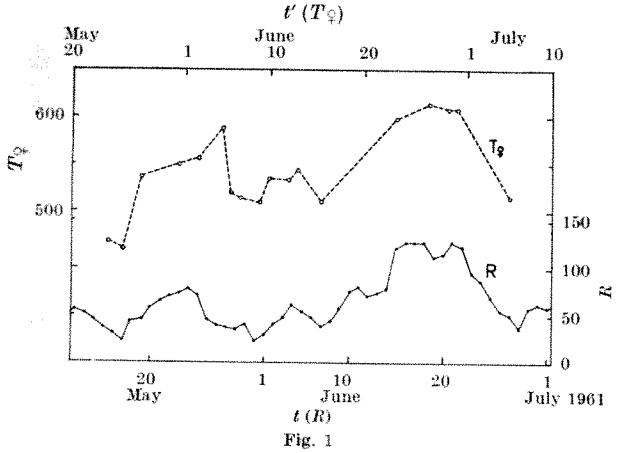


Fig. 1

In both the cases mentioned the trend of solar activity was decreasing in the direction of the conjunction (in 1956 observations were made before and in 1961 after the inferior conjunction). This led Bibikova *et al.* to suspect that the temperature was dependent on the phase of the planet. The actual independence of temperature from the phase suggests a high speed of rotation. However, the rotation of a planet with an atmosphere must induce a general circulation, with transport of energy through the atmosphere. It is very difficult to compare present models of the Venusian atmosphere with existing radio measurements, as has been shown by Danilov[7] and Jastrow and Rasool[8]. The dependence of the observed temperature on the solar activity indicates that the influence of far-ultra-violet and X-radiation, produced by the hot active regions in the solar corona, must now be taken into account in the construction of the models of the Venusian atmosphere. The most difficult aspect of this problem is the great retardation of about 8 days in the transformation of the radiation energy from the active regions into thermal energy in the form of radio emission from the planet.

More systematic, detailed and accurate observations at different wave-lengths during the rising part of the commencing solar cycle are now needed in order to verify the present results and to obtain more information on this phenomenon at other wave-lengths.

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Observations of the Low-energy Photon Fluxes during the Solar Approach of the Ikeya-Seki Comet

A BALLOON-BORNE omnidirectional scintillation detector was aloft over Palestine, Texas (L value about 1·6), during the solar approach of the Ikeya-Seki comet on October 20, 1965. One of the aims of this experiment was to observe any possible enhancements to the low-energy atmospheric photon flux induced by the passage of the comet. The perihelion of the comet occurred on the 'dark' side of the Sun with an orbital inclination of about 40° to the ecliptic and therefore had an apparent perihelion of about 1° around the Sun as observed from the surface of the Earth.

The detector consisted of a cylindrical sodium-iodide (thallium doped) scintillator of diameter 5 in. and depth ½ in., yielding a geometric factor for an isotropic flux of 76·2 cm². The axis of the detector was vertical, and a 5 in. photomultiplier tube observed the scintillations from below. Pulse height analysis techniques (which were compatible with FM/FM telemetry systems on both the balloon package and the ground station) enabled a study of the atmospheric photon component in the energy intervals 100 keV–325 keV, 100 keV–660 keV, energies

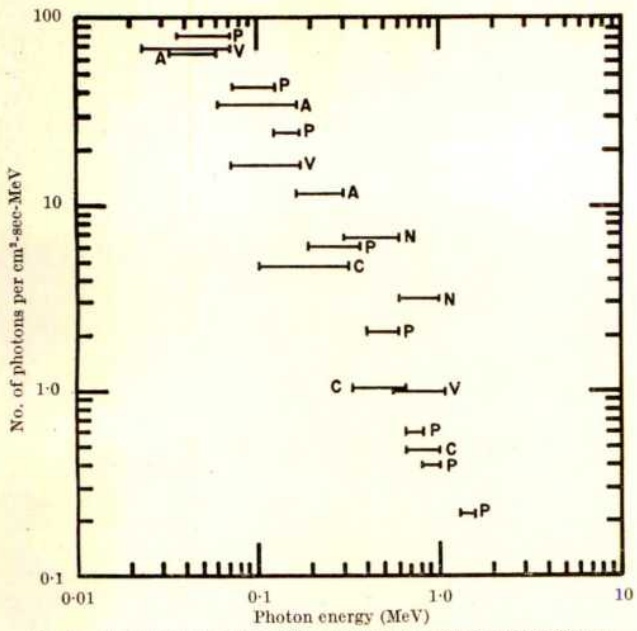


Fig. 1. A comparison of the differential photon fluxes obtained in the present investigation with those obtained by previous workers. The altitudes at which the various spectra were obtained are as follows: *P* (Peterson), 6 gcm^{-2}; *V* (Vette), 5·4 gcm^{-2}; *N* (Northrop *et al.*), rocket; *A* (Anderson), 6·5 gcm^{-2}; *C* (present results), 8·8 gcm^{-2}

above 660 keV, and 325 keV–660 kev (the details of this latter channel, which includes the positron annihilation photons, will be the subject of a later publication). At the float altitude of 8·8 gcm^{-2} the differential photon energy spectrum was determined to have the exponent $\left(-1{\cdot}80\ ^{+0{\cdot}2}_{-0{\cdot}1}\right)$.

Fig. 1 compares the results of some of our differential fluxes with the results of previous workers such as Peterson[1], Vette[2], Northrop and Hostetler[3], and Anderson[4]. It is readily seen from Fig. 1 that our results agree very favourably with the previously measured fluxes, albeit the latter were determined at slightly higher altitudes. In fact, this excellent agreement (any departures certainly lying completely within the differences in detector responses) is reason for concluding that the solar approach of the Ikeya-Seki comet produced no significant effects on the atmospheric photon flux.

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GEOLOGY

Devonian Fishes from Kashmir, India

THIS communication reports the finding of fragmentary remains of Devonian fishes from India. They were collected by one of us (V. J. G.) *in situ* near Margan Pass (33° 44′ N., 75° 32′ E.) in the Anantnag District of Kashmir, India. They were discovered in dark, calcareous shales that overlie the fossiliferous Naubug Beds and underlie the Muth Quartzite, which is exposed on the talus-covered slopes south-west of Mangan. The former are of Upper Silurian age, while the Muth Quartzite near Naubug has been assigned to the Middle Devonian.

The first specimen (Fig. 1) consists of crowded and stacked skull plates and scales. The plates are thick and their surfaces perforated by numerous pores—as in the cosmine of crossopterygians and lungfishes. They have a middle layer with numerous small canals or cavities, and a thick, clearly laminated basal layer. A few of the skull plates are only slightly displaced from their original articulation, and their small size in relation to the scales suggests that we are dealing with a primitive lungfish. These plates are comparable with, though not definitely identifiable with, plates of the *Dipterus* cranial roof; possibly they are X, Y_1, Y_2 and I_2, J of White's terminology[1]. They have relatively large lateral line pores, and the possible I_2 has a widely open pit-line groove. Other skull plates have not been determined even provisionally. The scales are broken but would seem to have been relatively large, thin and cycloidal. On part of their external surface they show fine radiating ridges which resemble those of the overlapped areas of *Dipterus* scales. One small area has more widely spaced, subparallel, fine ridges on a meshwork base; this resembles the exposed area of scales of a specimen of *Dipterus valenciennesi* (Field Museum, *PF* 1294) in which the cosmine layer is absent. The Kashmir specimen is without doubt a lungfish, probably of the family Dipteridae, but the genus and species must remain undetermined.

The other specimen (Fig. 2) shows scattered scales, a few of which are still articulated. The scales are smooth, more or less rhombic, and have an outer surface that is shiny and pierced by numerous fine pores as in cosmine. The anterior, overlapped area is relatively broad and lacks cosmine. These scales probably belong to a crossopterygian of the family Osteolepididae, but cannot be identified more specifically. The opposite surface of the same specimen shows a small part of a tail in which are

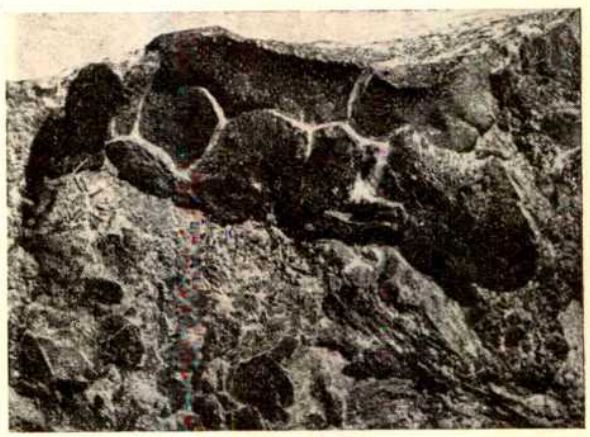


Fig. 1. Devonian lungfish from Kashmir, plates of cranial roof and broken scales (× *c.* 4)

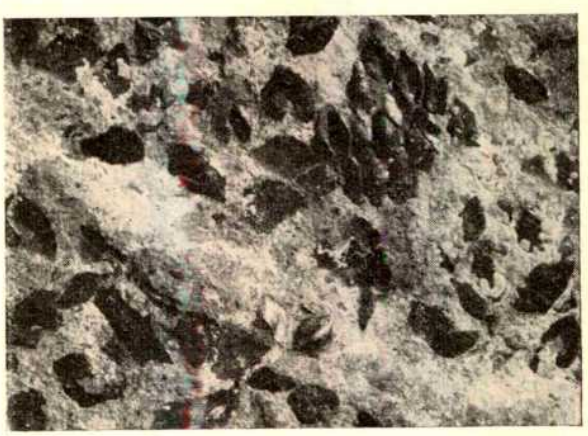


Fig. 2. Scales of a probable osteolepid (× *c.* 4)

preserved a few scales and fin rays. It is possible that it belongs to the same type of osteolepid.

These fishes are similar to the Middle Old Red Sandstone fishes of Scotland and suggest a Middle Devonian age for the shales which contain them. It is interesting that these shales have a very close lithologic resemblance to some of the beds of the Middle Old Red Sandstone, particularly of the Thurso Flagstone Group of Caithness, Scotland. The finding in India of osteolepids and dipterids, both typical families of fresh-water fishes, is of considerable palaeogeographical importance.

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Standard Cenozoic Stratigraphic Zonal Scheme

IN 1957 Bolli[1–3] published a detailed account of twenty-nine planktonic foraminiferal zones for the Trinidad Cenozoic. These zones have now been recognized and have been widely used in the stratigraphy of many other tropical and also to a lesser extent in the temperate regions[4–7]. I would like to comment briefly on a drastic revision and modification of Bolli's zonal scheme recently published in this journal[8].

One of the best documented evolutionary lineages in the Miocene planktonic Foraminifera was first described by Bolli[9] and is known as the *G. fohsi* lineage. Later, Banner and Blow[10] called it "the well known evolutionary lineage" and they provided considerable supporting evidence for the lineage in the form of excellent photomicrographs which demonstrated the development of the peripheral keel in the succeeding taxa. Banner and Blow[8] have now apparently revised the *G. fohsi* lineage to such an extent that none of Bolli's four original zones, based on the *G. fohsi* lineage taxa, appear in their revised zonal scheme.

In another communication[11] I have demonstrated to Banner, Blow and their colleagues that in the New Zealand Lower Miocene, *Globoquadrina dehiscens* appears stratigraphically lower than *Globorotalia kugleri*. A similar succession of species has also been reported by Wade[12] in South Australia. How then is it possible for "*G. dehiscens praedehiscens*" to evolve into "*G. dehiscens dehiscens*" after the extinction of *G. kugleri* in Zone N.5 as reported by Banner and Blow[8]? The co-authors[8] claimed to have examined Australian and New Zealand fossils, but they have obviously chosen to ignore well-documented published evidence of the stratigraphic succession of planktonic foraminiferal species from the Australasian region[13–15].

The use of *nomina nuda* in the zonal scheme proposed by Banner and Blow[8]? is bad taxonomic practice and the specific names do not appear to satisfy Article 13 of the International Code of Zoological Nomenclature.

One further point of criticism concerns the persistent placing by Banner and Blow of the *G. ampliapertura* and *G. opima* zones (or their now revised equivalent) in their so-called Miocene[8]. This is an integral part of the hypothesis of Eames, Banner, Blow and Clarke[16] that the "Oligocene" is missing in many parts of the world. The published evidence to the contrary and the opinion of most other workers is that the *G. ampliapertura* zone and part, if not the whole, of the succeeding *G. opima* zone form part of the Oligocene[17–20].

One of the best methods of appraisal of a new stratigraphic zonal subdivision is its usage and acceptance by other workers of the same discipline. Bolli's Trinidad zones have some drawbacks, as I have already noted elsewhere[21], but the zones have been workable units in the tropical areas. Berggren[22] made the following comment on Bolli's[1] Paleocene–lower Eocene zones: "This zonation has since (1957) found widespread utility and has served as a standard reference in the recognition of zones based on other pelagic groups, such as the nannoplankton". There is therefore considerable doubt in my mind as to the value and need for such a drastic change of Bolli's zonal scheme as that recently presented by Banner and Blow[8].

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METALLURGY

Fatigue—Generation of Vacancies

RECENTLY, it was reported that fatiguing (cyclic straining) at room temperature results in observable diffusion of silver into copper at that temperature[1]. This important effect was attributed to the athermal generation and the subsequent diffusion of vacancies which are greatly in excess of equilibrium.

We report here some preliminary results which are in agreement with the conclusions of Derrick Jones and Dover[1]: that large numbers of vacancies are generated by fatigue, and these vacancies can contribute to diffusive processes.

In the present instance, a precipitation reaction was used to study the influence of large numbers of vacancies. It is known that Guinier–Preston zones (clusters rich in solute) form at ambient temperatures at high rates owing to the supersaturation of vacancies which become frozen-in during the quench from the solution temperature[2]. The zones may be redissolved by heating above the ageing temperature for a short time—yet still within the two-phase region. This treatment is termed "reversion", and on recooling to the original ageing temperature, the zones form at a much lower rate owing to the smaller supersaturation of vacancies which are retained from the lower reversion temperature. In the experiments reported here, specimens in the reverted condition were cyclically strained and then aged.

Aluminium–4 wt. per cent copper was used. The treatment involved first quenching from 540° C to water, followed by ageing for 1 day at 30° C, then reverting at 200° C for 10 min, followed by acetone quenching. After the reversion treatment the specimens were mounted in a specially designed testing device and cycled at the temperature of liquid nitrogen[3]. The specimens were then

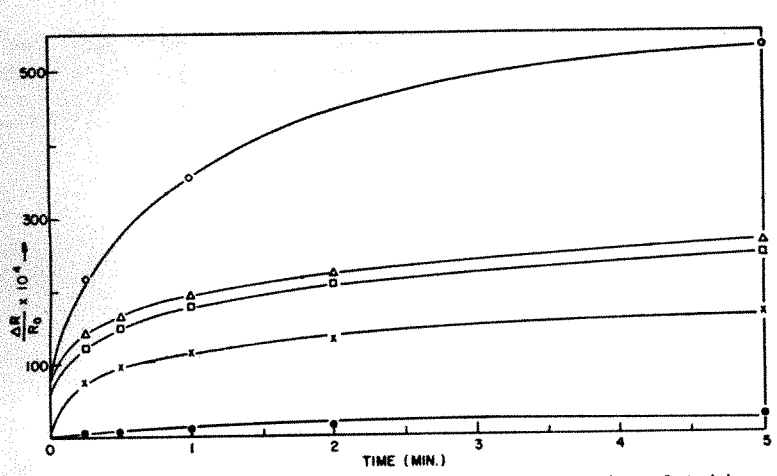


Fig. 1. Ageing curves at 30° C after quenching, reversion, and reversion and straining for various numbers of cycles (see text). ○, As quenched; ×, 10^2 cycles; □, 10^3 cycles; △, 10^4 cycles; ●, as reverted

re-aged at 30° C, and the electrical resistance determined in liquid nitrogen by means of an interruption technique.

The results for strain cycles of 0, 100, 1,000 and 10,000 are shown in Fig. 1. The maximum strain amplitude was ±0·0020 in./in. Data for the isothermal ageing of cyclically strained specimens are clearly representative of accelerated rates of zone formation—the rate increases with the number of cycles. It should be noted that the resistivity always initially increases during the formation of Guinier–Preston zones in this system[2].

We conclude that vacancies must be generated by cyclic straining of the reverted alloy and that these defects are able to contribute to diffusive processes, thus significantly aiding the transport of solute atoms to zones. An estimate of the excess vacancies (C_v) generated by cyclic straining can be obtained by applying the empirical relation of Seitz[4]: $C_v = 10^{-4}\varepsilon$, where ε is the total plastic strain. For 1,000 cycles, the atom fraction of vacancies thus obtained is of the order of 10^{-5}. This number is close to what would be expected for quenching from 500° C; however, it is certain that, before it is strained, the reverted alloy contains less than 10^{-8} atom fraction of vacancies[3].

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Room Temperature Stress Corrosion Cracking of Titanium Alloys

IT has been known for more than 10 years that titanium alloys suffer from intergranular stress corrosion cracking if they are in contact with chlorides at elevated temperatures (> 250° C), but it was only 18 months ago that Brown[1] discovered that stress corrosion failures could occur at room temperature in a 3 per cent solution of sodium chloride. Titanium alloys are highly resistant to pitting attack in chloride solutions, and in order to investigate crack propagation rather than crack initiation, Brown used specimens of titanium alloy in the form of notched rectangular bars which had been fatigued in air until fine cracks had formed at the base of the notch. He found with static load tests that under plain strain conditions a 3 per cent solution of sodium chloride caused a decrease in the resistance of the alloy to the further propagation of the crack. This was a very important result, as it indicated that the stress corrosion resistance of titanium alloys under marine conditions depended on the resistance of the surface oxide on the alloy to the solution and not on the intrinsic resistance of the alloy lattice to the propagation of a stress corrosion crack which is the only really safe criterion.

The situation is far more serious than suggested by the experiments of Brown, as we have recently found that stress corrosion cracking of a single phase α-titanium alloy (Ti-5Al-2·5 Sn) in 3 per cent solutions of sodium chloride does not require fine surface notches or fatigue cracks, and it can occur readily on flat chemically polished sheet specimens at room temperature.

By examining thin foils of the alloy by transmission electron microscopy, we had previously shown[2] that hydrides were formed in the alloy surface when the alloy was plastically deformed while immersed in a 3 per cent solution of sodium chloride. This occurred only in susceptible alloys and only in the specific cracking environment, and on the basis of these observations and other data we believe that hydride formation is part of the cracking mechanism. We had also found that at some stage during the chemical polishing of susceptible titanium alloys in a mixture of 40 per cent hydrogen fluoride and 72 per cent nitric acid in the volumetric ratio 2 : 3 the alloy surface became contaminated with hydrides. We therefore tested chemically polished Ti-5Al-2·5 Sn alloy by bending sheet specimens into a curved shape while immersed in a 3 per cent solution of sodium chloride acidified with hydrochloric acid to *p*H 0·75 and then clamping them. Within 6 weeks the specimens developed transgranular cracks on the outer surface of the bend. Specimens that were not chemically polished still show no signs of cracking even after exposure for 3 months.

We shall report on the mechanism of cracking and the crystallographic features of hydride precipitation on a later occasion. The purpose of this communication is to report that pre-existing notches are not required for stress corrosion cracking to occur in titanium alloys, and also to direct attention to the importance of surface preparation in determining susceptibility. The implications of these two points are important both in the determination of the cracking mechanism and in the future use of titanium alloys in marine engineering.

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CHEMISTRY

Pseudorutile—a New Mineral Intermediate between Ilmenite and Rutile in the N Alteration of Ilmenite

ALTERATION of the mineral ilmenite, $FeO{\cdot}TiO_2$ in nature involves the processes of oxidation and leaching whereby iron is progressively removed to give a residual product, essentially TiO_2. Several investigations have been reported of the alteration products of ilmenite, but apart from composites of known oxides of iron and titan-

ium, no distinct intermediate phase has been discovered. As a result of an investigation of several altered ilmenite concentrates by X-ray techniques hitherto not applied to this problem, a new crystalline phase has been identified as a major constituent of altered ilmenite. The new phase crystallizes in a disordered structure of hexagonal symmetry and has the theoretical composition $Fe_2O_3 \cdot 3TiO_2$. We propose the name 'pseudorutile' for this new mineral.

The samples used were all commercially available titanium mineral concentrates classed as 'Ilmenite', but are in fact largely composed of alteration products of the mineral ilmenite. (These materials are described at length separately[1].) Chemical and structural analyses of bulk samples used are given in Table 1. Water and oxides other than those of iron and titanium are omitted. The structural composition was derived from powder diffraction data subsequent to the identification of pseudorutile.

Table 1. CHEMICAL AND STRUCTURAL COMPOSITION OF ALTERED ILMENITE SAMPLES FROM VARIOUS LOCALITIES

Sample	Chemical composition in weight per cent			Estimated concentration of crystalline phases		
	FeO	Fe_2O_3	TiO_2	Ilmenite	Pseudorutile	Rutile
Florida ilmenite	4·1	25·2	64·2	Trace	High	Low
New Jersey ilmenite	0·1	32·2	62·7	Trace	High	Low
Quilon (Indian) ilmenite	9·4	25·4	60·0	Medium	High	Low
Brazilian ilmenite	5·7	31·5	57·1	Low	High	Low

Rotation of single grains of altered ilmenite about arbitrary axes in initial X-ray diffraction studies produced patterns characteristic of single crystals rather than of polycrystalline samples. Consequently, we treated small mineral grains of altered ilmenite like single crystals and recorded the diffraction patterns representing different orientations with rotating crystal, equi-inclination Weissenberg and precession camera techniques. Zirconium-filtered molybdenum $K\alpha$ radiation was used.

From these patterns it was determined that the major portion of the grains consisted of hexagonal pseudorutile with $a = 2{\cdot}872$ Å and $c = 4{\cdot}594$ Å, using the dimensions of rutile as an internal standard. Small amounts of rutile with $a = 4{\cdot}594$ Å and $c = 2{\cdot}958$ Å were present in all grains in a specific threefold orientation. Rhombohedral ilmenite with the hexagonal dimensions $a = 5{\cdot}078$ Å and $c = 14{\cdot}135$ Å was occasionally observed. The orientation relationship between ilmenite, pseudorutile and rutile is shown in Fig. 1.

In addition to sharp and well-defined diffraction spots present on all photographs, a number of diffuse streaks of considerable intensity were observed on $l = 2n + 1$ level photographs of pseudorutile. These streaks are caused by the disordered structure of pseudorutile and do not originate from another phase.

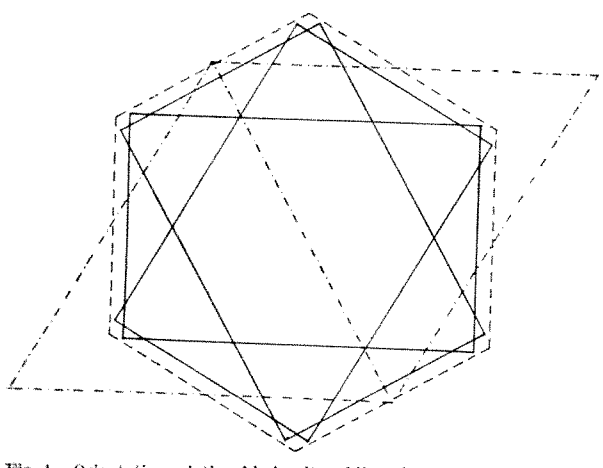


Fig. 1. Orientation relationship in altered ilmenite. ———, Ilmenite; — — — —, pseudorutile; ————, rutile triplet

The positions and thermal parameters of the atoms in pseudorutile were derived from diffraction data recorded by the Weissenberg technique and were refined by least-squares analysis. The best agreement between observed and calculated structure factors ($R = 4{\cdot}5$ per cent) was found with the oxygen atoms placed in a close-packed hexagonal arrangement. A fraction of the octahedral sites available in such a structure is statistically occupied with iron and titanium atoms according to the stoichiometry $Fe_2Ti_3O_9$.

The disordered structure of pseudorutile can be described as a transition phase between the atomic arrangements in ilmenite and rutile in which the nearly perfect close-packed hexagonal arrangement of oxygen atoms is but little affected during the transformation ilmenite → pseudorutile → rutile. The structural relationship between all three phases is apparent in the orientation relationship illustrated in Fig. 1.

The alteration of ilmenite can be formulated as a topochemical reaction in which the number and arrangement of oxygen atoms in ilmenite are essentially preserved in pseudorutile and rutile:

$$\underset{\text{Ilmenite}}{Fe^{II}Ti_3O_9} \xrightarrow{\text{Oxidation}} \underset{\text{Pseudorutile}}{Fe^{III}_2Ti_3O_9} + 1/2Fe^{III}_2O_3$$

Further removal of Fe_2O_3 from pseudorutile causes the direct formation of rutile without formation of other crystalline phases. Pseudobrookite and rutile are formed by heating pseudorutile to temperatures above 800° C:

$$\underset{\text{Pseudorutile}}{Fe^{III}_2Ti_3O_9} \xrightarrow{>800^\circ\,C} \underset{\text{Pseudobrookite}}{Fe^{III}_2TiO_5} + \underset{\text{Rutile}}{2TiO_2}$$

Two minerals of the composition $Fe_2Ti_3O_9$ have been reported: arizonite[2,3] and proarizonite[4]. In neither case are the published interplanar spacings and intensities identical with those of pseudorutile. With samples having a high rutile/pseudorutile ratio, the superposition of most of the powder lines might result in the presence of pseudorutile being overlooked—or mistaken for rutile.

Table 2. COMPARISON BETWEEN OBSERVED SPACINGS AND INTENSITIES OF NEW JERSEY ILMENITE WITH CALCULATED SPACINGS OF PSEUDORUTILE AND ASTM 4-0551 DATA REPORTED FOR RUTILE

New Jersey ilmenite		Pseudorutile		Rutile		
dÅ$_{obs.}$	I/I_1	dÅ$_{calc.}$	(hkl)	dÅ	I/I_1	(hkl)
3·847	*D*		*D*			
3·251	15			3·245	100	(110)
2·768	*D*		*D*			
2·484	60	2·489	(100)	2·489	41	(101)
2·301	4	2·297	(002)	2·297	7	(200)
2·187	50	2·187	(101)	2·188	22	(111)
2·057	1			2·054	9	(210)
1·688	100	1·688	(102)	1·687	50	(211)
1·632	*D*		*D*	1·624	16	(220)
1·469	*D*		*D*	1·480	8	(002)
1·437	30	1·436	(110)			
1·384	*D*		*D*	1·360	16	(301)
1·305	4	1·304	(103)	1·305	1	(311)
1·246	2	1·244	(200)	1·243	3	(202)
1·202	2	1·200	(201)	1·200	1	(212)
1·150	6	1·149	(004)	1·1485	4	(400)
1·094	12	1·094	(202)	1·0933	4	(222)
1·043	9	1·043	(104)	1·0424	5	(411)
0·8975	7	0·8970	(114)			
0·8711	6	0·8700	(212)			

Ilmenite $\{0001\}\,[11\bar{2}0]$ ‖ Pseudorutile $\{0001\}\,[10\bar{1}0]$ ‖ Rutile $\{010\}\,[001]$

Observed spacings (dÅ) and intensities (I/I_1) of an altered ilmenite sample are listed in Table 2. The spacings of the five principal diffuse bands are included. The sample represents a mixture of pseudorutile listed with calculated spacings and (hkl) indices together with a small amount of rutile (ASTM No. 4–0551). The diffuse bands are marked with a '*D*' to indicate that they

are an integral part of the X-ray diagram obtained from pseudorutile.

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Mechanism of the Acid Hydrolysis of Pentammine Cobalt Complex Ions

UNEQUIVOCAL evidence in support of a particular mechanism for the acid hydrolysis reaction (1) has not, as yet, been obtained.

$$[Co(NH_3)_5X^{2+}] + H_2O \rightleftarrows [Co(NH_3)_5H_2O^{3+}] + X^- \quad (1)$$

$$(X = F^-, Cl^-, Br^-, I^-, NO_3^-, H_2PO_4^-)$$

Several recent communications[1-4] serve to emphasize that the problem is still an open issue. In any kinetic investigation a knowledge of the species present is of prime importance, particularly, in a reaction of type (1), where ion-pairs are likely to occur; the acceleration of reaction (1) by added anions has been observed[5,6]. In a recent detailed investigation for ($X = Cl^-$), Laurie and Monk[7] attributed these accelerations to ion-pairs being more reactive than the free complex ion; the thermodynamic dissociation constants derived from the rate data for the proposed ion-pairs were in good agreement with values from investigations of the electromotive force[8]. The results are collected in Table 1. It is the purpose of this communication to show the importance of these results with regard to two misconceptions and to the mechanism of reaction (1).

According to Basolo and Pearson[9], increased reaction rates in the presence of added anions cannot be attributed solely to more reactive ion-pairs. Their conclusion is made invalid by the above results and by the findings of Davies[10] on other similar reactions. Moreover, Basolo[11] takes the increase in hydrolysis rate with decrease in positive charge on the complex to be indicative of an S_N1 reaction; for example, for similar low-spin d^6 complexes the decrease in reactivity follows the order Fe(II) > Co(III) > Pt(IV), but in such a sequence differences in ligand field strength will also be operative. For the species $[Co(NH_3)_5Cl]^{2+}$, $[Co(NH_3)_5Cl]L^+$, and $[Co(NH_3)_5Cl]M^0$, where L^- and M^{2-} are ion-pairing anions, ligand field strength differences are negligible and the observed[7] increase in hydrolysis rate along the series may be attributed solely to the lowering of the positive charge. These rate increases (Table 1) are, however, small (the activation energy changes by only 0·1 kcal on lowering the charge from +2 to 0) and can be interpreted in terms of an S_N2 mechanism in which the lowering of the positive charge promotes the removal of the chloride ligand (ion–ion interaction) but discourages attack by the nucleophilic water molecule (ion–dipole interaction). The former effect, being the greater, would predominate, giving the observed increases and at the same time preserving the well-established "bond breaking more important than bond making" order.

Table 1*

Ligand	Nitrate	Glycollate	Sulphate	Malonate	Phthalate
k_2/k_1	1·1	1·15	2·05	2·13†	2·95
K	0·09	0·03$_5$	0·0032	0·0050	0·0030

* Data taken from ref. 7.
† Incorrectly given as 2·08 in ref. 7. k_2/k_1 is the ratio of the reaction rate constant of the ion-pair to that of the free complex ion, at 35°. K is the thermodynamic dissociation constant at 35° C.

Langford's analysis[3] of the free-energy data suggests that for an S_N1 mechanism to occur the entering nucleophile must be previously present in the second co-ordination sphere of the Co(III) ion. If this is so, then the non-entry of the ion-pairing anion into the first co-ordination sphere must rule out an S_N1 process. By similar reasoning an S_N2-trans mechanism can be discounted.

The data presented comply with an S_N2-*cis*-type mechanism, in which the transition state involves an extended Co—X bond and an adjacent weak bond between the cobalt-atom and a water molecule present in the second co-ordination sphere, the reaction being completed by the breaking of the Co—X bond and the simultaneous entering of the water molecule. The inclusion of the extended Co—X bond is in accordance with Vlcek's deductions[12] from polarography measurements and means that expansion of the inner co-ordination sphere is not necessary. For minimum potential energy the ion-pairing anion must be "*trans*" to the departing ligand and hence would not be expected to enter the inner-co-ordination sphere on the basis of this mechanism. The proposed mechanism also fits in well with other observations on deuteration[13] and steric hindrance[14] effects and the findings that complexes of the type *cis*-$[Co(en)_2XCl^+]$, $X = NCS^-$, N_3^-, and NO_2^-, hydrolyse without stereochemical change[15].

Finally, it is to be noted that the mechanism proposed above is very similar to the "solvent-assisted dissociation" process favoured by others (for example, see ref. 2). Indeed, the difference may be one of semantics only.

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Enzymatic Etching of Single Crystals of Naphthalene

IT was previously observed[1] that when crystals of naphthalene, which had been grown from the vapour phase, were immersed in an aqueous suspension of soil pseudomonads for about 10 min at room temperatures, a small number (about 10^2 cm^{-2}) of well-defined, pyramidal pits were produced on the principal habit faces {00*l*}. This result was tentatively interpreted to mean that preferential attack of the naphthalene by the pseudomonads occurred at the regions of emergence of dislocations, a view which was consonant with: (i) the conclusions of Kochendorfer[2], who indicated that dislocations lying in {010} planes, having a Burgers vector in the [001] direction, might be possible in naphthalene; (ii) the results of Koslovskii[3], who observed growth spirals on the {00*l*} faces of certain naphthalene crystals; (iii) the more recent work of Sears[4], who also proffered evidence for dislocations emergent at {00*l*} faces. The result agreed

with the molecular interpretation of Davies and Evans[5] concerning the oxidative metabolism of naphthalene by microbial enzymes.

This communication reports some of the salient features of a more rigorous investigation which we have made of the enzymatic attack of naphthalene crystals produced in two distinct ways. Type I single crystals of 'scintillation grade' naphthalene were grown from the melt using the Bridgman technique by Thorn Electronics, Ltd. (Surbiton, Surrey). Type II single crystals were produced by slow sublimation of polycrystalline 'molecular weight grade' naphthalene. The etchant used consisted of an aqueous solution of the cell-free enzyme derived from pseudomonads as follows. Cells grown in a naphthalene–mineral salts medium under forced aeration were sonically disrupted in a phosphate buffer of *p*H 7 (0·05 M) to yield a supernatant (after centrifuging at 25,000*g*) which was active on naphthalene in the presence of reduced niacin adenine dinucleotide (NAD) as cofactor. Etch patterns on the {00*l*} faces only could be studied by transmission optical microscopy—a restriction imposed largely by the cleavage behaviour of naphthalene crystals.

In Fig. 1, which shows a {00*l*} face of a type I crystal after etching for 30 sec, there are numerous flat-bottomed etch-pits which tend to be hexagonal in outline and all of which are of the same crystallographic orientation. Fig. 2 is an enlargement of part of the surface shown in Fig. 1: the tendency for hexagonal etch-pits to be produced is more evident here. Type II crystals behave in essentially the same way, although there does appear to be rather more aptitude for triangular (flat-bottomed) pits to be produced than with type I crystals.

We are of the opinion that it is unlikely, but not impossible, that the etch-pits in Figs. 1 and 2 are nucleated at the termini of dislocations that glide in {0*l*0} planes (and would emerge at {00*l*} faces). (Gordon[6] has recently shown

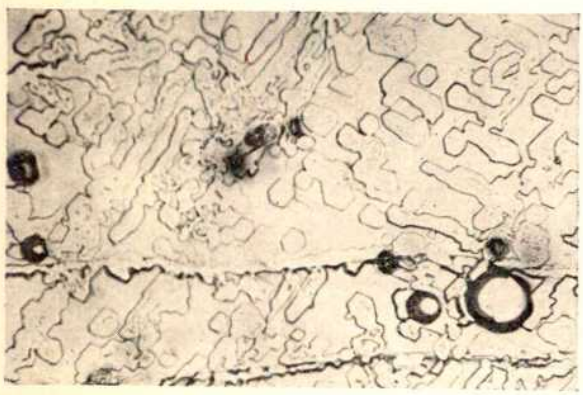


Fig. 1. Etch pattern on 00*l* face of naphthalene crystal after 30 sec exposure to enzymatic etchant (× *c*. 103)

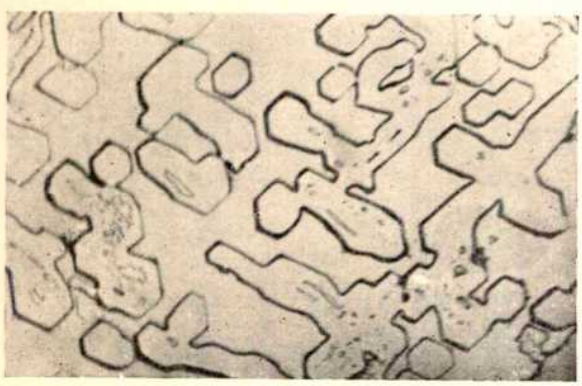


Fig. 2. Magnified area of another region of the crystal face shown in Fig. 1 (× *c*. 206)

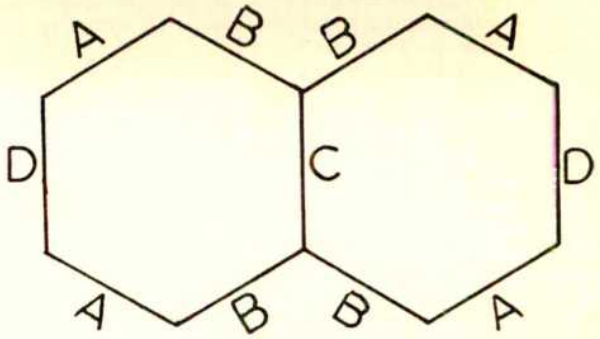


Fig. 3. The various bond types in naphthalene

that there is a greater likelihood for dislocations in naphthalene to glide in {00*l*} rather than in {010} planes.) We feel that they are more likely to be undersaturation etch-pits[7] which have been nucleated randomly on the {00*l*} faces. So far as the crystallographic orientation of the pits is concerned, it is possible to explain the results obtained with both types of crystals in terms of the anisotropy of reaction rates at various crystallographic planes of the naphthalene lattice. This anisotropy, in turn, devolves on differences of reactivity at various sites in the naphthalene molecule. In essence, our conclusions are that bonds *A* and *B* (see Fig. 3) are more susceptible to attack by pseudomonads than bonds *C* and *D*. A full account of this work is to be published elsewhere.

We wish to acknowledge the encouragement and advice of Prof. S. Peat.

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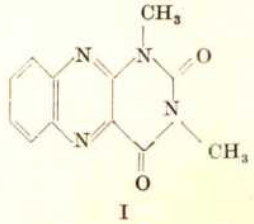
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Dimethylalloxazine as an Electron Donor and Acceptor

I WISH to report that 1,3-dimethylalloxazine (I) can form molecular complexes acting not only as an electron acceptor but also as a donor. It was possible to crystallize complexes from ethylene dichloride with the following compounds: pyrene, tetracyanoethylene (TCNE), and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone. The results of the elemental analysis indicate that these complexes contain dimethylalloxazine and the other component in a ratio of 2 : 1 regardless of whether the latter is a donor or acceptor. As the infra-red spectra of the complexes are very close to the superimposed spectra of the components,



I

there is no appreciable change in the intramolecular bonds by complex formation. Therefore, the ground states of the complexes are essentially non-bonding. The complexes are more deeply coloured than the components. For example, the pyrene and TCNE complexes are deep yellow. On the other hand, dimethylalloxazine is pale yellow and the other two components are colourless. As shown in Fig. 1, the appearance of a new absorption is clearly seen in these complexes. It can be concluded that these complexes are of the charge transfer type; dimethylalloxazine in the pyrene complex is acting as an electron acceptor and in the TCNE complex as a donor.

In the present complexes, which have essentially non-bonding ground states, the energies of the molecular orbitals may be little different from those in the separate components. In a naïve approach, the energy of the first charge transfer band in a given complex can be considered as the energy difference between the highest occupied molecular orbital of the donor and the lowest vacant molecular orbital of the acceptor[1]. Therefore, the relative positions of the molecular orbitals associated with the complex formation can be ascribed on the basis of the energies of the excitation within the component molecules and of the first charge transfer bands in the complexes. Strong donors are expected to have their highest occupied molecular orbitals at relatively high energies and strong acceptors are expected to have their lowest vacant molecular orbitals at relatively low energies.

In Fig. 2, the lowest vacant molecular orbitals of the acceptors, including dimethylalloxazine, were located relative to the highest occupied molecular orbital of pyrene by means of the energies of the charge transfer bands in the pyrene complexes. The positions of the remaining molecular orbitals were assigned on the basis of the first excitation energies in the component molecules. As any changes in energy of the molecular orbitals in forming the complex have been ignored, the present position is only approximate. Nevertheless, it is clearly shown that the highest occupied molecular orbital of dimethylalloxazine is located between those of the donor and the acceptors, and the lowest vacant molecular orbital of the alloxazine is located again between those of the donor and acceptors. In agreement with the observation reported here, the molecular orbital diagrams given in Fig. 2 indicate that dimethylalloxazine can be an electron donor when combined with strong acceptors and can be an acceptor when combined with strong donors.

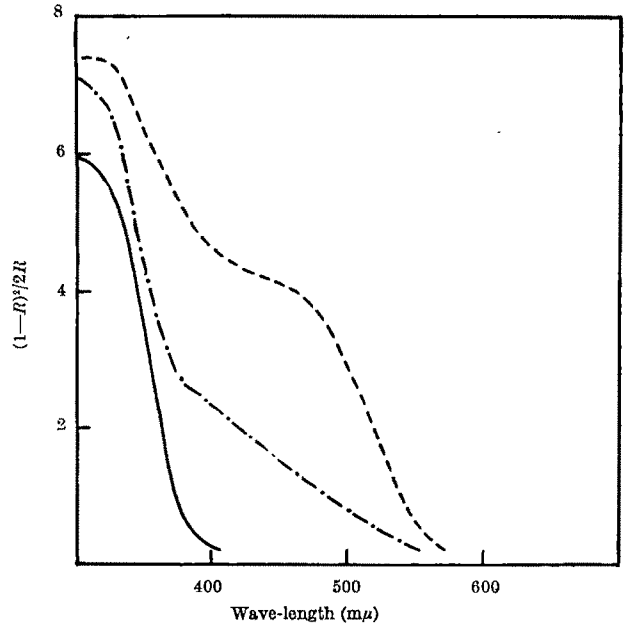


Fig. 1. Kubelka-Munk plots of diffuse reflectance spectra of dimethylalloxazine (——), the pyrene complex (- - -), and the TCNE complex (— · — · —)

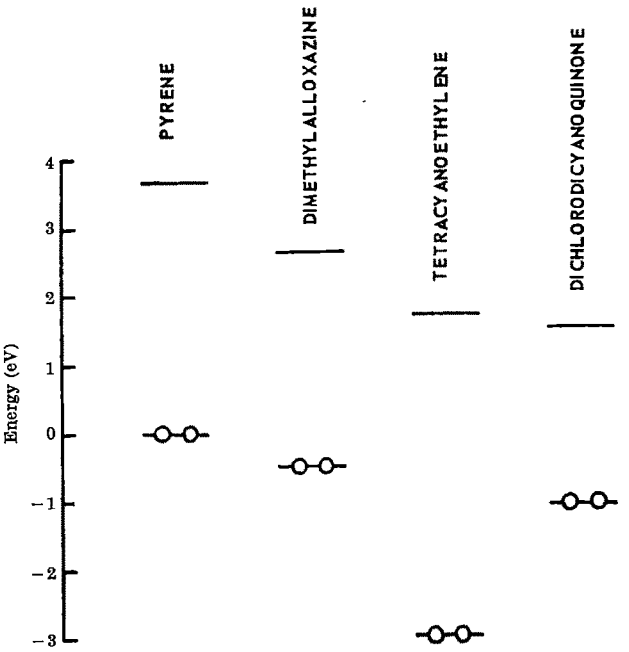


Fig. 2. Relative location of the highest occupied and the lowest vacant molecular orbitals in dimethylalloxazine and some other compounds

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Polymorphic Forms of the Diaminopyrene-*p*-chloranil and Related Molecular Complexes

I REPORTED earlier that most of the quinone complexes with electrical resistivities of 10^8 ohm cm or less have essentially dative ground states[1]. The only exceptions found were the 1,6-diaminopyrene complexes with *p*-chloranil and *p*-bromanil. It therefore seemed worthwhile to examine these complexes further. The resistivity values reported for the *p*-chloranil complex are scattered in a wide range, namely 10^3–10^4 ohm cm for the pressed polycrystalline samples[2,3] and 10^5–10^9 ohm cm in the three directions of the single crystal[4]. This communication reports the existence of some polymorphic forms of the 1,6-diaminopyrene-*p*-chloranil and related molecular complexes. The polymorphic forms are distinctly different in colour, diffuse reflectance spectrum, vibrational spectrum, X-ray diffraction, and also electrical resistivity. The scattered resistivity values appear to be well related to those of such forms.

The *p*-chloranil complex prepared in chloroform, the solvent from which Kronick and Labes grew their single crystal[4], is dark blue and green when pulverized. The composition is 1 : 1. Calculated (per cent): carbon 55·3, hydrogen, 2·5; nitrogen, 5·9; chlorine, 29·7. Found (per cent): carbon, 55·2; hydrogen, 2·4; nitrogen, 6·1; chlorine, 29·4. The diffuse reflectance spectrum shown in Fig. 1 indicates that, although the spectrum has an absorption minimum at about 530 mμ, the absorption extends to about 1·8 μ. The pressed pellet exhibits a resistivity of the order of 10^7 ohm cm. This value seems to be reasonable as the average for 10^5–10^9 ohm cm observed in the three directions of the single crystal. This green form can be transformed into the brown form (mentioned later) by recrystallization from benzene.

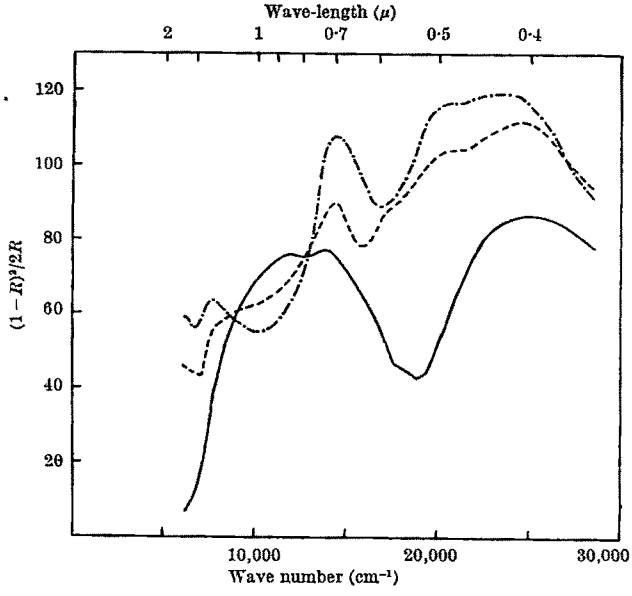


Fig. 1. Kubelka-Munk plots of diffuse reflectance spectra of diaminopyrene-*p*-chloranil complex, green (——), brown (- - -), and low-resistivity (— · — · —) forms

When benzene is used for the preparation, the brown form crystallizes. The composition is again 1 : 1. Found (per cent): carbon, 55·2; hydrogen, 2·8; nitrogen, 5·9; chlorine, 29·2. The resistivity of the pressed pellet prepared after careful drying of the sample is of the order of 10^5 ohm cm. This brown form can easily be transformed into a third form, exhibiting a resistivity of only a few ohm cm, by compression in the presence of benzene. Even a trace of the solvent remaining in the sample can result in a lowering of the resistivity of the pellet to about 10^3–10^4 ohm cm. It should be noted that the second and third forms show electronic absorption covering the visible and infra-red regions and extending beyond 2·5μ. The large difference in the optical and electrical properties found in these polymorphic forms shows the importance of the stacking of the component molecules as one of the factors determining the electronic properties of such a complex.

The vibrational spectra of the green and brown forms of the *p*-chloranil complex are similar to the superposed spectra of the components. Therefore, they appear to have essentially non-bonding ground states. However, the patterns in the region between 5·5 and 6·8μ are very characteristic of each form, and this indicates that the mode of intermolecular interaction is different in these forms (see Fig. 2*a* and 2*b*). The whole spectrum of the brown form was presented with those of the components in my previous note[1]. The spectrum of the low resistivity form is very dark, suggesting that the electronic absorption seen in Fig. 1 extends over the entire sodium chloride region. Although the vibrational bands of the various components are for the most preserved, their intensities are very much modified. Furthermore, the carbonyl stretching vibration band is shifted to longer wave-lengths by about 0·2 μ or 50 cm^{-1} in this form. This unusually large shift may indicate that the crystal structure is favourable for a strong interaction between the component molecules and that the contribution of the dative structure to the ground state is appreciable. It is suggested that the low resistivity form of the *p*-chloranil complex is near the border line between complexes with non-bonding ground states and those with dative ground states.

Green and brown forms were also found for the trichloromonobromo- and dichlorodibromo-*p*-benzoquinone complexes. The green forms, prepared in chloroform, would seem to be similar to the green form of the *p*-chloranil complex. On the other hand, the brown forms, crystallized from benzene, show infra-red spectra quite different from that of the brown form of the *p*-chloranil complex. Figs. 2*d* and 2*e* show, respectively, the spectra of the green and brown forms of the 2,5-dichloro-3,6-dibromo-*p*-benzoquinone complex.

Only the brown form is obtainable both from chloroform and from benzene for the monochlorotribromo-*p*-benzoquinone and *p*-bromanil complexes. All the brown complexes except the brown form of the *p*-chloranil complex are of similar nature and exhibit resistivities of the order of 10^3 ohm cm. This value is in accordance with that reported for the *p*-bromanil complex by Labes *et al.*[2].

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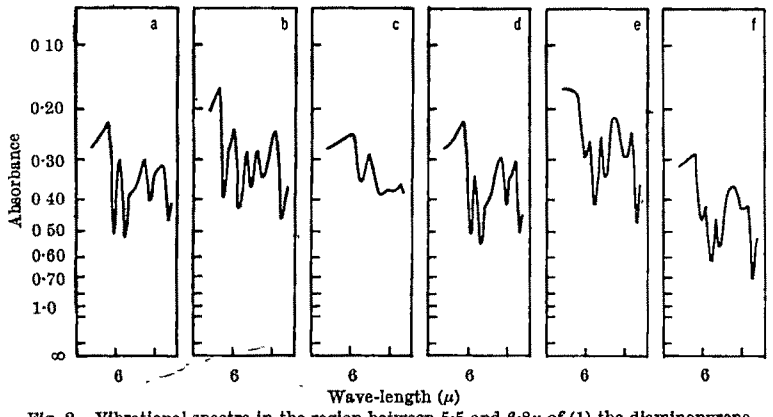


Fig. 2. Vibrational spectra in the region between 5·5 and 6·8μ of (1) the diaminopyrene-*p*-chloranil complex, (*a*) green, (*b*) brown and (*c*) low resistivity forms; (2) the 2,5-dichloro-3,6-dibromo-*p*-benzoquinone complex, (*d*) green and (*e*) brown forms; (3) (*f*) the *p*-bromanil complex

Transition State in the Inversion of the Sachse Hexagon

A FEW years ago, Hendrickson[1] suggested that the transition state in the inversion of the chair form of the Sachse hexagon in *cyclo*hexane was one in which half the molecule is in the planar Baeyer form and the other half in the Sachse chair form. This suggestion has recently been accepted by Anderson[2]. However, for various reasons, it is difficult to accept this idea.

For one thing, the two halves do not fit each other. The squared distances between opposite sides are, in the Baeyer and in the Sachse hexagons $2a^2(3/2)$ and $2a^2(4/3)$, respectively, and the squared distances between opposite corners $(36/9)a^2$ and $(33/9)a^2$, respectively, where a is the carbon–carbon distance. Secondly, there is nothing to prevent us from imagining forms of the carbon hexagon intermediate between the Baeyer and Sachse models, in which the angles are different and the partition of strain therefore more or less skew, as it is to an extreme degree in Hendrickson's model. But it is very questionable whether such non-symmetric structures can be formed from the highly symmetrical Sachse hexagon by stress-

ing forces from isotropic surroundings: and finally, if the partition of strain is skew, the internal molecular forces cannot balance each other, as they must.

So it is difficult to escape the conclusion that all six angles are equal in the strained hexagons, and that the transition state in question therefore must be the Baeyer form of *cyclo*hexane. In this form, the potential energy arises partly from the six angular strains and partly from the twisting of six pairs of valency triads from staggered into eclipsed orientations. In principle, it should thus be possible to calculate the work necessary to bring the *cyclo*hexane molecule from its normal strainless state into the transition state. The work of twisting has been determined by Pitzer[3] at about 2·8 kcals or about 12 kJ for each "mole" of a pair. The contribution from the work of twisting may therefore be estimated at about 72 kJ. The work, V, of extending each of the six angles from the tetrahedric value to 120° may be calculated from

$$2V = A\alpha^2$$

where A is the angular restoring force, and α the angular difference 10·53° = 0·1838 radian. In a well-known review, Westheimer[4] has accepted $A = 8 \times 10^{-12}$ erg/(radian)^2 for one angle in one molecule, while Wilson[5], on the basis of his investigation of the infra-red and Raman spectra of *cyclo*butane, derived $A = 7{\cdot}67 \times 10^{-12}$ for the same quantity. Westheimer's value yields for one mole

$$6V = 48{\cdot}82 \text{ kJ}$$

The total work of activation may therefore be estimated at about 120 kJ/mole, which means that the inversion of the Sachse chair form should be an extremely slow process at room temperature.

Tentative calculations based on the lowest frequency observed in the Raman spectrum[6,7] of *cyclo*hexane itself lead to an even higher work of activation of the reaction in question. Thus the evidence derived from infra-red and Raman spectra seems to disagree completely with that derived from nuclear magnetic resonance spectra. This situation calls urgently for a revision of the interpretation of both kinds of spectra. For example, one might ask, is the inversion of the Sachse hexagon the only possible explanation of the coalescence of a doublet in the nuclear magnetic resonance spectrum existing at lower temperature into a singlet at room temperatures[2] ? Or could the phenomenon be caused by the "thawing" of a vibration of the hydrogen atoms which is "frozen" at lower temperatures ?

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Electronic Transitions in Hexachloro-co-ordinated Titanium and Chromium

IN the crystal structure of α-titanium trichloride[1], the chlorine ions are in a hexagonal close-packed pattern, whereas in γ-titanium trichloride and chromium trichloride the chlorine ions are close-packed in a cubic pattern[1,2]. The metal ions occupy two-thirds of the octahedral holes between alternate layers of chlorine ions.

Wessel and Ydo[3] investigated the crystal structure of the isomorphous compounds $Cs_3Ti_2Cl_9$ and $Cs_3Cr_2Cl_9$. According to their results, units of $Ti_2Cl_9^{3-}$ and $Cr_2Cl_9^{3-}$ are present in the crystal in the form of two octahedra sharing a face.

The similarity of the octahedral surroundings in the compounds of the transition metal ions mentioned above induced us to study the optical transitions of α- and γ-titanium trichloride, $Cs_3Ti_2Cl_9$, chromium trichloride and $Cs_3Cr_2Cl_9$. The optical d–d transitions of transition metal ions placed in a crystal field of a certain symmetry can be interpreted on the basis of an electrostatic model in which the ligands are considered as point charges or point-shaped dipoles. According to the single-electron approximation the five-fold degenerate d-level of a transition metal ion will split into a three-fold degenerate and a two-fold degenerate level if the transition metal ion is placed in a crystal field of ideal octahedral symmetry.

Recently, we made observations of the electronic transitions of α-, β- and γ-titanium trichloride[4]. The spectra of α- and β-titanium trichloride have also been published by Clark[5]. However, our spectrum of α-titanium trichloride, reproduced in Fig. 1, curve 1, does not show the shoulder at 12,000 cm^{-1} observed by Clark.

The spectrum of γ-titanium trichloride is quite analogous, which is to be expected in view of the correspondence of the crystal structures of α- and γ-titanium trichloride. $Cs_3Ti_2Cl_9$ was prepared by fusing in a sealed quartz tube a mixture of caesium chloride and α-titanium trichloride (Stauffer *H.A.*) in the molar ratio of 3 : 2. After the tube had been heated at about 725° C for about 25 h the compound $Cs_3Ti_2Cl_6$ had formed and was identified by X-ray analysis.

In order to avoid contamination by oxygen and water the manipulations were carried out in a glove-box through which dry nitrogen was passed. The reflexion spectra were run by means of a 'Beckman *DK* 2' type spectrophotometer using magnesium oxide as a blank. The reflexion spectrum of $Cs_3Ti_2Cl_9$ is shown in Fig. 1, curve 2. The chromium trichloride used was sublimed chromium trichloride (Riedel-De Haen *A.G.*, Seelze-Hannover). The reflexion spectrum is shown in Fig. 2, curve 1. The complex $Cs_3Cr_2Cl_9$ was prepared and analysed in an analogous way as the complex $Cs_3Ti_2Cl_9$. The reflexion spectrum is given in Fig. 2, curve 2.

The titanium (III) ion has the electron configuration $A(3d)^1$. In ideal octahedral surroundings the $(3d)^1$ level splits in a three-fold degenerate t_{2g} and a two-fold degener-

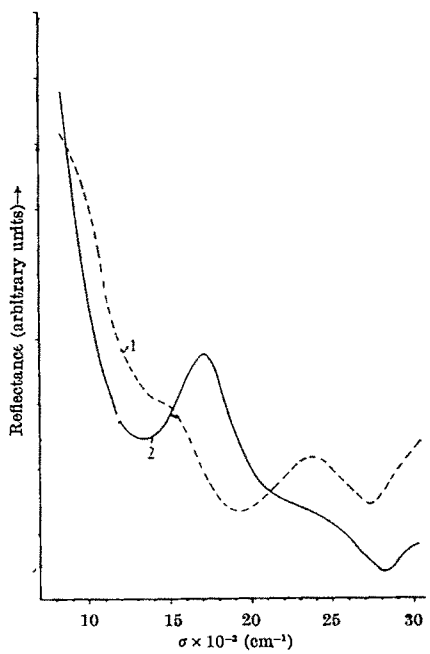


Fig 1. Reflexion spectrum of: 1, α-$TiCl_3$, 2, $Cs_3Ti_2Cl_9$

ate e_g level. From the knowledge of the crystal structure we conclude that in α- and γ-titanium trichloride the titanium ions are octahedrally surrounded by chlorine ions, whereas the deviations from the octahedral symmetry are relatively small. The absorption band at 14,300 cm^{-1} observed for α- and γ-titanium trichloride will correspond consequently to the transition $^2T_{2g} \rightarrow {}^2E_g$[4,5].

One might suggest that the bands observed for α- and γ-titanium trichloride at 14,300 cm^{-1} and 19,300 cm^{-1}, as well as the bands observed for $Cs_3Ti_2Cl_9$ at 13,400 cm^{-1} and 21,500 cm^{-1}, are caused by a splitting of the band formed by the transition $^2T_{2g} \rightarrow {}^2E_g$. The magnitude of such a split, however, seems too large to be caused by the Jahn-Teller effect or by deviations from the octahedral symmetry. Consequently, it would be reasonable to assign the band observed at 19,300 cm^{-1} for α-titanium trichloride and at 21,500 cm^{-1} for $Cs_3Ti_2Cl_9$ to charge-transfer transitions of the type $\pi \rightarrow t_{2g}$.

The chromium (III) ion has the electron configuration $A(3d)^3$. In ideal octahedral surroundings the strong field configuration $(t_{2g})^3$ gives rise to the states $^4A_{2g}$, ^2E_g, $^2T_{1g}$, and $^2T_{2g}$. The excited configuration $(t_{2g})^2(e_g)$ produces a.o. the states $^4T_{2g}$ and $^4T_{1g}$. It appears[6] that the level with $^4T_{2g}$ symmetry is lower in energy than the level with $^4T_{1g}$ symmetry. The only spin-allowed transitions are $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$. The transitions $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$ are observed in $Cr(H_2O)_6^{3+}$ at 17,400 and 24,500 cm^{-1} (ref. 7).

In chromium trichloride and $Cs_3Cr_2Cl_9$, the chromium ions are also octahedrally surrounded by chlorine ions. We therefore assign the bands observed for chromium trichloride at 13,600 cm^{-1} and 18,900 cm^{-1} and the bands observed for $Cs_3Cr_2Cl_9$ at 12,800 cm^{-1} and 18,000 cm^{-1} to the transition $^4A_{2g} \rightarrow {}^4T_{2g}$ and $^4A_{2g} \rightarrow {}^4T_{1g}$ respectively[5].

In Fig. 3 we give a picture of the discussed *d–d* transitions. The wave numbers of maximum absorption are summarized in Tables 1 and 2.

Table 1*

	α,γ-Titanium trichloride	$Cs_3Ti_2Cl_9$
$^2T_{2}\rightarrow{}^2E_g$	14,300	13,400
$\pi\rightarrow t_{2}$	19,300	21,500

Table 2*

	Chromium trichloride	$Cs_3Cr_2Cl_9$
$^4A_{2g}\rightarrow{}^4T_{2g}$	13,600	12,800
$^4A_{2g}\rightarrow{}^4T_{1g}$	18,900	18,000

* Wave numbers in cm^{-1}.

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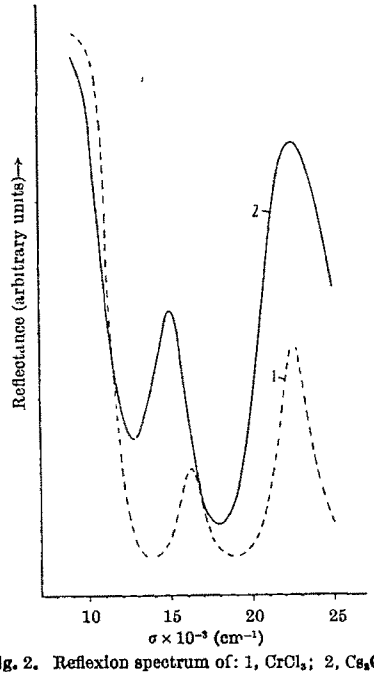


Fig. 2. Reflexion spectrum of: 1, $CrCl_3$; 2, $Cs_3Cr_2Cl_9$

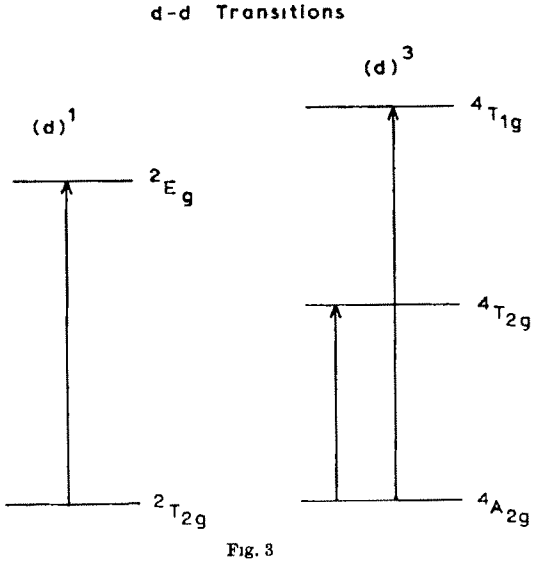
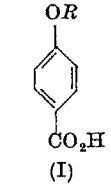


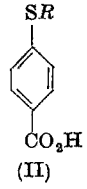
Fig. 3

Strengths of *p*-Alkoxy- and -Alkylthio-benzoic Acids

ALTHOUGH the literature contains references to the pK_a values of the acids (I)[1–3] and (II)[4], in neither case have they been determined in a common solvent; in each series, the group R has included only primary and secondary alkyl. Moreover, a discrepancy exists between the results of Jones and Speakman[1] and of Cavill, Gibson and Nyholm[2] for the *p*-alkoxybenzoic acids (I; R= Me, Et and Pr^i).



(I)



(II)

The apparent pK_a values for the acids (I) and (II), where R is Me, Et, Pr^i and Bu^t, respectively, and for benzoic acid, have been determined by potentiometric titration in 30 per cent v/v acetone–water. The choice of this solvent system was dictated largely by the lower solubilities of the acids (II) compared with those of (I).

Each alkoxybenzoic acid (I) is weaker than its alkylthio analogue (II); this is consistent with the more limited data of Baker, Barrett and Tweed[3]. Relative to benzoic acid, the variation in acid strength with variation in

R is for (I), Benzoic > Bu^t > Me ≳ Et > Pr^i, and for (II), Bu^t > Pr^i > Benzoic > Et ≳ Me. These trends confirm those observed by Cavill, Gibson and Nyholm[2] for (I; R=Me, Et, and Pr^i), and by Baliah, Shanmüganathan and Varadachari[4] for (II; R=Me, Et, Pr^i). The position of the Bu^t acid in each case is a clear demonstration of the operation of a steric effect involving the Bu^t group and the *o*-hydrogen atoms, which prevents the attainment of coplanarity of the bonds of the oxygen (or sulphur) atom and the benzene ring, and thereby destabilizes the acid relative to its anion. It would appear that a small, though real, steric effect operates in (II) even when R is Pr^i. The steric interactions present in (II; R=Pr^i and Bu^t, respectively) would appear to be so effective as to hinder the operation of the electron-releasing mesomeric effect of the sulphur atom to such a degree that the electron-withdrawing inductive effect of this atom becomes predominant in determining the free-energy difference between each of these acids and its anion.

A more complete interpretation must await the outcome of investigations into the esters of (I) and (II), and the corresponding *m*-alkoxy- and alkylthio-benzoic acids, and their esters.

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BIOCHEMISTRY

Incorporation of Histamine Ribonucleoside into Soluble Proteins

ISOTOPICALLY labelled histamine ribonucleoside can be detected in the urine of experimental animals after intraperitoneal administration of either 2(ring)-^{14}C-labelled histamine[1], or similarly labelled histamine-adenine-dinucleotides[2]. However, in such experiments the quantities of histamine ribonucleoside recovered from the urine represent only a small percentage of the expected amount. This apparent disappearance of histamine ribonucleoside *in vivo* suggested the possibility of its combination with proteins. The incorporation of amines into proteins, probably through transamidation, was studied by Waelsch *et al.*[3,4]. A similar system, that is, a 100,000*g* supernatant of a guinea-pig liver homogenate[3], was used in our investigations. Histamine ribonucleoside was prepared from histamine adenine dinucleotide phosphate[5], as already described[2]. The degree of purity of this material was checked ionophoretically[1,2], and only a single spot was observed in either *tris*-hydrochloric acid (*p*H 8·0) or sodium borate (*p*H 9·1). As shown in Table 1, there was appreciable incorporation of radioactivity into the acid insoluble fraction of the incubation mixtures when either histamine, or histamine ribonucleoside, or histamine-adenine-dinucleotide-phosphate was used. This incorporation was Ca^{2+}-dependent. Thus, in controls from which Ca^{2+} was omitted, very few scintillations were counted, and radioactivity approached background values (Table 1). When tested under conditions similar to those in Table 1, the guinea-pig liver supernatant used in our investigations was found free of activities capable of liberating inorganic phosphate from the dinucleotide. This may be partly due to the lack of the proper enzymes (that is, pyrophosphatases and phosphatases) and partly because of the lack of magnesium ions. In any event, this finding led us to the conclusion that the dinucleotide was probably attached as such on the protein molecule.

Table 1 INCORPORATION OF 2(RING)-^{14}C-LABELLED HISTAMINE AND CERTAIN OF ITS N(RING)-RIBOSYL DERIVATIVES IN THE ACID-INSOLUBLE FRACTION OF GUINEA-PIG LIVER PREPARATIONS

Compound	Ca^{++} (5 mM)	Radioactivity above background (d.p.m. per 2·7 mg of protein)
Histamine	+	5,688
Histamine	−	35
Histamine ribonucleoside	+	1,494
Histamine ribonucleoside	−	32
Histamine-adenine dinucleotide phosphate	+	1,840
Histamine-adenine dinucleotide phosphate	−	26

All compounds were identically labelled. Each amine (0·25 M, 50,000 d.p.m.) was incubated for 1 h at 37° C with an equal portion of 100,000*g* supernatant of a guinea-pig liver homogenate (ref. 3) corresponding to 2·7 mg of protein, as determined by the biuret method (ref. 8), in a total volume of 2·0 ml. The reaction was stopped by adding, in the cold, trichloroacetic acid to 5 per cent final concentration. The mixture was centrifuged and the supernatant was discarded. The precipitate was redissolved in 1·0 ml. of 0·2 N sodium hydroxide and reprecipitated with 3 ml. of 15 per cent trichloroacetic acid, in the cold. This operation was repeated five times. Finally, the washed precipitate was dissolved in 1·5 ml. of 'Hyamine' (Packard Instrument Co., Inc.) and the sample was prepared for counting in the scintillation counter as before (ref. 9). After counting, an appropriate quantity of ^{14}C-labeled toluene was added to the sample (internal standard) and counting was repeated. The efficiency of counting was computed from the difference of the two counts, and on this basis the results were converted to disintegrations per minute (d.p.m.). The background counts were about 30 c.p.m.

In the experiments shown in Table 1, incorporation of radioactivity into proteins from free histamine was greater than from its ribonucleoside derivatives. However, unlike histamine and other amines (see, for example, ref. 6), histamine ribonucleoside is not deaminated in mammalian tissues[1,2,7,10]. Because deaminated compounds would not react in the transamidation system, it follows that the histamine dinucleotides and histamine ribonucleoside would probably have a greater chance than histamine of becoming attached on to proteins if found in the proper *in vivo* environment (that is, in the presence of deaminases). These preliminary observations explain qualitatively previously found quantitative discrepancies and open the route for further experimentation concerning the significance of the formation and fate of histamine dinucleotides *in vivo*.

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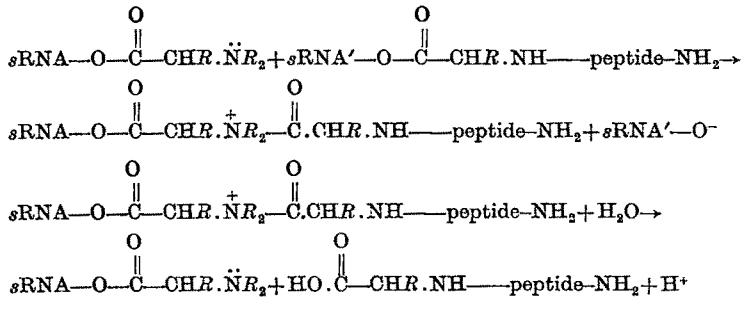
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Release of Peptide Chains from Rat-liver Polysomes by the *N*,*N*-Dimethyl-*p*-methoxy-L-phenylalanyl Analogue of Puromycin

THE synthesis of a peptide on the surface of a ribosome takes place in the presence of messenger RNA, aminoacyl-*s*RNA, transfer enzymes and guanosine triphosphate (GTP). During protein synthesis, ribosomes move along the message and become detached on completion of the peptide chain[1-4]. Release of the completed peptide chain appears to be rather complex in that (1) the peptide chain is always attached to a molecule of *s*RNA in all stages of

its growth and thus requires some special chemical or enzymatic process to release it from its *s*RNA carrier[5,6], and (ii) peptide chains are not normally released at intermediate stages of growth[4].

It appears reasonable to assume that there is a specific *s*RNA concerned with release of completed polypeptides. In this regard, Brenner[7] and his colleagues have recently provided evidence that this is the case, in their work on nonsense triplets for chain termination. If we then assume that there is a specific *s*RNA concerned with peptide chain release, we can imagine that it must carry an amino-acid of some particular type. Primary and secondary amino-acids cannot function here, as they would form stable peptide bonds with release of a polypeptide containing an additional amino-acid. However, if the releasing *s*RNA carries an amino-acid bearing a tertiary amino function, reaction can still occur, giving a quaternary linkage as shown in equations (1) and (2).



A quaternary bond of the type shown would be very unstable under aqueous conditions and would presumably be readily hydrolysed to the free peptide and tertiary aminoacyl-*s*RNA (2). It seems reasonable to suggest that a similar type of mechanism might be operating in protein synthesis.

Puromycin releases peptide chains from ribosomes at all stages of their growth by competing with and substituting for incoming aminoacyl–*s*RNA[8–11]. In this type of release, the primary amino group of puromycin forms a peptide bond with the growing peptide chain. If the aforementioned suggestion concerning peptide chain release by way of a tertiary aminoacyl–*s*RNA is feasible, it should be possible to demonstrate the release of peptide chains from ribosomes with a derivative of puromycin having its primary amino group changed to a tertiary amino function by introduction of two methyl groups. To test the above hypothesis, therefore, the *N*,*N*-dimethyl-*p*-methoxy-L-phenylalanyl analogue of puromycin (Fig. 3, I) was synthesized and its effect on protein synthesis and peptide chain release investigated.

In Table 1 are shown the results of the *N*,*N*-dimethyl-*p*-methoxy-L-phenylalanyl analogue (hereafter called *N*,*N*-dimethylpuromycin) and puromycin on protein synthesis in a system consisting of rat-liver polysomes and *p*H 5 enzyme. It is seen that the new derivative has approximately 58 per cent of the activity of puromycin (both at 5×10^{-4} M), and definitely inhibits protein synthesis. Further experiments were carried out with polysomes containing [^{14}C]-labelled peptide chains. Fig. 1 shows the effect of *N*,*N*-dimethylpuromycin on the release of peptide chains from pre-labelled polysomes. It can be seen that it has approximately 60 per cent of the activity of puromycin in releasing peptide chains at a concentration of 10^{-3} M. Further, it will be noted that increasing the concentration of the substituted puromycin above 10^{-3} M brings about no increase in its activity. *N*,*N*-Dimethylpuromycin has no effect on the integrity of polysome structure as evidenced by sucrose gradient centrifugation (Fig. 2), in conformity with the results obtained previously with puromycin[12].

The above results demonstrate that 6-dimethylamino-9-(3′-*N*,*N*-dimethyl-*p*-methoxy-L-phenylalanylamino-3′-deoxy-β-D-ribofuranosyl)-purine, a puromycin derivative bearing a dimethylamino-acid (tertiary), is capable of

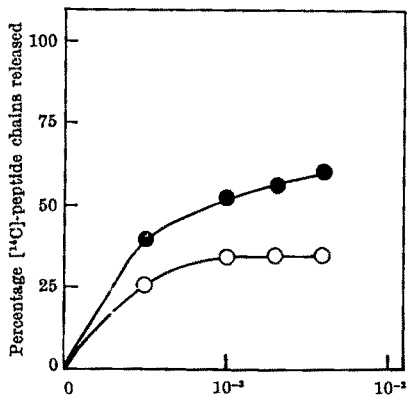


Fig. 1 Release of peptide chains from pre-labelled polysomes by the *N*,*N*-dimethyl-*p*-methoxy-L-phenylalanyl analogue of puromycin and puromycin. Polysomes were labelled in the incubation medium given in Table 1. Incubation was carried out for 10 min at 37° C. The labelled polysomes were diluted with medium *A* (0·25 M sucrose, 0·05 M *tris*-HCl (*p*H 7·6), 0·005 M magnesium chloride and 0·025 M potassium chloride) and isolated by centrifugation at 105,000*g* for 1 h. The resulting pellet was resuspended in medium *A*, and the suspension layered over 0·5 M sucrose (containing medium *A* buffer) and centrifuged at 99,000*g* for 2 h in the No. 30 rotor. The resulting pellet was taken up in medium *A*, and cleared of denatured material by low-speed centrifugation. Treatment with puromycin derivatives: aliquots of the [^{14}C]-labelled polysomes were treated with *N*,*N*-dimethylpuromycin and puromycin for 15 min at 37° C, cooled in ice, diluted with medium *A*, and then isolated by centrifugation at 105,000*g* for 1 h. They were resuspended in 0·02 M *tris*-HCl (*p*H 7·6), 0·004 M magnesium chloride, 0·05 M potassium chloride buffer (TKM buffer) and washed once again by centrifugation as described above. The pellets were finally suspended in TKM buffer and processed for [^{14}C] radioactivity by the 'Millipore' washing procedure of Nirenberg and Leder (ref. 14). Counting was carried out in the Packard scintillation spectrometer. Control polysomes gave 8,458 c.p.m./mμmole ribosome

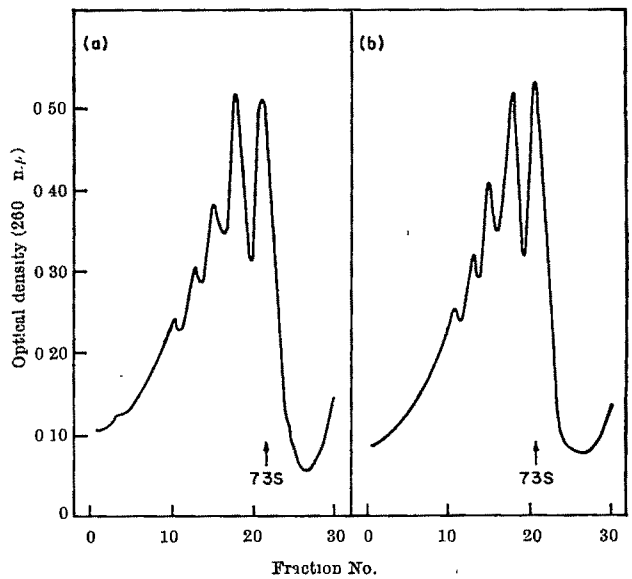


Fig. 2. Sucrose gradient analysis of polysomes pretreated with the *N*,*N*-dimethyl-*p*-methoxy-L-phenylalanyl analogue of puromycin. Polysomes (0·38 mμmoles ribonucleoprotein) in 1·0 ml. of medium *A* were incubated for 10 min at 37° C with and without *N*,*N*-dimethyl puromycin. They were cooled in ice, and then layered on to pre-cooled sucrose gradients (16–34 per cent w/v sucrose containing 0·05 M *tris*-HCl, *p*H 7·6 and 0·005 M magnesium chloride). Centrifugation was carried out in the '*SW* 25·1' swinging bucket rotor at 24,000 r.p.m. for 2·5 h. *a*, Profile of control polysomes; *b*, profile of polysomes pretreated with *N*,*N*-dimethylpuromycin (final concentration 10^{-3} M)

releasing peptide chains from polysomes in the absence of GTP and transfer enzyme. The possible chemical reactions depicting the release of peptide chains by *N,N*-dimethylpuromycin are shown in Fig. 3. We suggest that a similar type of reaction may possibly occur in normal protein synthesis with the releasing *s*RNA carrying a tertiary amino-acid.

Table 1. EFFECT OF THE *N,N*-DIMETHYL-*p*-METHOXY-L-PHENYLALANYL ANALOGUE OF PUROMYCIN AND PUROMYCIN ON PROTEIN SYNTHESIS BY RAT-LIVER POLYSOMES

Each reaction tube contained the following in a final volume of 1·1 ml.: 0·03 M *tris*-HCl (*p*H 7·6), 0·016 M potassium chloride, 0·006 M magnesium chloride, 0·14 M sucrose, ATP (3 μmoles), GTP (1·25 μmoles), phosphoenol pyruvate (12·5 μmoles, potassium salt), pyruvate kinase (60 μg), GSH (5 μmoles, *p*H 7·6), [^{14}C] yeast hydrolysate (1 μc.) (specific activity 1,200 μc./mg), polysomes (0·76 mg ribonucleoprotein), and *p*H 5 enzyme (2·6 mg protein) *N,N*-Dimethyl-*p*-methoxy-L-phenylalanyl-puromycin and puromycin were both dissolved in water and used as such. Incubations were carried out for 10 min at 37° C. Reactions were stopped by the addition of 1 ml. of 10 w/v trichloroacetic acid and the resulting precipitates washed by the procedure of Mans and Novelli (ref. 13). Labelled proteins were finally washed on to 'Millipore' filters with 5 per cent trichloroacetic acid. The filters were dried and counted for radioactivity in the Packard 'Tri-Carb' liquid scintillation spectrometer

Reaction conditions	[^{14}C]-labelled protein c.p.m /mg protein	Percentage inhibition
Control	10,860	—
Puromycin (5×10^{-4} M)	5,163	53
N,N-Dimethylpuromycin (5×10^{-4} M)	7,500	31
N,N-Dimethylpuromycin (10^{-3} M)	7,276	33

6-Dimethylamino-9-(3′-*N,N*-dimethyl-*p*-methoxy-L-phenylalanylamino-3′-deoxy-β-D-ribofuranosyl)-purine was prepared from puromycin dihydrochloride by reductive methylation (formaldehyde, hydrogen, palladium/carbon catalyst). The new compound was characterized by analysis and mass spectrometry. Details concerning its synthesis and mass spectra will be published shortly in conjunction with Dr S. H Eggers of these laboratories.

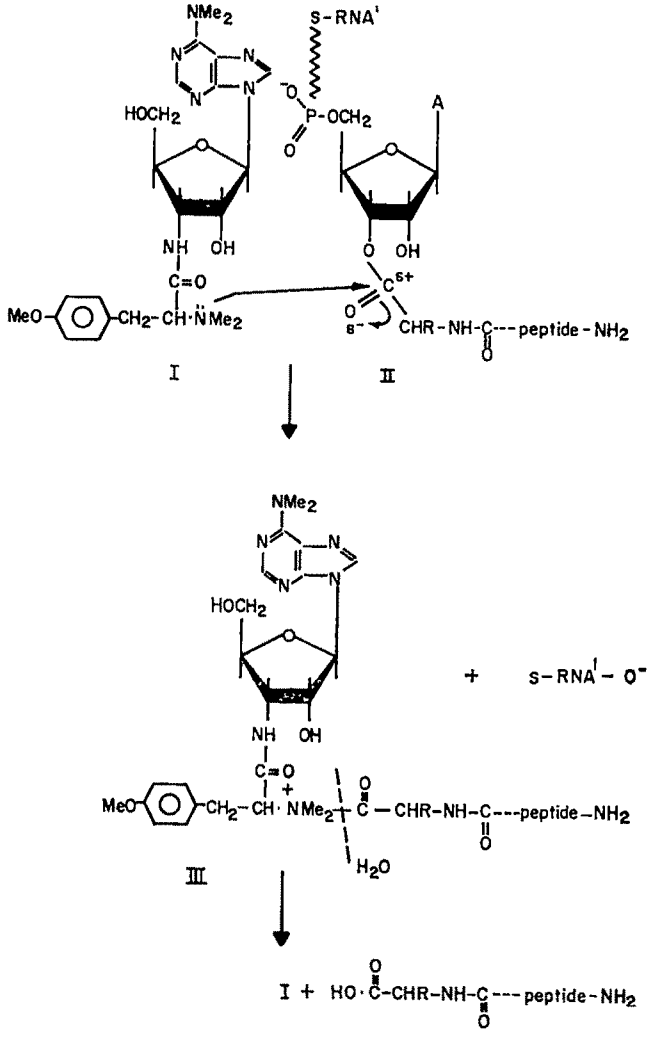


Fig. 3

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Nature of the Non-haem Iron in Ferredoxin and Rubredoxin

RECENTLY, Gillard, McKenzie, Mason, Mayhew, Peel and Stangroom[1] presented optical rotatory dispersion data on non-haem iron proteins from several sources. They found that whereas the bacterial ferredoxins from *Clostridium acidi-urici*, *C. pasteurianum* and *Peptostreptococcus elsdenii* and the rubredoxin from *P. elsdenii* have very similar optical rotatory dispersion spectra in both the oxidized and reduced forms, these spectra differ qualitatively from that obtained with oxidized plant (parsley and spinach) ferredoxin. No data were presented on reduced plant ferredoxin, and indeed it has been reported[2] that reduced plant ferredoxin has no optical activity in the visible region of the spectrum. Gillard *et al.* consequently suggested 'that the bonding of iron in plant ferredoxin is entirely different from that in bacterial ferredoxin and rubredoxin".

Recently, we obtained measurements of both optical rotatory dispersion and circular dichroism on plant ferredoxin[3] and confirmed the earlier data which Gillard *et al.* and earlier workers[2,4] had reported, with the exception that we were unable to find the trough at 330 mμ (see ref. 1, Fig. 3). Furthermore, we extended these measurements to reduced ferredoxin; in contrast to the earlier report[2], we found intense and detailed optical activity in the chemically reduced species in the visible region of the spectrum. However, this disappears slowly with a concomitant irreversible denaturation of the protein. The circular dichroism spectra indicate the presence of at least eight optically active transitions between 300 and 700 mμ in the oxidized and reduced proteins.

More pertinent, however, are some recent electron paramagnetic resonance (EPR) results which were not available to Gillard *et al.* Briefly, these can be summarized as follows. (i) Spinach ferredoxin exhibits no electron paramagnetic resonance spectrum in its oxidized form. On reduction, a spectrum typical of the $g = 1{\cdot}94$ class of iron proteins is obtained[5]. (ii) *C. pasteurianum* ferredoxin gives a variable amount of a $g = 2{\cdot}0$ signal in the oxidized form and develops strong resonance in the $g = 1{\cdot}94$ region on reduction[6]. However, whereas the line-shape of spinach ferredoxin is readily understood, that of the bacterial ferredoxin is not. (iii) *C. pasteurianum* rubredoxin exhibits a strong $g = 4{\cdot}3$ signal in the oxidized form and nothing on reduction[7,8]. Table 1 contains a summary of this and other pertinent data.

The prediction made by Gillard *et al.* on the basis of their optical rotatory dispersion results, that the binding of iron in rubredoxin should be quite similar to that of

Table 1

	Visible optical absorption		Visible optical activity		EPR* signals		Labile sulphide	Redox potential	Ref.
	Oxidized	Reduced	Oxidized	Reduced	Oxidized	Reduced			
Spinach ferredoxin	Strong	Strong	Strong	Strong	None	$g = 1.94$	Yes	−0·432	3, 5, 9
C. pasteurianum ferredoxin	Strong	Strong	Strong	Weak	$g = 2.0$ (variable)	"$g = 1.94$"	Yes	−0·42	1, 6, 10
C. pasteurianum rubredoxin	Strong	None	Strong	Weak	$g = 4.3$	None	No	−0·06	1, 7, 8, 11

* Electron paramagnetic resonance.

the bacterial ferredoxins, is not substantiated by our electron paramagnetic resonance data. The electron paramagnetic resonance spectrum of rubredoxin is of the high-spin type found with ferrichrome and transferrin[13]. However, the ferredoxins have spectra which would seem to be due to low-spin iron in tetrahedral symmetry. Furthermore, the contents of Table 1 indicate that the only satisfactory way to classify these three proteins is individually. However, as has been well known[15], the plant and bacterial ferredoxins have much more in common with each other than either has with rubredoxin; even so, the observed differences are real and cannot be rationalized at this time.

Attempts to determine the ligand environment of these proteins should not be based on the application of one experimental method alone. In particular the results of Mössbauer, magnetic resonance, optical and circular dichroism spectroscopy should all be considered in our attempts to define the molecular orbital configurations of this important new class of electron-transfer proteins.

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Chromogen Formation and Degradation of *N*-Acetylhexosamines and *N*-Acetylneuraminic Acid by Barium Hydroxide Treatment

ALKALINE degradation[1] has been used as a means of determining the structures of oligo- and poly-saccharides. Various alkalis have been used as degradation reagents. The barium hydroxide technique especially has been effectively used for the exploration of the chemical structure of the carbohydrate moieties of some sialomucoproteins[2–4]. Under the conditions of the barium hydroxide treatment, *N*-acetylglucosamine[5] and *N*-acetylneuraminic acid[2] are partly converted into chromogen I (ref. 5) and *N*-acetylhexosamine, respectively. However, none of the reactions of *N*-acetylhexosamines and *N*-acetylneuraminic acid with barium hydroxide has as yet been completely examined. The present work was undertaken to obtain more detailed knowledge of the chromogen formation and degradation of the *N*-acetylhexosamines and *N*-acetylneuraminic acid under the conditions of barium hydroxide treatment.

A solution of 20 mg of a sample in 4 ml. of 0·02 N barium hydroxide solution was heated at 80° C for 15 min and cooled. The cooled mixture was neutralized to *p*H 5·5 with 0·1 N sulphuric acid and centrifuged. The precipitate was washed with water and the washings and supernatant solution were combined. Half the combined solution was evaporated over phosphorus pentoxide in a vacuum desiccator. 0·3 ml. of water was added to the dry residue and the mixture centrifuged. The supernatant solution was subjected to the two-dimensional paper electrophoresis and chromatography as described in a previous article[6]. 0·5 ml. of 2 N sulphuric acid and 0·25 ml. of 2,4-dinitrophenylhydrazine reagent[7] was added to the other half of the combined solutions. The mixture was allowed to react overnight at 37° C and diluted to 20 ml. with water and then shaken with three successive portions of ethyl acetate—10, 5 and 5 ml. respectively. The combined solvents were washed with water and extracted with three 10-ml. portions of 3 per cent sodium bicarbonate. The solvent was washed with water and evaporated. The dry residue was designated as the neutral carbonyl compound fraction. The combined sodium bicarbonate extracts were acidified with 4 N sulphuric acid and re-extracted with three 10-ml. portions of ethyl acetate, and the solvents were washed with water and evaporated. The dry residue was designated as the keto acid fraction. The two fractions were subjected to ascending paper chromatography. (The solvent used for 2,4-dinitrophenylhydrazones of aliphatic carbonyl compound was methanol-hexane (1 : 1) (ref. 8). The solvents for keto acid 2,4-dinitrophenylhydrazones were *n*-butanol saturated with 3 per cent aqueous ammonia[9,10] and *n*-butanol-ethanol-water (5 : 1 : 4) (ref. 10).)

The results of the barium hydroxide treatment of *N*-acetylglucosamine, *N*-acetylmannosamine and *N*-acetylgalactosamine followed by two-dimensional separation of the reaction products are summarized in Table 1. Spots 1, 3, 4, 9 and 10, produced from the three *N*-acetylhexosamines, were classed as chromogens on the basis of their reactivities in colour tests 1 (the indirect Ehrlich reaction)[6] and 2 (the direct Ehrlich reaction)[6]. The chromogens appear to be furan derivatives, since these violet or pink colours fade within 1–2 h. Of these furan chromogens, spot 3 was the main chromogen at all times and identified as chromogen I (ref. 5) by its electrophoretic and chromatographic mobilities (Table 2). Spots 2, 5 and 7 were identified as *N*-acetylglucosamine, *N*-acetylgalactosamine and *N*-acetylmannosamine, respectively, on the basis of their reactivities in colour tests 1 and 2 and their mobilities. Spots 6 and 8 were unknown 2-acetamido-2-deoxy-aldoses. The results show that under the conditions of barium hydroxide treatment, the main reaction products of the three *N*-acetylhexosamines are furan chromogens which include chromogen I. Each *N*-acetylhexosamine gave an unidentified 2-acetamido-2-deoxy-aldose as a by-product and reversible epimerizations of *N*-acetylglucosamine and *N*-acetylmannosamine occur simultaneously as side reactions.

After *N*-acetylneuraminic acid was treated with barium hydroxide, the reaction products obtained were subjected to two-dimensional paper electrophoresis and chromatography. The results are shown in Table 3. Since spots 6–9 from *N*-acetylneuraminic acid gave pink violet colours which were stable for several days, these spots are likely to be *N*-acetylneuraminic acid and its derivatives, or pyrrole compounds derived from the acid by the barium hydroxide treatment. Of these spots, numbers 7 and 9 were identified as *N*-acetylneuraminic acid and pyrrole-2-carboxylic acid[11], respectively, by their electrophoretic and chromatographic mobilities. Spots 2, 5

Table 1. TWO-DIMENSIONAL PAPER ELECTROPHORESIS AND CHROMATOGRAPHY (REF. 6)* OF THE REACTION PRODUCTS OF THREE *N*-ACETYLHEXOSAMINES WITH BARIUM HYDROXIDE

Sample	Colour test	Colour and mobility	Spot separated on paper electropherochromatogram 1	2	3	4	5	6	7	8	9	10
Treated *N*-acetyl-glucosamine	1	Colour	l-P	V	int-V	int-V			l-V	l-V	l-P	l V
		EM	−1·4	1·8	2·5	2·7			7·2	7·3	7·3	7 6
		CM	16 7	8·9	12·8	0·5			4·6	0·3	2·4	12·7
	2	Colour	l-P		int-V	V					l-P	l-V
		EM	−1·4		2·6	2·8					7·3	7·6
		CM	16 3		12·7	0·5					2·0	12 5
Treated *N*-acetyl-mannosamine	1	Colour	P	l-V	int-V	int-V			l-V	l-V	l-P	f-V
		EM	−1·7	1·8	2·6	2·9			7·1	7·3	7·3	9·5
		CM	17·0	8·8	12·9	0·0			4·6	0 0	1·7	13·0
	2	Colour	l-P		int-V	l-P					l-P	f-V
		EM	−1·6		2·5	2·7					7·3	7·7
		CM	16 2		12·3	0·2					1 7	12·6
Treated *N*-acetyl-galactosamine	1	Colour	l-P		int-V		l-V	l-V				
		EM	−1 7		2·6		3·7	7·0				
		CM	16·3		12·9		5 0	3·6				
	2	Colour	l-P		int-V							
		EM	−1·6		2·6							
		CM	17·0		12·6							

* Whatman No. 3 *MM* paper strip (ref 6). Buffer for the first dimension (paper electrophoresis): 0 025 M sodium tetraborate, 200 V, 6 h. Solvent for the second dimension (paper chromatography): *n*-butanol–pyridine–water (2 : 2 · 1), 4–5° C, 16 h. Colour test 1 (refs. 6, 16): the indirect Ehrlich reaction. Colour test 2 (refs 6, 16): the direct Ehrlich reaction. EM, Electrophoretic mobility (cm); CM, chromatographic mobility (cm). All spots, except spot 1, were migrated to the anode f, Faint; int, Intense; l, light; P, pink; V, violet.

Table 2. TWO-DIMENSIONAL PAPER ELECTROPHORESIS AND CHROMATOGRAPHY OF REFERENCE COMPOUNDS

Reference	Colour and mobility		Reference	Colour and mobility	
	Colour	int-V		Colour	V
N-acetyl-glucosamine	EM	1·8	Chromogen I	EM	2 6
	CM	9·0		CM	12·6
	Colour	int-V		Colour	V
N-acetyl-mannosamine	EM	7·2	*N*-acetyl-neuraminic acid	EM	10·0
	CM	4·6		CM	0·0
	Colour	V		Colour	P
N-acetyl-galactosamine	EM	3·7	Pyrrole-2-carboxylic acid	EM	13·2
	CM	4·8		CM	8·7

Abbreviations as for Table 1.

Table 3. TWO-DIMENSIONAL PAPER ELECTROPHORESIS AND CHROMATOGRAPHY OF THE REACTION PRODUCTS OF *N*-ACETYLNEURAMINIC ACID WITH BARIUM HYDROXIDE

Sample	Colour test	Colour and mobility	Spot separated on paper electropherochromatogram 1	2	3	4	5	6	7	8	9
Treated *N*-acetyl-neuraminic acid	1	Colour	l-P	V	V	f-V	f-V	l-PV	l-PV	f-PV	f-PV
		EM	−1·4	1·7	2 6	2·6	7·2	9·0	10 0	12·8	13·4
		CM	16·2	8·7	12·8	0·0	4·5	2 0	0·3	0·0	8·6
	2	Colour	l-P		V	Very f-V		l-PV	l-PV	f-PV	f-PV
		EM	−1·6		2 7	2 5		9·1	10 3	13·0	13·2
		CM	16·5		12·6	0·0		1·8	0·0	0 0	8 6

Abbreviations as for Tables 1 and 2.

and 3, produced from *N*-acetylneuraminic acid, were identified as *N*-acetylglucosamine, *N*-acetylmannosamine and chromogen I, respectively, by their mobility and by their reactivities in colour tests 1 and 2. Paper chromatography of the neutral carbonyl compound and keto acid fractions obtained from the *N*-acetylneuraminic acid by the barium hydroxide treatment showed that *N*-acetylneuraminic acid produced pyruvic acid and acetaldehyde. These results indicate that under the conditions of the barium hydroxide treatment, *N*-acetylneuraminic acid is degraded into two groups, one of which consists of *N*-acetylmannosamine, *N*-acetylglucosamine, an unidentified 2-acetamido-2-deoxy-aldose, furan chromogens, including chromogen I, pyruvic acid and acetaldehyde, and the other is pyrrole chromogens, including pyrrole-2-carboxylic acid. Since it is clear[12] that the *N*-acetylhexosamine fragment present in the *N*-acetylneuraminic acid molecule is *N*-acetylmannosamine, *N*-acetylglucosamine formed in the degradation of the *N*-acetylneuraminic acid by the barium hydroxide treatment seems to be an epimerization product of the *N*-acetylmannosamine which derived from the acid by the treatment.

These findings indicate that in general the chromogen formation and epimerization of the *N*-acetylhexosamines in barium hydroxide solution are similar to those of the *N*-acetylhexosamines in sodium hydroxide[13], carbonate[5] and pyridine–nickelous acetate[13] solutions. Furthermore, the degradation of *N*-acetylneuraminic acid in barium hydroxide solution to *N*-acetylmannosamine, *N*-acetylglucosamine and pyruvic acid is similar to that of *N*-acetylneuraminic acid in sodium hydroxide[15] and pyridine–nickelous acetate[13] solutions. The reactions of the three *N*-acetylhexosamines and *N*-acetylneuraminic acid with barium hydroxide can be depicted as in Fig. 1.

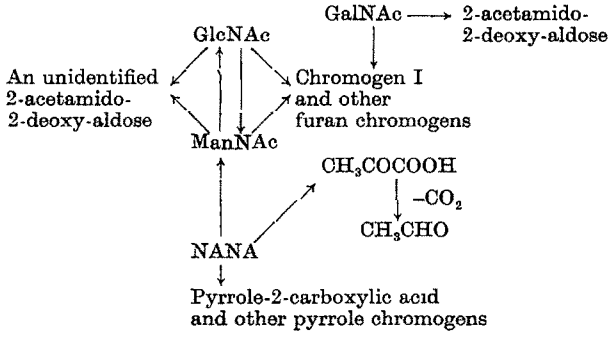


Fig. 1. Chromogen formation and degradation of *N*-acetylhexosamines and *N*-acetylneuraminic acid by barium hydroxide treatment. GlcNAc, *N*-acetylglucosamine; ManNAc, *N*-acetylmannosamine; GalNAc, N-acetylgalactosamine; NANA, N-acetylneuraminic acid

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Bone Marrow Sulphydryl Levels

DURING the course of other investigations the sulphydryl levels of rat bone marrow have been measured. Unlike other tissues examined, the bone marrow was found to show a high protein-bound sulphydryl level and a relatively low acid-soluble sulphydryl level. The investigation was extended to determine whether this unusual distribution of sulphydryl was a feature of other bone marrows.

All animal bone marrows were forced out of the femora of freshly killed animals by air pressure. The human specimens were obtained by sternal puncture. Estimations of total sulphydryl were made by the method of Calcutt and Doxey[1] using *p*-chloro-mercuribenzoic acid as the reactant. Acid-soluble sulphydryl was determined by the variation of the above method devised by Calcutt, Doxey and Coates[2], using trichloroacetic acid as the protein precipitant. The difference between the total sulphydryl and the acid-soluble sulphydryl levels as determined on two samples of the same marrow was taken as representing the protein-bound sulphydryl value.

The results for the animal experiments are shown in Table 1. It was noted that the rabbit marrow was extremely fatty in comparison with the others, and this may partly explain the lower total and protein-bound values obtained. It was also found that, proportionate to their size and weight, rats of the Wistar strain have considerably larger amounts of femoral marrow than do rats of the August strain.

Findings in respect of the human cases are given in Table 2. These samples were all taken from hospital patients (diagnoses are shown in the Table), and the possibility of the results being affected by the illness cannot be excluded. Nevertheless, the same general feature of high protein-bound sulphydryl and low acid-soluble sulphydryl is apparent.

The values for acid-soluble sulphydryl found in this work were lower than those previously obtained from animal livers but rather higher than those found for muscle, kidney, spleen or a range of animal tumours[3]. The present values are, however, considerably lower than those previously recorded by Lutwak-Mann for rat marrow (150–198 mg/100 g)[4]. This may arise from the fact that these last values were obtained by the non-specific iodometric method. An earlier investigation[5] gave 0·5 mg/100 g of glutathione for rabbit marrow. This is much lower than the present figures but, as no details of method were given, comment is not possible.

The protein-bound sulphydryl levels found in the present work are much higher than any previously found for other tissues. Liver has shown the highest values for normal tissues with levels up to about 7·5 μg sulphydryl per 100 mg wet weight[6], while one tumour has given 10·65 μg sulphydryl/100 mg wet weight[7]. This raises the question as to why bone marrow should have such unusually high levels of protein-bound sulphydryl. Possible explanations are: (1) marrow cells have protein with more than the normal number of cysteine residues; (2) the marrow cell protein has a greater than usual exposure of sulphydryl groups; or (3) the marrow cells contain much more protein than do other cells. Some further evidence is available in measurements of the total sulphydryl levels in denatured specimens of August bone marrow. A 1 per cent solution of 'Teepol' (Shell Chemical Co., Ltd.) was used to denature the homogenized marrow, this method having previously[6] been found to be a very effective one. Using marrow from six male rats, a mean value of 35·8 μg sulphydryl per 100 mg wet weight of tissue and a range of 24·8–50·9 were obtained. This value is only a little above the total sulphydryl values for male August rats and suggests that in bone marrow most of the protein-bound sulphydryl is normally exposed. This contrasts with previously[6] obtained values for liver and tumours where only one-twelfth to one-thirtieth of the protein-bound sulphydryl was normally found exposed.

These findings have a particular interest in respect of the great sensitivity of bone marrow to certain cytotoxic agents. It has already been suggested by Calcutt and Connors[7] that sensitivity to alkylating agents is correlated with a high ratio of protein-bound to acid-soluble sulphydryl. It now appears that bone marrow has such a high ratio and in fact one which is higher than for any other tissue so far examined. At the same time bone marrow sensitivity to alkylating agents is a limiting factor in their use.

Table 1. LEVELS OF SULPHYDRYL IN ANIMAL BONE MARROWS

Species	Sex	No. of samples	Total sulphydryl	Acid-soluble sulphydryl	Protein-bound sulphydryl
Rat (August)	M	10	29·9 (23·2–36·0)	5·9 (4·1–9·9)	24·0 (16·7–30·1)
Rat (August)	F	10	33·5 (29·3–39·0)	5·8 (2·3–8·4)	27·6 (21·6–33·6)
Rat (Wistar)	M	6	45·7 (40·6–52·3)	9·5 (6·0–13·1)	35·0 (28·6–44·5)
Rat (Wistar)	F	6	44·8 (31·1–53·7)	10·8 (7·7–14·0)	34·0 (19·7–41·3)
Guinea-pig	M	6	35·6 (28·9–46·1)	10·2 (7·6–12·3)	26·9 (18·5–35·4)
Rabbit	M	5	14·9 (12·3–16·2)	6·8 (3·5–9·5)	8·1 (6·1–9·8)

For each value the mean and range (in parentheses) are shown. All values are expressed as μg sulphydryl/100 mg wet weight of tissue.

Table 2. LEVELS OF SULPHYDRYL IN HUMAN BONE MARROW

Sex	Age	Diagnosis	Total sulphydryl	Acid-soluble sulphydryl	Protein-bound sulphydryl
M	57	Carcinoma of stomach	12·3	1·6	10·7
M	68	Lymphosarcoma	10·2	1·8	8·4
M	39	Melanoma	17·1	1·9	16·2
M	78	Mycosis fungoides	23·7	3·5	20·2
F	62	Plasmacytoma	15·9	1·3	14·6
F	47	Thyroid enlargement	18·6	3·1	15·5
F	20	Disseminated lupus erythematosus	9·2	2·1	7·1
F	78	Carcinoma of colon	9·4	1·1	8·3

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Normal Human Serum Fluoride Concentrations

RESULTS from a new method of analysis of fluoride from serum indicate that the generally accepted value for normal humans is too high by as much as a factor of ten. The average serum fluoride concentration of sixteen non-fasting individuals, all presumably drinking fluoridated water, was found to be 0·7 μM ± 0·4 *S.D.* (0·013 p.p.m.) and the average difference in duplicates was 0·18 μM. (The very low values found here for serum fluoride make p.p.m. an awkward unit. Therefore the shift has been made to the more useful and acceptable chemical concentration terms, mμmole (millimicromole) and μM (micromolar).) The generally accepted average serum fluoride value is 8 μM (0·15 p.p.m.) as found by Singer and Armstrong, regardless of the concentration of fluoride in the drinking water up to 130 μM (2·5 p.p.m.)[1].

The method is being published in detail elsewhere, but essential features are diffusion at 25° C from 3 N hydro.

chloric acid and an indicator which depends on the loss of fluorescence of a morin thorium complex. As little as 1 mμ mole of fluoride (0·02 μg) in a volume of 3 c.c. is able to reduce the fluorescence 50 per cent. Diffusion of the hydrogen fluoride into 0·1 N sodium bicarbonate is enhanced by means of a rotary shaker. The diffusion dishes are non-wettable polystyrene ('Falcon') with the septum in the outside compartment partially removed and the lids sealed with a silicone grease.

The crucial difference in this procedure as compared with that of Singer and Armstrong lies in the conditions for the diffusion process. Sera from six adults, run under conditions similar to those of Singer and Armstrong[2] (diffusion from 3 N perchloric acid to 2·5 N sodium hydroxide at 60° C), were found to have apparent fluoride values within the range that they find, 6–10 μM. One of these samples was then tested for heat stability using fluorine-18 as a control. After diffusion the sodium hydroxide solution was converted to a mixed carbonate–chloride salt which was fused in platinum crucibles over a burner. Blanks and standards were similarly treated and all were labelled with fluorine-18. Results showed only about a 10 per cent loss of fluorine-18, but there was a 90 per cent loss of apparent fluoride. Quadruplicate values for the non-fused serum samples ranged from 9 to 11 μM while the fused samples ranged from 0·9 to 1·7 μM (figures corrected for the loss during fusion). Standards treated the same way showed a 10 per cent loss of both fluorine-18 and fluorine-19. Serum samples diffused by the new method and then fused showed a 10 per cent loss of fluorine-18 and little or no loss in measured fluorine-19.

There has been other evidence, largely ignored, that the serum fluoride values reported by Singer and Armstrong are too high. Their results are most easily explained by a hypothesis that their serum fluoride values are too high by some large relatively constant error. The average serum fluoride values they report are the same whether the individuals are drinking 5 μM (0·1 p.p.m.) or 130 μM (2·5 p.p.m.) fluoride in the water[1]. This seems unreasonable, because with this difference of fluoride intake the incidence of dental mottling increases from 0 to 90 per cent[3] and the average bone[4] and the urine[5] fluoride concentrations change several-fold.

Data from their study on the clearance of radioactive fluoride, if applied to the serum fluoride values determined at the same time[6], indicate that the urine fluoride concentrations went as high as 800 μM (15 p.p.m.). Based on the amount of ingested fluoride and water this figure would have been expected to be less than 150 μM (3 p.p.m.) (ref. 7). The most likely explanation again seems to be that the serum value is in error by a factor of at least five.

Hall, using a new colorimetric method, which appears to be sensitive enough to detect serum fluoride-levels of about the magnitude that Singer and Armstrong claim, states that he rarely finds detectable amounts[8].

Smith, Gardner and Hodge in 1950 found average non-fasting blood fluoride-levels of 1·8 μM ± 1·2 *S.D.* (0·04 p.p.m.) in a community on fluoridated water[9]. Their method was cumbersome and they had to apply a correction factor due to losses occurring during the double distillation and ashing of the 50 c.c. of serum[10], but the work should receive more consideration than it has, because it is the only study to show a difference in serum fluoride concentrations of those living on fluoridated water (1·8 μM) as compared with those on non-fluoridated water (0·7 μM, '*P*' value of the difference given as 0·010). The range of values they found was 0–4 μM (0·072 p.p.m.). The difference between their average value and that reported here probably is due to analytical problems but may be due to sampling. The most likely reasons for sampling problems are a difference in the ages of the subjects and inclusion of subjects with sub-clinical kidney or bone disease. The group here averaged 24 years of age with a range of 17–40; their group averaged 42 years of age with a range of 27–53 (ref. 9).

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Effect of Arabolic Androgens on Tissue Replacement in the Ear of the Rabbit

IT was recently reported that, when tissue of 1 cm² area was excised through the full thickness of the pinna of the rabbit's ear, regeneration occurred. Furthermore, the rate of regeneration was significantly greater in males than in females[1,2]. It was suggested that the rate of regeneration might be influenced by male sex hormones. If this were so, then treatment of females with androgens should increase the rate of regeneration. Two androgens were used in experiments to test this hypothesis: (*a*) androst-4-en-17β-3-one-phenyl-propionate (testosterone phenylpropionate—TPP)—a steroid with highly androgenic and some anabolic activities, closely allied to the naturally occurring steroid testosterone; (*b*) 19-*nor*-androst-4-en-17β-3-one-phenyl-propionate (nandrolone phenylpropionate, NPP, or 'Durabolin')—a steroid with less androgenic but more anabolic activity than testosterone phenylpropionate, and therefore more suitable for the treatment of females.

Tissue of 1 cm² was removed from the ears of adult male and female rabbits under 'Nembutal' anaesthesia. Four groups of animals were given subcutaneous injections as follows: (1) six females, 25 mg TPP/3 kg body-weight, twice weekly; (2) six females, 1 mg TPP/3 kg body-weight, five times weekly; (3) six females, 17·5 mg NPP/3 kg body-weight, twice weekly; (4) six females, 1 mg NPP/3 kg body-weight, five times weekly. Group 5 (twelve females) and group 6 (eighteen males) were left untreated. The steroids injected were dissolved in sesame oil, to which 0·5 per cent phenol had been added as a bacteriostatic.

Photographs, taken every 7 days, recorded the progress of regeneration. The area of the new tissue and the degree of contraction were determined from transparencies by the 'square-counting' method described in a previous communication[1]. Growth measurements were commenced at 14 days, after shedding of the scab had revealed the junction between old and new tissue.

The rates of growth in area of the regenerates after treatment with TPP are shown in Fig. 1. In this, and in Fig. 2, each point on the graph represents the arithmetic mean of measurements at a particular time for all the members of a group, and the mean growth recorded was arrived at after correction for contraction. By means of analyses of variance it can be shown that treatment of female animals with TPP (groups 1 and 2) increases the growth of ear tissue significantly ($P < 0·005$ in both cases) in comparison with the amount of growth in un-

treated females (group 5). After 21 days there is a close similarity between the growth curves for untreated males (group 6) and for females treated with TPP at a high dose-level (group 1). This similarity may be coincidental, but it seems to indicate that the higher rate of tissue regeneration normally found in males is, in fact, due to their androgen-level.

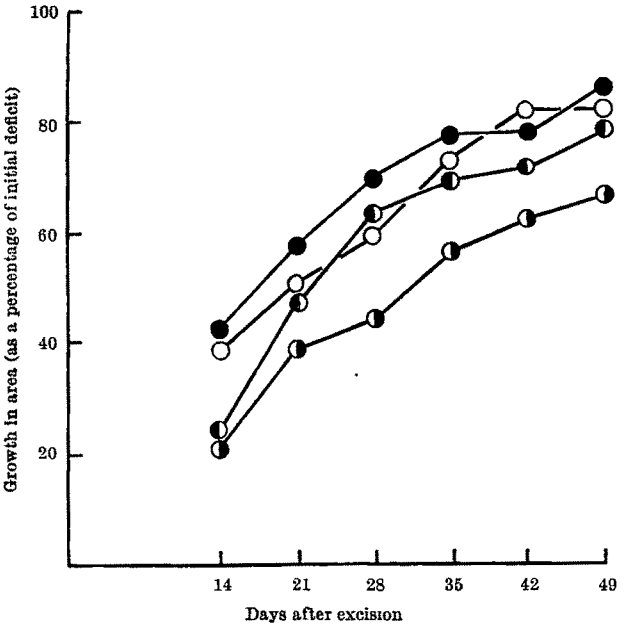


Fig. 1. Comparison of the rates of growth of the ear regenerates of TPP-treated animals and those of untreated animals. ●, High TPP female; ○, low TPP female; ◐, untreated male; ◑, untreated female

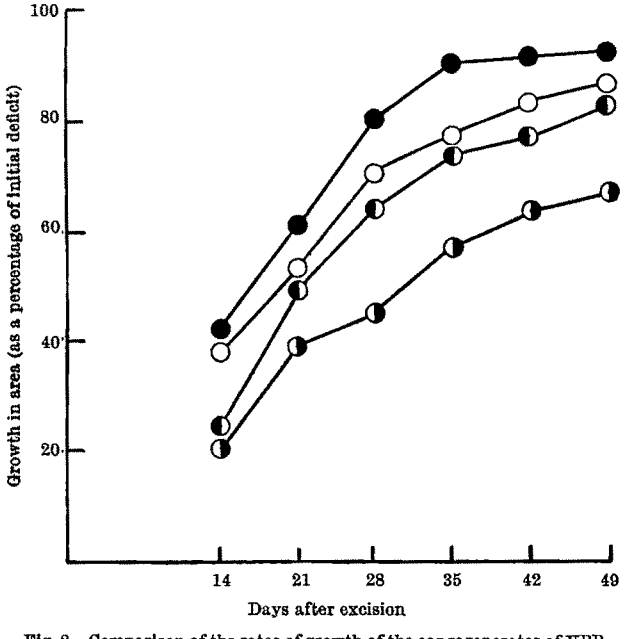


Fig 2. Comparison of the rates of growth of the ear regenerates of NPP-treated animals and those of untreated animals. ●, High NPP female; ○, low NPP female; ◐, untreated male; ◑, untreated female

The rates of growth of the regenerates after treatment with NPP are shown in Fig. 2. Analyses of variance show that treatment of female animals with NPP (groups 3 and 4) increases the growth of ear tissue significantly ($P < 0.005$ in both cases) in comparison with growth in untreated females (group 5). The effect of a low dose-level of NPP on the rate and extent of growth of the tissue regenerate of females closely resembles the effect of a high dose-level of TPP. The effect of NPP at a high dose-level was shown by analyses of variance to be significantly greater than the effect of TPP at either high or low dose-levels ($P < 0.005$ in both cases), and this, together with its lower androgenicity, makes it a more suitable growth-stimulating agent. The growth curves of untreated males, for females treated with low doses of NPP, and for females treated with high doses of TPP, are all very similar. Treatment with either steroid causes a steepening of the gradient of the linear portion of the growth curve (indicating a rate increase) and the point of inflexion occurs earlier than in untreated animals.

The following conclusions can be drawn from these experiments: (1) Androgens stimulate the rate of tissue regeneration in females. (2) It can be assumed that the higher rate of growth in males as compared with females is due to the higher androgen-level in the former. (3) Nandrolone phenyl propionate is more effective than testosterone phenyl propionate in increasing the rate of regeneration in females.

As a further test of validity of this hypothesis, the effect of lowering the androgen-level on the reparative rate in males is being investigated.

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Protective Effect of Some Neutral Solutes on the Inactivation of Myosin A-Adenosinetriphosphatase

MYOSIN-ATPASE is a labile enzyme the activity of which is easily destroyed by various physical or chemical operations during preparation such as heating[1-6], freezing and thawing[7], lyophilizing[8], and shifting the *p*H of the solution to the acid or alkaline side[6,9,10]. Hence, the only means of storage of this enzyme after preparation has been to keep it a little above 0° C and at a neutral *p*H. Under these conditions, it retains its activity for a few weeks.

Recent developments in methods of deep-freezing and lyophilization have made possible the storage of various biological materials while maintaining structural and functional integrity. It has been found empirically that several labile substances from living cells or tissues maintain their original biological functions through processes of freezing and thawing or lyophilization by the addition of certain inert organic compounds[7,11-14]. Though no explicit study has yet been undertaken, myosin also has been shown to behave in a similar way[8]. This communication reports investigations on the protective effect of some organic compounds containing hydroxyl group(s) (sugars and alcohols) on the inactivation of myosin A-ATPase caused by warming, freezing and thawing, lyophilization, and [H^+].

In Table 1 are illustrated the changes in the activity of myosin A before and after incubation at 35° C and *p*H 7·0 for 30–60 min in the presence of some sugars and alcohols. All compounds tested, except methyl alcohol and ethylene glycol, showed a protective effect against thermal inactivation of the enzyme. Although considerable inactivation

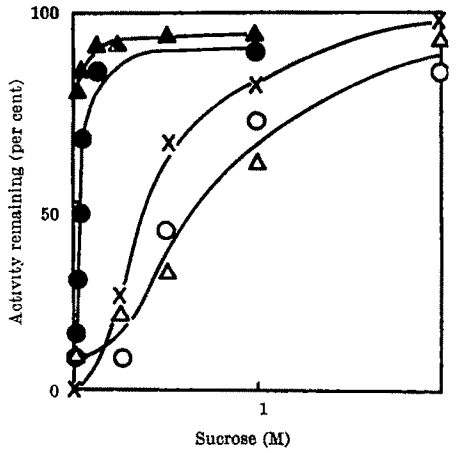


Fig. 1. Protective effect of sucrose on the inactivation of myosin A–ATPase activity. Freezing and thawing (▲), myosin A (2·0 mg/ml.) in 0·5 M potassium chloride, 50 mM *tris*-maleate buffer at *p*H 6·0 and 0–1·0 M sucrose was stored in a deep-freezer at −15° C for a week and thawed in a bath at 20° C within 5 min. Lyophilization (●), myosin A(~10 mg/ml.) in 0·5 M potassium chloride, 20 mM *tris*-maleate buffer at *p*H 6·0, and 0–1·0 M sucrose was lyophilized and stored at −15° C. When used, the samples were dissolved by distilled water in a volume equal to the original and equilibrated overnight at 3° C. Incubation at 35° C and *p*H 7·0 for 90 min. (△), Incubation mixture contained 2 mg/ml. myosin A 0·5 M potassium chloride, 20 mM *tris*-maleate buffer, and 0–2 M sucrose. Incubation at *p*H 6·0 and 3° C for 18 h. (○), Incubation mixture contained 2·0 mg/ml. myosin A, 50 mM *tris*-maleate buffer, 0·5 M potassium chloride and 0–2 M sucrose. Incubation at *p*H 10 3 and 3° C for 18 h. (×), Incubation system was the same as at *p*H 6 0 except that 50 mM glycine-potassium hydroxide buffer was used in place of *tris*-maleate buffer. All ATPase activity measurements were conducted in the following mixture at 21° C and *p*H 7·0: 0·5 M potassium chloride, 50 mM *tris*-maleate buffer, 5 mM calcium chloride, 1 mM ATP, 0·2 mg/ml. myosin A, and less than 0·2 M sucrose. P_i was determined by the same method as described in Table 1. Myosin A was prepared by the method of Perry (ref. 17)

of the enzyme was observed before incubation at 35° C when the activities were measured in the reaction system with high concentrations of the sugars and alcohols, the apparent inactivation was almost completely reversed, except in the case of methyl alcohol, as the concentrations of these organic compounds in the system were diminished by dilution. The same reversibility also held in the case of sucrose, fructose and glycerol even after incubation for 30 and 60 min at 35° C. Then the reversed activities were very close to those before thermal treatment, while a large percentage of the enzymatic activity of the control was lost by the same treatment.

On the other hand, despite its ability to cause the reversible inactivation of myosin A-ATPase before incubation, ethylene glycol has no influence over the thermal denaturation of the enzyme. The activity destroyed by incubation at 35° C is no longer reversible in this case. Since it has been suggested by Brahms and Kay[15,16] that the unfolding of the protein helical structure is intimately involved in the changes of the ATPase activity of myosin A examined in varying concentrations of ethylene glycol, the action of this alcohol differing from those of glycerol and the sugars to thermal inactivation of myosin A-ATPase may originate from this effect of ethylene glycol on the protein structure.

Fig. 1 summarizes the effects of sucrose on inactivation of myosin A. It protects against inactivation of myosin A by high (10·3) and low (6·0) *p*H, freezing and thawing, lyophilization, and heat. The differences in concentrations of sucrose necessary for protection against inactivation by *p*H and heat and inactivation by lyophilization and freezing and thawing may possibly be due to concentration of the solutions during freezing. The mechanism of the protective effect of these organic compounds on the inactivation of myosin A–ATPase is not yet known. Further investigations are in progress and will be published elsewhere.

Table 1. EFFECTS OF SUGARS AND ALCOHOLS ON MYOSIN A–ATPASE ACTIVITY BEFORE AND AFTER INCUBATION AT 35° C AND *p*H 7 0

Conc. of sugars or alcohols during incubation (M)		Conc. of sugars or alcohols in reaction mixture (M)	ATPase activity (μmoles P_i g^{-1} sec^{-1}) Before incubation	After incubation
Control	0	0 0	5 78	0·96*
Sucrose	2	2 0	0 62	0 62*
		1 0	2·93	2·76*
		0 2	5 58	5 58*
				5 49†
Fructose	2	2·0	0·56	0 56*
		0·2	5·65	5·41*
Glycerol	4	4·0	4 00	3·28†
		0·4	5·25	4·00†
Ethylene glycol	8	8·0	3·43	0·50†
		0·8	5·20	0 25†
	4	0·4	5·60	0·96 *
Methyl-alcohol	8	8·0	0·39	0 11†
		0·8	3·83	0·11†

* Incubation for 30 min. † Incubation for 60 min. Incubation system: 0·5 M potassium chloride, 20 mM *tris*-maleate buffer, and 2·0 mg myosin A/ml. Reaction system: 0·5 M potassium chloride, 5 mM calcium chloride, 20 mM *tris*-maleate buffer, 1 mM ATP, and 0·2 mg myosin A/ml. At 21° C and *p*H 7·0.

Both systems contained various amounts of organic compounds as indicated in Table 1. The ATPase reaction was stopped by addition of trichloroacetic acid in a final concentration of 5 per cent; P_i was determined by the method of Fiske and Sabbarow.

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Effect of Insulin on Purified Creatine Phosphokinase

THE effects of insulin can be readily demonstrated on a variety of metabolic processes in intact cells both *in vivo* and *in vitro*; however, the exact site of insulin action remains unexplained. Numerous investigators have proposed that the effects of insulin are mediated through its action on one enzyme or one enzyme system, but no such action has been consistently demonstrable. During an investigation involving the effects of insulin on certain aspects of creatine metabolism, I have found that insulin enhances the activity of a purified enzyme, creatine phosphokinase, *in vitro*. The reaction involved is the following:

$$\text{Creatine-PO}_4 + \text{ADP} \xrightarrow[\text{Mg++}]{\text{Insulin}} \text{creatine} + \text{ATP}$$

The physiological significance of this observation requires further enquiry; however, this *in vitro* effect is easily and consistently demonstrated.

The insulin effect was observed under a wide range of conditions. In most experiments 1·0 ml. of the following incubation mixture was used.

Creatine phosphokinase	About 1·0 μg/ml
Tris buffer	0·05 M
Creatine phosphate	0·02 M
Adenosine diphosphate	0·02 M
Magnesium acetate	0·02 M
Cysteine	0·02 M

The creatine phosphokinase was purified rabbit enzyme obtained from C. F. Boehringer und Soehne *G.m.b.H.*, Mannheim, Germany, or from Sigma Chemical Co., St. Louis, Missouri. Insulin was obtained from Eli Lilly and Co., Indianapolis, Indiana, or from E. R. Squibb and Sons, New York. The insulin was diluted to the required concentration with buffer before it was added to the incubation mixture. The entire mixture was prepared with the exception of the adenosine diphosphate (ADP), and the reaction was initiated by addition of the latter. A blank was included for each test and was identical to the incubation mixture except for the substitution of water for ADP. The incubation was carried out at 37° C and *p*H 7·40 for 10 min. After incubation, magnesium was chelated with a 10 per cent excess of ethylenediamine tetraacetic acid (EDTA), a barium hydroxide–zinc sulphate filtrate was made, and creatine was determined by a method previously described[1,2]. Subtraction of the blank value from the test value gave the creatine formed from creatine phosphate by the action of creatine phosphokinase. Fig. 1 illustrates the dose-response curve obtained under the described conditions.

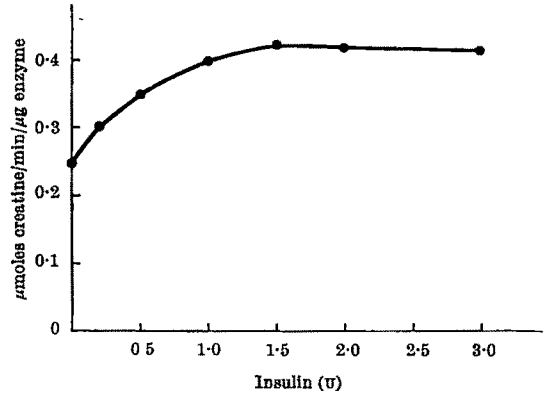


Fig. 1. The effect of varying amounts of insulin on creatine formation by purified creatine phosphokinase

An extensive series of experiments was undertaken in an attempt to rule out artefactitious causes for the observed effect. These experiments provided the following information: (1) Insulin after being heated with 1·0 N sodium hydroxide for 30 min was inactive. (2) Creatine phosphokinase and insulin from two independent sources gave similar results. (3) Zinc at concentrations found in insulin and at higher concentrations either had no effect or inhibited the reaction. (4) Glycerol and phenol, which were the preservatives in the insulin used, were without effect at the concentrations present. (5) Addition of purified bovine albumin instead of insulin had no effect. (6) None of the constituents in the incubation mixture except creatine had any effect on the reaction used for quantitation. Magnesium produced a slight interference but was removed with EDTA before measurements were made of the creatine. (7) No effect of insulin on the reverse reaction (ATP + creatine → ADP + creatine-phosphate) could be demonstrated.

The insulin effect was demonstrated in numerous experiments involving several hundred incubation mixtures. The enhancement of creatine phosphokinase activity usually represented a 60–100 per cent increase in rate of product formation. It is of interest to note that cysteine and other sulphydryl compounds have a similar activating effect on the forward reaction but not the reverse reaction[3]; however, it appears unlikely that the sulphydryl groups *per se* on the insulin chains are responsible for the observed effect as there is a several-thousand-fold excess of sulphydryl groups already present in the incubation mixture. Cysteine is not necessary in the incubation mixture for demonstration of the insulin effect, but its addition results in closer replicates and more linear dose-response curves. Calculation of the molar ratio between insulin and enzyme under the conditions of our experiments indicates that maximum effect was attained with a ratio of about 100 : 1. The conditions of incubation were not selected as the optimum conditions for the lowest insulin-enzyme ratio, however.

The relationship between the action of insulin on creatine phosphokinase and its known effects in the intact animal is now being investigated; however, I would like to suggest that a physiological significance for this action is not unreasonable. Insulin has been shown to affect numerous processes in the animal, including glucose uptake, glycogen synthesis, incorporation of amino-acids into protein and fatty acid synthesis. The diverse effects of insulin in the intact animal indicate that we must consider two possibilities in any attempt to formulate a hypothesis to explain its action. This diversity of effects may be caused by numerous discrete actions on different enzymes, enzyme systems or membranes, but a more attractive possibility is that insulin has only one basic action and that other observed phenomena are secondary to this. The common link between most of the known effects of insulin appears to be a requirement for energy, and it is clear that the net result of the demonstrated effect of insulin on creatine phosphokinase would be to increase the rate at which energy in the form of ATP is made available.

It is of interest that the tissue in which an insulin effect is most easily demonstrated is skeletal muscle, and it is this tissue which has the highest concentration of creatine phosphokinase. Liver, a tissue in which an insulin effect is less easily demonstrated, contains lesser amounts of creatine phosphokinase than does muscle. Since insulin has an effect on isolated adipose tissue, absence of creatine phosphokinase from this tissue would cast doubt on a possible physiological role for the observed action. Since no data on creatine phosphokinase activity in adipose tissue could be found, some preliminary experiments were undertaken to investigate this question. The dorsal fat pads of 250 g female rats were homogenized in a Vertis homogenizer with 40–80 volumes of *tris* buffer. The homogenates were tested in the system described above using homogenate instead of purified enzyme. Adipose tissue was found to possess appreciable creatine phosphokinase activity. Brown adipose tissue had a much higher creatine phosphokinase activity than did white adipose tissue. Thus, on the basis of available information, creatine phosphokinase is present in all tissues in which definite insulin effect can be demonstrated.

Numerous questions are immediately raised by the possibility that the effects of insulin in the intact animal might be mediated through its action on one enzyme, creatine phosphokinase. I am not aware of any experimental evidence which repudiates this hypothesis, and I believe that further experimental enquiry is warranted.

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Optical Rotation of Irradiated Rhodopsin Solution

It has been suggested from spectroscopic observations of rhodopsin[1-3] that the photoisomerization of chromophore—11-*cis* to all-*trans* retinal—must be followed by a conformational change in the protein moiety of the rhodopsin molecule, which is considered to play an important part in the initiation of visual impulses. Such a conformational change in the protein should be determined by direct measurement of the optical rotation, as this is believed to be closely related to the conformation of the protein[4-6]. The purpose of the present investigation was to determine how far the rotatory polarization of rhodopsin solution changes in response to bleaching by light.

The materials were mainly digitonin extracts of cattle rhodopsin[7], and in some experiments 'Tween 80' extracts[8] and sonicated rod particles[9]. The optical rotation of rhodopsin solution was determined by an automatic recording spectropolarimeter (Yanagimoto Manufacturing Co., Ltd.) and a Rudolph polarimeter (type 200); vessels with light paths 0·5, 1, 10 and 20 cm long were used, around which was circulated aqueous ethylene glycol at constant temperature. Each sample was adjusted to a desired *p*H with 1/15 M phosphate buffer.

The optical rotation of rhodopsin solution was recorded in the range 620–220 mμ before and after irradiation with 10,000 lux for 1 min—this was found to bleach rhodopsin completely. Fig. 1 shows curves of the optical rotatory dispersion in rhodopsin digitonin solution before and after irradiation. In the range of wave-lengths longer than 350 mμ, the 'dark' curve is above the 'irradiated' one; between 330 mμ and 340 mμ they cross over. In the neighbourhood of 450 mμ, the two curves come into contact with each other (Fig. 1*a*). As shown in Fig. 1*b*, we always observed a minimum in the optical rotatory dispersion curves

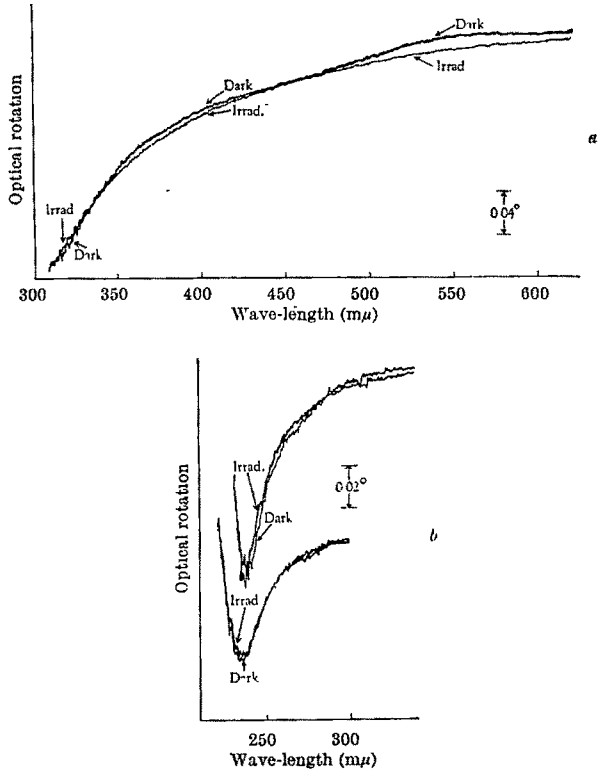


Fig 1. Curves for optical rotatory dispersion of rhodopsin digitonin solution before and after irradiation In Fig 1*a*, the optical density at 500 mμ is 1 98 (E_{500}/E_{400} · 0 32, *p*H 6 25, 15° C, irradiation · 10,000 lux, 1 min) In Fig 1*b*, the samples are diluted to 1/20 (upper two curves) and 1/40 (lower two curves)

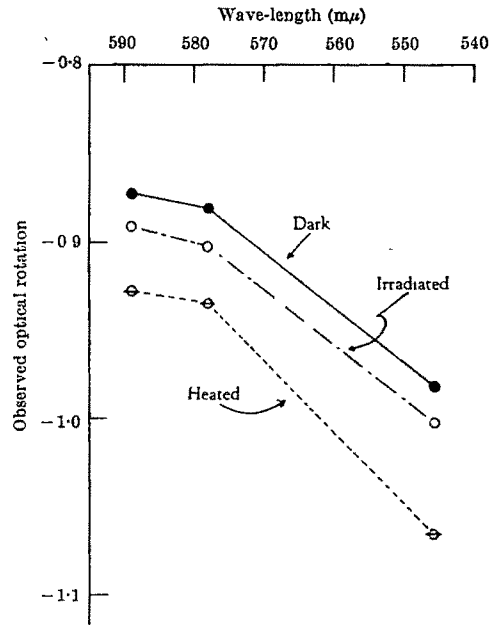


Fig. 2. Changes in observed optical rotation of rhodopsin solution after irradiation and after further heating at 80° C for 5 min (digitonin extract, *p*H 6·25; E_{500}, 0·45; light path, 10 cm; irradiation, 10,000 lux, 1 min, 28° C)

(Cotton effect). In the upper curves of Fig. 1*b*, the 'dark' curve is slightly lower than the irradiated one. It should be noticed that in this experiment the measurement in ranges shorter than 235 mμ was not accurate because of the deep absorbance of the sample, even though it was diluted to 1/20th for recording in the visible range. As for the lower curves in Fig. 1*b*, the sample was further diluted two-fold so that the measurement would be accurate down to 225 mμ. Thus a minimum of optical rotation is found at 235 mμ, although the difference in optical rotation between the dark sample and the one which is irradiated becomes less definite under these conditions.

The Cotton effect, which shows a minimum for optical rotatory dispersion at 235 mμ, is considered to result from the helical structure of the sample; after irradiation the optical rotation of the sample tends to increase in the ultra-violet range and to decrease in the visible range. These facts led us to suppose that the helical contents of rhodopsin might decrease in response to bleaching by light. Another noteworthy fact in the visible range is that the 'irradiated' curve seems to indicate a simple dispersion while the 'dark' curve indicates a rather complex one. In other words, the 'dark' curve appears to show another weak Cotton effect in the region of 450 mμ. Is this due to the chromophoric site giving rise to some optical activity ? This is unlikely, because the chromophoric site of rhodopsin is not considered to be optically active; (1) retinal itself is not optically active; (2) retinal is said to be bound with an amino group in rhodopsin[8], but it cannot interact with an optically active carbon to produce a new optical activity, because rhodopsin does not have a terminal α amino group[12], associated with an optically active carbon atom. Further experiments on this problem are in progress.

In order to compare the change of optical rotatory dispersion in rhodopsin with the results of conformational changes in other proteins, the optical rotation of the highly concentrated sample was measured between 610 mμ and 546 mμ. Fig. 2 shows the observed optical rotations of rhodopsin solution before and after irradiation at 28° C, and after heating at 80° C for a further 5 min. It can be seen from Fig. 2 that the optical rotation is decreased (increased in laevorotation) by irradiation and further

Table 1. $\Delta[\alpha]$, DIFFERENCE OF SPECIFIC OPTICAL ROTATORY POWER OF RHODOPSIN SOLUTION BOTH IN THE DARK AND WHEN IRRADIATED*

pH	Detergent	Temp. (° C)	E_{400}/E_{500}	E^{1cm}_{500}	Light path (cm)	Wave-length (mμ) 610	600	590	589	580	578	570	560	550	546
6·25	Digitonin	28	0·30	1·52	10	29	31	41		44		47	70	62	
6·25	'Tween 80'	28	0 48	0·54	20	17	33	43		43		49			
6·25	Digitonin (further heating 80° C, 5 min)†	28	0·33	0 45	10				40 (120)		49 (120)				100 (−80)
5·2	Digitonin	35	0·38	0·78	10	57				60			94	85	
5·2	Sonicated	34	0·45	0·33	10		69	69	76	69	76	107			100

* Details in the text.
† Calculated from the results shown in Fig. 2.

decreased by successive heatings at any measured wave-length.

As the heating condition (80° C, 5 min) is sure to denature opsin[10], it is more likely that, after heating, an increase in laevorotation reflects a change to a more random conformation in the protein moiety of rhodopsin—such as is observed in other proteins and peptides. A similar tendency was found in 'Tween 80' extracts and in sonicated rod particles.

In general the specific rotatory power is used to compare the magnitude of changes in the protein conformation. As rhodopsin cannot as yet be purified, it is difficult to determine its optical rotation. If the change in the optical rotation of the sample after irradiation, however, is considered to be dependent on rhodopsin only, the difference of specific rotatory power before and after irradiation, $\Delta[\alpha]$, may be given from the difference of the observed rotations divided by the gram concentration of rhodopsin, which can be estimated from the molar extinction of 40,600 at 500 mμ and the molecular weight (40,000) (ref. 13). Thus we can compare the effects of light on rhodopsin in different experimental circumstances by means of $\Delta[\alpha]$, and further discuss the results in comparison with the values from other denatured proteins.

Table 1 shows the differences in the specific rotatory powers before and after irradiation. From these, we notice that there is a general tendency such that the shorter the wave-length the larger the value of $\Delta[\alpha]$; furthermore, at any wave-length, the values are similar irrespective of optical purity. The latter fact may support the concept that the $\Delta[\alpha]$ value depends on conformational change only in rhodopsin caused by the action of light. ($\Delta[\alpha] = \frac{\Delta\alpha \times 100}{lc}$, where $\Delta\alpha$ is the difference of observed optical rotations, l is the light path in dm, and c is the gram concentration of the sample.) In many helical polypeptides, we find a value of 100° for $\Delta[\alpha]_D$ (D denotes sodium D line near 589 mμ), the difference between $[\alpha]_D$ observed in helical conformation and that in a random coiled one. The value of $\Delta[\alpha]_D$ may be referred to as the contribution of the helical structure[4]. Heat-denatured rhodopsin shows the large value of 120°, while the bleached form exhibits about a third of the value of $\Delta[\alpha]_D$ (*p*H 6·25). The values at *p*H 5·2 are larger than those at *p*H 6·25. Opsin is known to be denatured more easily than rhodopsin[10], and *p*H 6·25 may offer a better condition to prevent further denaturation of opsin after bleaching than *p*H 5·2. So the larger values at *p*H 5·2 may be explained by the contribution of further denaturation of opsin after bleaching by light.

Although we consider that changes in the optical rotation of rhodopsin directly correspond to changes in its helical structure, some recent findings suggest that the changes in the optical rotation are caused by changes in the hydrophobic interaction related to protein conformation[11], and also by changes in the antiparallel cross β-structure[6]. We cannot as yet conclude that the observed change in the optical rotation is entirely concerned with the helical structure in rhodopsin, but it must somehow be related to the change in conformation of opsin.

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Infra-red Spectroscopic Investigations of a New Acid Mucopolysaccharide isolated from Human Gastric Juice

IN our earlier investigations[1,2] we have tried to isolate and characterize the acid mucopolysaccharides from the gastric wall in the dog and from human gastric juice.

In the gastric wall, in addition to hyaluronic acid and chondroitin sulphuric acid *B*, traces of a different type of sulphated mucopolysaccharide were found. We thought it possible that this constituent was secreted mainly with the gastric mucus, and so we collected large amounts of human gastric juice. The acid mucopolysaccharides were precipitated from this gastric juice with cetylpyridinium chloride (CPC) after hydrolysis with papain. The material precipitated with CPC would distribute into four fractions which were soluble in 0·3 N sodium chloride, and in 0·5, 0·6 and 0·8 N magnesium chloride, respectively. The chemical analyses of the fractions revealed that they contained fucose, galactose, glucosamine, galactosamine, sialic acid and sulphate, but no hexuronic acids. The molar ratios of the monosaccharide components were nearly the same in all fractions, but the degree of sulphation increased in the above-mentioned fractions from 0·3 N sodium chloride to 0·8 N magnesium chloride in this order.

The infra-red spectra of these fractions were obtained on a Perkin–Elmer model 237 spectrophotometer using a potassium bromide technique. 1 mg of the mucopolysaccharide (as the sodium salt) was mixed with 150 mg potassium bromide. Examples of the spectra of the 0·3 and 0·6 fractions are shown in Fig. 1. The spectra of fractions 0·5 and 0·8 are similar except that the area of the absorption band of sulphate (1,230–1,260 cm^{-1}) in fraction 0·5 is smaller and in fraction 0·8 somewhat greater than in the 0·6 fraction.

These spectra very much resemble the earlier[3,4] published spectra of acid mucopolysaccharides, namely, those of hyaluronic acid and chondroitin sulphates. These polysaccharides have an absorption band at 1,420 cm^{-1} which is due to the ionized carboxyl group[3]. This absorp-

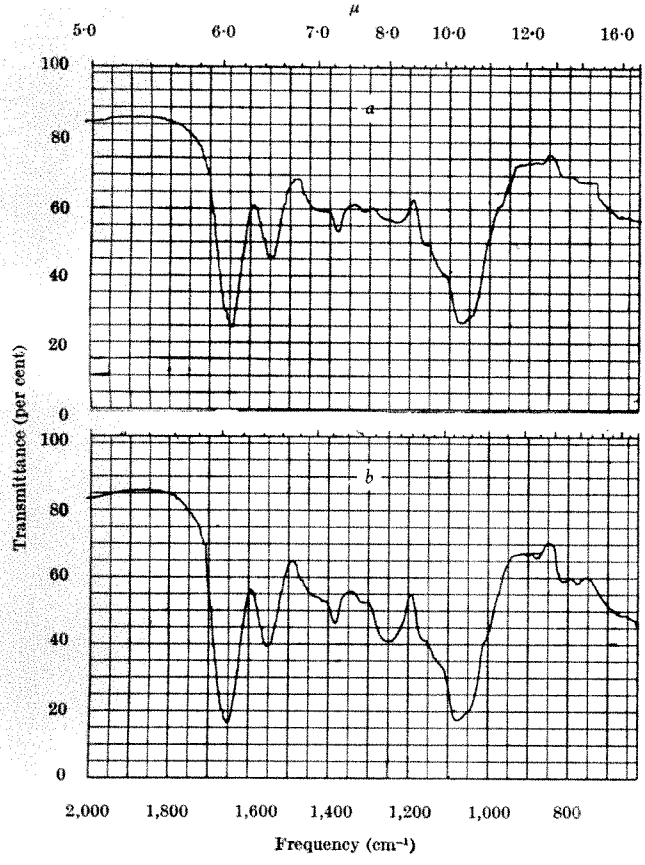


Fig. 1. Infra-red spectra of the fractions 0·3 (*a*) and 0·6 (*b*) of the mucopolysaccharide isolated from gastric juice

tion is quite weak in gastric juice polysaccharide (and may be due to sialic acid). The sharp band at 1,380 cm^{-1} may be due to the methyl group.

The absorption bands at 820 cm^{-1} and 775 cm^{-1} indicate that the polysaccharide from gastric juice resembles chondroitin sulphate *C* in this point. It may be that the sulphate groups occupy the C_6 position in the hexosamine moiety.

We conclude that these fractions represent various sulphation grades of the same polysaccharide. The non-sulphated form of this polysaccharide is not acidic by nature and is therefore not precipitable with CPC under the same conditions as are the actual acid mucopolysaccharides.

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Formation of a Phosphonolipid by Larvae of the Housefly, *Musca domestica*

THE presence of 2-aminoethylphosphonate has been reported in ciliates[1] and in two sea anemones *Anthopleura elegantissima*[2] and *Metridium dianthus*[3]. The finding of the phosphonate in hydrolysates of lipid extracts obtained from these sources and the identification of its glyceryl ester in alcoholic extracts of the sea anemone suggest the natural occurrence of a diacylglycerylphosphonolipid. Rouser *et al.*[4] isolated a phosphonolipid from the sea anemone and identified it as a sphingolipid on the basis of its hydrolysis products. Baer and Stanacev[5] have synthesized the phosphonic acid analogue of cephalin.

Larvae of the housefly, *Musca domestica*, are able to replace lipid-bound choline and ethanolamine with a number of other amino- and *N*-methylated amino-alcohols when these are added to the larval diet[6,7]. We have shown that 2-aminopropan-1-ol, 3-aminopropan-1-ol, 1-aminopropan-2-ol and 2-aminobutan-1-ol are all extensively incorporated into the phospholipid in place of ethanolamine. The implied lack of specificity of the enzyme systems responsible for phosphatidyl-choline and -ethanolamine synthesis led us to speculate that the reported occurrences of 2-aminoethylphosphonate in lipid extracts may be a consequence of this combined with the availability of the phosphonate to the organism. To test this, housefly larvae were allowed to grow under aseptic conditions on a casein medium as previously described[6]. 2-Aminoethylphosphonate (Calbiochem) was added to the medium (75 mg/7·5 g casein) in the absence or in the presence of choline. After 6 days, the lipids of the larvae were extracted using the procedure developed by Folch[8]. Equal portions of these lipid extracts were hydrolysed by heating in sealed tubes containing 6 N hydrochloric acid at 110° C for 24 h and examined for the presence of 2-aminoethylphosphonate. The phosphonate which is stable under these conditions can be separated from other ninhydrin-positive compounds likely to be present in a lipid hydrolysate and from α-glycerophosphate and *ortho*-phosphate by two-dimensional chromatography on thin layers of silica-gel *H* (Merck) using 77 : 23 (v/v) 96 per cent ethanol : 28 per cent ammonium hydroxide in the first direction and 50 : 15 : 5 : 30 (v/v) methanol : 28 per cent ammonium hydroxide : 10 per cent trichloroacetic acid : water in the second direction. Using this system, a zone giving a positive ninhydrin test and travelling to the same position on the plate as 2-aminoethylphosphonate was found in both hydrolysates. When the ninhydrin-positive zones were scraped from the plate and digested with perchloric acid, they were shown to contain phosphorus. These zones could not be detected in hydrolysed lipid extracts from larvae grown in the absence of 2-aminoethylphosphonate.

Equal portions of the lipid extracts were chromatographed on thin layers of silica-gel *H* using 65 : 35 : 5 (v/v) chloroform : methanol : 7 N ammonium hydroxide as solvent. The separated lipids were detected by exposure to iodine vapour and a new lipid zone was detected in the extracts of larvae fed on the phosphonate. This zone gave a positive test with ninhydrin and travelled slightly slower than phosphatidylethanolamine. Two-dimensional chromatography using the alkaline solvent in the first direction and 50 : 25 : 7 : 3 (v/v) chloroform : methanol : glacial acetic acid : water in the second (Fig. 1*a*) showed that the new lipid travelled slightly faster than phosphatidylethanolamine in the acid solvent. This new lipid zone when separated on silica-gel using the alkaline solvent was scraped from the plate and extracted from the absorbent in 1 per cent acetic acid in methanol. The extract was hydrolysed with 6 N hydrochloric acid for 24 h at 110° C. The separated phosphatidylethanolamine zone was similarly treated. Examination of the hydrolysis products for 2-aminoethylphosphonate using the chromatographic system described here showed that the phosphonate occurred only in the hydrolysates of the new lipid zone. The lipid extracts were freed from neutral lipids and then subjected to the mild alkaline hydrolysis procedure developed by Dawson[9]. This removes the fatty acid moieties from any diacylglycerylphosphatides yielding the water-soluble glycerylphosphoryl derivatives of the base. Sphingolipids, plasmalogens and glyceryl ethers are not hydrolysed (or are only partially so) and remain insoluble in water. The 6 N hydrochloric acid

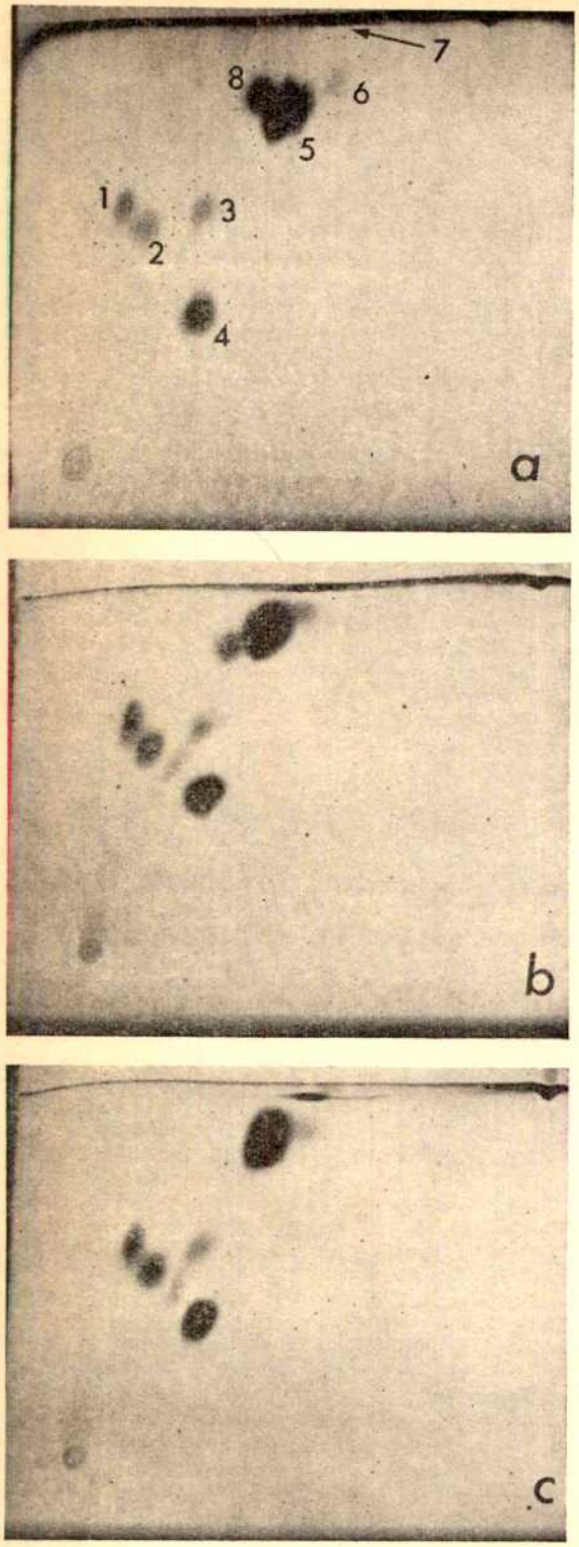


Fig. 1. Separation of phospholipids by two-dimensional chromatography on thin layers of silica-gel *H*. Horizontal direction: Chloroform followed by 65 : 35 : 5 (v/v) chloroform : methanol : 28 per cent ammonium hydroxide. Vertical direction: 50 : 25 : 7 : 3 (v/v) chloroform : methanol : glacial acetic acid : water. *a*, Lipid extract from larvae fed on medium containing choline and 2-aminoethylphosphonate. *b*, Lipid extract from larvae fed on medium containing choline and ethylamine hydrobromide. 10 μg of dimyristoyl-L-α-glyceryl-(2-aminoethyl) phosphonate added. *c*, As *b* but without addition of phosphonolipid. (1) Phosphatidylserine; (2) phosphatidylinositol; (3) lipid stable to dilute alkaline hydrolysis; (4) phosphatidylcholine; (5) phosphatidylethanolamine; (6) phosphatidylglycerol (?); (7) polyglycerophosphatide; (8) phosphonolipid

hydrolysates of the aqueous extracts after mild alkaline hydrolysis were shown to contain 2-aminoethylphosphonate whereas none could be detected in the hydrolysates of the lipids remaining after alkaline hydrolysis. A sample of dimyristoyl-L-α-glyceryl-(2-aminoethyl)-phosphonate (kindly supplied by Prof. E. Baer) had similar chromatographic properties to the new lipid (Fig. 1*b*). The lipid found in the larval extracts would seem to be a dialkylglyceryl-(2-aminoethyl)phosphonate and not a ceramide derivative, as such a compound would remain unhydrolysed by dilute alkali.

Table 1. PERCENTAGE DISTRIBUTION OF PHOSPHOLIPIDS CONTAINING ETHANOLAMINE AND CHOLINE AND OF THE PHOSPHONOLIPID IN EXTRACTS OF HOUSEFLY LARVAE FED ON DIETS CONTAINING ADDITIONS TO THE BASIC DIET AS INDICATED

Additions to diet	Percentage of total lipid phosphorus		
	Ethanolamine lipids	Choline lipid	Phosphono-lipid
None	80·5	1·7	0
2-Aminoethylphosphonate	70·9	2·7	5·1
Choline	65·4	17·0	0
Choline + 2-aminoethylphosphonate	54·6	18·1	8·4

Table 1 gives the percentage of the total lipid phosphorus determined in the phosphonolipid and phosphatidylcholine zones separated on thin layer. The values for lipid-bound ethanolamine were obtained after hydrolysis of the lipid extract and formation of the 2 : 4 dinitrophenyl-derivatives using the method of Axelrod *et al.*[10] (DNP-2-aminoethylphosphonate cannot be extracted with chloroform under the conditions used). The values obtained were expressed as a percentage of the total lipid phosphorus assuming a 1 : 1 molar ratio of ethanolamine to phosphorus. The smaller proportion of the phosphonolipid found in larvae reared in the absence of choline could be explained if the 2-aminoethylphosphonate were considered to be in competition with a larger amount of ethanolamine phosphate in such larvae compared with larvae in which choline is present in the diet. The larger percentage of phosphatidylethanolamine found in larvae reared in the absence of choline is consistent with this.

Inclusion of ethylamine hydrobromide in an equimolar concentration in place of 2-aminoethylphosphonate in the larval diet did not result in the formation of any phosphonolipid (Fig. 1*c*). Thus no enzyme, analogous to a phosphokinase but capable of producing a phosphonate, is present in housefly larvae. The present results together with our earlier observations show that a considerable lack of substrate specificity exists in the enzyme systems involving cytidine diphosphoryl bases for phospholipid synthesis. The experiments of Chojnacki[11] indicate that this is so for the rat, chicken and frog. The natural occurrence of phosphonolipids may well be a result of this and not of a requirement of any special metabolic function associated with this type of lipid.

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Protein-disulphide Reductase Activity in Yeast

A STRAIN of *Saccharomyces cerevisiae* which in batch culture demonstrated little tendency to elongate was shown to do so in continuous culture when the supply of nitrogen was limited[1]. Because fewer cells elongated when sulphydryl compounds or sodium selenate were added to the medium, the sulphydryl–disulphide balance in the cells seemed to be implicated. Later investigations with the same yeast[2] revealed that the change in cell shape was not closely paralleled by changes in total and individual carbohydrates, proteins, nucleic acids and lipids of the cells. Attention was therefore directed to enzymes thought to be important in cell extension. Nickerson *et al.* placed special emphasis on a protein-disulphide reductase system controlling the formation of disulphide bridges in mannan-protein of the wall. They demonstrated in bakers' yeast[3] and *Candida albicans*[4] that after oxidation with potassium ferricyanide of isolated cell wall protein, the disulphide linkages were reduced by isolated mitochondria. The protein-disulphide-reductase activity was, however, very low in a mutant strain of *C. albicans* with elongated cells[4]. It was of interest, therefore, to compare activities of mitochondria isolated from elongated and ovoid cells of our strain of bakers' yeast.

In preliminary experiments, both the oxidized mannan-protein representing the cell wall substrate and the mitochondria were prepared by methods resembling those of Nickerson and Falcone[3,4]. Batch-grown ovoid cells were the source of the mannan-protein. Mitochondria were isolated from both ovoid and elongated cells of the same strain which had been continuously cultured at 25° C in glucose-limiting and nitrogen-limiting media respectively[2]. In all cases the dilution rate was 0·1 h^{-1}. Protein-disulphide-reductase activity of the mitochondria was measured after incubation for 1 h at 25° C with the substrate. The method of Boyer[5] was used for the assay of sulphydryl groupings. Mitochondria from ovoid cells showed much greater activity than an equal mass from elongated cells. The incubation mixtures of Nickerson and Falcone contained a "liver coenzyme concentrate", but a similar addition was shown to be unnecessary in the present experiments. Activity was not promoted by adding dihydronicotinamide adenine dinucleotide phosphate (NADPH), while less than 10 per cent increase in reduction was stimulated by 0·5 μmole of dihydronicotinamide adenine dinucleotide (NADH).

Electrophoretically pure ovalbumin (Sigma grade) furnished an alternative and convenient substrate in place of mannan-protein. Ovalbumin and mitochondria derived as before from ovoid and elongated cells were incubated for 30 min, but in further work the incubation period was reduced to 10 min (Table 1). The results were supplemented by using mitochondria of ovoid cells continuously cultured in phosphate-limited medium. There was no substantial increase in activity when NADH was added to the various incubation mixtures.

Table 1

Culture medium	Cell shape	Incubation period (min)	—SH production*
Nitrogen limiting	Elongated	30	183·1
Glucose limiting	Ovoid	30	649·3
Nitrogen limiting	Elongated	10	98·2 ± 15·2
Glucose limiting	Ovoid	10	248·1 ± 25·0
Phosphate limiting	Ovoid	10	208·1 ± 28·4

* Expressed as mμmoles cysteine/mg protein, means of four results after subtraction of control values. Protein-levels of mitochondria estimated routinely as total nitrogen × 6·25 but confirmed in selected cases by the Folin–Ciocalteau method (ref. 6). Mitochondria in phosphate buffer (*p*H 7·0) (0·5 ml.) and ovalbumin solution adjusted to $E^{280}_{1\,cm} = 1·0$ (0·25 ml.) and incubated in an evacuated desiccator at 25° C.

Table 2

Culture medium	Cell shape	—SH production* Ovalbumin	MP(*E*)	MP(*O*)
Nitrogen limiting	Elongated	101·7 ± 11·9	0·00	0·00
Glucose limiting	Ovoid	375·4 ± 25·5	111·4	123·2
Phosphate limiting	Ovoid	312·8 ± 17·5	—	—

* 0·1 ml. mitochondrial supernatant and 0·1 ml. ovalbumin solution or 0·5 ml. mannan-protein incubated for 1 min at 25° C in air with results expressed as mμmoles cysteine/mg protein, mean of three results after subtraction of control values. MP(*E*) and MP(*O*) represent mannan-protein of elongated ovoid cells, respectively.

The results suggested either a considerable difference in protein-disulphide-reductase activity of the mitochondria of ovoid and elongated cells or differences in permeability of the mitochondrial membranes. Accordingly the experiments with ovalbumin and mannan-protein were repeated using mitochondria preparations which had been disrupted sonically and the membranes removed by centrifugation at 20,000*g* for 20 min. The activities shown in Table 2 were not significantly increased when NADH was added. They demonstrated for the first time that the difference in activity between mitochondria of elongated and ovoid cells was not due to permeability effects, and provided evidence that there is little difference between the mannan-protein of elongated and ovoid cells as substrates.

Activity of the mitochondrial supernatant was lost when 40–60 per cent saturated ammonium sulphate was added to the preparation. The material precipitated by the addition was taken up in 5 mM phosphate or *tris* buffer at *p*H 7·0 and dialysed overnight against similar buffer. When presented with ovalbumin (0·25 mg) as substrate, the dialysed material (adjusted to the usual absorbance) displayed no disulphide-reductase activity after 1 min at 25° C. Addition of 0·25 μmole NADH to the incubation mixture, however, brought about the production of 1,360 mμmoles cysteine/mg protein. It is clear, therefore, that while protein-disulphide-reductase in yeast mitochondria is not dependent on the mitochondrial membrane, it is closely coupled with either NADH or an alternative coenzyme.

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Trypsin Inhibition by Phenylmethylsulphonylfluoride with Reduced Wool as Substrate

WE are trying to dissolve native proteins from wool by the method of limited proteolysis[1,2]. Unreduced wool is not attacked by trypsin and so, in our experiments, wool has been reduced under the mildest possible conditions which will allow trypsin digestion. We believe that these conditions do not cause denaturation of the wool[3]. Apparently, large protein fragments are dissolved from the reduced wool by trypsin, and in a synthetic boundary run[4] in the Spinco model *E* ultracentrifuge, the unfractionated solution gave a schlieren peak, sedimentation coefficient of 3·1. One obstacle to further characterization of the dissolved proteins is the enzyme in the solution, which may continue to digest the fragments it has released from the fibre. This communication describes experiments which show that the inhibitor phenylmethylsulphonylfluoride (PMSF)[5] will arrest the reaction of trypsin with reduced wool.

Accurately weighed samples of wool were reduced under controlled conditions, rinsed and treated with trypsin solutions. Fig. 1 shows the results of a series of experiments in which different amounts of inhibitor were added to trypsin solutions at different times before introducing the reduced wool sample. A comparison was made between the amount of protein dissolved by the inhibited

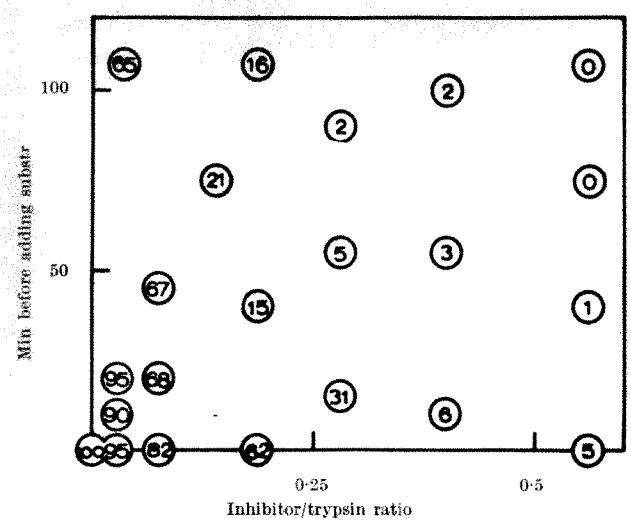


Fig. 1. The numbers in the circles indicate the amount of protein dissolved from reduced wool by trypsin solutions inhibited by PMSF, as a percentage of the amount dissolved by the uninhibited trypsin solution. Lincoln wool was purified by extraction with methylene chloride, dried at room temperature, and conditioned at 65 per cent relative humidity and 22° C so that it absorbed 15 per cent of water. Samples of about 0·2 g were accurately weighed out and reduced for 18 h at 4° C in 0·2 M thioglycollic acid, 0·1 M disodium hydrogen phosphate adjusted with sodium hydroxide to *p*H 9·75. In this treatment 19 per cent of the wool dissolved. The reduced wool was rinsed in 0·1 M disodium hydrogen phosphate at *p*H 8·0, and then placed in 4 ml. of a trypsin solution (wool : enzyme ratio, 50 : 1) in the same buffer, to which different amounts of PMSF dissolved in 45 per cent *i*-propanol–water had been added at various times before the wool was added. Digestion was allowed to occur for 50 min at 20° C after which the wool was removed and the solution centrifuged at 100,000*g* for 15 min. The concentration of protein in solution was measured in a Zeiss interferometer. If inhibitor is not added to the trypsin solution, 64 per cent of the reduced wool is digested

trypsin solutions and the uninhibited solution. Trypsin activity is almost completely inhibited if, for example, an amount of PMSF equal to 0·28 times the weight of enzyme is allowed to react with the enzyme for 55 min at *p*H 8·0 and 20° C.

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IMMUNOLOGY

Antibody Production in Bursectomized Chickens given Repeated Injections of Antigen

CHICKENS which have had their bursa of Fabricius removed at birth are unable to produce adequate quantities of antibody[1]. Assay of spleen sections from such bursectomized birds has revealed an almost complete failure of the pyroninophilic cells to proliferate after an intravenous injection of antigen[2]. In chickens, many basophilic lymphoid cells are situated outside the spleen and the bursa, and cells of the plasmacytic series are found scattered throughout the wall of the intestine, or accumulated in the tonsilla caecalis[3]. For this reason, it cannot be assumed that the deficiency in antibody formation of bursectomized chickens is absolute. Indeed, the present experiment demonstrates that the compromised antibody function of surgically bursectomized birds can be repaired by multiple injections of antigen.

Rhode Island Red chickens were used. Surgical bursectomy was performed on one-day-old chickens. All operated birds were checked at autopsy for bursal remnants, and only those in which no residual bursal tissues were to be found were taken into consideration. One group of bursectomized and unoperated birds was immunized when 8 weeks old, and another group was immunized when 6 months old. 2·0 ml. of a 10 per cent suspension of washed human O red blood cells were injected into the wing veins of the chickens; a further three injections were given with an interval of 24–26 days between injections. Blood samples were taken 9 days after antigen injection and sera examined for the presence of any haemagglutinins. All sera were assayed for mercaptoethanol-sensitive antibody according to the method previously described[4]. Thymus, spleen, bursa, tonsilla caecalis and fragments of intestine were taken for histological examination.

Table 1. HAEMAGGLUTININ PRODUCTION (LOG$_2$ MEAN TITRE) IN NEONATALLY BURSECTOMIZED CHICKENS HYPERIMMUNIZED WITH HUMAN ERYTHROCYTES

Group	Age at time of primary challenge	Primary response	Secondary response	Tertiary response	Quaternary response
Unoperated	8 weeks	7·7 (14/14)*	8·0 (14/14)	8·6 (12/12)	9·2 (12/12)
Bursectomized	8 weeks	0 (0/13)	4·5 (10/13)	5·7 (10/13)	6·6 (10/12)
Unoperated	6 months	8·8 (8/8)	10·0 (8/8)	—	—
Bursectomized	6 months	0 (0/10)	6·1 (10/10)	—	—

* In parenthesis, *nominator*, No. of chickens with haemagglutinins; *denominator*, No. of chickens in group.

Table 1 summarizes the results of antibody production in hyperimmunized unoperated and bursectomized chickens. The titre of haemagglutinin increases as the hyperimmunization progresses in control groups. Birds of both bursectomized groups failed to produce any detectable quantities of circulating antibodies after primary immunization. However, they did respond to some extent to the second injection of antigen, and the third and fourth injections caused a further increase in antibody production.

Histological examination of the spleen sections of unoperated chickens has revealed large areas of mature and immature plasmacellular elements[2]. In the spleen of hyperimmunized bursectomized chickens the size of the area occupied by the proliferating pyroninophilic cells varied from individual to individual. The spindle-shaped thickenings of arterial subterminal branches were surrounded by a large number of pyronin-positive cells. An increase in the number of plasmacellular elements was also noted in the tonsilla caecalis and intestine of unoperated and bursectomized chickens after multiple injections of antigen. Plasma cells were found in the thymus of chickens from all experimental groups; in six cases round-shaped aggregates of cells, which closely resembled the germinal centres seen in the spleen and caecal tonsil, were observed in the medullary portion of the thymus (Fig. 1). No apparent alterations were noted in the bursa of hyperimmunized control chickens. These morphological changes in the spleen, tonsilla caecalis and gut, and probably in the thymus, following multiple immunological stimulations could well account for the increase in level of circulating antibodies during hyperimmunization.

After primary immunization, haemagglutinins in the circulation of normal chickens were predominantly mercaptoethanol-resistant, and antibodies produced in the course of hyperimmunization continued to be of this type. On the other hand, a preponderant production of mercaptoethanol-sensitive antibodies by bursectomized chickens was noted. Using a soluble antigen, Benedict *et al.*[5] showed that the sera obtained after secondary antigen injection have some antibodies which were

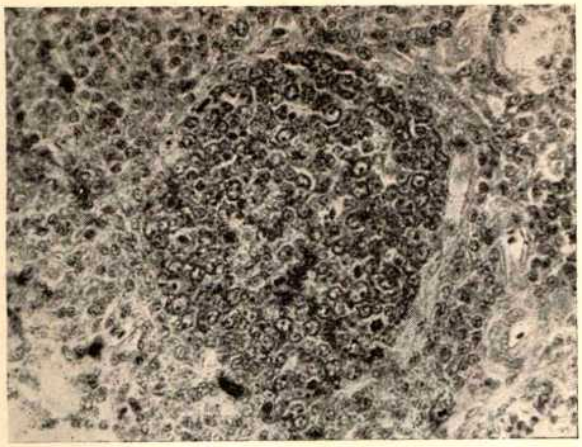


Fig. 1. Germinal centre in the thymus of a bursectomized hyperimmunized chicken. (Stained with methyl green–pyronin)

resistant to mercaptoethanol and sedimented as 7*S* immunoglobulin.

It has been shown by others[6] that lymphoid tissue of the gut is capable of producing antibodies, and that the intestinal bacterial flora probably continuously stimulates the lymphoid tissue in the gut. The absence of lymphoid masses in the intestinal wall of germ-free animals gives an indication of the important part played by the intestinal flora and its products in the multiplication and differentiation of intestinal lymphoid cells[7]. The question still remains, however, as to why these cells (which are present in large numbers in the intestine of bursectomized chickens) do not manufacture circulating antibodies following the first intravenous injection of antigen. So far as histological changes in the spleen and gut are concerned, it would also be intriguing to know why the proliferation of pyronin-positive cells of bursa-less chickens occurs solely after the secondary and subsequent antigenic stimulations. It can further be assumed, however, that in bursectomized chickens there is an inhibitor of antibody synthesis which is particularly related to the primary response, or that bursa-less birds are deficient in some factors (hormone ?) necessary for the initiation of antibody formation. Consequently, the priming capacity of antigen in unoperated chickens is superior to that in bursectomized ones. In any event, the phenomenon described requires careful scrutiny in any discussion of the transient vulnerability of antibody-synthesizing mechanism due to extirpation of the bursa which can be repaired by hyperimmunization, regardless of whether or not there are more than two primary organs (thymus and bursa) in birds which control the immunological machinery of the body.

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Immunochemical Reactions of Conalbumin and its Metal Complexes

IMMUNOCHEMICAL methods have been extensively used in protein chemistry for the elucidation of structural changes in proteins. More recently, this method has been successfully used to demonstrate possible conformational and configurational changes in the protein molecule as a result of its interaction with small molecules and prosthetic groups[1,2]. Immunochemical differences, for example, have been reported for horse oxyhaemoglobin and deoxyhaemoglobin, as well as for metmyoglobin and apomyoglobin[1,2].

These investigations have prompted our interest in the application of immunochemical methods for the investigation of the antigenic properties of metallo-proteins as compared with the apo-proteins. We found earlier that even small structural changes cause measurable changes in the rate of the antigen–antibody reaction[3]. The rate of antigen–antibody reaction can be conveniently followed by measuring the change in the light scattering of the antigen–antibody reaction mixture. The method of measuring light scattering also has the advantage that it is a direct measure of the antigen–antibody interaction, which requires no alteration of antigen or antibody by labelling, or by the addition of precipitating agents as other techniques do.

The protein chosen for this investigation was conalbumin (CA)—a well-known metal-binding protein from egg albumen[4]. CA forms a red-coloured stable complex with two atoms of Fe^{3+} per molecule of protein. It also forms weaker complexes with Cu^{2+} and Zn^{2+}. The metals are readily dissociated from the protein at *p*H values below 6. Tyrosine phenolic and histidine imidazole groups have been implicated at the metal-binding sites of this protein[5–7]. Three-times crystallized CA was prepared essentially as in earlier investigations, use being made of the carboxymethylcellulose cation exchanger[8].

Bovine and anti-conalbumin (ACA) was prepared as described earlier[3] and contained 3·7 mg antibody protein/ml. antiserum. All measurements of light scatter were performed with reagents which had been passed through 'Millipore' filters. Various concentrations of the antigens were mixed with a constant amount of 25 × diluted ACA in 0·1 M borate buffer (*p*H 8·2) directly in a 1 × 1 cm rectangular quartz cuvette. The light scatter of the antigen–antibody reaction mixture was measured in an Aminco–Bowman spectrophotofluorometer at 90° angles. The incident beam was at 334 mμ and the scattered beam at 668 mμ. The light intensity of the scattered light was directly recorded on a Honeywell Electronic 19 recorder with a chart speed of 1 in./min. The results are expressed as the increment in light scatter (read on the transmittance scale ΔT) in the first 2 min of the reaction. The light scatter increased almost linearly with the antigen concentration in the 'antibody excess' zone; therefore, comparisons of the immunoreactivity of the systems examined were made in the 'slight antibody' excess zone. Fig. 1 illustrates typical results from such experiments.

Because the most significant decrease in the rate of the antigen–antibody reaction was caused by FeCA, we investigated the possible effect of Fe^{3+} itself on antibody–antigen reaction. The results are given in Table 1.

These experiments were conducted under the same conditions as those in Fig. 1. The free Fe^{3+} added was equal to the stoichiometric amount needed for the FeCA complex formation (2 moles Fe^{3+}/mole CA). It is evident from Table 1 that the addition of Fe^{3+} to the ACA before, after, or simultaneously with the addition of CA or FeCA does not influence the rate of the reaction. This indicates that the free Fe^{3+} in the applied concentration, does not interfere with the antibody or with the immuno-reaction itself. These results also indicate that the antigen–antibody reaction occurs much faster than the Fe^{3+}

Table 1. EFFECT OF Fe^{3+} ON THE CA–ACA REACTION

No.	Reaction mixture*	ΔT†
1	ACA + CA	13·0
2	ACA + FeCA	5·0
3	ACA + CA + Fe^{3+}	14·0
4	ACA + FeCA + Fe^{3+}	4·5
5	ACA + Fe^{3+} + CA	12·0
6	ACA + Fe^{3+} + FeCA	6·5
7	ACA + { Fe^{3+}, CA simultaneous	13·0
8	ACA + { Fe^{3+}, FeCA simultaneous	5·0

Protocol: 1·0 ml. antibody + 0·1 ml. antigen + 0·1 ml. Fe^{3+} $\frac{\text{mixed}}{\text{in 6–10 sec}}$ light scatter recorded.

* The composition of the reaction mixtures was the same as for Fig. 1. The extra Fe^{3+} added was $3{\cdot}74 \times 10^{-4}$ μmole per ml. of reaction mixture.

† ΔT, increment in the light scatter between 0 and 2 min of the reaction.

binding by CA—at least under the conditions used in this experiment.

The increase in the light scatter during an immunochemical reaction is an indication of the combining properties of the antigen with its antibody and thus represents molecular events which are occurring during this interaction[9,10]. It is, therefore, expected that alteration in the structure of antigen may affect these interactions. The decreased antigenic reactivity of metal–CA complexes as compared with CA may, therefore, reflect the alteration in the protein structure as a result of chelation of metal ions by the protein. The interaction of metal with CA may result in configurational changes of the protein molecule, as well as in masking of important functional sites (antigenic sites) which may be required for a maximum antigenic reaction.

These immunochemical observations are in agreement with information obtained on the structure of CA and its metal complexes by other techniques. Previous investigations on the metal-binding activity of CA have demonstrated that FeCA is significantly more resistant to proteolysis, thermal denaturation, as well as to chemical modifications, as compared with CA (refs. 11 and 12). Investigations of optical rotatory dispersion have demonstrated an anomalous effect for FeCA, as compared with a plain rotatory dispersion for CA (ref. 13). This suggests that the interaction of metal with CA produces important changes in the protein molecule which affect its specific metal-binding functional groups as well as its overall structure.

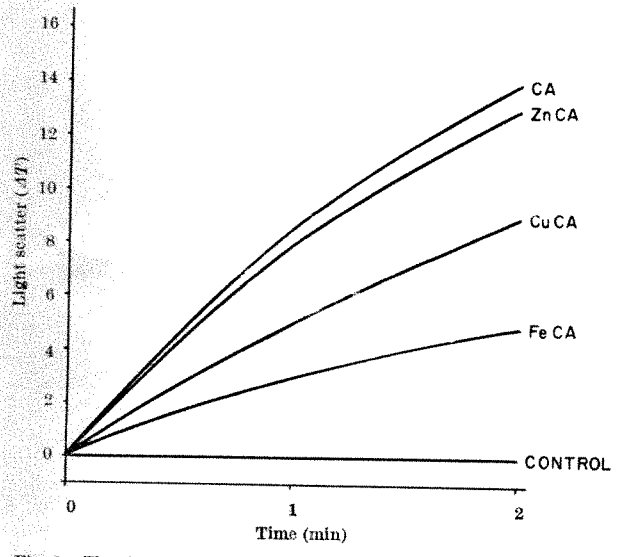


Fig. 1. The change of light scatter in the reaction of CA and its metal complexes with ACA. Protocol: 1·0 ml. antibody + 0·1 ml. antigen $\frac{\text{mixed}}{\text{in 3–5 sec}}$ light scatter recorded. Antibody: 25 × diluted ACA in 0·1 M borate buffer pH 8·2 ($0{\cdot}1 \times 10^{-4}$ μmole/ml. antibody protein, 3·2 mg/ml. total serum protein). Antigen: CA, FeCA, CuCA or ZnCA ($1{\cdot}87 \times 10^{-4}$ μmole/ml. in 0·1 M borate buffer, pH 8·2). The experiments were conducted at 21° C. The curves shown are redrawn from actual recorded tracings

The present investigation offers evidence for a change in the antigenic reactivity of a protein on metal binding. The light scattering technique, although it measures the secondary antigen–antibody interaction, is an even more sensitive method for the detection of changes in the antigenic reactivity than techniques which measure the primary antigen–antibody interactions[14]. Investigations are being carried out to determine whether or not this change in antigenic reactivity is caused by configurational or conformational change in the protein molecule.

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MICROBIOLOGY

Inhibitory Effect of *Lactobacillus acidophilus*

SABINE[1] demonstrated the antibiotic-like effect of *Lactobacillus acidophilus* by growing it in a solid medium in holes cut in a plate seeded with a staphylococcus and *Escherichia coli* as test organisms. He stated that the inhibition observed was not a result of acidity, since there was no difference in the *p*H of the agar in the cup, in the inhibition zone or in the outer zone. The antibiotic substance was said to be extremely labile and had not yet been isolated.

We examined the inhibitory effect of *L. acidophilus* (Aplin and Barrett, Ltd., Yeovil) against *E. coli* by a procedure based on that of Ritter[2]. 50 ml. of milk agar was poured into a 6-in. plastic Petri dish and allowed to solidify. Another 30 ml. of milk agar inoculated with 3 ml. of a 10^{-4} dilution of 1–2-day-old nutrient broth culture of *E. coli* I was then poured on top of the solidified agar and allowed to set. Using a sterile hollow steel borer, 15 mm in diameter, the required number of holes were cut into the medium and the bottom of each hole was sealed with a few drops of milk agar. 0·3 ml. quantities of the test solutions were then pipetted into these holes. After 3 h of pre-diffusion at 5° C the plates were incubated for 18 h at 30° C.

Using this technique we found that a 48-h-old *L. acidophilus* culture in a tomato dextrose broth (TDB) was usually about *p*H 3·5 and inhibitory. Raising the *p*H reduced the inhibition, which almost disappeared above *p*H 4·5. A neutralized and non-inhibitory TDB became inhibitory again on reacidification with lactic acid, but far less so when hydrochloric acid was used. The inhibitor was found to be present in a cell-free (membrane-filtered) filtrate and to be heat-stable. We photographed a plate (Fig. 1) showing the inhibitory effect of sterile TDB adjusted with either lactic acid or hydrochloric acid to

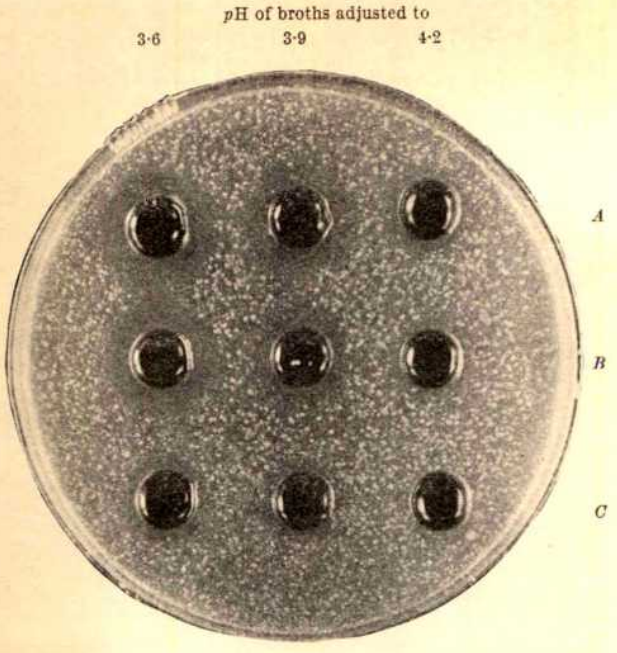


Fig. 1. Inhibition of *E. coli* by various broths. Plate seeded with *E. coli*; cups filled with broth. Prediffusion for 3 h at 5° C followed by 18 h at 30° C. *A*, Cell-free filtrate from *L. acidophilus* culture in tomato dextrose broth; *B*, tomato dextrose broth acidified with 2·3 N lactic acid; *C*, tomato dextrose broth acidified with 0·5 N hydrochloric acid

*p*H 3·6, 3·9 and 4·2, and also that of a cell-free filtrate of an *L. acidophilus* culture of *p*H 3·6 and adjusted with caustic soda to 3·9 and 4·2. As can be seen, the zones of inhibition diminish markedly when the *p*H of the broths is raised in fairly small stages. While the inhibitory effect of the *L. acidophilus* filtrate is almost unequalled by that of the broth acidified with lactic acid, the broth acidified to corresponding *p*Hs with hydrochloric acid produces only little inhibition. By incorporating a mixed indicator consisting of 0·25 per cent methyl red and 0·5 per cent bromthymol blue (1 : 1) it was found that the zones of inhibition coincided with the extent of spread of acidity from the cups as judged by the orange colour of the indicator. The lactic acid spreads much farther into the medium than the hydrochloric acid–acidified broth, apparently governing the size of the zones of inhibition observed.

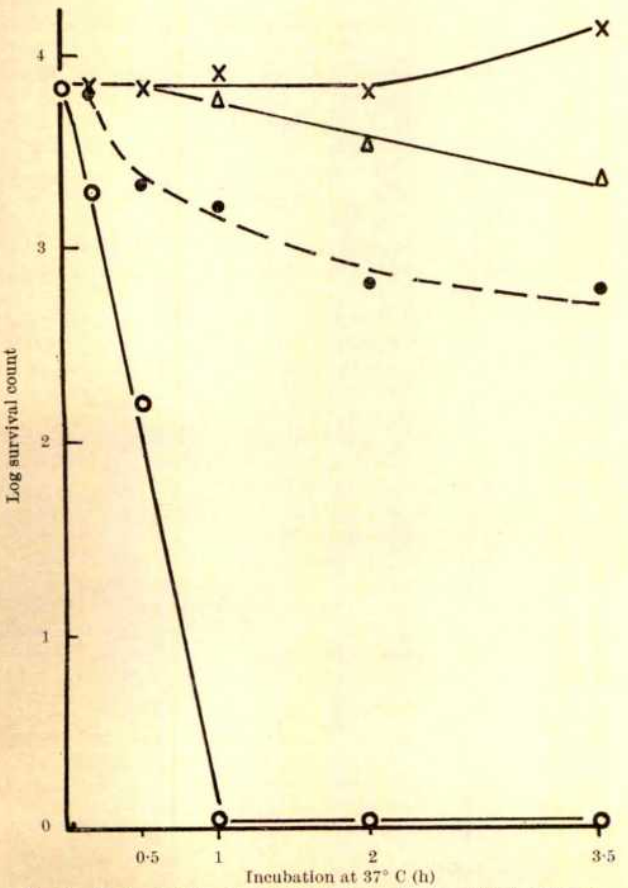


Fig. 2. Survival of *E. coli* in tomato dextrose broth acidified with lactic acid to *p*H 3·6 (○), 4·2 (△), and 4·9 (×) and with hydrochloric acid to *p*H 3·6 (●)

However, if the incubation time was extended beyond 18 h or the incubation temperature raised to 37° C, the *p*H of the whole agar changed gradually towards alkalinity with only some diminution of the zones of inhibition. Thus the time of measuring the *p*H is fairly critical, and Sabine's finding (after 24 h at 37° C) that the *p*H was the same throughout the plate may well be explained in this way.

Since the zones of inhibition produced by lactic acid remained clear even though the whole plate had become uniformly slightly alkaline, we next examined the relative lethal effect of lactic acid and of hydrochloric acid on *E. coli*. TDB acidified with either acid was inoculated and plate counts were carried out after incubation at 37° C. The results given in Fig. 2 show that lactic acid at *p*H 3·6 causes practically 100 per cent mortality of *E. coli* in TDB at 37° C in 1 h, whereas hydrochloric acid at the same *p*H reduces the viable count by only 60 per cent in that time and by 90 per cent in 3·5 h. Only very little, if any, kill occurred at 5° C in 3·5 h with either acid. These results are in good agreement with some earlier findings of Shillinglaw and Levine[3].

Lactic acid is thus shown to be strongly germicidal and possibly largely responsible for the inhibitory effect of *L. acidophilus*. Our plate cup experiments, notably the small inhibition zone produced by *L. acidophilus* culture even when its *p*H is raised to 4·2, suggest the possibility that some other inhibitor is also formed.

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Incidence of *Clostridium botulinum* Type *E* in Fish Products in the United Kingdom

UNLIKE other areas of the world, such as the United States, Japan and Scandinavia, the British Isles, until recently, appeared to be free from the presence of *Clostridium botulinum* type *E*. Early surveys by Meyer and Dubovsky[1], Leighton and Buxton[2], Haines[3], and more recently by Dolman[4] and Cann *et al.*[5], failed to demonstrate its presence either in soil in the British Isles or in sea muds and fish from traditional British fishing grounds. A recent examination by Hobbs *et al.*[6] of more than two hundred commercial vacuum-packed fish purchased in the United Kingdom and of the intestines of a hundred fresh North Sea herring gave no toxic samples. Nevertheless, type *E* botulism could arise from the ingestion of fish imported from areas where there is a high incidence of *Cl. botulinum* type *E*.[5,7].

In continuing a survey of fish and fish products in the United Kingdom for the presence of *Cl. botulinum* type *E*, 646 samples of vacuum-packed fish and samples of fresh herring were examined. The vacuum-packed fish were purchased at random from shops in England. The techniques of examination are reported elsewhere[8]. The herring were part of cargoes landed at British ports

by foreign vessels thought to have been fishing in areas where there is a high incidence of *Cl. botulinum* type *E*. These were sampled in two ways. In the first, contents of the lower intestine of each fish were cultured anaerobically after dissection under aseptic conditions and the broths were incubated for 3–6 days at 30° C. In the second, herring were vacuum-packed whole and incubated for 6 days at 30° C before testing for the presence of toxin.

Of the 646 samples of vacuum-packed fish examined, five contained *Cl. botulinum* type *E*. These were kippers which were all purchased in the same area in early summer 1965. Further attempts to isolate *Cl. botulinum* type *E* from fishery products met with no success until February 1966, when out of one sample of twenty-six Storsild herring, landed at Aberdeen by a Scandinavian carrier ship, eleven were toxic, and out of a second sample of sixteen herring five were toxic.

One of these catches is known to have been taken just off the coast of Norway east of the Viking bank. In spring and early summer there is a large herring fishery off the coast of Norway and it is possible that the five positive isolates of *Cl. botulinum* type *E* made in 1965 came from herring landings from this area.

Table 1. INCIDENCE OF *Clostridium botulinum* TYPE *E*

Source	Type of sample	No. tested	No. containing *Cl. botulinum* type *E*
Shops in Britain	Vacuum-packed fish	646	5
Skagerrak	White fish (intestines)	130	0
Skagerrak	White fish (whole)	130	0
North Sea	White fish (intestines)	96	0
North Sea	Herring (intestines)	200	0
Norwegian Sea	Herring (whole)	29	19
Norwegian Sea	Herring (intestines)	14	0
Viking Bank	Herring (whole)	18	8
Viking Bank	Herring (intestines)	8	3
Fish room of Swedish trawler carrying contaminated fish	Swabs and ice samples	16	0
North Sea west of Haugesund	Herring (whole)	12	0

As a consequence of the demonstration of such a high incidence of *Cl. botulinum* type *E* in the Storsild herring, further batches of fish landed by Scandinavian trawlers were examined. The results are shown in Table 1. The incidence of the organism was sufficiently high to attempt viable counts using 'most probable number' techniques with the demonstration of toxin in a culture indicating the presence of at least one viable spore. Of six fish tested, one gave a count of one spore/16 g and two gave counts of one spore/200 g of whole fish.

Previous work on the distribution of *Cl. botulinum* type *E* in sea-bottom deposits had shown the organism to be present off the coast of Norway so far north as 59° 59′ N., 4° 53′ E. (unpublished data) but had not covered the areas in which the above-mentioned fish were known to have been caught. The research trawler, *Sir William Hardy*, was therefore sent to sample the off-shore areas between the Viking Bank and the towns of Ålesund and Kristiansund. In all, twenty-eight samples of sea bottom deposit were examined with negative results.

The source of contamination of the herring, therefore, is not known, but the sporadic incidence of *Cl. botulinum* type *E* in herring indicates the probable presence of the organism in the sea in concentrated pockets, and although it is still true to say that *Cl. botulinum* type *E* has not been demonstrated in materials indigenous to Britain there is now no doubt that the organism is present in some fish sold in British shops.

There is little danger of botulism due to the ingestion of this type of product, as the cooking times recommended by manufacturers will generally inactivate any toxin which may be formed as a result of bad storage (unpublished data). The main danger lies in products such as smoked salmon which are eaten without further cooking. It is, therefore, essential that all fish products, whether vacuum-packed or not, should be stored below 4° C or preferably frozen from the time they are processed until they are eaten, and all packs should be clearly labelled to this effect.

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An Enrichment Method for Auxotrophic Yeast Mutants using the Antibiotic 'Nystatin'

ENRICHMENT methods have greatly facilitated the work of geneticists in obtaining desired mutants. The various methods in use depend on the selective removal or killing of growing cells relative to non-growing mutants. Moat *et al.*[1] showed that several antifungal agents could be used with yeast to increase the proportion of mutant survivors in artificial mixtures of mutant and wild-type cells. Although 'Nystatin' was not so effective as 'Endomycin' or 'Amphotericin *B*' in their experiments, it seemed a desirable antibiotic with which to undertake further work, as a great deal is known about the physiology of its action on yeast[2–4] and especially as it had been reported that actively metabolizing cells take up much more of it than glucose-starved cells[5].

Preliminary experiments were carried out to determine the killing action of 'Nystatin' on growing and non-growing cells. Haploid strain *S288C* of *Saccharomyces cerevisiae* (obtained from Dr. R. K. Mortimer) was grown with shaking at 30° C until stationary phase in YEP (1 per cent yeast extract, 2 per cent Bacto-peptone, 2 per cent glucose). The culture was washed twice with water, resuspended in a synthetic minimal medium (Difco yeast nitrogen base, without amino-acids) from which the nitrogen source, ammonium sulphate, was omitted, and incubated for a further 6 h. After washing twice with water, the nitrogen-starved culture was adjusted to about 10^8 cells/ml. 1 ml. of this suspension was added to a flask containing 8·0 ml. of the minimal medium with ammonium sulphate (S medium), and to a second flask containing 8·0 ml. of the nitrogen-free medium (S—NH_4 medium). The two flasks were incubated for 3·5 h before 1·0 ml. of a 100 μg/ml. 'Nystatin' solution was added to each. The 'Nystatin' solution (Squibb) was made by diluting a 1 mg/ml. solution in 95 per cent alcohol 1 : 10 with sterile distilled water, and was used without additional sterilization. Samples were removed at 0, 20, 40 and 60 min, and plated for viability in YEP agar. The results are given in Table 1. The viability of the cells in S—NH_4 dropped about 20 per cent after 60 min, but only 0·003 per cent of the cells in S were alive after 40 min, and less than 0·0005 per cent were alive after 60 min.

Table 1. VIABILITY OF NITROGEN-STARVED YEAST CELLS EXPOSED TO 10 μG/ML. 'NYSTATIN' IN MINIMAL MEDIUM WITH AND WITHOUT NITROGEN

	'Nystatin' treatment before plating (min)	Viability (per cent)
Cells in S—NH_4 medium	0	100
	20	85
	40	85
	60	81
Cells in S medium	0	100
	20	0·04
	40	0·003
	60	< 0·0005

Several experiments were carried out to determine the best conditions for killing prototrophic cells. The following are our conclusions. (1) At least 3 h of nitrogen starvation should be given, but the period may be extended to 24 h if desired with no significant loss of viability through starvation. (2) Starvation for glucose for 6 h did not prevent killing by the antibiotic—presumably the cells were still metabolizing sufficiently to be sensitive to its action. (3) Maximum killing was found when 3–4 h growth in S was allowed between nitrogen starvation and addition of 'Nystatin'. During this time, cell number increased 10–15 per cent, and microscopic examination showed that most cells had started budding. (4) An exposure of at least 1 h to the antibiotic should be given. Longer treatments (up to 2·5 h) resulted in somewhat greater killing, but they also resulted in increased killing of non-growing cells and possibly some mutants may be lost. (5) The amount of killing is rather less at 10^8 cells/ml. than at 10^7/ml.; however, even with the higher cell concentration viability was reduced by almost thirty after treatment for 1 h.

To determine whether the proportion of mutant cells could be increased relative to wild-type, an experiment similar to those of Moat *et al.*[1] was undertaken. *S288C* and a red adenine-requiring strain of the same mating-type (*A708C*) were separately grown in YEP and treated as before. After nitrogen starvation and washing they were mixed by volume in the proportion of one mutant to 1,000 wild-type cells, and then suspended in S. The *A708C* cells would not grow because the medium lacked adenine. After incubation for 4 h in S, 'Nystatin' was added to a final concentration of 10 μg/ml. as before, and platings were made at intervals of 30 min on to S-agar supplemented with 20 μg/ml. adenine. On this medium, the red adenine-requiring colonies could easily be distinguished from the white wild-type colonies. Our results are given in Table 2 as the change in ratio of red to white colonies. There was a maximum calculated enrichment of about 10,000-fold for mutant cells relative to wild-type cells. However, this figure is not very reliable as there were very few red colonies in the 0 min platings. A more reasonable estimate would be at least a 1,000-fold enrichment for mutants among survivors.

It thus became necessary to determine whether the method could be used with newly induced mutants. *S288C* was grown in YEP as before, washed, suspended at 10^7 cells/ml. in *tris* buffer at *p*H 7·4, and ethylmethanesulphonate (EMS) was added to a final concentration of 1 per cent. After 3 h incubation the cells were washed, suspended in 6 per cent sodium thiosulphate for 10 min, and washed twice more. Survival after treatment with EMS was about 50 per cent. The treated cells were suspended in YEP and grown for about 18 h, then subjected to nitrogen starvation and 'Nystatin' selection as before. Survival after treatment with 'Nystatin' was 0·61 per cent. Platings of suitable dilutions were made on YEP agar. Almost 10 per cent of the resulting colonies failed to grow when replicated to S-agar. The requirements of the various auxotrophic strains isolated were satisfied by arginine, histidine, leucine, lysine, isoleucine, isoleucine + valine, methionine, phenylalanine, threonine, tryptophan, tyrosine and adenine. Because an intermediate growth period in YEP was allowed, many of the colonies which required a particular substance were without doubt clonal repeats of one mutational event.

The method can be used to select specific auxotrophic mutants by allowing growth after mutagen treatment in S supplemented with the specific growth factor. One cycle of 'Nystatin' selection may be sufficient in such cases, but if the mutation is rare it is advantageous to go through a second selection cycle (after washing and a second intermediate growth period) in order to increase the mutant yield. When using this two-cycle method to select specifically for histidine auxotrophs, 5–10 per cent of surviving colonies regularly proved to be the required mutant types.

Table 2. CHANGE IN THE PROPORTION OF ADENINE-REQUIRING (RED) TO WILD-TYPE (WHITE) COLONIES IN A 1 : 1,000 MIXTURE (BY VOLUME) TREATED IN MINIMAL MEDIUM CONTAINING 10 μG/ML. 'NYSTATIN'

'Nystatin' treatment before plating (min)	Ratio red : white colonies	Enrichment factor
0	1 : 470	—
30	1·24 : 1	580
60	9·2 : 1	4,300
90	25·2 : 1	11,800
120	11·7 : 1	5,500

The proportion of petite colonies is increased after 'Nystatin' selection, but in practice this has not been a serious disadvantage. They can be eliminated by plating on a medium containing a non-fermentable carbon source such as glycerol, or identified by replicating to such a medium.

A period of growth after mutagen treatment seems necessary to 'fix' the mutation. No auxotrophs were obtained in one experiment in which 'Nystatin' selection was used immediately after EMS treatment. The desirability of intermediate growth has also been noted when using the penicillin method with bacteria[6]. As is also the case with penicillin selection, it is desirable to allow the prototrophs to grow vigorously before the antibiotic is added (Moat, personal communication). Lampen *et al.*[4] have shown that log phase cells bind more 'Nystatin' than stationary phase cells.

The uptake of 'Nystatin' by yeast is influenced by *p*H, the maximum being at about *p*H 4 (ref. 5). Because the minimal medium used had a *p*H of about 4·2, no adjustment was made. If media with higher *p*H's are used adjustment would probably be necessary to ensure that the antibiotic has maximum effect.

The 'Nystatin'-selection method is analogous to the penicillin-selection method widely used in bacteria[6–8]. Although the mutant yield does not reach that of the penicillin method, 'Nystatin' selection has nevertheless proved a convenient way to isolate a wide variety of auxotrophic yeast strains.

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PHYSIOLOGY

Kininase Activity in Experimental Pancreatitis

THE role of vasoactive polypeptides as humoral mediators in acute pancreatitis has been the subject of much recent speculation. Both trypsin and kallikrein, normally present in the pancreas in an inactive form, are activated in acute pancreatitis, and these enzymes are able in turn to split the polypeptides bradykinin and kallidin from the alpha 2 globulins to which they are bound in the blood. The active peptides have well-known pharmacological properties which include pain production, increased capillary permeability, smooth muscle stimulation, production of hypotension and leucocyte aggregation. The severe pain, unique retroperitoneal oedema, hypotension, and shock encountered in acute pancreatitis suggest the presence of such a substance.

The kinins are rapidly destroyed in the normal subject by peptidases which split off a terminal peptide leaving

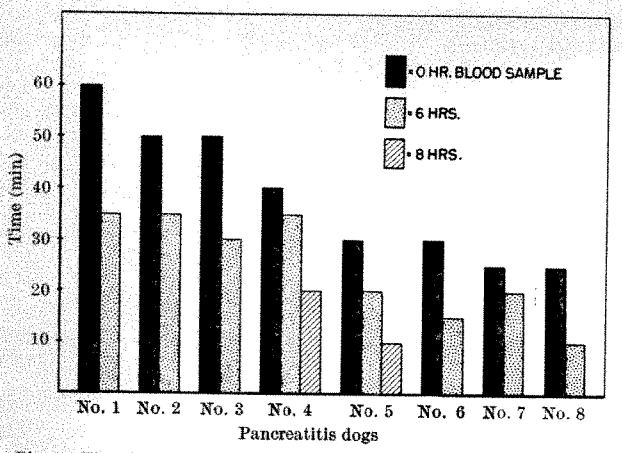


Fig. 1. Time for complete inhibition of 0·05 mcg of bradykinin in 0, 6-h, and 8-h blood samples from eight dogs with bile pancreatitis

an inactive form[1]. The balance between available kininase and kinin production thus becomes extremely important in this disease.

Reports have been made of increased kinin activity[2-4] and of decreased kinin precursor[3] during experimental pancreatitis in the dog, but to our knowledge changes in plasma kininase levels have not been studied.

Eight mongrel dogs were anaesthetized with sodium pentothal (10·5 mg per lb.). Bile pancreatitis was produced by injecting 10 c.c. of the bile of the dogs into the major pancreatic duct under pressure. The portal vein was cannulated through the splenic vein and blood was collected at 0, 2, 4 and 6 h; in two dogs a specimen was also collected at 8 h.

5 c.c. of oxalated blood was centrifuged at 37° C and 40,000 r.p.m. for 15 min; 0·5 c.c. of plasma was then diluted with 19·5 c.c. of buffered saline solution, and 5 c.c. of this mixture was incubated with an equal amount of a standard solution containing 0·05 mcg of bradykinin (kindly supplied by Sandoz Pharmaceuticals, Hanover, New Jersey). In a rat uterus bioassay system, unresponsive to histamine, serotonin and catecholamines, vasoactivity of the incubated mixture was determined at 5-min intervals until no activity was detectable.

Plasma kininase levels increased during the first 8 h of pancreatitis in all the dogs. The time for total inhibition of 0·05 mcg of bradykinin decreased from a mean control time of 39 min to a mean of 23 min in the 6-h pancreatitis specimen (Fig. 1). No change in the level of plasma kininase was detected during sham pancreatitis.

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Alveolar Deposition of Aerosols on the Moon and in Outer Space

IT was recently suggested[1] that, on the Moon, man will be more susceptible to infection by aerosols containing bacteria because the smaller gravitational field will lower the filtration efficiency of the upper respiratory tract. The reduction in upper respiratory tract deposition will be caused by a reduction in the sedimentation velocity

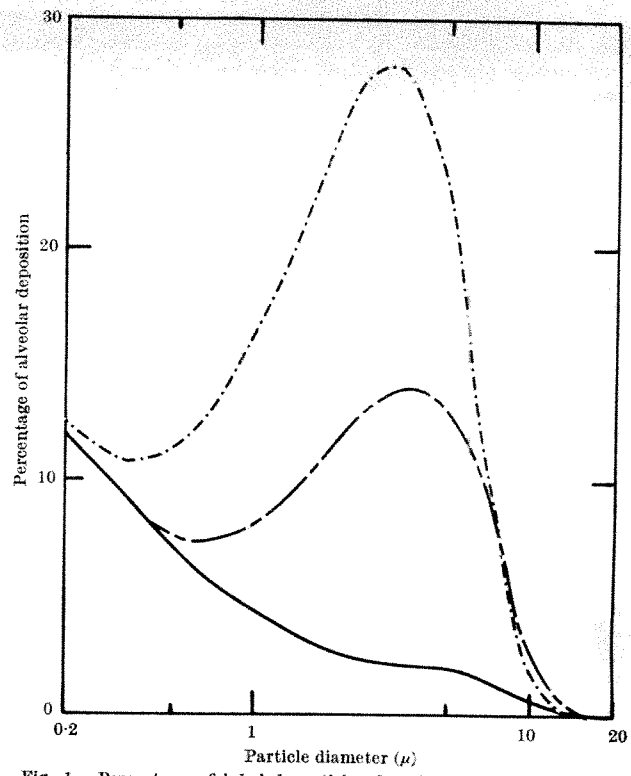


Fig. 1. Percentage of inhaled particles deposited in the alveolated regions of the lung as a function of particle size, for unit density spheres. *Upper curve*, Terrestrial deposition; *middle curve*, lunar deposition; *lower curve*, deposition in the absence of gravity

of the aerosol particles. The other mechanisms for upper respiratory tract deposition, Brownian diffusion and impaction, will be unaffected, but the former is of negligible importance for particles greater than about 1μ.

I recently published details of a method for calculating alveolar deposition as a function of particle size[2,3]. The computations were performed on an electronic computer, and recalculations based on a different gravitational acceleration were easily performed. The results are shown in Fig. 1, for a tidal volume of 500 c.c., a 3-sec linear symmetrical breathing cycle without pauses, and a functional residual capacity of 2 l. It is seen that alveolar deposition on the Moon does exceed terrestrial alveolar deposition, as predicted, in the region of particle-sizes greater than 7μ. However, the difference is not so great as might have been expected, and this is due to the fact that, on the Moon, a higher proportion of those particles which penetrate the upper respiratory tract are exhaled from the lower respiratory tract without being deposited there. This is even more true in the complete absence of gravity, and alveolar deposition in this case is everywhere lower than it would be on either the Moon or the Earth.

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Technique for investigating Binding of Labelled Thyroxine by Human Serum

TECHNIQUES using two-dimensional electrophoresis for the investigation of the proteins of human serum have been reported in the past[1,2]. However, these techniques are rarely used because they do not offer advantages over other procedures[3]. The present communication contains a report of a new two-dimensional technique of paper electrophoresis which was used to achieve a better separation of the proteins of human serum. The same technique

was afterwards used to investigate the binding of thyroxine labelled with iodine-131 (T4*) by human serum proteins.

The new technique is based on the ordinary hanging-strip paper electrophoresis procedure in a 'Spinco' cell, with some modifications. A special 30 × 30 cm sheet of Whatman 3 *MM* paper was prepared to replace the eight paper strips which go in the folding rack. Eight holes were punched in the sheet on each of the four sides coinciding with the pegs of the folding rack. Also, for the first-dimensional electrophoresis, one of the edges of the sheet was folded under to allow for the introduction of one narrow paper strip in the folding rack in addition to the sheet. The purpose of the paper strip was to have a control of the migration of the serum proteins in the first-dimensional electrophoresis. It also became important to choose proper buffers for the procedure. Veronal and *tris*-maleate[4], both at *p*H 8·6, were chosen. These buffers were interchanged at different steps of the procedure. Veronal was selected for the second-dimensional electrophoresis when *tris*-maleate had been used in the first dimension and vice versa.

In the first-dimensional electrophoresis, the folding rack with the sheet and one paper strip was placed in the 'Spinco' cell; the papers were then wetted with the buffer, and electrophoresis was started after equilibration. After approximately 16 h, the procedure was stopped, and the papers were taken out of the cell and dried at room temperature. Only the paper strip was stained and fixed in order to obtain information on the migration of the proteins of the serum in the first-dimensional electrophoresis.

In the second-dimensional electrophoresis, the sheet of paper was unfolded, rotated through 90° and placed back with the folding rack in the 'Spinco' cell. The alternate buffer, as stated before, was used. Every step in this part of the procedure followed exactly those of the first procedure. After 16 h of electrophoresis, the sheet was taken out from the cell, dried and stained[3]. The staining revealed five bands in the sheet. These represented albumin (A), alpha 1, alpha 2, beta and gamma globulin when veronal was used as the first and *tris*-maleate as the second buffer (Fig. 1). Only four bands identified as albumin (A), alpha, beta and gamma globulin were stained when *tris*-maleate was the first and veronal the second buffer (Fig. 2). It became evident from this part of the experiment that separation between alpha 1 and alpha 2 globulins could only be obtained under one of the experimental conditions, namely, when veronal was the first buffer and *tris*-maleate the second. The reason for the different behaviours of the proteins of the serum with the two buffers is unknown at present.

It has been shown in the first part of this experiment that the new two-dimensional electrophoretic method produced a distinct separation in two dimensions of the proteins of human serum. This separation is, of course, better than the one obtained in one dimension only.

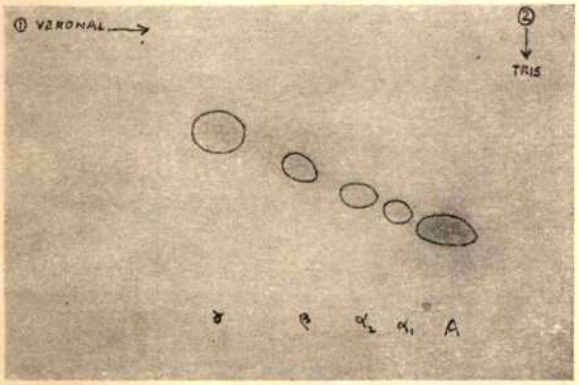


Fig. 1. Two-dimensional electrophoretogram of human serum with veronal as the first and *tris*-maleate as the second buffer, both at *p*H 8·6

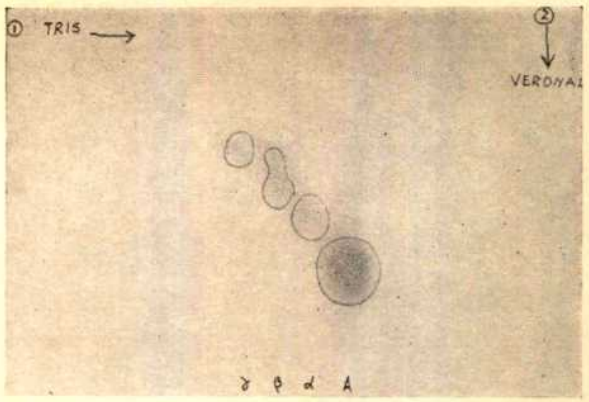


Fig. 2. Two-dimensional electrophoretogram of human serum with *tris*-maleate as the first and veronal as the second buffer, both at *p*H 8·6

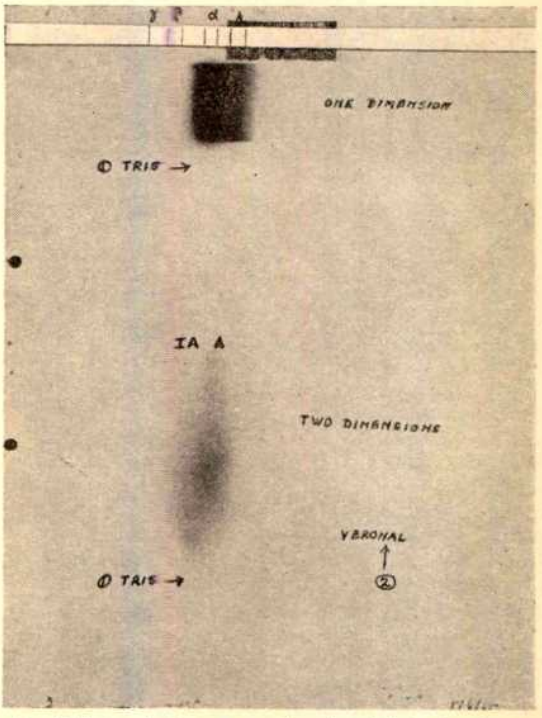


Fig. 3. At the top is shown a one-dimensional electrophoretogram of human serum in *tris*-maleate buffer at *p*H 8·6. Immediately below is the autoradiogram of a one-dimensional electrophoretogram of human serum plus iodine-131-labelled thyroxine in *tris*-maleate buffer. In the lower part is the autoradiogram of a two-dimensional electrophoretogram of human serum plus iodine-131-labelled thyroxine with *tris*-maleate as the first and veronal as the second buffer, both at *p*H 8·6

These results encouraged us to undertake the second part of this experiment, in which binding of iodine-131-labelled thyroxine (T4*) by the proteins of human serum was investigated in two-dimensional electrophoresis.

For this purpose a fresh batch of T4*, specific activity 42·8 mc./mg (0·0049 mg T4/ml.), was purchased from Abbott Laboratories, Oak Ridge, Tennessee. 3 μc. of this T4* were then mixed thoroughly in a test-tube with 1 ml. of normal human serum, and 10 μl. of the mixture were used for each of the two dimensions of the electrophoretic procedure. The materials and methods used were the same as those previously described. Another step was introduced which consisted of the preparation of autoradiograms from the electrophoretograms[5]. It was found necessary to take 2–4 days over this process if clear and defined autoradiograms of the electrophoretograms were to be obtained. In the autoradiograms, T4* was bound in three areas which corresponded to the pre-albumin (PA), albumin (A) and inter-alpha (IA) regions of the human serum when veronal was the first buffer

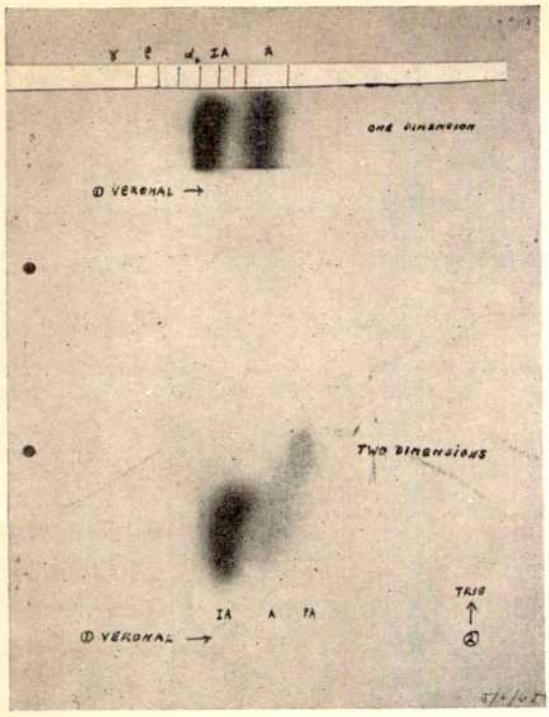


Fig. 4. At the top is shown a one-dimensional electrophoretogram of human serum in veronal buffer at *p*H 8·6. Immediately below is shown an autoradiogram of a one-dimensional electrophoretogram of human serum plus iodine-131-labelled thyroxine with veronal buffer at *p*H 8·6. In the lower part is the autoradiogram of a two-dimensional electrophoretogram of human serum plus iodine-131-labelled thyroxine with veronal as the first and *tris*-maleate as the second buffer, both at *p*H 8·6

used and *tris*-maleate the second (Fig. 3). Poor separation between the bands of albumin (A) and inter-alpha (IA) globulin was obtained when *tris*-maleate was the first buffer and veronal the second. No binding of T4* could be detected in this instance in the pre-albumin area (Fig. 4). It is known that T4* binds in the albumin and inter-alpha regions when veronal is the buffer[6], and that T4* also binds in the pre-albumin area when *tris*-maleate is the buffer. It is the first time, however, that T4* has been shown to bind in the pre-albumin area in two-dimensional electrophoresis using veronal as the first and *tris*-maleate as the second buffer. Since pre-albumin shows T4* binding in *tris*-maleate buffer, the results are not surprising. The second run merely washed all veronal away with the *tris*-maleate buffer so that the inhibitor of T4* binding by pre-albumin (that is, veronal) was no longer present.

More experiments are now in progress in which the binding of T4* by the human serum and tissues is under investigation with the aid of the new two-dimensional electrophoretic technique. In these studies autoradiograms of the electrophoretograms are made and counting of the radioactivities in the sheets is also carried out. It is expected that more progress in this field will be made in the future with the new technique.

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BIOLOGY

Translocation of Amino-acids in Sugar Beet

STUDIES of translocation have been largely concerned with movement of sugars or photosynthate, and investigations of transport of nitrogenous material have been limited. Organic nitrogen has been found in root exudates[1,2], but these consist of material transported in xylem. Analyses of phloem contents have revealed nitrogenous material[3], but large samples are difficult to obtain. Indirect methods for investigation of phloem transport must often be used. An earlier report[4] described specific movement of amino-acids from roots to young leaves in phloem. Further experiments on nitrogen movement are now reported.

Sugar beet were used for this work, although some similar phenomena were observed with seedlings of cucumber, tobacco and vine. Methods of growth and detection of radioactivity were as described earlier[4,5].

When supplied to the roots of intact plants, a hydrolysate of carbon-14-labelled protein (complete mixture of protein amino-acids) was translocated preferentially to young leaves over periods of 6–24 h, as were glutamine and glycine, reported earlier[4]. Sarcosine, betaine and β-alanine were more generally distributed to all leaves, as reported for α-amino-isobutyric acid. In an attempt to investigate the mechanism of selective distribution, intact plants were supplied for 30 min with carbon-14-labelled protein hydrolysate, then subjected to the treatments shown in Table 1. In the subsequent 2·5 h in unlabelled nutrient solution considerable amounts of carbon-14 were translocated into young leaves. Removal of old leaves had relatively little effect on accumulation of carbon-14 by young leaves, whereas removal of roots markedly decreased translocation of label (transfer of material to the insoluble fraction accounts for the fact that the soluble fractions in treatment 4 are lower than in 1). It is likely, therefore, that the specific distribution involves discrimination and transfer to phloem in the roots, rather than a general transport to all leaves and redistribution from old to young. However, this latter mechanism could be made to operate in abnormal circumstances. When seedling tops were supplied with amino-acids through the cut hypocotyl, all leaves became labelled in the first 30 min and there was a subsequent redistribution of label to young leaves.

Phloem translocation of amino-acids from older leaves could be demonstrated. Carbon-14-labelled glutamate, -glycine and -protein hydrolysate, applied in drops to the upper surface of leaves, were translocated into growing leaves. After 24 h the typical distribution was to the nearest young leaf, often only the adjacent half, as shown in Fig. 1. This resembles the distribution of photosynthate from mature leaves[5]. A greater proportion of the absorbed activity was translocated when the treated leaf was nearing senescence. 5–12 per cent of the absorbed amino-acid was translocated from the treated leaf in 18 h. Translocation was stopped by petiole steaming. The non-protein amino-acids α-amino-isobutyric acid and D-alanine were absorbed by treated leaves but very little was translocated to young leaves.

The date presented here and earlier show that phloem transport of organic nitrogen is readily carried out by the plant, and that the mechanism and resulting distribution

Table 1. RADIOACTIVITY IN LEAVES OF SUGAR BEET SEEDLINGS (EIGHT LEAF STAGE) AFTER SUPPLYING ^{14}C-AMINO-ACID MIXTURE TO THE ROOTS

Amino-acids were supplied for 30 min, then plants were treated as shown. Leaves were extracted with hot 70 per cent ethanol

	Radioactivity (c.p.s.)			
	Leaves 1–3 (growing)		Leaves 4–6 (mature)	
Further treatment:	Soluble fraction	Insoluble	Soluble fraction	Insoluble
(1) Immediate harvest	1,097	827	2,008	1,470
(2) Transferred to inactive nutrient for further 150 min	3,402	4,533	1,736	1,267
(3) As 2, but older leaves (4–8) removed	3,054	—	(1,850 at 30 min)	—
(4) As 2, but fibrous roots and lower tap root removed	551	—	1,158	—

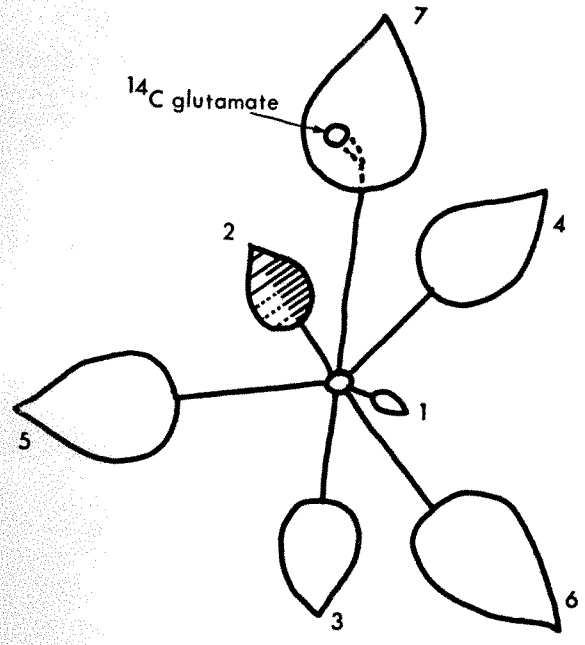


Fig. 1. Translocation of carbon-14 to a young leaf after supplying carbon-14-labelled glutamate to a mature leaf of beet for 18 h. The glutamate solution was placed in a small well (a ring of 'Tygon' tubing) sealed to the leaf with lanolin, and covered with cellulose acetate film to restrict evaporation. Diagram based on autoradiographs

are quite different from those of xylem transport. It remains to be shown whether phloem translocation of amino-acids can contribute a significant amount of nitrogen for young leaf growth.

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Variations in Gestation Length of Mink (*Mustela vison*)

MINK reproduction is characterized by several remarkable features, among which the variability of gestation length is of outstanding interest. Hansson[1] and Enders[2] have emphasized this variability (from 37 to 91 days) in their classic studies on mink reproduction. It is generally accepted that this range in gestation length is due to varying periods of delayed implantation, when the fertilized ova reside in the uterus for an extended period of time before nidation occurs. This communication reports some investigations which I made of the normal variations of gestation length in four modern colour phases (strains) of mink and the effects of the age of the female, the date of mating and litter size on gestation length.

Ninety mink of the four colour phases were used. The genetic formulae of these colour phases were: 'Pearl' (*pp bpbp alal*), 'Cameo' (*alal bm^{H}bm^H*), 'Dawn Pastel' (*bb bsbs*) and 'Violet' (*pp alal bmbm*). The mink were raised under normal ranch conditions in the Great Lakes region of the United States. All mink received a standard ration consisting of 55 per cent meat, 12 per cent cereal and 33 per cent water. This ration was supplemented with vitamin A (1,560,000 I.U. per ton of dry feed) and a bacterial-fungal enzyme (Nopco Chemical Co., Harrison, New Jersey) (1·8 kg per ton of dry feed). Some of the mink received an additional supplement of ethylene dichloride extracted wheat germ oil (Dukelow[3]). This treatment did not significantly affect gestation length, and therefore data from mink receiving this treatment were pooled with data from untreated mink.

Table 1. GESTATION LENGTH OF YEARLING AND OLDER MINK OF FOUR COLOUR PHASES

Factor	No. of gestations	Mean gestation length (days)
(1) Colour phase		
(*a*) 'Pearl'	41	52·5 ± 4·0
(*b*) 'Cameo'	20	50·8 ± 5·2
(*c*) 'Dawn Pastel'	22	53·3 ± 3·9
(*d*) 'Violet'	7	52·0 ± 5·1
(2) Age of female		
(*a*) Yearling	60	52·0 ± 4·1
(*b*) Older	30	52·9 ± 4·5

Table 2. GESTATION LENGTH OF MINK BASED ON TIME OF BREEDING

Date of breeding	No. of gestations	Mean gestation length (days)
March 10–14	12	56·8 ± 2·3
March 15–19	46	53·0 ± 2·7
March 20–24	25	50·3 ± 4·5
March 25–29	7	46·4 ± 4·4

Table 3. GESTATION LENGTH OF MINK IN RELATION TO LITTER SIZE

Gestation length range (days)	No. of gestations	Mean gestation length (days)	Mean litter size
42–45	8	43·1	4·00 ± 1·82
46–49	11	47·5	4·00 ± 2·14
50–53	33	51·5	3·88 ± 2·18
54–57	29	55·1	3·59 ± 2·21
58–61	9	58·7	3·33 ± 1·66

Matings were started on March 10, 1963. All females were mated only once and did not accept mating at a later date. Because of this procedure it can be assumed that these mink were of higher fertility than the remainder of the herd which afterwards accepted a later mating. The gestation length was measured from the date of mating to the date of birth. All data were treated by analysis of variance. Differences in gestation length between colour phases and yearling and aged females were not statistically significant (Table 1). Hansson[1] reported gestation length means of 51·3 ± 5·4 days for 'Eastern' mink and 49·0 ± 4·2 days for 'Alaskan' mink. The lack of a significant difference between yearling and older mink in gestation length is in agreement with Apelgren[4], but conflicts with Enders[2], who believed that the gestation length was longer in yearlings than in the same individuals when older. A highly significant difference ($P < 0.01$) was observed between gestation lengths based on the date of mating (Table 2). Generally, the later in the breeding season that mating occurred, the shorter was the gestation period.

Hansson[1] reported that the length of gestation decreases greatly when the date of mating is moved forward. He also reported a decrease in gestation length from matings on March 10–March 29 of 6·5 days compared with 10·4 in the present investigations. Table 3 illustrates the relationship between the length of the gestation period and the number of kits born. The shorter gestations tended to be associated with larger litters. Hansson[1] showed a similar but irregular tendency. The cause of this phenomenon is not known. Early workers believed that the presence of a larger number of foetuses resulted in an earlier stimulus to parturition and a shorter gestation length. However, modern endocrinological theories of the onset of parturition are in conflict with this. A more logical explanation lies in the higher mortality of zygotes during the period of delayed implantation associated with longer gestations.

I thank Mr. Nobe Hall, Woodland Mink Ranch, Grand Rapids, Minnesota, for his assistance and co-operation in these studies.

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Ascent of Calcium in Intact and De-rooted Bean Plants

CALCIUM ions are known to be practically immobile in the phloem of plants and are thus not re-translocated from older leaves[1]. Other elements manifesting low phloem mobility are zinc, boron and iron; the last-mentioned is also immobilized in the xylem in various circumstances[2]. Apparently, it has not been suggested that elements other than iron may be immobilized in the xylem[2].



Fig. 1. Autoradiographs of (*A*) intact bean plant, and (*B*) similar but de-rooted bean plant. Both absorbed calcium-45 from a labelled 10^{-4} *N* calcium chloride solution during 24 h, at 25° C, in a photoperiod of 18 h light and 6 h darkness

In the present investigation, upward movement of calcium in de-rooted and in intact bean plants (*Phaseolus vulgaris* L.) was compared. Calcium was delivered to the whole plant when absorbed by roots of intact plants; it was, however, retained in the basal stem segment when absorbed by cut surfaces of similar but de-rooted plants (Fig. 1). Other mineral ions such as chloride[3], sulphate and potassium, as well as an acid dye—fuchsin—were found to be distributed throughout the whole plant when absorbed by de-rooted beans. Immediate microscopic examinations of hand-sections made from stems of de-rooted plants after absorption of acid fuchsin showed that the flow of the solutions was restricted to the xylem vessels. These observations seem to indicate that the mobility of calcium ions in the xylem vessels of bean plants is increased by the passage of these ions through the roots.

Iron chelates have been found in exudates from soybean[4] and tobacco[5] plants; it was therefore suggested that chelated iron is the form translocated in intact plants. The effect of bean roots on the mobility of calcium ions in the xylem may also result from the binding of these ions to organic ligands during permeation of the roots, and thus translocation in the xylem as metal organic calcium compounds. This hypothesis seems to be confirmed by further experiments, the results of which will be published in detail elsewhere.

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RADIOBIOLOGY

Roles of Phosphate and Respiration in Nuclear Damage in Irradiated Rat Thymocytes

THE highly radiosensitive thymic lymphocyte (or thymocyte) suffers true physiological death shortly after irradiation with relatively low doses of X-rays. The most striking symptom of radiation damage is the loss of nuclear structure (pycnosis); the prominent chromatin granules and threads of the interphase nucleus disperse and form a structurally homogeneous basophilic mass[1].

The development of nuclear homogeneity is prevented, or reduced, by the inhibition of respiration either with inhibitors such as cyanide and high concentrations of nicotinamide, or by incubating the irradiated cells in an anaerobic environment[2,3]. Changing the phosphate concentration of the medium also has a profound effect on the post-irradiation rate of nuclear homogenization[1,4]. This communication describes the relationships between respiration rate, phosphate concentration and nuclear structural homogenization (pycnosis). It also presents evidence to support the hypothesis that nuclear homogenization is caused by phosphate.

Thymocytes (from 1-month-old Sprague–Dawley rats) were suspended in a glucose-phosphate medium buffered at *p*H 7·2 with *tris* (hydroxymethyl) aminomethane. Phosphate was added in the form of sodium monohydrogen phosphate, and variation of its concentration required that the concentration of sodium chloride also be varied to keep the total Na^+ concentration at 130 m.equiv./l.; the rate of nuclear homogenization is strongly influenced by the concentration of sodium ions[1]. This medium and the method of preparing thymocyte suspensions have already been described in full[1,2,4,5].

Thymocyte suspensions were irradiated at 37° C with 1,000 r. (at 100 r. min^{-1}) of 250-kV X-rays produced by a Seifert 'Isovolt' X-ray machine. After irradiation, 2 ml. samples were removed from the suspensions (containing about 1×10^8 cells/ml.) and placed in respirometer flasks. After irradiation, oxygen consumption was measured for 2 h with a Warburg constant volume respirometer. The gas phase in the flasks was air. The cells were then fixed in neutral formalin and stained with Delafield's haematoxylin according to the method developed by Whitfield *et al.*[1].

The relation between respiration rate and nuclear homogenization was determined by suspending irradiated cells in medium containing 15 mM sodium monohydrogen phosphate and varying their respiration rate by incubating them at various temperatures from 25° to 37° C. The rate of nuclear homogenization was very sensitive to changes in the respiration rate between 8 and 24 μl. oxygen consumed per 10^8 cells/h (Fig. 1). When the oxygen consumption fell below 8 μl./10^8 cells/h, the development of nuclear homogeneity was completely inhibited.

Therefore, when the phosphate concentration is constant, the rate of nuclear homogenization is determined by the respiration rate. However, experiments with 2,4-dinitrophenol clearly showed that respiration itself was not directly responsible for the nuclear changes. When dinitrophenol was added to cell suspensions (in 15 mM phosphate medium; incubated at 37° C) at a concentration

of 10^{-4} M immediately after irradiation, there was no inhibition of respiration, but the development of nuclear homogeneity was strongly inhibited. For example, the respiration rate in the presence of dinitrophenol was 24·1 μl. oxygen/10^6 cells/h while in the control it was 20·6. However, dinitrophenol reduced the percentage of cells with homogeneous nuclei 2 h after irradiation from the control value of 25·0 per cent to 1·9 per cent. Higher concentrations of dinitrophenol (5×10^{-4} and 10^{-3} M) halved the respiration rate and inhibited nuclear homogenization.

Concentrations of dinitrophenol which do not inhibit respiration inhibit the active transport of inorganic phosphate[6]. Because active transport of phosphate into cells and mitochondria is driven by respiration[7,8] and the post-irradiation rate of nuclear homogenization is strongly affected by the concentration of inorganic phosphate in the medium[1,4,5], it is very likely to be the phosphate which is somehow responsible for the nuclear changes. Respiration would influence nuclear changes only indirectly by way of its role in active transport of phosphate.

To relate the respiration and phosphate effects, we incubated irradiated cells at 37° C in media containing various concentrations of sodium monohydrogen phosphate. Raising the concentration of sodium monohydrogen phosphate from 0 to 2·0 mM increased the respiration rate (Fig. 2*A*), but there was no change in the rate of nuclear homogenization (Fig. 2*B*). However, the rate of nuclear homogenization was directly and linearly proportional to the phosphate concentration between 2·0 and 30·0 mM while the respiration rate remained essentially constant (Fig. 2*A* and *B*). Therefore, it is probable that phosphate is directly responsible for nuclear homogenization.

On the basis of cytological[1,4,5,9] and biochemical[10–13] observations, we have already suggested[1,2,5] that nuclear homogenization in irradiated thymocytes arises from a phosphate-induced separation of the chromatin-condensing histones from the deoxyribonucleic acids of the nucleoproteins which constitute the chromatin. It has, in fact, been clearly and directly shown that phosphate compounds are responsible for structural homogenization in isolated nuclei[9,12].

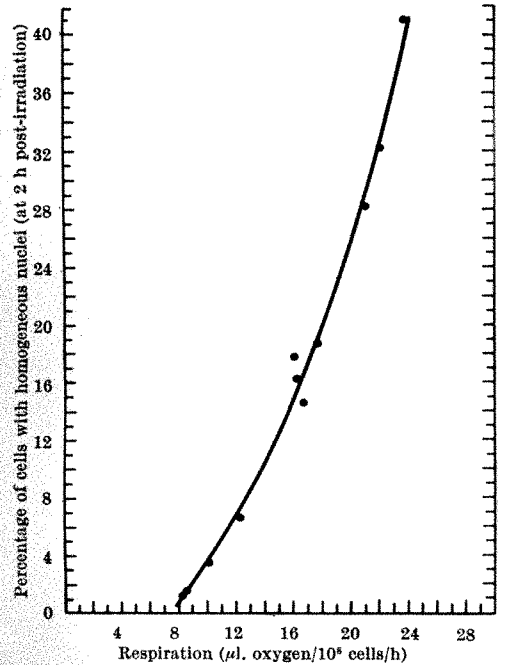


Fig. 1. Relation between the rates of nuclear homogenization and respiration in irradiated cultures of rat thymocytes. The respiration rate was varied by incubating the cells at different temperatures (25° – 37° C)

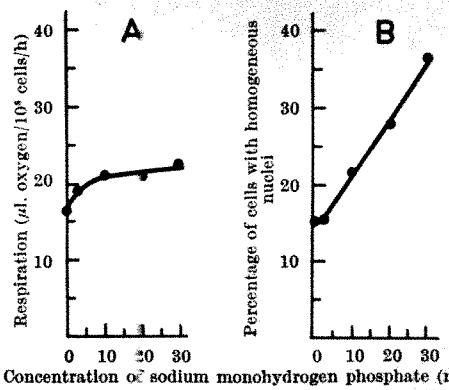


Fig. 2. Effect of varying the phosphate concentration in the culture medium on (*A*) the respiration rate and (*B*) the rate of homogenization of nuclear structure in populations of irradiated rat thymocytes

A satisfactory explanation of the present observations could therefore be formulated in the following way. During the first hour after irradiation, respiration[2] and phosphate uptake[14] proceed normally; however, the ability of the irradiated nuclei to combine inorganic phosphate with adenosine diphosphate to form adenosine triphosphate is impaired or inhibited[14,15]. For this reason, free phosphate accumulates and releases the histones from the irradiated, and thus unstable[16], deoxyribonucleoproteins of the chromatin aggregates. Therefore, variation of the respiration rate affects the post-irradiation development of nuclear changes indirectly by way of a change in the amount of phosphate which is drawn into the cell during the first hour after irradiation.

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Strontium-90 in Finnish and some Imported Cereals during the Harvest Period 1963–64

MILK and cereals provide the major part of the calcium, and consequently of the strontium-90, in the Finnish diet. According to the statistics of the Food and Agriculture Organization[1], the contribution of cereals to the calcium supply is calculated at 6 per cent; this is much less than the corresponding figure for milk, 87 per cent. However, the ratio of strontium-90 to calcium is so much greater in cereals than in milk that the cereals approach milk in importance as a supplier of radio-strontium, especially in a country like Finland, where no calcium is added to cereals.

Samples of wheat and rye from the 1963 harvest (harvest period 1963–64, the main part being consumed during 1964) were delivered by the State Grain Storage

from four storage sites (Districts Nos. 1, 2, 3 and 4). District No. 1 is an area which contributes about 80 per cent of all Finnish wheat production, and nearly half the rye production. The proportion of wheat from District No. 2 is about 6 per cent. Districts 3 and 4 represent only local production in a narrow area. The State Grain Storage also collected the samples of imported cereals; these samples were taken from cereals imported during the winter 1963–64—their histories are unknown apart from their countries of origin, namely, the U.S.S.R., the United States, Canada and Argentina.

The determinations were made in respect of whole grain and flour. The flour was separated in a laboratory mill with a yield of 55 per cent for wheat flour and 80 per cent for rye flour. The analyses were made as double determinations. 1 kg of flour and 0·5 kg of whole grain were taken for each determination. The analytical method used, with some modifications, is given in ref. 2. The yttrium daughter of strontium-90 was precipitated as oxalate, and the radioactivity was counted in an anti-coincidence β-counter, of which the background was about 0·7 c.p.m. and the efficiency about 50 per cent for yttrium-90. The chemical yield of strontium was determined with an atomic absorption spectrophotometer, and the yield of yttrium determined gravimetrically.

It can be seen that there is a distinct difference between the strontium-90 activities of the Finnish and the imported cereals. The reason for this is not clear, as the histories of the imported samples are unknown. These may be from the harvests of earlier years. If this were the reason for lower levels of activity, they should have been harvested in 1961 or earlier—Finnish cereals of the 1962 harvest[3] were of appreciably higher activity than the imported material of 1963–64 examined in this investigation. When the levels of activity of the 1963 Finnish harvest are compared with the Danish results from the same year, it is found that the level of strontium-90 in Danish wheat is very similar to the activity found in the Finnish wheat; the mean level of strontium-90 in whole grain according to the Danish determinations[4] was 846 S.U.—the corresponding Finnish value was 890 S.U. In the Danish rye, on the other hand, the strontium-90 content was appreciably higher than in the Finnish: 1195 and 637 S.U. respectively. The West German results from 1962 (ref. 5) are also rather similar to the corresponding Finnish figures for the same year. Thus, at least so far as the neighbouring countries are concerned, the contamination of cereals is rather similar to that

Table 1. STRONTIUM-90 IN WHEAT AND RYE IN THE HARVEST PERIOD 1963–64

Sample	Moisture	Ca-content (g/kg fresh weight)	Strontium-90 activity (pc./kg fresh weight)	(pc./g calcium)	Deviation from the mean of the double determinations (%)
		Wheat			
Whole grain					
District 1	12·26	0·380	328	863	0·34
District 2	12·44	0·420	446	1,063	5·61
District 3	11·44	0·545	357	655	1·30
District 4	13·13	0·405	397	981	0·34
Russian	10·86	0·385	46	120	2·27
Soft White	12·11	0·350	73	208	5·42
Manitoba	12·45	0·280	78	279	3·40
Flour					
District 1	14·00	0·155	33	212	7·50
District 2	14·21	0·155	42	269	0·58
District 3	14·01	0·190	44	229	3·45
District 4	13·88	0·190	82	433	1·06
Russian	13·98	0·140	12·0	86	0·17
Soft White	13·60	0·200	13·5	67	2·15
Manitoba	14·02	0·130	11·5	88	0·87
		Rye			
Whole grain					
District 1	12·26	0·340	174	513	1·26
District 2	12·64	0·360	294	816	7·26
District 3	12·24	0·345	139	403	3·96
District 4	13·83	0·400	326	816	1·68
Russian	11·56	0·350	109	310	0·23
Argentinean	11·26	0·380	45	119	4·47
Flour					
District 1	11·61	0·290	145	500	7·38
District 2	11·85	0·220	224	1,018	0·46
District 3	11·39	0·260	129	496	3·81
District 4	12·20	0·320	269	840	1·26
Russian	11·24	0·300	86	288	0·39
Argentinean	11·13	0·420	42	100	3·00

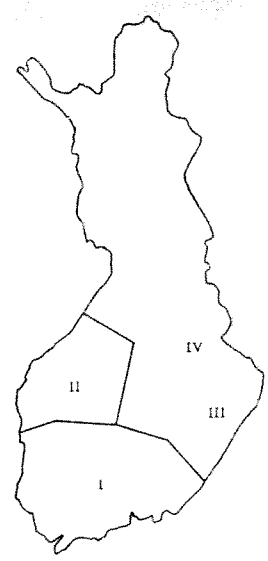


Fig. 1. The grain collecting areas

in Finland, and it seems probable that the reason for the lower levels of contamination of the imported cereals depends not so much on a difference in location but rather on a difference in the time of harvesting.

We thank the State Grain Storage for the important contribution it made to this investigation in collecting the samples.

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ENTOMOLOGY

Chromosome Numbers of some Psocid Genera (Psocoptera)

PSOCID chromosomes have been studied by Boring[1], who has described in detail the behaviour of the chromosomes of *Cerastipsocus venosus* (Kolbe) during meiosis in spermatogenesis. The chromosome number for this species of the family Psocidae was established as $2n = 16 + 1$ in the male. We have examined testis squash preparations of a number of Hong Kong species, both in order to verify Boring's description and interpretation of the mechanism of sex determination, and also to establish the chromosome numbers of representatives of as many genera and families as possible.

Specimens of twenty-one species, representing seventeen genera in twelve families of Psocoptera, were collected in Hong Kong throughout the year. Males were used in this investigation; female oogonial meiosis and embryonic mitotic figures were attempted, but without success.

The insects were lightly etherized, and the testes were removed by dissection in 0·7 per cent sodium chloride containing a little detergent to reduce surface tension, and transferred into a drop of aceto-carmine to fix and stain for 20 min on an albuminized slide[2]. The testis squashes were made according to the method described

Table 1. DIPLOID NUMBER OF CHROMOSOMES OF VARIOUS SPECIES OF HONG KONG PSOCIDS

Sub-order	Division	Family	Species	2n in male
Trogiomorpha	Atropetae	Trogiidae	*Lepinotus inquilinus* Heyden	16+1
		Psoquillidae	*Psoquilla marginepunctata* Hagen	18+1
	Psocatropetae	Psyllipsocidae	*Psocatropos* sp.	28+1
Troctomorpha	Amphientometae	Amphientomidae	*Seopsis* sp.	14+1
Psocomorpha	Caecilietae	Caeciliidae	*Caecilius singaporensis* Enderlein	16+1
			Caecilius sp.	16+1
			Dypsocus sp.	16+1
	Homilopsocidea I	Mesopsocidae	*Mesopsocus hongkongensis* Thornton	16+1
		Philotarsidae	*Haplophallus orientalis* Thornton	16+1
	Homilopsocidea II	Peripsocidae	*Ectopsocopsis cryptomeriae* (Enderlein)	16+1
			Ectopsocus maindroni Badonnel	16+1
			Peripsocus fasciatus Thornton	16+1
			Peripsocus quercicola Enderlein	16+1
			Peripsocus sp.	16+1
		Hemipsocidae	*Hemipsocus* sp.	16+1
		Pseudocaeciliidae	*Pseudocaecilius hirsutus* Thornton	16+1
		Archipsocidae	*Archipsocus* sp.	16+1
	Psocetae	Psocidae	**Cerastipsocus venosus* (Kolbe)	16+1
			Psococerastis sinensis Thornton	16+1
			Ptycta incurvata Thornton	16+1
			Trichadenotecnum medium Thornton	16+1
			Trichadenotecnum nudum Thornton	16+1

* Boring's determination.

by Darlington and La Cour[3]. The slides were made permanent by removing the cover glass using dry ice[4].

The chromosomes of psocid spermatogonia are short and small (Fig. 1), and it was not possible to interpret their morphology from the preparations.

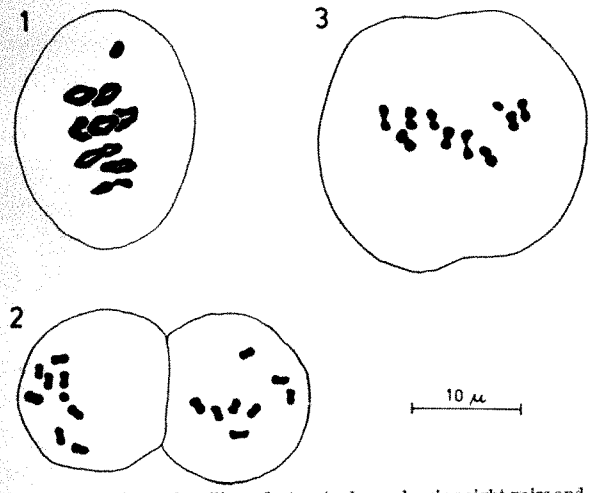


Fig. 1. (1) *Lepinotus inquilinus*, first metaphase, showing eight pairs and one odd chromosome. (2) *Hemipsocus* sp., second metaphase, showing eight chromosomes in one cell, nine in the other. (3) *Psoquilla marginepunctata*, first metaphase, showing nine pairs and one odd chromosome. All drawings to same scale

In all species examined an odd chromosome was present. Boring's interpretation of the sex-determining mechanism is therefore confirmed, and it seems likely that the *XO* type of sex determination is general in the Psocoptera.

The chromosome numbers found for the different species are listed in Table 1, in which the classification adopted is that of Badonnel[5].

The constant number of $2n = 16 + 1$ for eighteen representatives of fourteen genera of eight families of the sub-order Psocomorpha is noteworthy, particularly in view of the fact that departures from this were found in the two remaining sub-orders.

The three sub-orders are regarded on morphological grounds as fairly discrete taxonomic groups; the present investigation would appear to confirm this, although an investigation of more representatives of other families of the Trogiomorpha and Troctomorpha, usually regarded as more primitive than the Psocomorpha, is needed before any evolutionary conclusions can be drawn.

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A Neural Fat-body Sheath in a Phytophagous Insect (*Carausius morosus*)

RECENT experiments have demonstrated rapid exchanges of inorganic ions and molecules between the haemolymph and the tissues of the central nervous system in insect species[1–3]. These exchanges occur through the peripheral cellular and fibrous nerve sheath, which in most species is bathed directly by the surrounding haemolymph. In certain regions, however, as in the cockroach, the surface of the abdominal nerve cord may be overlaid by patches of fat-body. Electron microscopic investigation has shown that in the cockroach the fat-body cells exhibit a very close relation with the underlying neural lamella[4]. In view of the relative permeability of the neural lamella it was suggested[4] that the metabolic processes of the fat-body cells might be intimately linked with those of the underlying perineurium, which is thought to be involved in the mobilization of nutrient substances by the central nervous system[5].

In an insect such as the cockroach, the deposits of closely adhering fat-body cells are confined as small patches on the surface of ganglia and are absent from the inter-ganglionic connectives. Recent investigation has shown that in the stick insect (*Carausius morosus*), however, the fat-body cells are modified to form a continuous sheath which completely encloses both the ganglia and connectives of the ventral nerve cord (Fig. 1). The fat-body cells overlying the ganglia tend to be thinner and less closely packed than those associated with the connectives; the continuity of the sheath is, however, maintained by the basement membrane. The ultrastructural organization of these fat-body cells is essentially similar to those found in other parts of the insect. The disposition of the fat-body cells appears to differ from that found in the cockroach ganglia in that they do not seem to be closely applied to the surface of the neural lamella. The environment contained between the fat-body sheath and the neural lamella has also been found to contain haemocytes.

The discovery of the neural fat-body sheath surrounding the central nervous system in *Carausius* is of interest in view of the extremely specialized composition of the haemolymph in this insect. The haemolymph of this phytophagous species is characterized by an extremely low sodium content, of about 20 mmole/l. (refs. 3, 6–9), and a relatively high magnesium level, averaging 60 mmole/l. The haemolymph is also relatively dilute, with a freezing point depression of only 0·53° C (ref. 8). It is possible that this neural fat-body sheath may be involved in the ionic regulation of the central nervous tissues. It might, for example, contribute to the relatively large, rapidly exchanging, sodium fraction demonstrated in a previous investigation[3]. Preliminary experiments have shown that there is a potential gradient of between 15–20 mV across the fat-body sheath, the inner surface being positive with respect to the exterior. The un-

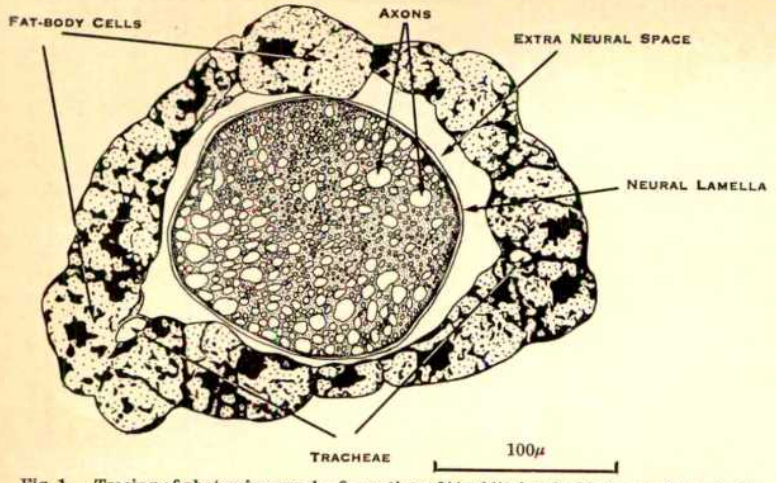


Fig. 1. Tracing of photomicrograph of a section of 'Araldite'-embedded material stained with methylene blue (ref. 10). The diagram shows the sheath formed by fat-body cells around a connective between pro- and meso-thoracic ganglia

specialized nature of the fat-body cells in this sheath is, however, difficult to correlate with any extensive role in ionic regulation. Furthermore, nervous conduction has been shown to occur in the absence of the fat-body sheath in preparations bathed in low sodium solutions.

The possibility exists that this fat-body sheath may represent a device to maintain an adequate concentration of appropriate nutrient molecules in the immediate vicinity of the nerve cord surface. Such a specialization might also be correlated with the relatively low osmotic concentration of the haemolymph in this species.

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AGRICULTURE

Influence of the Level of Nitrogenous Fertilizer on the Yield and Dry Matter Content of Marrow Stem Kale at Various Sowing Dates

ALTHOUGH nitrogen appears to be the major element affecting the yield of kale[1], there is marked variation in response[2], which depends on the level of soil fertility[3], rainfall[4] and the dates of harvesting[4,5] and sowing, some authors finding a greater response to nitrogen when sowing occurs after early June[2]. It is also known that higher rates of nitrogen reduce the dry matter content[4,5] and increase the number of lodged and rotted plants in marrow stem kale, especially with very late harvesting[5]. In 1962, 1963 and 1964 the effects of nitrogen level and plant population on the yield and dry matter content of Cannells marrow stem kale were investigated in respect of varied dates of sowing and two dates of cutting (Table 1). Five levels of nitrogen were used: (*a*) no nitrogen, 1963 and 1964; (*b*) 2 cwt. nitro-chalk (21 per cent nitrogen) per acre, late sowing 1964; (*c*) 4 cwt. nitro-chalk/acre, 1962, 1963 and 1964; (*d*) 6 cwt. nitro-chalk/acre, early sowing 1964; (*e*) 8 cwt. nitro-chalk/acre, 1962, 1963 and 1964. All nitrogen was broadcast immediately after drilling and each treatment received a basal dressing of 5 cwt./acre of a 20 per cent phosphorus pentoxide compound, 20 per cent potassium monoxide compound which contained no nitrogen. Graded seed was precision drilled at 1 and 2 in. spacings in rows 8, 16 (1962) and 24 in. apart.

In all years the response to nitrogen level obeyed the law of diminishing returns, but the magnitude of the response depended on the date of sowing and declined as sowing was delayed. The actual yield of dry matter (Table 1) and the response in terms of lb. dry matter/unit nitrogen applied (Table 2) were thus very large with the early sowings and lower rates of nitrogen application, but were small or even negative with late sowing and a high rate of nitrogen application. The response to a high nitrogen level was also greater at the second harvest, except for (*e*) in 1963.

Table 1. WEIGHT OF DRY MATTER/UNIT AREA (CWT./ACRE) AND PERCENTAGE DRY MATTER OF LEAF AND STEM

Treatments		Weight of dry matter (cwt./acre)		Dry matter (per cent) Leaf		Stem	
				1962			
Date of cut		25.9	14.11	25.9	14.11	25.9	14.11
Date of sowing	Nitrogen level*						
E, 16.4	*c*	77·7	88·7	15·03	14·60	15·42	17·13
	e	85·8	100·9	15·31	13·98	13·76	14·43
M, 30.5	*c*	71·8	85·8	13·25	13·36	10·89	12·77
	e	73·6	91·5	13·37	13·15	10·38	12·20
L, 10.7	*c*	37·4	51·1	10·29	12·22	7·86	9·73
	e	35·4	49·5	9·91	11·70	7·61	8·74
				1963			
Date of cut		25.9	10.12	25.9	10.12	25.9	10.12
E, 7.5	*a*	53·4	60·0	14·10	14·17	18·42	20·95
	c	86·0	104·8	14·13	12·79	16·06	17·55
	e	96·7	111·9	13·50	12·22	13·72	15·45
L, 3.7	*a*	34·7	53·6	10·77	12·17	9·87	13·84
	c	40·9	60·7	9·98	11·91	8·37	11·73
	e	42·6	58·9	8·95	11·32	7·40	10·02
				1964			
Date of cut		22.9	8.12	22.9	8.12	22.9	10.12
E, 7.5	*a*	40·0	35·3	18·62	14·11	20·94	20·02
	c	61·4	59·8	16·79	12·48	17·19	15·74
	d	68·6	74·2	16·20	11·95	15·80	14·89
	e	70·0	72·7	15·70	11·32	14·61	13·19
L, 2.7	*a*	30·9	43·4	14·20	12·87	11·96	16·06
	b	35·2	50·8	13·46	11·74	11·15	13·52
	c	36·1	52·2	13·05	11·59	10·33	12·35
	e	40·0	56·4	12·24	10·39	9·50	10·39

Sowing date: *E*, early; *L*, late.

* *a*, No nitrogen applied; *b*, 2 cwt./acre nitro-chalk applied; *c*, 4 cwt./acre nitro-chalk applied; *d*, 6 cwt./acre nitro-chalk applied; *e*, 8 cwt./acre nitro-chalk applied. The nitro-chalk used contained 21 per cent nitrogen.

Table 2. RESPONSE IN LB./UNIT NITROGEN FOR THE FIRST AND SECOND 4 CWT. APPLICATIONS OF NITRO-CHALK 21 PER CENT NITROGEN (84 UNITS OF NITROGEN)

Treatment	Early cut 1962	1963	1964	Mean	Late cut 1962	1963	1964	Mean
E, *c*	—	43·5	28·5	36·0	—	59·7	32·7	46·2
e	10·8	14·3	11·5	12·2	16·3	9·5	17·2	14·3
M, *e*	2·4	—	—	2·4	7·6	—	—	7·6
L, *c*	—	8·3	6·9	7·6	—	9·5	14·7	12·1
e	−2·7	2·3	5·2	1·6	2·1	−2·1	5·6	1·9

The visible effects of nitrogen on growth depended on sowing date and on season. The plots sown late and receiving no nitrogen (*a*) were affected only mildly, but the corresponding plots sown early exhibited severe symptoms of nitrogen deficiency and hard stunted growth. This effect was accentuated by severe drought in July, August and September 1964, when only 3·95 in. of rain fell, compared with 9·59 and 7·14 in. in 1962 and 1963 respectively. The 1964 drought also affected autumn growth with early sowing, when only the (*d*) and (*e*) levels of nitrogen gave any further increase in weight of dry matter at the second harvest. The yield of the (*a*) and (*c*) levels was actually lower.

Dry matter content was affected by date of sowing and nitrogen level, although differences were far greater in the stem than in the leaf. Dry matter content was greatest at the early sowings and the lowest nitrogen level, and declined as sowing was delayed and as nitrogen increased. The effect of the date of harvest depended on the season. In all sowings except the early sowing in 1964 when drought advanced maturity excessively, the content of dry matter rose substantially at the late harvest.

The effects of spacing, relative to nitrogen, were usually small and there were no significant interactions between the two. However, closer spacing gave a somewhat higher content of dry matter and a shorter crop; this is an important consideration when grazing kale.

This investigation shows that in conditions of field practice the level of nitrogen application to marrow stem kale should be related to the date of sowing so that the level is reduced as sowing is delayed. The results obtained in 1964 suggest that, in an arable rotation, 126 units is likely to be the maximum amount profitable for sowings before mid-May, while for sowings in early July the nitrogen should be restricted to about 60 units. If the crop is sown at the latter date, high levels of nitrogen result in severe reductions in the content of dry matter in the stem and reduced production of dry matter may occur in a normal year. However, in an extremely dry year higher rates of nitrogen application give a slightly better response. With late sowing it is normally better to drill closely rather than to give heavy applications of nitrogen.

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Absorption of Ethylene by Self-indicating Soda-lime

During investigations on the effects and interactions of carbon dioxide and ethylene on the physiology of flowers after cutting, an absorbent was required which would remove respiratory carbon dioxide but not ethylene. Investigations by Walls[1] and Potter and Griffiths[2] indicated that soda-lime absorbs fruit volatiles but probably not ethylene. Petri dishes of carbon dioxide absorbents (150 g) were placed in large glass tanks of capacity about 70 l., sealed, and then injected with sufficient ethylene through a rubber cap ('Suba-seal') to give a concentration of approximately 1·0 p.p.m. After allowing time for mixing, gas samples (1·0 ml.) were withdrawn at intervals and the concentration of ethylene determined by gas chromatography using a flame-ionization detector as described by Meigh[3]. The absorbents investigated were soda asbestos, sodium hydroxide, and self-indicating ('Carbosorb', BDH; 'Indicarb', Hopkin and Williams) and non-indicating soda-limes. Sodium hydroxide proved the most suitable for the investigation in hand.

However, it became clear that there was a substantial difference between the two soda-lime formulations; the self-indicating forms rapidly absorbed the ethylene, whereas the non-indicating forms did not. These observations were confirmed by repeating the experiments using small quantities of soda-lime (2·5 g) wrapped in cotton cloth inserted into winchester quart bottles. The bottles were then stoppered with 'Suba-seals' and treated with ethylene and sampled. The results are shown in Fig. 1.

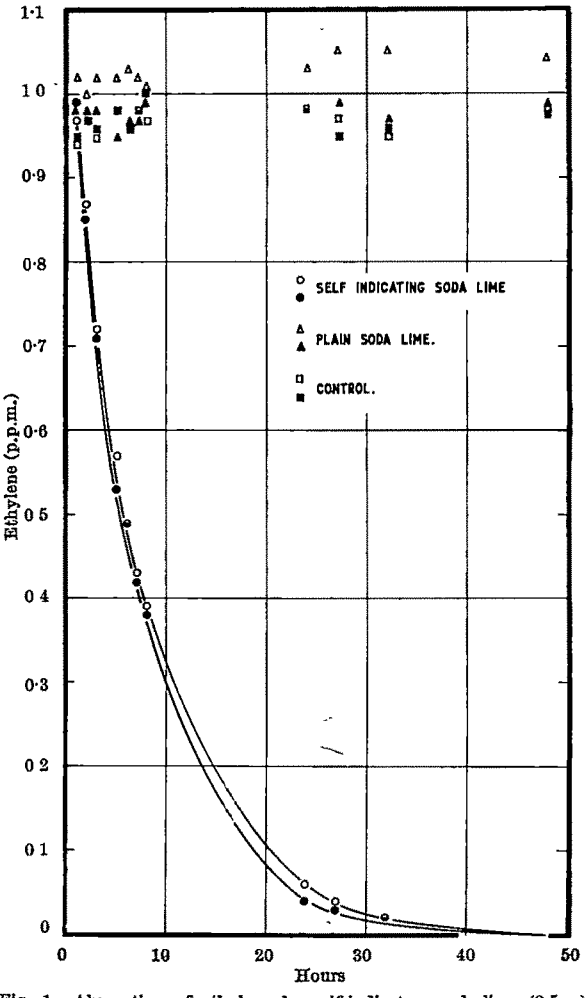


Fig. 1. Absorption of ethylene by self-indicating soda-lime (2·5 g) enclosed in winchester quart bottles. Initial concentration of ethylene approximately 1·0 p.p.m (≏2·75 μl.)

Two chemical supply houses suggested that there were no physical or chemical differences in their soda-limes apart from the addition of the indicator. One of the firms disclosed that the indicator is a manganate. A possible explanation of the phenomenon is that ethylene is oxidized to ethylene glycol, either by the manganate or by permanganate formed in the presence of carbon dioxide and water.

The important point is that recently there has been considerable interest in the physiological interaction of carbon dioxide and ethylene[4]. This is well recognized for fruits and has been reported for cut flowers[5], and is supported by work in this laboratory. Thus the observation that the commonly used self-indicating form of soda-lime absorbs both carbon dioxide and traces of ethylene at concentrations which are physiologically active may indicate a possible source of error in interpreting results in investigations in this field. The possibility of using some form of manganate for absorption of ethylene would merit further investigation.

We thank British Drug Houses Ltd. for disclosing and allowing us to publish the indicator in 'Carbosorb'.

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PSYCHOLOGY

Obstacle Detection using Ambient or Self-generated Noise

OBJECT ranging can be achieved by humans using the sound reflected or scattered from obstacles. For a range of wave-lengths short compared with the size of its surfaces, and long compared with the size of the irregularities in these surfaces, specular reflexion will be obtained and an acoustic image of the sound source will be formed analogous to an optical image. In many situations, however, such as the approach of an external corner, an acoustic image may not be audible from the observer's position. In these cases sound scattered from the edges and irregularities may, or frequently may not, give the necessary warning. Such signals are, however, weak compared with those of the acoustic image with which we are chiefly concerned.

In the neighbourhood of such an obstacle, sound reaches the ears of an observer both directly and by reflexion from the obstacle, as if originating from the acoustic image. Interference between these signals introduces a series of maxima and minima into the frequency spectrum, the spacings of which depend on the path difference. A sound of broad bandwidth develops a characteristic tone the pitch of which becomes higher as the obstacle is approached[1–3]. This tone is not normally perceived as such; blind subjects generally claim to 'feel' the presence of an obstacle directly[4]. It is, however, readily perceivable under laboratory conditions.

Blind people are reputed to find difficulty in negotiating obstacles in the immediate vicinity of intense noise sources[5]. There would appear to be several reasons for this difficulty.

First, people accustomed to using self-generated noises such as their own footsteps may find such noises masked and be unaware that reflexion of the extraneous noise itself could give them the information that they require.

For people who normally make use of the ambient noise, there are some further causes of difficulty when the source is nearby. The depth of modulation of the spectrum as they approach an obstacle will depend on the relative amplitude of the direct and reflected waves. For large source distances and high reflexion coefficients, these will be nearly equal and the depth of modulation will be high. Nearby sources, however, in which the ratio of the path lengths is large may give modulation depths below threshold.

The pitch itself may also be very different from that obtaining for a distant source so that, for example, while maintaining a parallel course with a wall they would hear a changing pitch. Naturally, this could lead to the disorientation of the observer. Moreover, when the source lies between the observer and the obstacle, it can be seen that the pitch will be determined predominantly by the position of the source and very little by the position of the observer. This is, however, unimportant in practice as the reflecting surface would not be considered as an obstacle until the source of sound itself had been negotiated.

The main object of this article is to point out the potential importance of rates of change of pitch in giving additional ranging information, enabling several ambiguities to be overcome. The first of these is the occurrence of octave mismatches when this type of signal is matched in pitch to a sine wave, indicating the possibility of more than one estimate for the distance. The second arises when the source of sound lies at an angle remote from the normal to the reflecting surface, giving a higher value of pitch than for angles near the normal ($f = \frac{c}{2x\cos\theta}$ where c is the velocity of sound, x the distance of the observer's ear from the plane of the reflecting surface, and θ the angle between the source of sound and the normal to the surface).

In both cases, if pitch is defined in the musical sense of log frequency, it can be seen that the rate of change of pitch with movement of the observer, $\frac{df/f}{ds}$, will lead to unique evaluation of the distance of the obstacle in the direction of motion. Specifically, $\frac{df/f}{ds} = -\frac{1}{s}$ regardless of the angle of approach; so that the motion required to change the pitch by a given small amount is a direct measure of obstacle distance. This will, of course, be true only if the distance of the source from the surface is large compared with that of the observer. For distances that are comparable, the expression for the path difference is more complex; but, as before, the rates of change can be calculated by differentiating this. Despite the apparent complexity of this expression, the rate of change of pitch tends toward the simple value given above as the obstacle is approached.

It would seem then that, although a blind man must inevitably encounter difficulty and disorientation in the neighbourhood of a sound source, nevertheless if he is prepared to carry on his original course the appropriate ranging information will reappear and become more definite and more accurate as he gets nearer to the obstacle.

At higher speeds of travel, greater distances will be covered before the observer can come to a halt. The higher speeds, however, will also lead to greater rates of change of pitch with respect to time or conversely to a greater distance at which a predetermined rate of change is achieved.

As the rate of change of pitch is proportional to velocity, and inversely proportional to distance in the direction of motion, it follows that once a certain critical value of rate of change of pitch has been reached, the observer, whatever his velocity, will have a constant time, T, before collision if he maintains this velocity. An experiment of Leonard and Wycherley[6] gave stopping distances proportional to speed up to 5 m.p.h., at about 1 ft./m.p.h. (0·7 ft./ft./sec). The critical value for rate of change for this subject would therefore be about 2 octaves/sec, giving him 0·7 sec before collision if he takes no avoiding action or, assuming uniform retardation, 1·4 sec to stop.

The human auditory system is likely to be particularly sensitive to rates of change of this order, as they lie well within the range of rates encountered in the rise and fall of the formant frequencies of speech. It is interesting to note that, in the case of the cat, cortical neurones have been found that respond only to certain ranges of changing pitch[7]. Although the frequencies are much higher than can be perceived by humans, the rates of change concerned are comparable (1–5 octaves/sec).

Furthermore, most subjects have expressed the opinion that the pitch of this type of signal is much more readily perceived when it is changing.

It would appear that our auditory system and possibly those of some animals are well suited to the problems of detection and avoidance of obstacles.

I thank the Science Research Council for supporting this work.

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1 Wood, A., *Acoustics*, 200 (Blackie, London, 1940).
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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Wednesday, July 13

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (at University College, Gower Street, London, W.C.1), at 2.30 p.m.—Symposium on "What are the Criteria by which the Content of Sixth Form Mathematics Syllabuses should be Judged?"

BRITISH INTERPLANETARY SOCIETY (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 6.30 p.m.—Academician A. A. Blagonravov and Mr. S. N. Vernov (U.S.S.R.): "The Soviet Space Programme".

Monday, July 18—Friday, July 22

ROYAL MICROSCOPICAL SOCIETY (at Imperial College, London, S.W.7), at 10 a.m. daily—Conference on "The Role of the Microscope in Scientific Investigation".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

JUNIOR LECTURER (graduate with medical or science qualifications in pharmacology, biochemistry or physiology) IN PHARMACOLOGY—The Registrar, King's College (University of London), Strand, London, W.C.2 (July 11).

LECTURER IN EXPERIMENTAL PHYSICS—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (July 12).

EXPERIMENTAL OFFICER (graduate in geology, physics or chemistry) IN THE DEPARTMENT OF GEOLOGY, to take charge of and operate the X-ray laboratory, including scanning electron probe microanalyser and X-ray diffractometer—The Registrar, The University, Leicester (July 14).

LECTURER IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, The University, Liverpool, quoting Ref. CV/118/N (July 15).

LECTURER or ASSISTANT LECTURER (with a good honours degree in an appropriate subject and experience in the field of either experimental pharmacology or biochemical pharmacology) IN PHARMACOLOGY in the School of Pharmacology—The Registrar, Bradford Institute of Technology, Bradford, 7 (July 15).

SENIOR DEMONSTRATOR (graduate, preferably with a higher degree and research experience in industry, a university or government research establishment) IN THE SCHOOL OF PHYSICS, to undertake original research in collaboration in one of the research groups operating in the School of Physics and to take an active part in the undergraduate and postgraduate teaching programmes—The Registrar, The University, Newcastle upon Tyne, 2 (July 15).

SENIOR LECTURER (medically qualified) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, 3, quoting Ref. CV/119/N (July 15).

ASSISTANT LECTURER IN MATHEMATICAL LOGIC AND THE THEORY OF PROBABILITY IN THE DEPARTMENT OF HISTORY AND PHILOSOPHY OF SCIENCE—The Secretary, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 18).

DEMONSTRATOR (preferably with a special interest in taxonomy or ecology) IN THE DEPARTMENT OF BOTANY—The Registrar, University College of Wales, Aberystwyth (July 18).

LECTURER or ASSISTANT LECTURER (graduate in fuel science or technology, chemical engineering, physics or physical chemistry) IN THE DEPARTMENT OF FUEL SCIENCE, The Houldsworth School of Applied Science—The Registrar, The University, Leeds, 2 (July 18).

LECTURER (with a special research interest in genetics) IN THE DEPARTMENT OF BOTANY—The Deputy Secretary, The University, Southampton (July 18).

LIBRARIAN (graduate with a professional qualification in librarianship, and appropriate senior experience)—The Secretary, Royal College of Advanced Technology, Salford, 5, Lancs, quoting Ref. LY/19 (July 18).

TECHNICIAN IN THE ELECTRON MICROSCOPE LABORATORY at the Hammersmith Annexe, working mainly in the fields of biology and polymer physics—The Superintendent of the Physics Laboratories, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (July 18).

ASSISTANT LECTURER or LECTURER (with a good honours degree and experience in the fields of digital computers and electronics) IN THE DEPARTMENT OF ELECTRICAL AND CONTROL ENGINEERING—The Academic Registrar (LFG), Battersea College of Technology, London, S.W.11 (July 22).

LECTURER or ASSISTANT LECTURER (with good qualifications in astronomy with some research experience, or qualifications in mathematics or physics with experience in astronomy) IN ASTRONOMY—The Secretary, University College, Gower Street, London, W.C.1 (July 22).

ASSISTANT LECTURER (preferably with theoretical or experimental experience in plasma or laser physics) IN THE DEPARTMENT OF NATURAL PHILOSOPHY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 27/66 (July 23).

LECTURER (with a good honours degree and professional and/or research experience) IN CIVIL ENGINEERING—The Secretary, The University, Aberdeen (July 23).

LECTURER (with qualifications in entomology and preferably research experience in ecology) IN ENTOMOLOGY at the University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 25).

SENIOR LECTURER, LECTURER or ASSISTANT LECTURER (honours graduate) IN MATERIALS SCIENCE/PHYSICAL ELECTRONICS; and a LECTURER or ASSISTANT LECTURER (preferably with experience in modern semiconductor circuit design) IN COMMUNICATIONS ENGINEERING—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (July 25).

CHAIR OF MATHEMATICS; and CHAIR OF PHILOSOPHY—The Registrar, University of York, Heslington, York (July 29).

SENIOR LECTURER or LECTURER (suitably qualified and interested in any of the following research topics: solid state studies, radio investigations of the upper atmosphere such as meteor distribution, auroral zone detection and studies of air-glow and of the lower ionosphere) IN PHYSICS at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 29).

TECHNICIAN (with a pass degree, A.I.M.L.T., H.N.C. or equivalent qualifications, interested in intermediary metabolism and willing to handle large farm animals) IN THE DEPARTMENT OF BIOCHEMISTRY—The Secretary, Institute for Research on Animal Diseases, A.R.C., Compton, Near Newbury, Berkshire (July 30).

LECTURERS/ASSISTANT LECTURERS (with a degree in medicine or science, suitable teaching and research experience, and preferably with a special interest in enzymology or biophysical chemistry) IN BIOCHEMISTRY at the University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, July 31).

MICROBIOLOGIST (with a first- or upper second-class honours degree with microbiology or bacteriology as one of the main subjects, together with research experience and preferably a Ph.D.) at the Food Research Institute, Norwich, for fundamental research concerning food spoilage bacteria—The Secretary, Low Temperature Research Station, Downing Street, Cambridge, quoting Ref. 66/2 (July 31).

READER/SENIOR LECTURER or LECTURER/ASSISTANT LECTURER (graduate in electrical engineering or any other related discipline with research experience in the field of electronic engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING SCIENCE—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (July 31).

SENIOR LECTURER or LECTURER IN PREHISTORY IN THE DEPARTMENT OF ANTHROPOLOGY, University of Auckland, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, July 31).

RESEARCH ASSISTANT (with a good honours degree in zoology and preferably with interests in palaeontology, sedimentary petrology or structural geology) IN GEOLOGY IN THE DEPARTMENT OF BIOLOGY AND GEOLOGY—The Secretary, Norwood Technical College, Knight's Hill, London, S.E.27 (August 1)

SENIOR LECTURER (preferably medically qualified pharmacologist) IN THE DEPARTMENT OF PHARMACOLOGY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 1).

VETERINARY OFFICERS (men and women aged at least 23, M.R.C.V.S., with at least one year's appropriate postgraduate experience) with the Ministry of Agriculture, Fisheries and Food, for posts in various parts of the country—Civil Service Commission, Savile Row, London, W.1, quoting Ref. 130/66/1 (August 1).

LECTURER IN BIOCHEMISTRY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 5).

LECTURER (preferably with experience and interests in the fields of control engineering or energy conversion) IN ELECTRICAL ENGINEERING at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, August 8).

LECTURER (preferably with several years postgraduate experience and specialized in the field of theoretical elementary particle physics, and capable of supervising M.Sc. and Ph.D. students) IN MATHEMATICAL PHYSICS at the University of Adelaide—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Registrar of the University (Australia, August 8).

LECTURER (graduate of a British Commonwealth University or of equivalent status, preferably corporate member of the Institution of Mechanical Engineers, and teaching experience at university level) IN MECHANICAL ENGINEERING at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, August 15).

LECTURER or ASSISTANT LECTURER (preferably with research experience in plant metabolism including radio-tracer techniques) IN BOTANY—The Registrar, University College of North Wales, Bangor, North Wales (August 15).

LECTURER (preferably with a special interest in high energy nuclear physics) IN EXPERIMENTAL PHYSICS—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (August 15).

SENIOR LECTURERS/LECTURER IN PHYSICS in the School of Mathematics and Physics, Macquarie University, Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 26).

CHAIR OF MATERIALS SCIENCE at Monash University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or the Academic Registrar, Monash University, Clayton, Victoria, Australia (Australia and London, August 31).

CHAIR OF PSYCHOLOGY at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

LECTURER or ASSISTANT LECTURER IN THE NEW DEPARTMENT OF CERAMICS, Houldsworth School of Applied Science—The Registrar, The University, Leeds, 2 (August 31).

LECTURER (preferably with an interest in material science, physical electronics or monolithic circuits) IN ELECTRICAL ENGINEERING at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

SECOND CHAIR OF ELECTRICAL ENGINEERING at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

SENIOR LECTURER IN PHYSICS at the University of Cape Town, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, and The Registrar, University of Cape Town, Private Bag, Rondebosch, Cape Town, South Africa (August 31).

SENIOR LECTURER (with wide research experience in ionospheric or radio physics) IN THE DEPARTMENT OF PHYSICS (R.A.A.F. Academy), University of Melbourne, Australia, to engage full-time in research and postgraduate training—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 3).

ASSISTANT LECTURER (with experience either in the biochemistry or reproduction in vertebrates or in mechanisms of photosynthesis) IN BIOCHEMISTRY, to participate in the teaching and research programmes of the Department—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 26/66.

ASSOCIATE PROFESSOR IN THE FIELD OF MOLECULAR BIOCHEMISTRY—Prof. B. E. Conway, Chairman, Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada.

BIOCHEMIST (preferably graduate in chemistry or biochemistry) IN THE PATHOLOGY DEPARTMENT—The Group Secretary, Princess Margaret Hospital, Swindon, Wiltshire.

BIOCHEMIST (well-qualified with suitable research experience) IN THE PHYSIOLOGY DEPARTMENT to lead a small group investigating the biochemistry of the mammary gland and related topics—The Secretary, National

Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 66/N/15.

DEPARTMENTAL DEMONSTRATOR (preferably with some experience in X-ray crystallography or in physical methods of rock and mineral analysis) IN MINERALOGY—The Reader in Mineralogy, Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford.

EDITOR OF *Chemistry in Britain*—The Secretary and Registrar, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1.

LECTURER (preferably with the ability to assist with biochemistry) IN MICROBIOLOGY to teach the subject to university degree level—The Principal, College of Technology, Headington, Oxford.

MATHEMATICIANS to work on a wide range of problems arising from research into the utilization and distribution of electricity (topics include: arc physics, electro-chemical reaction kinetics, solid state devices, magnetism, electrical distribution systems, heat transfer, and the application of mathematics to life science)—D. C. Page, Head of Personnel Services, The Electricity Council, Research Centre, Capenhurst, Chester, quoting Ref. N/29.

READER (with high academic qualifications, together with an established record of research in an appropriate field such as vibrations, materials, applied thermodynamics or fluid mechanics) IN MECHANICAL ENGINEERING—The Academic Registrar, Hatfield College of Technology, Hatfield, Hertfordshire.

RESEARCH ASSISTANT OR EXPERIENCED SENIOR RESEARCH TECHNICIAN IN THE PHYSIOLOGY DEPARTMENT (Division of Biochemistry) for research on lipid and carbohydrate metabolism—The Assistant Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1.

RESEARCH FELLOW IN CHEMISTRY for work which will involve the application of far infra-red spectroscopy to problems in inorganic and physical chemistry, under the direction of Dr. A. Finch—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Council for Scientific Policy—University Grants Committee. A Report of a Joint Working Group on Computers for Research. Pp. vii+80. (Cmnd. 2883.) (London: H.M. Stationery Office, 1966) 6*s* 6*d*. net. [224

The British Industrial Biological Research Association Annual Report 1965. Pp. 43. (Carshalton, Surrey: The British Industrial Biological Research Association, 1966.) [254

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The Zoological Record. Vol. 100, Section 4 (1963). Coelenterata. Compiled by Dr. H. Dighton Thomas. Pp 30. 12*s*. 6*d*.; 1 80 dollars. Vol. 100, Section 15 (1963): Pisces. Compiled by G. Palmer and H. A. Toombs. Pp. 98. 30*s*; 4.23 dollars. (London: The Zoological Society of London, 1966) [254

Building Research Station Digest No. 69 (Second Series): Applications and Durability of Plastics. Pp. 8. (London: H.M. Stationery Office, 1966.) 4*d*. [254

River Pollution and the Farmer. Pp. 12. (Edinburgh: Department of Agriculture and Fisheries for Scotland, 1966) [264

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BBC Engineering Monograph No. 60: Colorimetric Analysis of Interference in Colour Television. By K. Hacking. Pp 28. (London: British Broadcasting Corporation, 1966) 5*s*. [274

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Heveacrumb: a Standard Malaysian Rubber in a New Form. Pp. 24. (London: Malayan Rubber Fund Board, 1966) [294

English Electric Valve Company By Precision to Perfection Revised edition Pp. 36 (Chelmsford: English Electric Valve Company, 1966.) [294

Astronomical Contributions from the University of Manchester. Series III, No. 128: Three Colour Photoelectric Photometry of the Moon. By G. L. Roberts. Pp. 80. (Manchester: The University, 1966) [294

Proceedings of the University of Newcastle upon Tyne Philosophical Society. Vol. 1, No. 4: The History of the Pottery Firm of W. H. Goss of Stoke-on-Trent, together with a Review of Some of the Wares Produced. By M. J. W. Willis-Fear. Pp. 36–51. (Newcastle upon Tyne: The University, 1965) [25

The British Coal Utilisation Research Association. Annual Report 1965. Pp. iv+106. (Leatherhead: The British Coal Utilisation Research Association, 1966) [25

The Commonwealth Forestry Institute, University of Oxford Forty-first Annual Report, 1964–65 Pp. 61. (Oxford: The Commonwealth Forestry Institute, The University, 1966.) [35

The Council of Engineering Institutions. Education and Training 1966. Statement No. 1: The Council's Examination. Part 1: Syllabuses and Specimen Papers. Pp. 36. (London: The Council of Engineering Institutions, 1966.) 2*s*. [35

The Kent Incorporated Society for Promoting Experiments in Horticulture. Annual Report of the East Malling Research Station for 1964 (1st October 1964 to 30th September 1965). Pp. xxiv+210. (A49 Fifty-third Year.) (Maidstone: East Malling Research Station, 1966.) 21*s*.; 3 dollars. [45

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1102, Vol. 259 (5 May 1966): Wind Effects on the Water in a Narrow Two-Layered Lake. Part 1: Theoretical Analysis. By N. S. Heaps. Part 2: Analysis of Observations from Windermere By N. S Heaps and A. E. Ramsbottom. Part 3: Application of the Theory to Windermere. By N. S. Heaps. Pp. 391–340. 15*s* 2.25 dollars. No. 1103, Vol. 259 (5 May 1966): Propagation of Ocean Swell Across the Pacific. By F. E. Snodgrass, G. W. Groves, K F. Hasselmann, G. R. Miller, W. H. Munk and W. H. Powers. Pp. 431–497. 27*s*; 4.05 dollars. (London: The Royal Society, 1966.) [55

Imperial Cancer Research Fund. Research Using Transplanted Tumours of Laboratory Animals. a Cross Referenced Bibliography—II. By D. C. Roberts. Pp. 127. (London: Imperial Cancer Research Fund, 1966.) [55

Office of Health Economics. The Common Illness of Our Time: a Study of the Problem of Ischaemic Heart Disease. Pp. 43. (London: Office of Health Economics, 1966.) 2*s*. 6*d*. [55

Other Countries

Académie Royale de Belgique. Mémoires. Tome 36, Fascicule 5: Propriétés Électriques Superficielles des Diélectriques Solides. Par R Goffaux. Pp. 74. 120 francs. Tome 36, Fascicule 6: l'Astronomie Royal de Turin, Giovanni Plana (1781–1864)—Un Homme, Une Carrière, un Destin. Par Dr Albert Maquet Pp. 254. 300 francs Tome 36, Fascicule 7 Évolution de la Végétation Épiphytique du Peuplier en Relation avec l'Age et les Modifications de l'Écorce. Par Jean Maigot. Pp. 82. 140 francs (Bruxelles: Académie Royale de Belgique, 1965) [254

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Malaysia: Ministry of Agriculture and Co-operatives. Division of Agriculture Bulletin No. 119: Soil Survey Manual for Malayan Conditions. By M. L. Leamy and W. P. Panton. Pp. x+226. (Kuala Lumpur: Division of Agriculture, Ministry of Agriculture and Co-operatives, 1966.) 5 Malayan dollars; 11*s*. 8*d*. [264

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United States Department of the Interior: Geological Survey Geophysical Abstracts, No. 231 (April, 1966). By James W. Clarke, Dorothy B. Vitaliano, Virginia S. Neuschel and others. Pp ii+299–396. 35 cents. Water Supply Paper 1767. Delaware Water. By W. C. Rasmussen, J W. Odell and N. H. Beamer. Pp. vii+106. 70 cents. Water-Supply Paper 1815: Gunpowder Falls, Maryland: Uses of a Water Resource Today and Tomorrow. By Deric O'Bryan and Russell L McAvoy. Pp. v+90. 45 cents. Professional Paper 502-A: The Hydrology and Mineralogy of Deep Springs Lake, Inyo County, California. By Blair F. Jones. Pp. iv+56+plates 1 and 2. Professional Paper 542-B: Effects of the Earthquake of March 27, 1964, at Whittier, Alaska. By Reuben Kachadoorian. Pp. vi+21+plates 1–3. (Washington, D.C: Government Printing Office, 1965 and 1966) [264

Smithsonian Institution: United States National Museum. Bulletin 245: Cincinnati Locomotive Builders, 1845–1868. By John H. White. Pp. ix+167. (Washington, D C.: Government Printing Office, 1965.) 2 dollars. [284

Glossary of Terms Frequently Used in Cloud Physics. Pp. 40. (New York: American Institute of Physics, 1966.) 1 dollar. [284

A Field Key to the Savanna Trees of Nigeria. By Dr. Brian Hopkins and D. P. Stanfield Pp. viii+39. (Ibadan: Ibadan University Press, 1966) 5*s*. 6*d*. [284

Bulletin of the American Museum of Natural History. Vol. 131, Article 4: Phyletic Studies of Teleostean Fishes, with a Provisional Classification of Living Forms. By P. Humphrey Greenwood, Donn E. Rosen, Stanley H. Weitzman and George S. Myers. Pp. 339–456+plates 21–23. (New York: American Museum of Natural History, 1966) 3 dollars [284

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HOW MUCH SCIENCE?

DECIDING what part of a nation's resources should be spent on scientific research and development remains unfortunately just beyond the reach of rational discussion. Though there is now a great volume of public literature on the various problems of estimating and allocating scientific resources, and though no self-respecting nation will embark on a year of public administration without some declaration of its intentions towards science, rules of thumb are still as conspicuous as more objective yardsticks in the making of scientific budgets. Recently the most familiar, and the most fashionable, of all these rules is the doctrine that total expenditure on research and development should amount to a certain proportion of the Gross National Product. In Britain, for example, this proportion is something like 2·7 per cent, and those who fashion science policy comfort themselves that this is only a little less than the corresponding proportion in the United States, and that it exceeds the percentage of the Gross National Product being spent on development in other European countries. But one nation's comfort is another's goad, and it is in this spirit that the most recent edition of the French Plan declares that expenditure on research and development must be at least 2·5 per cent of the Gross National Product by 1970.

The most obvious difficulty, of course, is that the percentage of a nation's Gross National Product being spent on research and development is only a rough-and-ready guide to the real volume of its scientific activity. Much depends, for example, on how much of the total is spent on military research and development, for in such activities it is known that qualified scientists can run through comparatively large sums of money in a year. The balance between academic and industrial research can also vary enormously within the confines of a fixed proportion of the Gross National Product, which also suggests that the use of the Gross National Product as an indicator can conceal great variations in the real volume of a nation's scientific effort—and great differences in the consequential benefits which it is entitled to expect. All this implies that yardsticks constructed from the Gross National Product have strictly limited value.

They can probably be used with confidence to suggest that nations spending a mere 0·3 per cent of their wealth on research and development are probably being too niggardly, and that nations spending—or trying to spend—30 per cent of their Gross National Product in the same way are being unreasonably ambitious. They are probably no more valuable than other indices—such as those which indicate the allocation of a nation's skilled manpower to different kinds of activities—in deciding whether the balance of activity is right or wrong. Indeed, the index based on the Gross National Product is likely to be misleading, and dangerously so, when used to suggest that all must be well with a nation's policies because the proportion of its Gross National Product being spent on research and development is such and such a percentage.

But if the Gross National Product is an insufficient yardstick for deciding science policy, what yardsticks should be used instead ? This was one of the questions implicit in the recent report of the Council on Scientific Policy in Britain, for it is plain that the Council's argument with the Treasury in the months ahead is going to depend in a crucial manner on the degree to which its claims on behalf of scientific research can be sustained by objective arguments. The question has also been raised explicitly in the United States, and one response has been the great volume of detailed study, under the National Academy of Sciences, which has now led to the publication of assessments of the various branches of science and of possibilities which the immediate future has to offer. Among these documents the report on chemistry (the Westheimer Report) stands out because of its calculation that the economic importance of the chemical industry in the United States should somehow be reflected arithmetically in the funds devoted to academic chemical research at American universities. On the face of things, this looks like the design of an objective yardstick of a novel kind, though in the event only the chemists have welcomed it wholeheartedly. In general, of course, the attempt to indicate where opportunities now exist for investing profitably in scientific research must be as much an embarrassment as a help to those who must allocate resources, for there is nothing to suggest how priorities should be decided among the possibilities which have been described.

The construction of objective yardsticks is plainly a problem of international importance. How is it to be begun ? The ideal, of course, would be some grand theory linking the investment of funds in scientific research and development with the economic value of the consequences. If only it were possible to show that a million dollars spent on research in high-energy physics must eventually result in, say, the addition of ten million dollars a year to that nation's Gross National Product, the difficulties of financing scientific research would melt away. The trouble is that the connexion between research and economic prosperity is by no means simple. Research is not always profitable. There are, for example, some circumstances in which it may be economically profitable not to carry out research but to use results obtained by other people, at least in the short run. There may also be circumstances in which a rapidly growing research programme may drain skilled people away from productive jobs, and may thus diminish a nation's productivity—in the short run, again.

The use of educational principles in constructing an objective yardstick for deciding expenditure on university research is perhaps a more hopeful possibility.

In Britain, thanks to the detailed description of the university system provided by documents such as the Robbins Report, there are good foundations for calculating how many people must be employed on science faculties at universities. For example, with an undergraduate population similar to that in 1964, and on the assumption that one undergraduate in three stays on to do research, it is easy to conclude that university research in Britain should cost something like £130 million a year, at least on the assumption that each member of a scientific faculty costs roughly £10,000 a year by the time he has been provided with a salary, somewhere to work and with adequate assistance and materials. As it happens, this figure is not very different from that which the British Government at present spends on university research, either through the grants of the research councils or by the subventions of the University Grants Committee. The immediate interest, however, is that this line of argument could in principle be made to yield a link between the nation's demand on the educational system and the cost of scientific research in the universities. It would in particular show that the greatest cost in increasing the population of post-graduate students will be that of providing the students and those who teach them with opportunities for research.

Another yardstick could in principle be designed to ensure that a nation could hold on to a certain proportion of its senior scientists. Though not enough is known about the reasons why people leave nations in Western Europe to take permanent jobs in the United States for example, there is no question that one of the decisive factors is the availability of facilities for research. It follows that a nation wishing to keep a greater proportion of its skilled people at home must spend more on competitive facilities. With persistence, this line of argument could be made to yield criteria for deciding how much money should be spent on capital equipment of various kinds. Like the educational yardsticks, those based on attempts to prevent emigration at a certain level would necessarily be incomplete. They would, however, have the advantage of being ways of linking the cost of scientific research with tangible decisions about public policy in a wider field. At a time when the gross yardsticks based on Gross National Product are evidently too crude, and when treasuries everywhere are anxious to see further growth of science justified objectively, they obviously have great value.

ELDO RECONSTITUTED

THE new agreement on ELDO (European Launcher Development Organization) reached at Paris on July 8 seems to have papered over the cracks previously apparent in the structure of that organization, at least so far as the members are concerned. At the same time, the reputation of the British Government has been saved because the new agreement, operative from January 1, 1967, ensures that the British share of the cost will amount to 27 per cent, or just about two-thirds of the original levy. The fact that the West German contribution will, in future, be the same should further help to make it appear as if the Government has been fighting for nothing but the interests of its taxpayers in the past few months. In reality, however, the so-called apogee–perigee system to which British representatives were objecting vigorously on grounds of cost only a few months ago is now firmly written into an extended programme that will run until 1970.

The heads of the agreement at Paris include a determination to extend the ELDO programme so as to make possible a stationary communications satellite system of the kind proposed by the European Conference on Satellite Communications (CETS). On to the original ELDO-*A* programme are to be grafted a number of subsidiary programmes, including some work on inertial guidance, the development of a launching site near the Equator in French Guiana (which has done little to comfort the Australians), and the development of two rocket systems to be fired at the perigee and then the apogee of an elliptical orbit so as to carry a load of as much as 200 kilograms into a stationary orbit about the Earth. It is expected that the cost of the four years of work from January 1967 will amount to 330 million dollars, or rather more than the sum that will have been spent by the end of 1966. By way of a watchdog, ELDO is to be provided with a force of financial inspectors. The central organization will in future be free to place contracts directly with potential contractors, but will have to suffer more frequent meetings of its controlling committees.

To the extent that an efficiently managed programme is better than one that is muddled, the last of this reorganization of ELDO is obviously something of a gain. Yet the suspicion remains that ELDO is awkwardly incomplete and inadequately constructed. For one thing, there is no guarantee and only a small likelihood of a commercial demand in 1970 for the kinds of communications satellites that ELDO will then be able to construct. And whatever successes the years ahead may bring, much more will be determined by what happens within the COMSAT organization in the United States, and in the development of its international links with other nations. In this sense, there is much in the view that the best insurance of European freedom in international telecommunications will be to drive tough bargains on landing rights from communications satellites—not to follow the American development of communications satellites proper. This is probably too cynical an argument, and may be wrong as well, but it would be a great mistake if the new excitement about ELDO were to blind its sponsors to the need for working out practical but permanent arrangements for European participation in international satellite communications, if necessary without the participation of European rockets.

NEWS AND VIEWS

Successful Physics

THE Institute of Physics and the Physical Society seems to have had a successful and busy year. In its annual report for 1965, the Institute says that membership increased by 1,086 to a total of 11,273 at the end of the year. This is a larger increase than in recent years, and is attributed in part to the energy which has been devoted to recruitment and in part to the introduction of the new licentiate grade of membership. The buoyant membership has been reflected in a buoyant revenue, and the Society was able to transfer more than £9,000 to its general reserve during 1965. It is proving to be particularly successful as a publishing organization and showed a surplus of some £50,000 on the sales of books and journals during 1965—a figure that should comfortably cover the cost of salaries and other overheads. Even the annual exhibition of instruments produced a cheerful surplus of more than £5,000, though in this respect 1965 was not so successful as 1964.

These surpluses are evidently not going to be wasted. During 1965, the Institute of Physics and the Physical Society finally acquired the Fulmer Research Institute and thus set up in business as a research contractor in its own right. It also provided its members with advice on what are described as "various aspects of professional conduct". Though the four documents concerned are supposed to be confidential to the membership, enquiry shows that they are concerned with the terms of employment which working physicists should be prepared to accept, the terms and conditions on which outside consultancy work should be carried out, the rules to be followed by physicists wishing to write for the Press or to appear on television, and the precautions to be taken by those about to sign contracts with publishers. Clearly, the Institute of Physics and the Physical Society now feels able to pass on to its membership some of the secrets of its own success in combining business with learning. It remains curiously anomalous that this go-ahead organization still cannot settle for one half or the other of its composite title, and cannot call itself either the Institute of Physics or the Physical Society.

World Atmospheric Study

THE meteorologists have tentatively settled on 1972 for a comprehensive study of weather patterns and their predictability in both hemispheres to be known as the Global Atmospheric Research Programme. The decision was taken at the second meeting of the Committee on Atmospheric Sciences of the International Union of Geodesy and Geophysics (IUGG) held in Geneva at the end of April. According to Prof. P. A. Sheppard of the Imperial College of Science and Technology, a member of the IUGG Committee, the aim is nothing less than an observation of the whole atmosphere up to 30 km in order to determine how far ahead—perhaps a few weeks—the evolution of weather systems can be worked out from data available at a given instant. "We see the possibility and we should try to turn it into fact."

Talk of the research programme has been in the air for the past two years. Part of the stimulus for it has been a wish to see whether the atmosphere is really determinate on larger time scales as some theories of numerical prediction now suggest. Weather satellites provide further opportunities. Because of the planning which has to be done, 1972 is the earliest year in which the experiment can be mounted. It is intended that intensive studies in that year will be well co-ordinated with the World Weather Watch being sponsored by the World Meteorological Organization. The atmospheric programme is, however, intended to be very much more complete and, indeed, it owes its existence in part to the impatience of some meteorologists with the difficulty of using the work of the World Weather Watch to make a critical assessment of the value of their theories in computer programmes.

It is probable that by 1972 there will be in operation a number of meteorological balloons, able to drift at fixed heights of up to 30 km, and equipped to transmit information by means of communications satellites. According to one plan, these balloons would be launched into eight or nine distinct levels in the atmosphere; they would be sufficiently permanent, and their spacing sufficiently close, to provide the detailed information about the free atmosphere on which accurate long-range computer forecasting must eventually be based.

Atomic Energy in Latin America

THE Organization of American States appears to be doing some constructive work at last. The Inter-American Nuclear Energy Commission of the Organization met for its sixth annual meeting in Washington, D.C., at the end of June. Five resolutions were passed encouraging research and co-operation in the economic uses of atomic power, but the Organization has not yet chosen to back these resolutions with hard cash. Before the June meeting, the approved programme for 1966–1967 provided only for the holding of a conference on radiochemistry and a special legal committee meeting. At the meeting itself, four countries pledged 35,000 dollars, including 20,000 from the United States, and other member states stated their intention to make contributions during the year. The additional funds will be used for technical conferences on radioisotopes in agriculture and food preservation, reactors for the production of electric power and water, and problems related to health and radiological protection. Some funds will also be available for assisting member states in the fields of insect control, the assessment of water resources, food preservation and the determination of soil needs. Additional funds would make it possible to expand these programmes, to implement study groups to deal with specific topics, and to initiate training programmes in accordance with interests expressed by the member states.

The meeting agreed that "the importance of these programmes may not have been adequately recognized at all stages of the budgetary process of the Organization of American States" and resolved "to recommend that the Secretary-General of the Organization give greater consideration to the growing importance of science and technology in the balanced development of the countries of the hemisphere, and particularly to more adequate support of reasonable programmes in the field of nuclear energy". The 1967–1968 budget that the meeting submitted for approval includes

68,000 dollars to support conferences, study groups, training, and assistance to member states—a slightly larger figure.

National Academy *versus* Fat

THE United States National Academy of Sciences has now published a further edition of the report of the Committee on Fats of the Food and Nutrition Board, and has in the process moved a tiny step closer to a declaration against fatty acids and phospholipids as components of human diet. Without saying in as many words that dietary fat disposes a person to atherosclerosis, the report wags a finger at diets containing excessive fat—a category which can be counted as including the American diet, with 40 per cent of its calories derived from fat. Though it lends some support to the view that there is a link between the concentration of cholesterol in the blood and the likelihood of contracting atherosclerosis, this evidence is described as "considerable but not conclusive".

On the issue of whether poly-unsaturated fats are the best fats to eat, the report comes down, without much emphasis, on the side of those who hold that margarine is better than butter. First, it acknowledges the validity of the dietary experiments which show that the substitution of "unsaturated vegetable oils for animal fats" leads to a reduction in the concentrations of cholesterol in the circulating plasma. The report goes on to argue, however, that there is no evidence to show whether some unsaturated acids are more effective than others in bringing about a reduction of plasma cholesterol, and no evidence to show which natural animal fats are most productive of high concentrations of cholesterol. There is also continuing uncertainty about the extent to which the concentration of cholesterol, as distinct from the concentration of triglycerides in plasma, or some combination of them both, may be relevant to the causation of atherosclerosis. The evidence of epidemiological studies is, at the same time, inconclusive; in some parts of the world where all the fats in diet are animal fats, the amounts of cholesterol in the blood are low. In the circumstances it is no wonder that the report comes out most strongly against over-eating as such.

Material for Materials Science

TWO new journals have stepped in to fill what they feel is a gap in the literature. The *Journal of Materials Science* and *Materials Science and Engineering* have appeared within five months of each other; the *Journal*, first published in February, states that its purpose "is to promote an inter-disciplinary approach to the study of the relationships between the structure, properties and uses of materials". The editorial staff is based in England with Prof. R. W. Cahn, professor of materials science at the University of Sussex, as chairman. The first issue of *Materials Science and Engineering* came out this May; its sub-title is "An International Journal" and its associate editors and advisory board represent the United States, Britain, The Netherlands, Germany, France, Italy, the Soviet Union, Japan, Australia, Canada, Sweden, and Argentina. Prof. Robert Madden, director of the School of Metallurgical Engineering of the University of Pennsylvania, is editor-in-chief; Dr. A. H. Cottrell is a member of the Advisory Board. Like the *Journal*, *Materials Science* "has been founded in the belief that much depends on a deeper understanding of the structure and synthesis of materials, and of their engineering application. . . . It is their [the editors and publishers] hope that *Materials Science and Engineering* will become the chosen publication medium for significant and forward-looking work in this broad field."

If these two journals are to become complementary and not merely competitive, the operative word in the above statement must be 'broad'. And from the introductory issues there is some hope that this will be the case; *Journal of Materials Science* seems to be concentrating more on the chemical properties while *Materials Science and Engineering* is placing more emphasis on physical and engineering aspects of the science.

Parliament in Britain

IN a written answer in the House of Commons on June 26 to a request for a list of advanced technical and scientific projects which Great Britain is undertaking in collaboration with European nations, Mr. G. Thomson, on behalf of the Secretary of State for Foreign Affairs, listed twenty-five projects. Three of those are under the European Nuclear Energy Agency, four under the European Space Research Organization, in addition to the Sounding Rocket Programme 1964–67, four aviation projects in collaboration with France, and two natural gas projects. The list also includes the *Vulcain* project, the European Launcher Development Organization, the European Organization for Nuclear Research, the Channel Tunnel, an Anglo–Dutch project, *Sea Dart*, an Anglo–French–German project for a lightweight jet lift engine, an Anglo–German–Canadian project for a reconnaissance drone system, the International Agency for Research on Cancer, and the Cross-Channel Current Link. There are also close relations between many British and European bodies in the field of advanced science and technology.

IN reply to questions in the House of Commons on June 28, the Prime Minister, Mr. H. Wilson, said that it was the duty of the Committees on Manpower Reserves for Science and Technology to look at the whole field, including manpower employed in defence. Although much work was due on this subject in relation to the National Plan, there was no special entry or section dealing with it. Sir Solly Zuckerman was now looking intensively at all questions arising out of the co-ordination of scientific and technological matters, and manpower was one of them. It was the Government's policy to transfer far more scientific reserves to civil purposes from defence, and the transfer of much of the Ministry of Aviation to the Ministry of Technology would further this process. Expenditure on civil research and development had increased in 2 years from £194 million to £289 million on the Civil Votes.

IN replying in an adjournment debate on water supplies for Manchester in the House of Commons on June 27, the Minister of Housing and Local Government, Mr. R. Crossman, stated that after the new Water Order at present with the printers, there would be no further abstraction from Ullswater in the Lake District, and no more artificial resources such as was

proposed for the Winster Valley. In adding that it was necessary to look at the lower reaches of the rivers in this region, he appeared to have accepted an earlier recommendation of the Nature Conservancy. Mr. Crossman went on to say that it would be sensible to rule out barrages across Morecambe Bay and the Solway Firth as sources of water supply before 1980. Mr. Crossman said also that the Water Resources Board had a Northern Committee which was assessing long-term demands and resources for the whole of the north of England, and at his request this Committee was preparing a short list of schemes for Manchester and south-west Lancashire on which urgent detailed investigations must be concentrated. This Committee was also doing the exploratory work which formerly had been left to individual water undertakers or had been neglected.

IN a written answer in the House of Commons on June 27, the Secretary of State for Education and Science, Mr. A. Crosland, listed thirty-two institutions registered by him as charities whose purposes concerned him in connexion with his general functions relating to education. The list included the Battersea College of Technology, the Bradford Institute of Technology, Bristol College of Science and Technology, Chelsea College of Science and Technology, College of Aeronautics, Institute of Electronics, Institute of Transport, Loughborough College of Science and Technology, Royal Aeronautical Society, Royal College of Advanced Technology, Salford, and Woolwich Polytechnic.

University and College News:

Battersea College of Technology

DR. P. R. DAVIS, reader in anatomy in the Royal Free Hospital School of Medicine, has been appointed to the chair of human biology.

Heriot-Watt

DR. B. GLOVER has been appointed to the chair of chemistry in succession to Prof. F. Bell.

Massachusetts Institute of Technology

GIFTS exceeding 40 million dollars have been received by the Massachusetts Institute of Technology during the past year, according to Dr. James R. Killian, jun., chairman of the Corporation. This is the largest sum ever received in one year and exceeds the total amount received by the Institute during its first fifty years. Reflecting on the growth of the Institute, Dr. Killian said that the academic staff has grown from 300 to 2,800 in fifty years, that graduate enrolment has increased 50 times, and that expenditure is 250 times larger than half a century ago.

Nottingham

PROF. A. D. M. GREENFIELD, at present professor of physiology in St. Mary's Hospital Medical School, has been appointed first dean and professor of physiology in the new University of Nottingham Medical School.

Sheffield

DR. W. GALBRAITH, at present group leader, High Energy Physics Group, Nuclear Physics Division, Atomic Energy Research Establishment, has been appointed professor of physics.

Appointments

PROF. K. DUNHAM, at present professor of geology in the University of Durham, has been appointed director of the Institute of Geological Sciences in succession to Sir James Stubblefield.

DR. D. W. KENT-JONES has been appointed chairman of the Council of the British Industrial Biological Research Association, in succession to Sir Owen Wansbrough-Jones.

DR. S. Q. DUNTLEY has been appointed president of the Optical Society of America to fill the unexpired term for 1966 of the late Dr. Van Zandt Williams.

Announcements

AT the ordinary meeting of the Royal Society of Edinburgh on July 4 the following were elected honorary fellows of the Society: PROF. P. M. S. BLACKETT, emeritus professor of physics in the University of London; DR. F. H. C. CRICK, member of staff of the Medical Research Council Laboratory of Molecular Biology; SIR PETER MEDAWAR, director of the National Institute for Medical Research; LORD TODD OF TRUMPINGTON, Master of Christ's College, Cambridge; PROF. F. BLOCH, Department of Physics, Stanford University; PROF. K. KURATOWSKI, Faculty of Mathematics and Physics, University of Warsaw; PROF. J. MONOD, Faculty of Science, University of Paris at the Sorbonne; DR. T. B. NOLAN, formerly director, United States of America Geological Survey; PROF. L. C. PAULING, Santa Barbara, California; ACADEMICIAN N. N. SEMENOV, director of the Institute of Chemical Physics, Moscow.

AT the annual general meeting of the Institute of Physics and the Physical Society on July 5, the following were elected to honorary offices and as members of the Council: SIR JAMES TAYLOR, president; DR. J. V. DUNWORTH, vice-president; P. T. MENZIES, honorary treasurer; DR. R. PRESS, honorary secretary.

AT the annual meeting of the Board of Trustees and the members of the Corporation of the Woods Hole Oceanographic Institution held during June 22–23, the following were elected officers of the Corporation: P. M. FYE, president of the Corporation; N. B. MCLEAN, chairman of the Board of Trustees; E. D. BROOKS, JUN., treasurer.

MR. C. COCKERELL has been awarded the gold Albert Medal of the Royal Society of Arts for 1966, in recognition of his work in connexion with the technical development of the hovercraft.

THE eighth international conference on "Semiconductors" will be held in Kyoto during September 8–13. Further information can be obtained from Dr. M. G. Hatoyama, The Research Institute of Sony Corporation, c/o Japan Physical Society, P.O. Box 28, Hongo Post Office, Tokyo.

AN international symposium on "Atherosclerosis and Reticulo-Endothelial System" will be held in Como during September 8–10. Further information can be obtained from Prof. R. Paoletti, Institute of Pharmacology, 32 Via Vanvitelli, Milan.

THE ninth international Congress of Internal Medicine will be held in Amsterdam during September 7–10. Further information can be obtained from the Secretariat of the Congress, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague.

THE ninth congress of the International Society of Geographical Pathology will be held in the State University at Leiden during September 7–10. Further information can be obtained from the Secretariat of the Congress, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague.

A SYMPOSIUM on "Synthesis" will be held by the Organic Division of the Chemical Institute of Canada at the University of Alberta, during August 31–September 2. Further information can be obtained from Dr. F. W. Bachelow, Symposium Secretary, C.I.C., Department of Chemistry, University of Alberta, Calgary, Alberta.

THE third international congress of psychosomatic medicine on "The Psychosomatic Approach to Fatigue" will be held in Paris during September 15–18. Further information can be obtained from the Société Française de Médecine Psychosomatique, Hôpital Cochin, 27 rue du Faubourg-Saint-Jacques, Paris 14.

THE inaugural meeting of the new Radiochemical Methods Group of the Society for Analytical Chemistry will be held jointly with the Microchemical Methods Group at the University of Kent, Canterbury, during September 16–17. Further information can be obtained from the Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

A TWO-DAY meeting, organized by the Mass Spectroscopy Group, will be held in the University of Birmingham during September 14–15. Further information can be obtained from R. M. Elliott, Associated Electrical Industries, Ltd., Scientific Apparatus Department, Barton Dock Road, Urmston, Manchester.

THE seventh international Machine Tool Design and Research Conference will be held in the University of Birmingham during September 12–16. Further information can be obtained from the Organizing Secretary of the Conference, Department of Mechanical Engineering, The University, Edgbaston, Birmingham 15.

A CONFERENCE on "Electrical Network Theory and Design" will be held in the University of Newcastle upon Tyne during September 11–18. Further information can be obtained from Dr. A. G. J. Holt, Department of Electrical Engineering, Merz Court, The University, Newcastle upon Tyne 1.

A CONFERENCE on "Instrumental Optics and Optical Design", arranged by the Optical Group of the Institute of Physics and the Physical Society, will be held at Chelsea College of Science and Technology during September 19–21. Further information can be obtained from the Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A TWO-DAY conference on "Use of Rubber in Engineering Applications", organized by the Natural Rubber Producers' Research Association, will be held at the Imperial College of Science and Technology during September 15–16. Further information can be obtained from the Natural Rubber Producers' Research Association, 19 Buckingham Street, London, W.C.2.

THE second decennial conference on "Advances in Cell, Tissue, and Organ Culture", sponsored by the Tissue Culture Association and supported in part by the American Cancer Society, will be held at Bedford, Pennsylvania, during September 11–15. Further information can be obtained from Dr. V. J. Evans, National Cancer Institute, Bethesda, Maryland.

THE sixth international conference on "Microwave and Optical Generation and Amplification", sponsored jointly by the Electronics Division of the Institution of Electrical Engineers and the Institution of Electronic and Radio Engineers, will be held in the University of Cambridge during September 12–16. Further information can be obtained from the Joint Conference Secretariat, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

A CONFERENCE on "Radiation Measurements in Nuclear Power", arranged by the Berkeley Nuclear Laboratories of the Central Electricity Generating Board in collaboration with the Institute of Physics and the Physical Society, will be held at Berkeley during September 12–16. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE annual meeting of the Conference on Electrical Insulation of the National Academy of Sciences–National Research Council will be held in Pocono Manor, Pennsylvania, during October 4–5. Further information can be obtained from Mr. D. W. Thornhill, Staff Executive, Conference on Electrical Insulation, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C.

CORRESPONDENCE

Independence for Universities

SIR,—Much of your leader of July 2, 1966, is timely and will find support in the universities. A few points call for comment, however.

The main point which will not find support is the suggestion that the vice-chancellors must represent the universities in the outside world, unless all you mean by that is that, in a given area, it is the local vice-chancellor who will be the main P.R.O. for his university. It is undoubtedly time that there was a body which could state the case for the universities, and this body should contain representatives of the committee of vice-chancellors as top administrators and of my Association as the actual teachers who bear the brunt of work-pressures with inadequate facilities in too many cases. At the moment anyone, whether he be malicious or well-intentioned, can make any criticism of the universities without drawing an official comment or explanation: this is sheer folly on the part of the universities, in my view. The fact remains, however, that the committee of vice-chancellors alone can never speak for the universities, nor would they claim to do so. Many apparently administrative matters are in the last resort dependent on academic decisions and the voice of the academic should be clearly heard in any public pronouncements.

May I add a few other points briefly? The 'really big' university, if that means the American-type mammoth organization, presents major problems of student/staff relations in the context of the British concept of university education. This Association did, it should be noted, submit to the Robbins Committee that there was room for some large universities in our system, but may not have meant by 'large' what you have in mind. As for the staff/student ratio, this does in fact differ very much from faculty to faculty and from subject to subject.

You raise a most important question at the end of the article. Robbins estimated the need for university places on the basis of student demand, because that was the only basis sufficiently documented at the time—and that is still largely true to-day. That student demand and national needs must sooner or later be reconciled is obvious, and my Executive has promised to prepare and submit to the University Grants Committee at a later date some observations on this and on possible methods of reconciling them. The main problem may be to avoid suggesting to the unfortunate sixth-former that he may be directed to study a subject which he has no desire to study. Much long-term planning (and publicity) is required to avoid universities and schools reacting to trends in such a way as to produce permanent imbalance of need and demand.

Yours faithfully,

K. URWIN

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X-RAY DIFFRACTION STUDIES OF DOUBLE HELICAL RIBONUCLEIC ACID

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THIS article presents a preliminary account of X-ray diffraction studies of helical RNA molecules and of how they crystallize. The results lead to suggestions of how helical regions in RNA molecules may play an essential part in protein synthesis.

The first diffraction patterns of RNA were obtained by Rich and Watson[1]. These patterns were not very well defined but suggested that RNA had a helical structure with some resemblance to that of DNA. Improved, semicrystalline patterns (Fig. 1) were obtained by some of us[2] from yeast RNA which was first believed to be intact transfer RNA but which was later shown by one of us to be fragmented RNA[3] probably of ribosomal origin[4]. The conclusions drawn from these patterns were that there are strong similarities between the helical structure of RNA and the *A* conformation of double-helical DNA, and that the helices are very similar in all kinds of RNA. By analogy with the *A* DNA structure and because there was a faint 2·5 Å reflexion on the meridian or close to it, the internucleotide spacing was taken as 2·5 Å. The helix-pitch varied from 28 Å to 30 Å as the humidity varied from 66 per cent to 92 per cent, implying eleven to twelve nucleotide pairs per helix turn. The base tilt (angle between the normal to the base-pair plane and the helix axis) appeared to be about 20°. Much more accurate and detailed structure analysis became possible when Langridge and Gomatos[5] obtained crystalline diffraction patterns from reovirus RNA. Their preliminary analysis[5] indicated that the RNA helix might be ten-fold (that is, with ten nucleotide pairs per helix turn) with internucleotide spacing of $3{\cdot}05 \pm 0{\cdot}05$ Å and base tilt of 10–15°. Similar results were obtained by Tomita and Rich[6] with wound tumour virus RNA and by Langridge *et al.* with *MS*2 virus replicative intermediate RNA[7]. The present, more extensive, analysis of reovirus RNA patterns shows that an eleven-fold helical model (base tilt 17°) is in as good agreement with the data as a ten-fold model (base tilt 11°).

It appears that viral RNA has two crystal forms, which we call α and β. RNA diffraction patterns of α-type (Fig. 2) have already been published[5,6] and indexed[6]. The β-form obtained by one of us (R. L.) with reovirus RNA (Fig. 3) has crystalline perfection approaching that of DNA (Langridge and Gomatos, to be published). Recently, using rice virus RNA, similar α- and β-patterns have been observed and analysed[8]. (We thank Prof. M. Tsuboi for kindly showing us his results before publication.) There is no appreciable difference between the overall diffraction intensity distributions in the crystalline patterns of α- and β-RNA and in the less-well-defined patterns of fragmented yeast RNA. This shows that the helical structure is, within small limits, the same in all three cases. We give here a preliminary account of quantitative diffraction data obtained in two dimensions from the yeast RNA patterns and in three dimensions from α and β reovirus RNA patterns. The molecular packing in RNA crystals is more complicated than for DNA and the analysis required to determine the crystal structure and the precise molecular conformation is more elaborate than that previously applied to fibre diffraction patterns. Preliminary molecular models derived from these data are described. In fibres of α- and β-RNA, the molecules are

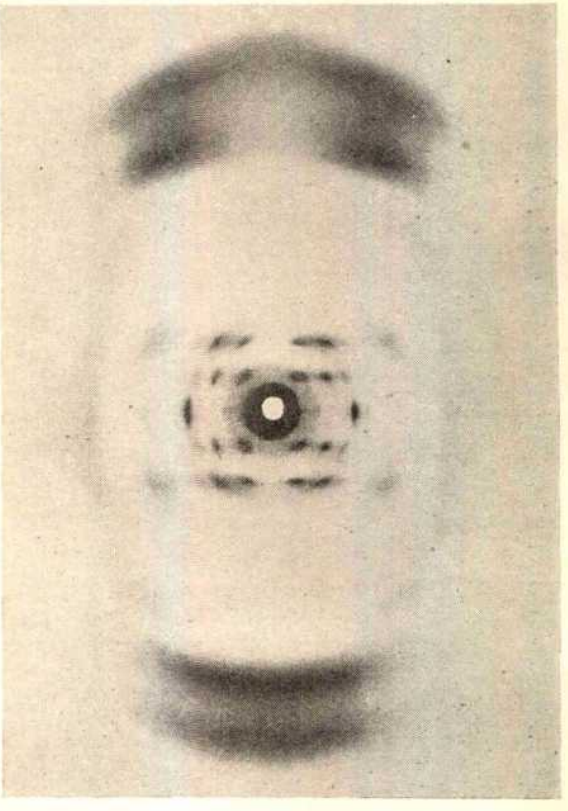


Fig. 1. Diffraction pattern of fragmented yeast RNA[2] (prepared by Dr. G. L. Brown), fibre at 77° to X-ray beam. 92 per cent relative humidity

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arranged in groups of three which appear to be the same in both cases. We find many indications that hydrogen bonds hold the molecules together in these groups. We suggest the possibility that such hydrogen bonds may be involved in the interaction of transfer RNA (*t*RNA), messenger RNA and the ribosome.

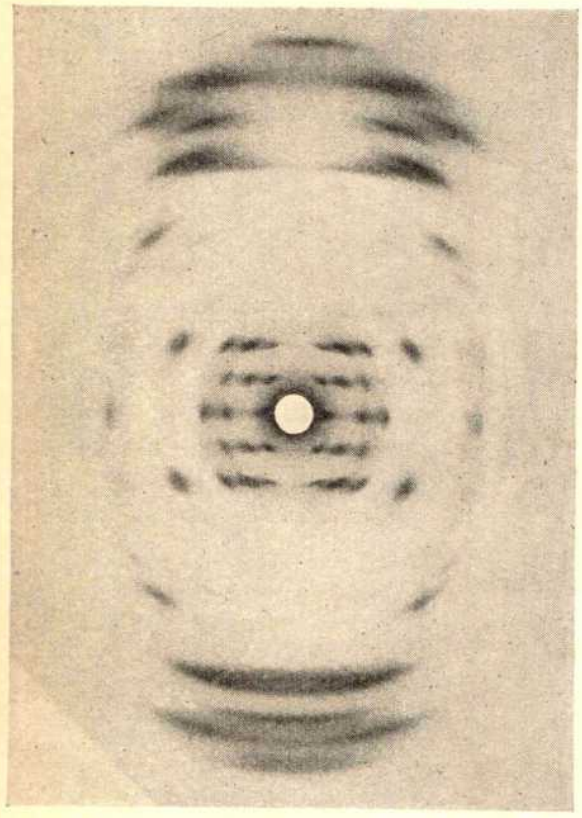


Fig. 2. Diffraction pattern of reovirus RNA (prepared by Dr. P. J. Gomatos), α-type fibre at 81° to X-ray beam. 75 per cent relative humidity. Obtained at Children's Cancer Research Foundation by R. Langridge

Analysis of the Diffraction Patterns of Fragmented Yeast RNA

Densitometry of the pattern shown in Fig. 1 gives the X-ray intensity data shown in Fig. 4. The lattice is hexagonal with three molecules passing through the unit cell at positions $(\frac{1}{3}, \frac{1}{3}, \frac{1}{3})$, $(\frac{2}{3}, 0, \frac{2}{3})$, $(0, \frac{2}{3}, 0)$. The crystallinity is optimum when the fibre humidity is between 75 per cent and 92 per cent; at 75 per cent the unit cell dimensions are $a = 40{\cdot}7 \pm 0{\cdot}2$ Å, $c = 29{\cdot}0 \pm 0{\cdot}2$ Å, the c axis being in the helix axis direction. Because of disorder, the outer parts of the pattern consist of continuous diffraction. Measurements of this part were related to those of the sharp spots in the inner part by a procedure[9] applied to DNA patterns. As a result, curves were derived of the scattered intensity from a single molecule (Fig. 4).

When the relative humidity surrounding the RNA fibres is increased the fibre swells and the separation between molecules increases. The resulting considerable variations of intensity of the 110 and 220 reflexions show that the molecular scattering curve passes through zero at $R = 0{\cdot}05$ Å^{-1} and $0{\cdot}10$ Å^{-1} approximately, as is the case for *A* DNA[10].

Preliminary molecular models. The similarity of the diffraction patterns of helical RNA and *A* DNA strongly suggests that the RNA helix is basically like that of DNA[2]: it consists of two anti-parallel polynucleotide chains joined by Watson–Crick base pairs, a conclusion supported by the base ratios of the viral RNAs[13,14]. A series of maxima and minima on the scattering curve on the equator (including the zeros referred to in the previous paragraph) and on the second layer line shows, as for *A* DNA, that the phosphate and sugar groups in RNA are both at a radius of about 9 Å[10]. When molecular models are built and adjusted to fit the X-ray data (using procedures already described[11]) it becomes clear that the middle of the base-pairs is displaced about 5 Å from the helix axis and that the phosphates in the two chains almost superimpose when viewed parallel to the helix axis.

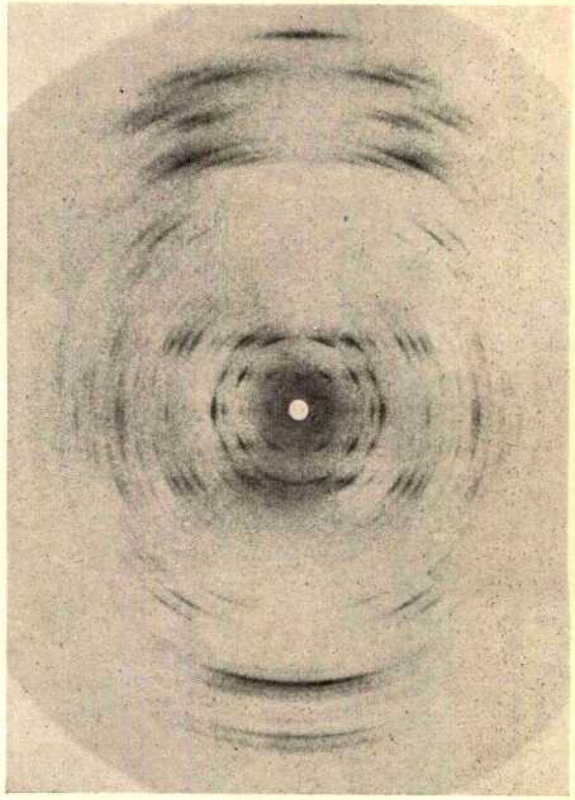


Fig. 3. Diffraction pattern of reovirus RNA (prepared by Dr. P. J. Gomatos), β-type fibre at 76° to X-ray beam. 75 per cent relative humidity. Obtained at Children's Cancer Research Foundation by R. Langridge

Because the upper layer lines of the fragmented yeast RNA pattern are diffuse it is not possible to derive unambiguously from the pattern the number of nucleotide pairs per turn of the helix. It was first suggested[2] that the number lay between eleven and twelve (depending on the relative humidity) and that the base tilt was about 20°. The crystalline reovirus RNA patterns were much sharper; and the fact that on the tenth layer line there was a reflexion which appeared to be meridional strongly suggested that there were ten nucleotide pairs per turn[5,6], and preliminary consideration of models suggested[5,6] that the base-pairs were tilted at 10°–15°. However, when we built various types of models we discovered, rather to our surprise, that very similar intensity distributions could be produced with different degrees of twisting and extending of the helix. Except for the meridional reflexions, the effect of changing the helix twist could largely be

compensated by changing the tilt and position of the bases. It is true that the meridional reflexion appeared on different layer lines for different twists, but it was not strong. Unless a meridional reflexion is strong, it cannot reliably be used to identify the number of residues per helix turn because we cannot safely assume that all the unit cell contents are in a regular helix. For these reasons and because the observed reflexion on the tenth layer line is not unambiguously meridional, the number of nucleotide pairs per turn could not be established with certainty. Nevertheless, the allowed variation of helix twist is small enough for the relative arrangement of the atoms in each nucleotide to be determined to within a few tenths of an Ångström. In this sense the conformation of the molecule is already fairly well defined. Fig. 5 shows a preliminary ten-fold model built on the basis of the fragmented yeast RNA data (and taking into account the published analysis[5,6] of α-RNA crystalline patterns and the general appearance of the unpublished reovirus β-pattern obtained by one of us (R. L.)), and an eleven-fold model developed during the present analysis of crystalline reovirus RNA data. Comparison of observed and calculated diffraction is shown in Fig. 4.

The free OH group. One feature of the RNA models is of special interest. Although helical models can be constructed with the ribose OH hydrogen-bonded to another part of the same molecule, so far we have not succeeded in building a model of this kind which is both stereochemically satisfactory *and* compatible with the X-ray data. Therefore, although not yet firmly established, it appears very likely that the OH does not form intramolecular hydrogen bonds. As a result, the OH is free to form external hydrogen bonds. Such bonds to phosphate groups on other RNA molecules could influence crystallization of helical RNA, as will be discussed later.

If the ribose OH hydrogen were to form a hydrogen bond within the RNA helix this would provide an explanation, which is otherwise so far lacking, of why RNA double helices are stable at higher temperatures than DNA double helices of equivalent base composition[15]. The present work concerns RNA structure in the solid state; we cannot exclude the possibility that the RNA helix in dilute solution (when its stability is assessed) contains intramolecular hydrogen bonds and that when the RNA solution is concentrated the helical conformation alters reversibly and intermolecular hydrogen bonds form.

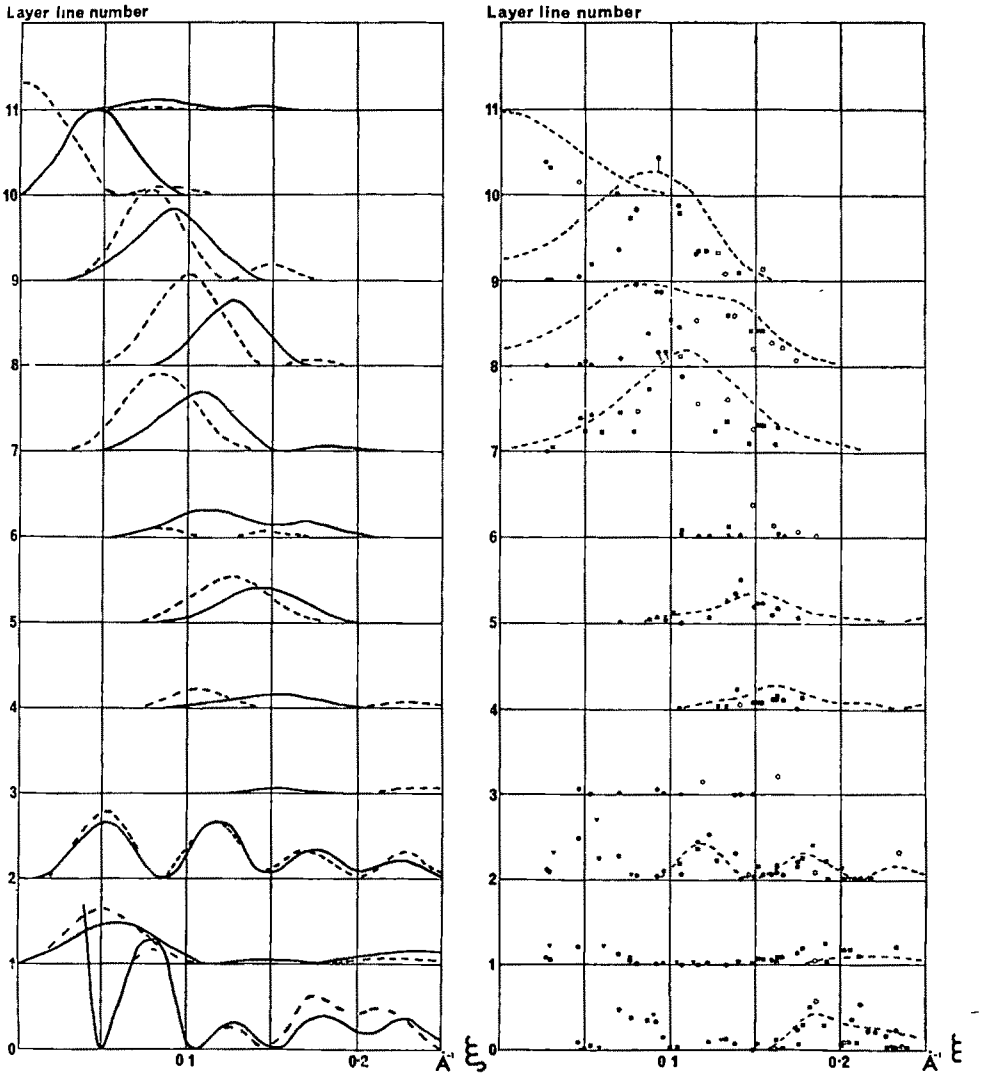


Fig. 4. *Right*, Observed diffraction intensities corrected to show intensity of scattering from a single RNA helix. Yeast RNA: ▼, sharp reflexions; - - - -, continuous diffraction. Reovirus RNA α, ● (○, less accurate observations). Reovirus RNA β, ▣ (□, less accurate observations). (Note: The yeast RNA intensities (▼) are less accurate than the reovirus intensities (■) and (●).) *Left*, ——, Corresponding calculated diffraction from ten-fold helical model; - - - -, corresponding calculated diffraction from eleven-fold helical model

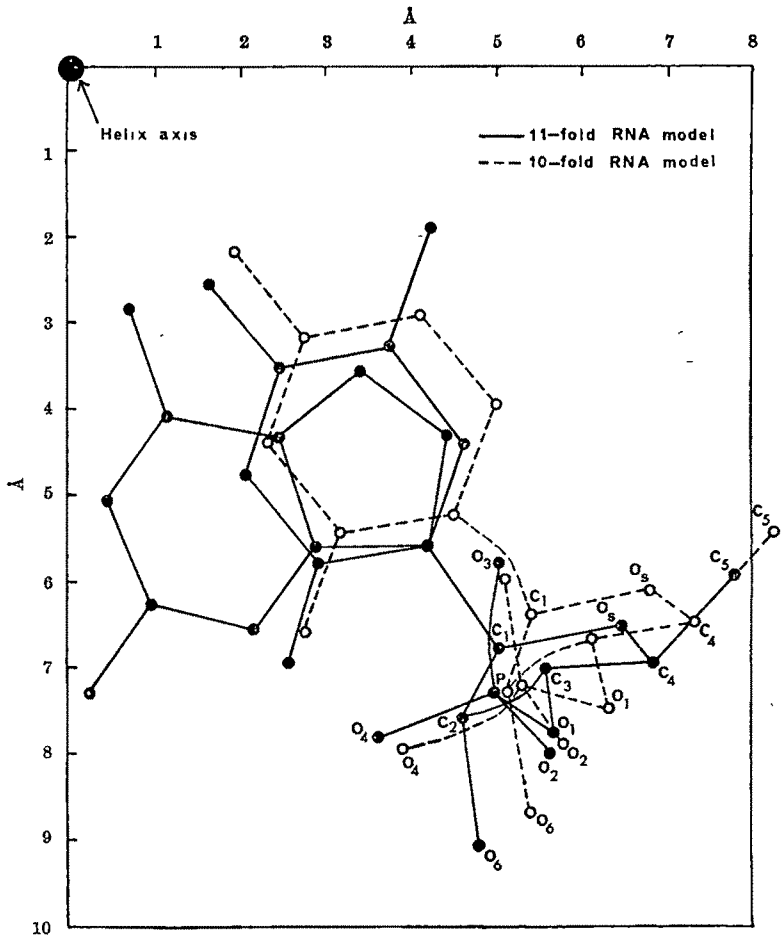


Fig. 5. Similarity of ten- and eleven-fold RNA models. Projection down helix axis of a nucleotide (the purine base of the ten-fold model is omitted for clarity)

The Crystalline Virus RNA Patterns

Three-fold arrangement of molecules. The best-defined diffraction patterns were obtained by one of us (R. L.) from reovirus RNA prepared by Dr. P. J. Gomatos[5]. One of the α-patterns measured (Fig. 2) has been published[5]. The β-pattern is shown in Fig. 3.

Both types of patterns can be indexed on hexagonal lattices:

for α, $a=b=43{\cdot}9\pm0{\cdot}2$ Å, $c=30{\cdot}0\pm0{\cdot}2$ Å (at 75 per cent relative humidity);

for β, $a=b=39{\cdot}8_7\pm0{\cdot}1_5$ Å, $c=29{\cdot}9_5\pm0{\cdot}1_5$ Å (at 75 per cent relative humidity).

The α-lattice is very similar to that already derived for wound tumour virus RNA[6]. The fact that the lattices are hexagonal suggests that the contents of the unit cells have trigonal or hexagonal symmetry. The intensity relations described below are consistent with this symmetry and support its assignment. The intensities exclude the possibility that the RNA molecule itself has three-fold symmetry. Therefore, because the unit cell is too small to accommodate six molecules, it appears that it has passing through it three molecules related either by a triad axis, or by a screw triad. Intensities were measured with a densitometer[11].

When the α- and β-patterns of reovirus RNA are carefully indexed special intensity relations become apparent. These are of key importance because they reveal the nature of the molecular packing and thus enable one to compare the observed diffraction intensities with those calculated for a given molecular model. The relations also show that only ten-fold and eleven-fold models need be considered.

Allowing for the form of the scattering curve for a single molecule, and particularly bearing in mind where the curve falls to zero, the intensities follow the rules:

When $l=0$, or 3 (for the inner part of the pattern) and when $l=5$, 8, for α, in the main, reflexions with both h and k even are strong and others weak; for β, reflexions with $h-k=3n$ are strong and others weak.

When $l=1$, 2 (for the inner part of the pattern) and when $l=7$, 9, for α, in the main, reflexions with both h and k even are absent and others present; for β, reflexions with $h-k=3n$ are weak and others strong.

The rules arise as follows. Assume that the unit cell contains three right-handed ten- or eleven-fold helical molecules. The diffracted amplitude from one molecule is given by the sum of Bessel function terms[12]. For the inner part of the pattern ($l=0$–3) only the Bessel function terms with $m=0$ (see ref. 12) are appreciable. When l is not much different from 10 or 11, only the terms with $m=1$ are appreciable (for our models this applies for $l=5$–11). The Bessel function terms from each of the three molecules interfere to give the various $F(hkl)$. Let the jth molecule be at position (ax_j, by_j, cz_j) in the unit cell. First let us consider the equator ($l=0$). It may readily be seen that when $x_j=y_j=\frac{1}{3}$ (as for some forms of DNA[9]) the intensity rules are $h-k=3n$ strong and $h-k\neq3n$ absent. For β-RNA the $h-k\neq3n$ reflexions are not absent, but are weak; this shows that $x_j\sim y_j\sim\frac{1}{3}$. The α-RNA rules apply when $x_j\sim y_j\sim\frac{1}{2}$.

Considering other layer lines, if it is assumed that the Bessel function terms with $m=0$ need only be considered when $l=0$, 1, 2, 3 and those with $m=1$ when $l=7$, 8, 9, it may be shown that the observed rules apply only for

three right-handed ten-fold helices related by a left-handed screw triad axis or for three eleven-fold helices related by a triad axis. Nine-fold and twelve-fold helices are excluded. These deductions can be made because in much of the pattern one set of Bessel function terms only is appreciable. When more than one set is appreciable (that is, on outer parts of layer lines) intensity rules are complicated.

The molecular packing and identity of molecular triads in α and β. The molecular packing is defined by the molecular dimensions, the dimensions of the molecular triad, its orientation in the unit cell and the orientation of the molecules in the triad. The geometry of the packing is essentially the same for both α- and β-crystals, but they differ in the value of x (see Fig. 6) which is about $\frac{1}{2}$ in α and about $\frac{1}{3}$ in β. In β-RNA the molecules are approximately hexagonally close packed. α-RNA has a surprising feature: there are large 'holes' in the structure—the structure may be regarded as approximately hexagonally close packed, with one quarter of the RNA sites occupied only by water and ions. Values of x, probably accurate to within 0·01, can be obtained from the diffraction data by least-squares analysis. This requires that a molecular model be assumed. However, the value of x was insensitive to the model chosen: in β-RNA a ten-fold model gave 0·360 and an eleven-fold gave 0·365. The value for α-RNA was 0·46. It was assumed that the triads were oriented so that, in both crystals, molecules lay on the unit cell edges and the short diagonal. That this was correct was confirmed by varying the triad orientation.

It is noteworthy that the molecular triads in the α- and β-crystals appear to be identical: although the values of a and of x are different for the two structures, the dimensions of the triads are the same, and there is good evidence that the orientation of the molecules is the same. This orientation was determined by comparing the agreement between

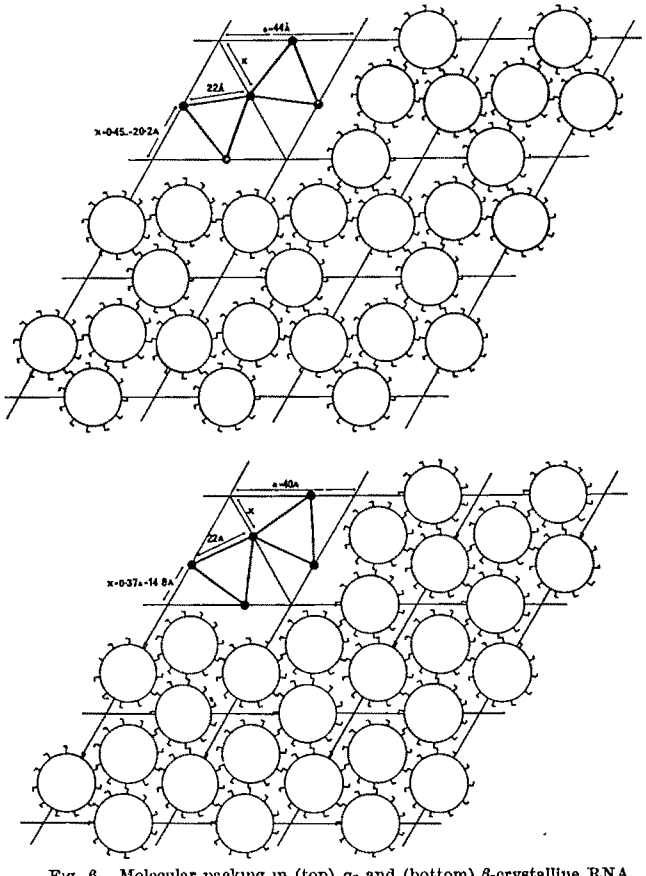


Fig. 6. Molecular packing in (top) α- and (bottom) β-crystalline RNA structures. Vacant sites may be seen in the α structure. The molecules are shown as eleven-fold

observed and calculated diffraction intensities (as measured by conventional crystallographic residual) for different molecular orientations. With an eleven-fold model the agreement varied markedly with orientation and the best orientation was defined within a few degrees for both α and β. For the ten-fold model the measure of agreement for both α and β varied little with orientation and therefore the best orientation was poorly defined. The best agreement which could be obtained for the ten-fold model in both α and β was slightly inferior to that obtained with the eleven-fold model. The difference was not sufficient to exclude the possibility that an improved ten-fold model could be built with better agreement than that obtained by an eleven-fold model.

In addition to the identity of the molecular triads in α and β, the crystals exhibit another special feature—one which may provide a clue to the origin of the two crystal forms. If an eleven-fold molecular model is assumed, it follows from the values of x established for both α- and β-RNA that every molecule in both crystal forms makes identical contacts with each of its four nearest neighbours. This equivalence of contacts is possible only for triadic arrangements where the triads are arranged so that the molecules lie on the unit cell sides and on minor diagonals where the values of x are related to the number of molecular residues per helix turn. A similar equivalence would occur for ten-fold helices only if x were 0·50 in α-RNA and 0·41 in β-RNA. It thus appears that only an eleven-fold helical RNA molecule gives the same contacts throughout both α and β crystals.

It was hoped that consideration of interatomic distances between molecules in close contact in α- and β-crystals might enable one to choose between ten-fold and eleven-fold models. Neither, however, could be eliminated. Intermolecular interatomic distances were calculated as a function of molecular orientation. The stereochemically permissible orientations (assuming normal van der Waals radii) were in agreement with those derived from the X-ray data for both ten-fold and eleven-fold helices.

Fourier synthesis. Fourier studies, particularly difference syntheses of the c-axis projection, were made using phases derived from both molecular models. The molecular orientation previously determined for the eleven-fold model was confirmed, but in some of the Fourier maps for the ten-fold model discrepancies persisted of a kind which suggested that no ten-fold model might be acceptable.

Is the RNA helix ten- or eleven-fold? The main reason for thinking that the helix might be ten-fold is that there is a fairly strong reflexion on the tenth layer line on or close to the meridian in both α- and β-patterns. Furthermore, the distribution of intensity on the seventh, eighth, ninth and tenth layer lines of the RNA patterns closely resembles that on the ninth, tenth, eleventh and twelfth layer lines of the pattern of the complex of polyinosinic and polycytidylic acids (poly I : poly C)[16] (also Langridge and Chamberlin, in preparation). The twelfth layer line of the poly I : poly C pattern is closely meridional and the helix is twelve-fold. It may be valid to compare corresponding parts of the diffraction pattern of a twelve-fold helix with that of a ten-fold. In the present case, such a comparison indicates that the RNA helix is ten-fold. However, we have shown that, if the helix is ten-fold, a screw triad axis must relate three molecules (though this cannot be proved exactly) and, as a result, the meridional (0,0,10) reflexion, due to the helices, on the tenth layer line must be absent. If a meridional reflexion is present it must be produced by some part of the structure other than the ten-fold helical molecule, (that is, ions, water, or perturbation of the helices) and, moreover, by a part of the structure which is not operated on by the screw triad axis (the same is true for a meridional reflexion on the second layer line of some α-patterns). A ten-fold helix could give (1,0,10) diffraction which would be difficult to distinguish from the true meridional (0,0,10),

because the patterns are not sufficiently well defined. While it does not appear that an unperturbed eleven-fold helix can give diffraction on the tenth layer line as close to the meridian as is observed (allowing for disorientation), a perturbed eleven-fold helix (as discussed later) could do so.

Other considerations relevant to the question—is the helix ten- or eleven-fold ?—may be summarized as follows. If the overall agreement between observed and calculated diffraction intensities is considered, there is no clear preference for the eleven-fold model. However, the geometry of the α- and β-crystals is neatly explained if the helices are eleven-fold but not if they are ten-fold. If one accepts the idea that RNA molecules should crystallize with only one intermolecular stereochemical relation between molecules in contact, then the structure must be eleven-fold and not ten-fold. However, such an argument cannot be relied on to establish the structure. To do this it is very desirable to obtain X-ray diffraction data of still higher resolution than those described here.

Hydrogen bonding in the RNA crystallites. The viral RNA crystallizes in forms more complicated than those of DNA. Intermolecular hydrogen bonding could be responsible for this. Calculation of interatomic distances between neighbouring molecules shows that, for both ten- and eleven-fold models, hydrogen bonding is likely. Because the positions of atoms may be in error by 0·2 Å or more, the results cannot be interpreted very strictly. However, they show that with eleven-fold helices oriented as indicated by the α and β X-ray data, two hydrogen bonds per helix turn between two molecules are possibilities. The distance between the relevant oxygen atoms of the ribose OH and phosphate groups is correct (~2·7 Å) for hydrogen bonding. Non-bonding atoms are in contact but not too close. By varying the orientation of the molecules by an amount within the error of the X-ray determination, one OH direction may be made to lie along one hydrogen bond. There is a deviation of 60° for the other. (Hydrogen bond angles were derived by Dr. J. H. Venable.) With a ten-fold model the situation is much the same. It does not seem possible to adjust a regular helical model so that the deviations from linearity of both hydrogen bonds are reduced to reasonable values, that is, less than 20°. We are, therefore, at present investigating the possibility that the RNA helix is distorted slightly by the hydrogen bonding. Reasonably linear hydrogen bonds could be achieved if the sugar phosphate chains in the region of the hydrogen bonds were displaced (~1 Å) in a direction parallel to the helix axis. Systematic distortions of a regular eleven-fold helix could also result in meridional diffraction on $l=10$ and broadening of the transform peaks on $l=7$, 8 and 9, as observed.

Possible Biological Implications

Study of the RNA crystallites in the fibre specimens suggests that helical regions of RNA molecules join together in a specific way by hydrogen bonding from a ribose OH group of one helix to a phosphate group of another. There is no direct evidence from the diffraction patterns of fragmented RNA that the molecules are hydrogen bonded together. However, the peaks on higher layer lines (particularly on $l=8$) are broadened as in the crystalline reovirus patterns. As already discussed, peak broadening could result from distortion of the regular helix. If this were so, it could be argued that distortion was unlikely to occur unless there were specific intermolecular interactions as in α- β-RNA. We will briefly discuss possible roles of this hydrogen bonding in protein synthesis. Specific attachment of a *t*RNA molecule to a ribosome carrying messenger-RNA is believed to involve Watson-Crick hydrogen bonding between the bases in the codon and in the anticodon. The RNA in intact ribosomes probably contains regular helical regions like fragmented yeast RNA helices. *t*RNA also contains helical regions. Some of the ribosomal RNA helices may be sufficiently free of protein to come in contact with the *t*RNA helices, especially when the repulsion between phosphates of the ribosomal RNA and of *t*RNA is reduced by cation concentration and presence of basic groups in ribosomal protein. Ribose OH hydrogen bonding might then occur between the helices of ribosomal RNA and of *t*RNA. Bonding of a *t*RNA molecule specific for a particular amino-acid would depend on the additional binding resulting from the codon-anticodon interaction. The inter-helix ribose OH hydrogen bonding would hold the *t*RNA molecule to the ribosome in a highly specific way; this might facilitate codon-anticodon interaction. Furthermore, ribose OH hydrogen bonding might be one of the factors involved in holding, in correct relation to the ribosome, the codon in the single-chain messenger RNA molecule. In order that inter-base hydrogen bonding should cause formation of Watson-Crick pairs and not others it may be necessary for the glycosidic bonds in each base pair to be held relative to each other at the correct angle and separation. This could be achieved by ribose OH hydrogen bonding of both *t*RNA and codon to the ribosomal RNA.

Conclusion

We find that both ten-fold and eleven-fold helical RNA models are in reasonable agreement with the X-ray data. Because these data are limited and the structures complex we cannot yet decide between the two types of model. Furthermore, our conclusion that there are hydrogen bonds between adjacent helical molecules is tentative. That these bonds exist is indicated by the following:

(1) In both ten-fold and eleven-fold models described here the ribose OH groups are not hydrogen bonded to any part of the same molecule.

(2) The distances calculated between atoms in neighbouring helices in virus RNA crystallites following derivation of the orientation of the RNA molecules in the reovirus RNA crystallites are such as to permit intermolecular hydrogen bonding.

(3) The geometry of the α- and β-crystal forms suggests that RNA helices have specific directional interaction.

If the inter-helix bonds exist, their discovery is probably the most significant part of this work. To obtain results which are more definite, diffraction patterns with still higher resolution should be obtained and analysed. The main obstacle to doing this is the lack of suitable RNA in sufficient quantities.

One of us (F. H.) held a Guggenheim Memorial fellowship at the time of the work. We thank Miss A. Kernaghan, Miss J. Graham and Mr. Z. Gabor for technical help. The work in Boston was supported by grants from the U.S. Public Health Service and National Science Foundation to R. Langridge. The reovirus RNA was prepared by P. J. Gomatos and the fragmented RNA by G. L. Brown.

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BOOK REVIEWS

THE METHOD

The Methods of Contemporary Thought

By J. M. Bocheński. Translated from the German by Peter Caws. Pp. x+135. (Dordrecht: D. Reidel Publishing Company, 1965.) 18 D.fl.

In this slight volume we have everything essential for the understanding of the methods of rational thought and, moreover, completely up to date. As a subject, this is distinct from both philosophy and logic, and claims attention in its own right. Basically, methodology has not been enriched appreciably from British sources; its study and impact came largely from the Continent and the United States. The translator, Dr. Peter Caws, has produced a clear text to which he has added a few footnotes of his own, in one or two of which he has slight reservations about the original text to make. All this is to the good, resulting in a book of exceptional value, because of the author's erudition both as a historian of logic and as a teacher of wide experience. He never leaves the reader in the air, however difficult the theme may be. There are not too many examples: they occur at exactly the right place, not to make things easier, but to clinch the argument.

Before going into detail, a general point will be noticed at once, namely, that in science there is little need for a complex system of methodology; what is required is fairly obvious, and the means of obtaining it admit of little argument. In philosophy, however, method is more important, and may well determine the outcome. However, this is by no means all—the major scientific achievements of the twentieth century, the theory of relativity and the quantum theory, have thrown up problems which are fundamentally philosophical, and to advance in these fields a deliberate *methodik* is essential. Neither Prof. Bocheński nor the translator say so, but both classical thermodynamics (including Nernst's theorem) and Carathéodory's axiomatic surfaces of adiabatic isolation are in the same position; the traditional cycles are very clumsy, and the implications of the concepts of Kelvin and Clausius for a formal deductive process are still scarcely resolved.

All this renders essential a firmer grasp *au fond:* no amount of mere technological expertise will help in the least, nor will greater refinement of experimental skills, except in so far that events knowable in principle (that is, not physically meaningless, like velocities in excess of that of light) may in time become factual knowledge.

After a few pages of introduction, which are definitive for such necessary elements as terminology and so forth, *The Methods of Contemporary Thought* consists of four parts: the phenomenological method, semiotic methods, the axiological method, and reductive methods. At the end is an epilogue, containing a guide to future reading, a bibliography and a couple of indexes; everything, in fact, which a careful reader needs. At the outset, great emphasis is placed on the necessity to keep the disciplines of formal logic, methodology and the philosophy of logic separate. Confusion has arisen in the past on this score. Meanwhile, Prof. Bocheński's four divisions may be discussed in detail. The name 'phenomenology' seems to stem from J. H. Lambert's *Neues Organon* of 1764, but little of permanent moment appeared until E. Husserl first used it in an exact context, since when it has become largely the working creed of the existentialists. Methodologically speaking, this means seeing things intellectually, which lies at the root of all true cognition, the putting into practice of the German *ding-an-sich*. From the observer this demands a rigid type of contemplation, utterly devoid of any axiological content, still less of any teleological element. This does not entirely exclude value-judgments as such (as Scheler and Otto have demonstrated), but only that the basic detachment must be supreme. Incidentally, one suspects that herein lies the aesthetic appeal of phenomenology.

Husserl himself, always ready (perhaps a little too ready) to do battle with psychologism, was radical in his exclusion of knowledge by testimony, deductions, hypotheses and so forth. In a word, for him tradition was suspect, and that brought him nearly into line with a tenet already accepted some six centuries earlier when St. Thomas Aquinas gave the lowest rating to argument from human authority. It is noteworthy that in this, if nowhere else, modern thought clasps hands with the *philosophia perennis*.

We now proceed to the study of semistics, or the analysis of language Historically, this subject was known to the Stoics (next to nothing of whose contribution now exists), and to the scholastics, of whose work in this field little is known. As we regard it to-day, such learning is attributable to Charles Morris (1938). Among its conquests are acceptable solutions of several of the antimonies, including the famous paradox of the liar, a sore vexation since the time of Plato. It is now recognized that such sentences are incapable of resolution in terms of syntax alone: they entail the use of a meta-language. Why should this be? Because everything that needs to be said about a science is sayable (if at all) not in the language of that science, but in its meta-language. It is almost impossible to over-value the importance of this process. Perhaps the most telling illustration of its power is the well-known attempt of Hilbert to lay the foundation of mathematics by means of such a meta-discipline, only to be followed by Gödel's discovery of the impossibility of the completeness of an axiomatic system (for example, arithmetic), probably the greatest advance in logic since Aristotle.

This leads naturally to the consideration of the axiomatic method itself. Its history is revealed with great lucidity: Leibniz as a precursor, followed by Boole, de Morgan and others, culminating in *Principia Mathematica*. Then another leap forward, which produced results neither Aristotelian nor Stoic, namely, the many-valued logic of Lukasiewicz (1931), and the work of the intuitionists. Here occurs perhaps the only slight obscurity in the book, the 'natural logics' of Gentzen and Jackowsky, which consist of rules only. Surely, these have nothing to do with the 'natural logic' of p. 13, a naïve affair suited to the needs of elementary science. But we are not told in so many words what this is. However, an axiomatic system must be complete and consistent; the latter requires proof that no contradiction can occur: it is not enough to show that no contradiction is discoverable. Aesthetically, the urge is to reduce the number of axioms, perhaps even to one.

Finally come reductive methods, of special importance to the sciences, and again originating with Aristotle, in spite of his preoccupation with deduction. To us, the basis seems to be Francis Bacon's *tabulae*, but they were in fact scarcely such as to aid research or discovery. Broadly, with emphasis on induction, we witness the rise of natural science, and the establishment of history as a scientific activity, differing only (in principle) in the assessment of the stage at which protocol statements can be made.

In sum, this elementary treatise of Prof. Bocheński is a little masterpiece. There is no branch of learning, scientific or humanistic, into which methodology does not enter. Here is a specific contribution to the unity of knowledge.

F. I. G. RAWLINS

THEORY FOR EXPERIMENTALISTS

The Physics of Elementary Particles

By H. Muirhead. Pp. xv+737. (London and New York: Pergamon Press, Ltd., 1965.) 140*s*. net.

EXPERIMENTAL workers in elementary particle physics are almost always conscious of never having been able to devote the time they would have wished to mastering the basic theory of the subject. This is partly due to the inadequacy of the typical first degree course, which does not bring them to the stage where they can readily progress naturally to an understanding of the theory without the help and stimulus of formal postgraduate courses. Happily, these latter courses are now becoming more and more common. Also, in such a competitive and rapidly moving field where the student is invariably working in a fairly large team, there is a tendency for many students to be involved immediately in the development of advanced techniques and, at an early stage, to make an immediate contribution to an often complicated experiment, the choice of experiments being determined by the more senior members of the team.

It is therefore an event of great interest when a comprehensive treatise, written by an experimentalist for experimentalists, is published. It will be hoped that here is a book which can be used as a basis for postgraduate courses, and as a reference book by the students and indeed by their mentors. How far does Dr. Muirhead's book meet these expectations? As may be expected from the sheer size, it could certainly be called comprehensive. In addition to dealing with topics with which the fresh research student will be unfamiliar, an excellent résumé of the special theory of relativity is given and also the Euler-Lagrange equation and Hamilton's equations are derived starting from the Principle of Least Action. The bold statement of this principle, as the starting point of dynamics without further discussion, illustrates one of my main criticisms of the treatment. Other examples of an unstated axiomatic approach in which physical requirements follow from the mathematical formalism instead of determining that formalism are "Thus the invariance of the Lagrangian under gauge transformation of the first kind can lead to charge conservation", and "The linearity of the space also implies the principle of superposition. . . . This properly is of great importance in quantum mechanics". How different from Dirac! The principle of superposition is the physical reason for choosing the mathematical formalism of linear vector spaces.

Of the more difficult, less familiar parts of the theory, the author's stated intention to keep mathematics as simple as possible has not, despite some sacrifice of mathematical elegance, made for easy reading, and most experimental readers will have to work hard to master the subject. I have a slight feeling that an apparently more demanding, but more elegant approach may have been easier for the reader. This is not to imply that any effort made by the student will not be amply rewarded. I have learnt much from this book, not without some effort; the conservation of energy implies a minimum expenditure of effort to reach a given height, and free airline tickets are not easy to come by. To counteract the criticisms made above, the merits of the book should be clearly stated. Not only are there many excellent parts (Chapter 6, for example), but also the completeness of the treatment offers an excellent reference to the whole subject. Many parts are sufficiently self-contained to make for easy reference to, and brushing up of, particular topics. The book is relatively free from typographical errors, considering the size. A second edition could remove the more glaring and irritating of these and also the few examples of confusing English.

All in all, Dr. Muirhead is to be admired and congratulated on the industry and knowledge which have added such a useful volume to the literature.

E. H. BELLAMY

MODERN GAS DYNAMICS

Atomic Theory of Gas Dynamics

By John W. Bond, Jr., Kenneth M. Watson and Jasper A. Welch, Jr. (Addison-Wesley Series in Aerospace Science.) Pp. x+518. (Reading, Mass., and London: Addison-Wesley Publishing Company, Inc., 1965.) 136*s*.

IT is almost certain that a review of *Atomic Theory of Gas Dynamics* will betray, by its criticisms, the allegiance of the reviewer, for it is still usual to identify oneself by exhibiting a label of some kind, 'aerodynamicist' or 'physicist' or 'chemist' or 'applied mathematician', for example, even though such labels no longer define the occupation and interests of their owners with any degree of precision. Indeed, the book by Bond, Watson and Welch provides an illustration of the necessity nowadays for a good measure of expertise in all the disciplines mentioned here, since they have become so closely interwoven as a result of the exigencies arising, principally, from flight through the atmosphere at super- and hypersonic speeds, although, to be sure, the subject matter has many applications in other fields. The thirteen chapter headings will perhaps illustrate the point; they are, in order: "Thermodynamic Properties of a Gas"; "Shock Hydrodynamics"; "Continuum Hydrodynamics"; "Atomic and Molecular Physics"; "Equation of State"; "Kinetic Theory of Gases"; "General Theory of Transport Processes and Hydrodynamics"; "Dissociation and Ionization in a Gas"; "Kinetic Theory of Transport Processes in Multi-component Gases"; "Radiation Transport Theory"; "Opacity"; "Radiation Transport Applications"; and, finally, "Shock-Front Structure".

It is quite clearly not possible, within the compass of only five hundred or so pages, to satisfy all of the people all of the time where such a wide variety of topics is concerned. The specialist practitioner in one or other of the foregoing arts is almost sure to find some omission or imprecision in a book of this kind which will upset him, and it is sometimes necessary to exercise a more-than-average degree of tolerance in order to avoid criticizing the authors unfairly. Indeed it would be difficult to criticize those parts of the book which deal with the more physical aspects of the problem. The description of a wide variety of particle interactions and their cross-sections and of the associated rate processes constitutes a most useful and readable contribution to the subject. The same is true of the later chapters, which concentrate primarily on the problems arising in radiating gases, although, inevitably, the rapid advances being made in this area must leave the information contained therein rather dated. From my point of view, then, the exercise of tolerance was not necessary here. A mild application was required for the discussions of kinetic theory, since I felt that these were rather disjointed and would have benefited from a more unified treatment. This is especially true of the derivation of the equations of change from the Boltzmann equation. It is possible to carry this through with a very high degree of generality without ever entering into detailed discussions of the collision terms in the latter equation and without any implied restriction to binary collision processes, or implied approximation if ternary and higher-order collisions actually are involved. A matter of rather more importance is the quite unnecessary confusion of Δ, δ and d operators which

occurs in the earlier chapters. This lack of precision is also too evident to avoid comment in parts of the chapter on thermodynamics. For example, $p\delta V$ [*sic*] is only the work done in an infinitesimal expansion of the system when that work is done reversibly.

Thus far the criticisms are of a comparatively minor nature. It is when one comes to the chapters on shock and continuum hydrodynamics that the important deficiencies occur. At best these parts of the book are amateurish, at worst downright incorrect. Most especially I refer to the section on sound waves, where a particular solution of the inhomogeneous wave equation is derived on the basis of a gross confusion between what are commonly known as 'body forces' and the forces actually experienced by a body immersed in a continuum supersonic stream. The distinction between them is known to every undergraduate who has anything to do with fluid mechanics and the appearance of such an error in any text-book is unforgivable. To summarize, the present book can be read with profit for its atomic theory but can hardly be recommended for its gas dynamics. JOHN F. CLARKE

SOVIET OPERATIONAL CALCULUS

Integral Transforms and Operational Calculus

By V. A. Ditkin and A. P. Prudnikov. Translated by D. E. Brown. English translation edited by Ian N. Sneddon. (International Series of Monographs in Pure and Applied Mathematics, Vol. 78.) Pp. xi+529. (London and New York: Pergamon Press, Ltd., 1965.) 100*s*. net.

INTEGRAL transforms, as a technique for solving differential equations, are a familiar part of the education of every mathematician. The technique is only really effective for some amenable linear differential and integral equations and provided that the space-time boundary conditions of the problem are sufficiently specialized. It is not viewed with great favour by modern pure mathematicians, as it aims at producing results rather than understanding. Yet, despite these manifest limitations, it will always be studied and books about it will continue to be written, because it rapidly and elegantly yields formulae of basic importance throughout the whole of linear physics and engineering. Indeed, its theoretical foundations have recently been the object of intense study by Russian mathematicians. Operational calculus was developed systematically in the middle of the nineteenth century, and then successfully applied by Oliver Heaviside to the solution of various problems arising from electromagnetic oscillation theory. Heaviside's point of view was afterwards displaced by the integral transform methods of Bromwich (not mentioned in this book) and others. Now the wheel has turned full cycle, and, as the authors point out in their foreword, "A complete return to the original operator view-point was made by Mikusinskii. He has provided a strict operator basis for Heaviside's operational calculus without any reference to the theory of the Laplace transform. When describing his theory, it occurred to Mikusinskii to introduce different notations for a function and for its value at a given point. For instance, 2 is the number, while {2} is the function taking the constant value 2.

"The definition of convolution given in Chapter V differs from that of Mikusinskii in that there is no need to distinguish between constants and functions of a constant. Since the various transformations and calculations connected with finding the operational formulae are considerably simplified in a number of cases when the Laplace integral is used, an indication is given here of the connexion between our calculus and the Laplace transform."

This chapter, entitled "Operational Calculus", is by far the most recondite in the book, and is evidently addressed to a more exclusive audience than are the other chapters. It does, however, contain remarks on difference equations, and on differential equations with phase lag, that should be of general interest. The treatment throughout the book is fairly straightforward, though one may sometimes wonder what distinguishes a "theorem" from a "property", and why dates are mentioned in the enunciation of some theorems and not in others. Reasonable familiarity with the special functions of analysis is assumed: Bessel and Gamma functions appear without warning on page 23; Green's function appears without explanation on page 62; MacDonald's function is introduced without a definition on page 75. A number of minor errors and misprints will be spotted by the reader without difficulty.

These five chapters, taking up some 150 pages, comprise only Part I of the book. They are in effect an introduction to Part II, which is a 350-page compilation of well-classified transforms drawn up partly from existing tables and partly from the solution of concrete problems known to the authors. Most of the latter are probably unknown outside the Soviet Union. The tables are prefaced by a list of some 150 special functions encountered in the text. Russian functional symbolism is used, but differs only slightly from our own as indicated on p. 147. About 250 references are cited.

This is not a book for the beginner, or even for the amateur, but specialists should find it a mine of useful information about developments in this field.

M. A. JASWON

TECHNETIUM AND RHENIUM

The Chemistry of Technetium and Rhenium

By R. D. Peacock. (Topics in Inorganic and General Chemistry, Monograph 6.) Pp. ix+137. (Amsterdam, London and New York: Elsevier Publishing Company, 1966.) 55*s*.

The Chemistry of Technetium and Rhenium is a fascinating example of recent developments in inorganic chemistry, and the majority of the references cited at the end of each chapter are drawn from the literature of the past 15 years. Rhenium, discovered in 1925 by Noddack by means of its characteristic X-ray spectrum, is widely dispersed in nature and this factor undoubtedly delayed its discovery and impeded the study of its chemistry. In 1937 Perrier and Segre produced a radioactive isotope of element No. 43 by deuteron bombardment of molybdenum and appropriately named the element technetium. Earlier attempts to discover "primordial" technetium were thwarted by the instability of all its isotopes, although "natural" technetium-99, generated by spontaneous fission of uranium, has recently been detected. The development of large-scale nuclear reactors has produced reasonable quantities of technetium-99 as a by-product and has accordingly stimulated the investigation of the chemistry of this element.

Technetium and rhenium are transition elements *par excellence*, and show oxidation states from 0 to 7 and co-ordination numbers from 4 to 7 with a wide variety of ligands including fluoride, nitride, hydride, *π-cyclo*-pentadienyl, acetylacetonate, diarsine, triphenylphosphine oxide, cyanide and carbonyl, not forgetting some fascinating polynuclear chlorides containing metal–metal bonds.

Prof. Peacock has done justice to this interesting field with a concisely written monograph which is a mine of information and interesting to read. Following the two opening chapters on the discovery and isolation of the elements and their general and elementary properties, the descriptive chemistry of these elements is dealt with in a series of chapters on oxides, halides, cyanides, etc., and the final chapter summarizes analytical methods. For good measure there are appendixes on the laboratory handling of technetium, bibliography and further references. This method of organization seems successful,

and the subject index makes reference to particular compounds agreeably speedy. A valuable feature of this book is the critical approach adopted by the author to the material taken from the literature.

It seems a pity that with Colton's book, *The Chemistry of Rhenium and Technetium*, published in 1965, there are now two very similar books covering the same ground.

D. C. BRADLEY

GAS CHROMATOGRAPHY

Gas Chromatography 1964

Edited by A. Goldup. (Proceedings of the Fifth Symposium organized by the Gas Chromatography Discussion Group of the Institute of Petroleum held at Brighton, September 8–10, 1964.) Pp. xii+386. (London: The Institute of Petroleum, 1965.) 90*s*.

THIS is an account of the proceedings of the fifth symposium organized by the Gas Chromatography Discussion Group held at Brighton during September 8–10, 1964.

The book is on similar lines to previous volumes with the exception of an additional section dealing with the informal discussion periods which were an innovation at this symposium. The main content, however, is a collection of the twenty papers presented and verbatim reports of discussions following each paper. These papers are presented under three headings, dealing with (*a*) column design, (*b*) techniques of separation and identification and (*c*) exploitation of molecular interaction.

Apart from one paper dealing with the preparation and operation of high-efficiency columns, section (*a*) is devoted to theoretical aspects of column evaluation and design. Although bridging the gulf between theory and practice will present many formidable problems, the possibility of very-high-efficiency columns of the order of a million theoretical plates performing separations within minutes is fascinating. The theory is aimed in general at extending established principles and correlating them with practice. The non-mathematical student of gas-chromatography must sometimes be perplexed with the number of parameters required to express the theory and he is unlikely to welcome the addition to this list of a "performance parameter". This section also contains a comprehensive appraisal of packed capillary columns, a relatively new aspect of column technology.

The second section begins with a philosophical discussion of the gas-chromatographic process of separation in terms of the entropy of a hypothetical system. The remainder, in contrast, is entirely practical in aspect. For example, pyrolysis, normally a "finger print" technique, is discussed in a paper which describes an apparatus claimed to give better reproducibility than a mass spectrometer. Because of the time factor involved, normal elution chromatography is not always a satisfactory method when applied to process control, and a number of alternative techniques involving continuous sample flow through a column are discussed. Other papers include the complementary use of thin-layer chromatography and mass spectrometry. This symposium, which has always been a forum for the introduction of innovations, includes papers describing the adaptation of the flame ionization and argon detectors for monitoring the effluent of non-volatile substances in solid/liquid chromatography.

The third section on exploitation of molecular interaction is mainly concerned with the growing potential applications of gas/solid chromatography. Appreciations of present views on molecular interactions in liquids and solutions, and solid interfaces, are made, with particular reference to their application to the interpretation of gas solid chromatography. The relationship between the surface chemistry of solids and retention data is also discussed at some length. Considerable point is made that one of the great virtues of gas/solid chromatography is the stability of many of the types of absorbants over relatively wide temperature ranges. Several developments in this direction are described and the impression emerges that here there is a whole new area for research worthy of greater attention than has hitherto been given, particularly with modified types of absorbants.

The editor, A. Goldup, is to be congratulated on maintaining the high standard one has come to expect of this biennial publication. The book should be of great value both to research and analytical chemists, particularly those who wish to remain in step with developments in the sphere of gas chromatography

E. A. WALKER

A MODERN *LIBER DE ARTE DISTIL?*

Distillation

Edited by E. S. Perry and A. Weissberger. Second completely revised and augmented edition. (Technique of Organic Chemistry, Vol. 4.) Pp. xx+838. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 180*s*.

DESPITE the large number of books and papers published over the years on various aspects of distillation processes and apparatus, there is still room for works which either make an original contribution to the field or else provide a comprehensive account of the state of knowledge.

With the relatively few fundamental advances in distillation theory over the past 40 years, there has been a tendency to treat the details of the latest apparatus and techniques in some isolation, without presenting a critical appraisal of the application of theory to practical problems.

The appearance of a comprehensive work which covers the field of laboratory distillation in both its theoretical and practical aspects, and which includes a wealth of details of apparatus and operating techniques, is therefore particularly welcome.

The new edition of *Distillation*, volume 4 of the Interscience "Technique of Organic Chemistry" series, provides much useful material in addition to that given in the first edition published in 1951. This volume in its augmented form covers all aspects of experimental distillation theory and practice from laboratory to pilot scale, and not only constitutes a valuable and comprehensive handbook for all those directly involved in such experimental work but also presents an admirable and concise summary of basic distillation theory.

The contents cover basic distillation theory, with particular reference to laboratory distillation. The theoretical basis and experimental determination of vapour-liquid equilibria, apparatus and procedures for fractional distillation under a wide range of conditions from low-temperature to high-vacuum with particular emphasis in the latter case on ancillary apparatus and the special requirements of extractive and azeotropic distillation and sublimation, are all dealt with.

Of particular value are the new sections on continuous distillation, pilot plant distillation and automation in distillation.

The theoretical section, written by Arthur and Elizabeth Rose, occupies more than 200 pages and provides a thoroughly detailed account of the development of distillation theory and its application to the practical problems. Of particular importance is the emphasis on batch distillation and fractionation and the methods of determining the number and height of theoretical plates, with details of still sampling techniques, and the use of specialized test mixtures for which detailed vapour-liquid equilibrium, density and refractive index data are given. One further aspect which is dealt with and which is particularly important in laboratory work is the effect of hold-up on performance. This is of interest not only

in determining batch distillation performance but, more vitally, in the calculation of equilibration time for different types of column.

This aspect is extended to a general investigation of the effects of process variables in batch distillation and the recommendation of optimum operating conditions. It also covers the use of digital computers in calculating the separation of multicomponent mixtures. Typical of the thoroughness of this section is the comparison of the Lewis, Chilton and Colburn, Westhaven and Kuhn, the Cohen, Marshall-Pigford and Bowman equations governing the approach to equilibrium. The section concludes with an extensive bibliography.

The treatment by Deal of vapour-liquid equilibria is concise and yet contains a valuable survey of the relation between molecular structure and activity coefficients. The treatment of experimental techniques and the design of the equilibrium is still perhaps excessively brief, as is the coverage of consistency tests, which are so important in the assessment of the reliability of experimental data. Williams on the other hand gives a wealth of detail on the performance and construction of both wetted-wall, packed and plate columns and ancillary apparatus such as temperature controllers, pressure controllers and reflux dividers. The operating procedure is also covered in detail.

The section on extractive and azeotropic distillation by Carlson and Steward might have suffered from the problem inevitable in a book of this type, namely the duplication of material covered by other contributors. However, the treatment of equilibrium is still so much more comprehensive than Deal's that all one can do is to criticize the editor for the allocation of section headings. The actual techniques of extractive and azeotropic distillation are well covered, although one would like to see a more extensive list of specific solvents and entrainers and a more detailed analysis of the relative merits of the two separation systems.

The section by Tipson on vacuum distillation gives much useful information on column design and vacuum techniques including the application of mechanical stills. Perry provides a detailed account of molecular distillation which is complemented by Hecker's contribution on all aspects of the performance of the distillation apparatus and ancillaries under vacuum.

The three new sections in this edition provide a valuable extension to the literature of laboratory distillation. Continuous laboratory distillation is treated somewhat briefly by Williams but is particularly interesting despite the lack of details on reboilers, feed pumps and flow meters. Orgen's treatment of pilot plant distillation covers the sizing of plant and ancillaries and is therefore valuable in itself and also serves as an introduction to the engineering of distillation plant and thus serves as a useful bridge between chemists and chemical engineers. This is particularly important at the present time when the control of full-scale plants is changing from that of the simple physical parameters of temperature, pressure and flow to the more elaborate and challenging idea of direct control of product composition by means of chromatography, mass spectrometry and infra-red and ultra-violet spectrometry. In this area the chemists become directly involved in commercial scale processing techniques.

The final section by Williams on automation and control in distillation therefore serves as a fitting conclusion. The chemist should find the coverage of these control techniques of both interest and use. Fundamental to control is the phenomenon of the dynamic responses in fractionation columns which is also covered and is a much-needed reminder to all those involved in distillation that the operation of fractionating plant is essentially an unsteady state in stable equilibrium on which are superimposed transient responses to external conditions.

This book covers a very wide range of topics of interest and value to the worker in the field, and will certainly earn a place on his bookshelf. M. G. Royston

THE BALD FACTS

Die Menschliche Glatze im Altersformwandel der Behaarten Kopfhaut

Morphologische Studie. Von Klaus Goerttler. Unter Mitarbeit von Peter Gordel. (Zwanglose Abhandlungen aus dem Gebiet der Normalen und Pathologischen Anatomie, Heft 17.) Pp. viii+48. (Stuttgart: Georg Thieme Verlag, 1965.) 23 D.M.

This short monograph, an account of the way the "glatze" or bald pate develops during ageing, is based on a pathological investigation of 118 scalps of both sexes, classed by decade of life and ranging from birth to old age. The material was taken from several regions—top, sides and in-between—and examined not only by traditional histological methods but also by the preparation of epidermal sheets.

In the newly-born, it seems that the dermo-epidermal junction is uncorrugated, but as the lanugo is replaced by terminal hair, the interface becomes crenulated to form the pattern of the rete-pegs. This development reaches a maximum in the second and third decades, when even the hair follicles become involved in the interdigitation of dermis and epidermis. By the fourth decade there are already signs of decline in those regions which were previously most active; the hair bulbs still appear relatively strong, but the hair shafts become feeble. From the fifth decade onwards, first at the side and crown, later in the intermediate regions, the dermo-epidermal junction flattens and the hair follicles diminish and shorten. For a time the sebaceous glands resist change, or even enlarge, but ultimately they, too, succumb to ageing; only the sweat glands remain unaffected and continue to function in old age.

This work is beautifully illustrated with photographs and constitutes a classic anatomical description of the relentless ageing of the scalp, a field in which surmise has often been a substitute for evidence. A full understanding of the process in dynamic terms awaits other investigators. The prognosis may appear somewhat depressing, but a faint optimism may be engendered by the reports of Drs. Papa and Kligman of Philadelphia that, in spite of the undoubted implication of male hormones in the development of hereditary baldness, the process can sometimes be mysteriously reversed by the local application of androgens. And if the hair follicle of the scalp, of all human organs, appears to be the one least well designed for a lifetime of service, one can at least take comfort from the thought that this is but a recognition by natural selection of its vital redundancy.

R. E. Church
F. J. Ebling

Forest and Savanna

An Introduction to Tropical Plant Ecology with Special Reference to West Africa. By Brian Hopkins. Pp. xii+100+5 plates. (London: Heinemann Educational Books, Ltd., 1965. 18*s*. net.

Dr. Hopkins devotes the first few pages of *Forest and Savanna* to an explanation of what is meant by ecology and he then gives background information about West African population, geology, soil and climate. This information is clearly set out at an elementary level and it would serve beginners at college, as well as the interested layman or others unfamiliar with the West African scene. The real meat is to be found in Chapters 3 and 4, which deal with forest and savanna respectively from an ecological point of view. They provide sufficient detail to instruct without overloading the beginner. On the other hand, as the author points out in his preface, he has in some sections reached the limit of our knowledge simply because the study of tropical ecology is still relatively neglected. Things are changing in this respect,

however, partly as a result of Hopkins's own valuable researches, and it is to be hoped that this book may hasten the progress by interesting African nationals in the subject. Conservation of natural vegetation can only be achieved effectively by those who live in the country concerned having an intelligent appreciation of the need for it. *Forest and Savanna* should have a part to play in this all-important educational aspect. In particular the fifth chapter, which deals with the relationship between forest and savanna, brings out many points concerning land management that are vital to the inter-related aspects of agriculture, forestry and conservation. Two appendixes, dealing with field methods for vegetation analysis and suggested student projects, will be of special value to the instructor. References and an index conclude this small text-book which should rapidly find an ecological niche in the curricula of school sixth-forms and first-year university courses.

F. N. HEPPER

Mathematical Analysis

Differentiation and Integration. By I. G. Aramanovich, R. S. Guter, L. A. Lyusternik, I. L. Raukhvarger, M. I. Skanavi and A. R. Yanpol'skii. Translated by H. Moss. English edition edited by I. N. Sneddon. (International Series of Monographs in Pure and Applied Mathematics, Vol. 81.) Pp. xi+322. (London and New York: Pergamon Press, Ltd., 1965.) 63*s*. net.

THIS book contains a concise account of the properties of differentiation, differentials, Riemann integration in one and several dimensions, together with implicit function theory and the effect of transformations in differentiation and integration theory. The Stieltjes integral is briefly introduced.

There are no proofs, examples, or exercises, but there are several counter-examples to show where and why the theory will not extend. This renders the book useful for reference for the non-specialist, and it might be valuable to a lecturer planning a course, but not to a student.

The fact that different authors have written different parts is apparent in an inconsistent notation. Thus, for example, $y[n](x)$ and $y(n)(x)$ are used to denote the Schwartzian derivative, and elsewhere both $y(n)(x)$ and $y^n(x)$ denote the ordinary derivative. The book concludes with useful lists of integrals, etc., and a few tables of higher transcendental functions.

R. L. PERRY

Die Tierische Zelle in Zellkultur

Von R. Schindler. (Fortschritte der Krebsforschung. Recent Results in Cancer Research, Vol. 1.) Pp. vi+88. (Berlin: Springer-Verlag, 1965.) 16 D.M.

To assess the value of cell culture in cancer research, it is clearly necessary to consider many different aspects. In the series on "Recent Results in Cancer Research", published by Springer, the author, Dr. R. Schindler, has discussed the culture of animal cells rather generally. This book deals with various culture methods, basic medium and biochemical requirements of cells, pharmacological studies, cytogenetics, specific cell functions, the mitotic cycle, effects of ionizing radiation and changes brought about by viruses. The main description of cancer cells in culture is given in the last chapter. There is an extensive bibliography of more than 700 references. The book therefore provides good background reading and a good bibliography for people interested in taking up cell culture work on cancer problems. A great deal of work has been done on cancer-cell culture—both hanging-drop cultures and cell lines in monolayer culture, and more recently in organ culture. One could wish that the author had covered these subjects in more detail. For the study of human cancer, where it is impossible to carry out experimental work *in vivo*, with one or two exceptions involving transplantation of tissues into animals, the main future is likely to lie in the use of cultures for experimental work.

A. HADDOW

MEETINGS

GEOPHYSICS AND COMPUTERS

THE Department of Applied Mathematics and Theoretical Physics at Cambridge was host from June 27 to July 5 to the Third International Symposium on Geophysical Theory and Computers, an activity of the I.U.G.G. Upper Mantle Committee.

About eighty-five attended the symposium, and more than forty papers were given, each between half an hour and an hour in length. It would be impossible to mention every paper—invidious though it is to mention particular contributions, certain recurrent new themes emerged which are specially worthy of report.

The major problem, one might say the only problem, in geophysics is that of inversion. Given a set or sets of data, what can be deduced about continuum parameters of specified regions of the Earth? And is that information a sufficient guide to the composition and thermodynamics of the region? The question of uniqueness of interpretation has until recently been treated in a rather indefinite way in seismology. Whereas it is fairly easy to show that surface observations of gravity and magnetic fields are insufficient to give unique distributions of density and magnetization beneath the surface, the uniqueness or otherwise of seismic data (which seems likely to be the principal source of information about the deep interior of the Earth for some time) is much more difficult to establish.

Prof. V. I. Keilis-Borok and two colleagues from the U.S.S.R. gave summaries of the present situation with regard to body wave travel times, where uniqueness has been a pressing problem. It is demonstrable that in the presence of a 'low velocity layer', two different subsurface velocity distributions may yield the same travel time curve. However, the situation is in practice bedevilled by the difficulties of positively identifying body wave signals on actual seismograms. A lively debate between Miss I. Lehmann (Copenhagen) and Dr. E. A. Flinn (Alexandria, Virginia) on work which the latter had presented using array processing techniques epitomized this problem. However, as Flinn and others, notably Dr. D. H. Weichert (Ottawa), Dr. E. J. Kelly (Lexington, Massachusetts) and Dr. R. A. Phinney (Princeton) showed, array processing is becoming a valuable tool in mantle and core geophysics.

The uniqueness, or otherwise, of normal mode data (from free oscillations of the Earth, Rayleigh waves and Love waves) has been an equally thorny problem. A notable paper by Prof. G. E. Backus and Prof. F. Gilbert (La Jolla, California) showed, among other things, that more than one density distribution can satisfy the same data to any prescribed degree of accuracy. This paper, with the Russian contributions, clearly showed the necessity of developing further criteria for the acceptance of Earth models.

One such consideration is seismic amplitude data, and much more reliable information about amplitude as a function of range from source is being compiled by the U.K. Atomic Energy Authority group, and was presented by Dr. A. Douglas (Aldermaston).

Another consideration is the plausibility of an Earth model on solid-state criteria. Prof. D. L. Anderson (California Institute of Technology) indicated the questions which we would wish to ask of solid-state physics, and Prof. L. Knopoff (University of California, Los Angeles) indicated the possible uses of solid-state physics in combination with seismology in the determination of reasonable composition models.

A seismological parameter of increasing importance and whose significance is still not fully understood is dissipation. There is no doubt that this too will have to be taken into account in any unified view of the Earth's structure.

Constant, or nearly constant, Q, the dimensionless attenuation, is commonly observed in laboratory tests on many materials.

Papers by Dr. E. Strick (Houston, Texas) and Dr. M. Caputo (Trieste) examined consequences of these observations. In different ways they both come to the same conclusion—that Q is very nearly frequency independent. This approach from an experimental point of view was complemented by a paper from Dr. B. M. Naimark (Moscow) in which it was shown how the effect of specific mathematical models of anelasticity on stress distributions could be calculated.

Not all the conference was devoted to seismology, however, and there were other impressive contributions. Dr. A. H. Cook (National Physical Laboratory) gave an authoritative account of the present status of gravitational potential determinations by satellite observations. There were several papers on magnetic and gravity fields and the application of computers to their analysis. Dr. N. O. Weiss (Cambridge) and Dr. M. G. Wurtele (University of California, Los Angeles) both demonstrated the power of the computer to solve problems in magneto-hydrodynamics and meteorological fluid mechanics.

D. DAVIES

NON-ELASTIC MANTLE

A SYMPOSIUM on "Non-Elastic Processes in the Mantle" was held in the School of Physics of the University of Newcastle upon Tyne during February 21–25. The symposium, which was organized at the request of the Upper Mantle Committee, was one of several to have been arranged in conjunction with the 'Upper Mantle Project', an attempt to co-ordinate internationally research on the outer 1,000 km of the Earth.

It is a good indication of the present explosive growth of seismological research and interest in the deformation of the Earth on a geological time scale that an international conference with more than a hundred participants can now be convened on such a specialized subject. As organizer of this symposium, my chief aim was to bring together physicists and mechanical engineers whose special interest is the deformation of solids and those geophysicists whose observational or theoretical interests entail some knowledge of the rheology of the mantle of the Earth. It is becoming ever more apparent to geophysicists that elasticity is only a convenient conceptual subdivision of a description of the total rheological behaviour of a material, and that it should not be given special significance. This has been obvious to solid-state physicists and professional rheologists for many years, and this remark is an indication of the need for much closer intellectual contact between these disciplines.

After an introductory lecture by Sir Harold Jeffreys on "Rheological Problems in Geophysics", the first one and a half days of the symposium were devoted to experimental and theoretical work on deformation in mineral and rock systems. Prof. P. L. Pratt (Imperial College of Science and Technology) reviewed current theories relating the microstructure of ionic solids to their plastic flow, creep and fracture. In ionic as opposed to covalent solids there is a more rapid decline with decreasing temperature of the number of slip systems, and this, together with the inhibition of dislocation multiplication mechanisms, accounts for the marked increase in brittleness of these materials at low temperatures. As the temperature is increased, the relative importance of deformation mechanisms based on diffusion processes increases and, as a result, permanent deformation occurs at very low applied stresses. A process of continuous deformation, usually referred to as steady-state creep, is observable in which hardening by deformation and thermal recovery are in a state of balance. Prof. Pratt commented that, so far as the interpretation of laboratory investigations is concerned, the transition from low temperature deformation by dislocation glide mechanisms to the high temperature thermally activated processes occurs at a temperature in the neighbourhood of 0·5–0·7 of the absolute melting temperature. (This figure may be somewhat less for deformation under low stresses applied on a geological time scale.)

Dr. Paterson (Australian National University, Canberra) discussed the general effect of confining pressure on the non-hydrostatic stress–strain relationship. He described how the different susceptibilities to such a pressure of various mechanisms of flow and fracture in a general solid can be used to elucidate the most important deformation mechanisms in at least some materials. Prof. Griggs (University of California, Los Angeles) reviewed the present evidence of weakening in rocks and minerals at high pressures by water, and described in some detail his recent experiments with Blacic on the behaviour of synthetic quartz in such conditions. Above a critical temperature, which is itself dependent on the water content (10^{-3}–10^{-1} per cent in the experiments), dislocation glide movement within the crystals appears to be enhanced by hydroxyl ions. Electron micrographs have demonstrated the generation and propagation of dislocations in the weakened quartz similar to those in unweakened quartz at higher stresses. Prof. Griggs considered that the occurrence of this and other hydrolytic weakening processes in silicates at such low concentrations of water is of considerable geophysical significance. Prof. Gordon (Yale University) reviewed the evidence pertaining to diffusional creep and, using the result that in certain circumstances these processes can be represented by a Newtonian viscosity, he described the derivation of a relationship of viscosity with depth in the mantle.

In this connexion there was a recurring debate throughout the symposium about the concentration of sources and sinks of point defects in mantle material, a quantity which enters directly into calculations of diffusional creep rates. Grain boundaries are often identified with such sources and sinks, but, judging from the discordant estimates of grain size that were made at the symposium, there is certainly a need for experimental work on high temperature, low stress creep in likely mantle material. A considerable problem here will be the acquisition of representative mantle material.

Dr. Raleigh (Australian National University, Canberra) presented papers on an experimental study of glide in olivine and pyroxene minerals, and on a study of the strength and ductility of serpentinite in a range of conditions up to 700° C and a confining pressure of 5 kilobars. In addition to a considerable weakening at temperatures exceeding 500° C, there was an accompanying increase of brittleness. It is believed that both effects are associated with the dehydration reaction, the embrittlement being due to growth of pore pressure which is known to decrease the effectiveness of a confining pressure. These experiments are thought to be of considerable significance for theories of the tectonic emplacement of serpentinites, and possibly may also bear on theories of deep earthquakes if the concept of a serpentinized oceanic upper mantle can be further substantiated.

During the session on fracture phenomena, an interesting theoretical paper was presented by B. V. Kostrov and L. V. Nikitin (Institute of Physics of the Earth, Moscow) on the equilibrium of a crack under the application of a uniform longitudinal shear at infinity in an elasto-plastic medium; the yield was determined by the Von Mises–Hencky theory. A full solution was obtained for both the pre-limiting and limiting stages of crack equilibrium and the results were compared with brittle fracture theory. It would seem that in the elasto-plastic theory it is impossible to decide whether crack propagation is unstable at stresses higher than the limiting stress.

In planning the sessions of specific geophysical interest, it was decided to consider deformations of progressively

greater period. Papers were presented by Prof. Anderson (Seismological Laboratory, Pasadena), Prof. Kovach (Stanford University) and Prof. Slichter (University of California, Los Angeles) on the observation of damping of seismic body-waves and free oscillations and their application in computing the specific dissipation function Q as a function of depth in the mantle. Prof. Orowan (Massachusetts Institute of Technology) reviewed data on amplitude and frequency dependence of the damping of oscillatory motions in solids in the light of observations of Q for the mantle obtained by various methods. He emphasized the fact that distinct physical processes are thought to be involved in damping at seismic frequencies and in viscous creep, and it is difficult to derive from seismology information about the long-term response of the mantle to non-hydrostatic stress.

Two papers by Jobert (Paris) and Melchoir (Brussels) described attempts to deduce the anelastic response of the upper mantle to oceanic tidal loads. Although regional differences have been detected which indicate non-elastic response of the crust, no data suitable for use in a model of upper mantle anelasticity have yet been obtained.

Another set of papers concerned the process of isostatic adjustment. Prof. Bott (University of Durham) emphasized the role of non-Newtonian effects accompanying adjustment, and Dr. Artyushkov (Institute of Physics of the Earth, Moscow) the great decrease of viscosity with depth in the outermost layers of the Earth. The latter can now be understood without direct appeal to partial melting in the upper mantle. Prof. V. Magnitsky (Institute of the Earth Sciences, Moscow) spoke of the possibility of accounting for the viscosities derived from post-glacial uplift theory in terms of a diffusional creep process. Dr. Crittenden (U.S. Geological Survey) described further data on isostatic adjustment from Lake Bonneville and Glacier Bay, Alaska, and emphasized that the inhomogeneity of the Earth with respect to viscosity could well explain the apparent permanence of certain topographic features and the disappearance of others.

A session of papers on the theory of mantle convection was enlivened by a vigorously critical paper by Dr. Lyustikh (Institute of Physics of the Earth, Moscow) on the shortcomings of present theories of mantle convection, and the evidence for continental drift. In particular, the simple assumptions that are made connecting surface topography and mantle convection were criticized and the blind use of spherical harmonics as an analytical tool was commented on. Prof. Knopoff (University of California, Los Angeles) described an investigation which he had made of the gravity field specifically associated with convection patterns. He concluded that there is general agreement on the magnitude of the observed departures of the geoid from a hydrostatic shape and those expected using the convection hypothesis. Prof. Orowan (Massachusetts Institute of Technology) described a theory which attempts to explain the arcuate form of certain island chains in terms of a redistribution of a sub-crustal flow and its associated stress system in the crust after an initial crustal fracture. An interesting feature of the theory is the use of the observations of hydrolytic weakening in serpentinite by Raleigh and Paterson to predict underthrusting by material on the upstream side of the fracture. If the flow of oceanic material towards continents is accepted, observed island structures in the Pacific basin are in significant agreement with this theory. Dr. Tozer (University of Newcastle upon Tyne) reported some model experiments on plane layers with internal heat generation. These show cellular flows at high values of the Rayleigh number that are greatly elongated in the horizontal direction. A ratio of horizontal to vertical length scales greater than five has been observed at values of the Rayleigh number of interest to mantle convection theorists. It was suggested that this feature overcomes objections that have been made to upper mantle convection on the grounds that the horizontal length scale is insufficient to account for continental drift. Other convection papers were presented by Dr. Oxburgh (Oxford) and Prof. Bott (Durham), who has attempted to deduce the pattern of heat flow on the Earth's external surface by a convection theory. He believes that a low heat on the flanks of oceanic ridges is a manifestation of non-Newtonian flow.

D. C. TOZER

RADIOSENSITIVITY AND REPAIR

A MEETING to consider current ideas about the mechanisms of repair of radiation damage was convened at the Vienna headquarters of the International Atomic Energy Agency during April 18–22.

The first session was devoted to repair at the chromosome level. Dr. S. Wolff (United States) reviewed evidence for the existence of repair mechanisms for radiation-induced chromosomal damage. It has been clearly established that metabolic and enzymatic activity is required for breakage and reunion, and evidence that proteins play an important part in the maintenance of chromosome integrity is increasing. Dr. J. R. K. Savage (United Kingdom), using data from fractionated dose experiments with *Tradescantia*, presented evidence that the initial decrease in exchange yield is consistent with the repair of partially damaged chromosome exchange sites to pre-irradiation sensitivity. The non-linearity of the X-ray dose-response curve for true chromatid discontinuities, and its bearing on the exchange hypothesis, was discussed by Dr. S. H. Revell (United Kingdom). Prof. H. J. Evans presented new data from dose fractionation effects in human leucocyte cultures, and challenged the classic breakage/reunion interpretation for chromosome aberrations. As an alternative hypothesis, he suggested that the observed changes are the result of "mis-repair".

There is much evidence to indicate that some genetic mutations can also be repaired, and in the second session Prof. F. H. Sobels (The Netherlands) reported some contrasting processes of post-irradiation modifications of genetic damage in *Drosophila*. After irradiation under anoxic conditions, treatment with nitrogen favours repair in mature spermatozoa as compared with treatment with oxygen. Precisely the reverse situation is obtained in early spermatids, where repair is favoured by post-treatment with oxygen. In another paper on *Drosophila*, Dr. H. Traut (Federal Republic of Germany) concluded that differential radiosensitivity during gametogenesis can be explained by metabolic repair of pre-mutational damage. Dr. S. Igali (Hungary) reported an antimutagenic effect in the bacterium *E. coli* which occurred when homologous DNA extract was present in the medium during irradiation.

In the third session, the various changes at the molecular level known to result in detectable mutation were summarized by Prof. M. Errera (Belgium), and he discussed the processes of mutation reversion and suppression on the basis of what is known about amino-acid sequences in specific proteins. Because most of the detailed knowledge of repair mechanisms has been derived from work with phages and bacteria, a number of the papers were concerned with this field. Dr. R. Devoret (France) reported some investigations of the effect of ultra-violet damage repair on the multiplication of phage λ, and he showed that the 'capacity' of the cell to support phage growth was less affected by the ultra-violet damage of the phage-synthesizing bacterial structures than by bacterial repair processes, which modified indirectly the expression of the information content carried by the phage. The genetic and enzymatic control of radiation sensitivity in *E. coli* formed the basis of a paper by Dr. A. Rörsch (The Netherlands). Five different classes of radiation-sensitive mutants are known, and these are associated with several different linkage groups on the

bacterial chromosome. He also reported details of a cell-free system for the demonstration of repair of ultra-violet-irradiated DNA *in vitro*. Dr. P. C. Hanawalt (United States) dealt with known repair/replication mechanisms in the bacterial genome. It is possible that some form of non-conservative DNA replication mechanism has a necessary function in the normal replication and transcription process, and that it may also function in the repair of a variety of defects in DNA. Repair of damage may also be related to the normal processes involved in genetic recombination, and this problem was reviewed by Dr. B. M. Wilkins (United Kingdom).

Prof. D. Shugar (Poland) outlined the fundamental chemical changes known to be induced by ultra-violet light in pyrimidine analogues and in model oligo- and polynucleotides. The processes involved in the reversal of a number of these changes are now well understood, and attempts may be made to relate them to photochemical transformations in nucleic acids. Dr. S. Robev (Bulgaria) discussed X-ray damage to some informational micromolecules. Investigations of the formation, after irradiation, of a DNA/*m*RNA hybrid indicate a similar intrinsic radiosensitivity for both molecules, and it is possible to explain many apparent differences on the grounds of the number and rapid turnover of *m*RNA molecules.

The last session of the panel took the form of a general discussion of some of the major topics which arose from the papers presented, and an attempt was made to interrelate observations at the chromosomal, genetic and molecular levels. The first question to be considered was concerned with the nature of the basic lesions in nucleic acids brought about by ultra-violet light, ionizing radiation and chemical mutagens. Work using ultra-violet light forms the current basis for the interpretation of much of the radiation chemistry of nucleic acids, and the panel emphasized the importance of pursuing comparable investigations with ionizing radiations.

The relative contributions of the various types of damage to such end-points as cell lethality and mutations were considered, and while some answers could be given for phages and bacteria, no assessment could be made for more complex systems. The panel then discussed known repair processes and how they operate. On almost all occasions, repair is of an enzymatic nature, and several genetic loci seem to be involved in its control. The DNA synthesis required in repair will occur in the absence of normal DNA replication.

Much information has been obtained from investigations of the factors which influence repair mechanisms (for example, metabolic inhibitors). In some systems these factors act by preventing the formation of enzymes required for repair, although in most cases these enzymes are already present. There is also a possibility that some radioprotective agents may exert their effects by way of promoting repair, rather than by radical scavenging.

The panel next examined models recently proposed for genetic recombination, and their possible relationships with known repair systems for irradiation damage. All these models involve single DNA molecules, and one of the major obstacles in any attempt to extend them to events at meiosis in higher organisms is an almost complete lack of information as to the fine structure of large chromosomes, which contain not only DNA but at least two types of protein and some RNA. There is increasing cytological evidence that such chromosomes are multi-strand, despite the fact that genetically they behave like a single DNA molecule.

Finally, consideration was given to radiation-induced achromatic chromosomal lesions (gaps) which appear to be completely reparable. Further progress in understanding their nature requires biochemical investigation, and in particular, whether DNA loss is involved in their formation.

MORTON W. MILLER
JOHN R. K. SAVAGE

OBITUARIES

Dirk Brouwer

WITH the unexpected death of Dirk Brouwer on January 31, 1966, the subject of celestial mechanics suffered an irreparable loss. It is almost entirely due to Brouwer, directly or through his former students, that this mainly nineteenth-century discipline has been able to meet the quite incredible demands made upon it by recent space research. In addition to his many contributions to what may be termed the classical branch of the subject, he pioneered and developed the methods of numerical integration which must still be the mainstay of current orbital calculations for artificial satellites; and he gave a completely general solution, of great practical as well as theoretical interest, of the effect of the oblateness of the Earth on the motions of satellites. Not content with his unique personal contributions, he created in 1959 an annual series of summer institutes in dynamical astronomy, supported by the National Science Foundation, from which have already come over 600 workers in this field, mainly in the space sciences.

Brouwer was born on September 1, 1902, in Rotterdam, and in due course studied mathematics and astronomy at Leiden, where he was greatly influenced by Willem de Sitter. After taking his doctorate (in celestial mechanics) he went to the United States in September 1927 with a post-doctoral fellowship for study at the University of California at Berkeley, and later at Yale. He there became research assistant to E. W. Brown, and, remaining at Yale, became in turn professor, director of the University Observatory (in 1941), chairman of the Department of Astronomy, and Hunson Professor (in 1944). He was to carry forward brilliantly the work of both his famous mentors and, moreover, to prove a worthy successor of Frank Schlesinger as director of the Observatory. Yale's traditional observational activity has been in the field of astrometry, particularly in the construction of photographic star catalogues and the derivation of proper motions. Brouwer pursued this activity with characteristic thoroughness and vigour, both scientifically and administratively. He himself developed new techniques of observation and measurement, as well as pioneering the reference system of the minor planets.

With his unique experience, Brouwer was in constant demand as chairman of or adviser to commissions and, more recently, to various ambitious projects. He served as President of Commissions 7 (Celestial Mechanics) and 20 (Minor Planets) of the International Astronomical Union; and on many special committees, such as those on astronomical constants. He was editor of the *Astronomical Journal* from 194_ and its rapid growth owes much to his influence. But, far from being overwhelmed by such administrative demands, he seemed to thrive on the additional work and responsibility.

Probably the most significant of Brouwer's many contributions to celestial mechanics are his work on planetary theory, using rectangular co-ordinates (1944); his discussion of the secular variations in the solar system (1950); his share of the numerical integration of the orbits of the five outer planets (1951); and the explanation of the "asteroid gaps" in the distribution of the minor planets (1963). But his work on astronomical constants, stretching from his completion of de Sitter's work in 1938 through the introduction of Ephemeris Time (1950) to the IAU System of Astronomical Constants in 1964 must not be overlooked.

Brouwer was a friendly and approachable man, willing at all times to discuss problems with his research associates and students, and capable of inspiring leadership; it is to be hoped that his monumental work at Yale can be continued, and even expanded, to meet still further challenges from space research, for this is surely what he would have most wished.

D. H. SADLER

ORIGIN OF ANTIBODY VARIATION

By DR. S. BRENNER and DR. C. MILSTEIN
Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge

VARIATION of the structure of both κ (refs. 1–3) and λ (ref. 4) light chains of antibody molecules appears to be confined to the N-terminal halves of the proteins while the C-terminal sequences are invariant. All theories of the origin of the variation of antibody proteins must account for this remarkable structural feature of the molecules. Lederberg[5] proposed that the variety of antibodies is a consequence of a high rate of mutation confined to a few genes in the somatic line of antibody producing cells. It is generally believed that hypermutation theories are unlikely to be correct because of the difficulty of defining a mechanism which operates only on part of a gene[6]. The emphasis has therefore been placed on hypotheses which realize the observed restriction of sequence changes by postulating genetic incorporation or polypeptide fusion. Smithies[7] has proposed that the information specifying the variable region is carried by a virus which integrates into a gene determining the invariable region. In an analogous theory, Dreyer and Bennett[8] suggest that the constant region is specified by a virus or episome which is incorporated into many different genetic sites. Another suggestion is that the two halves of the polypeptide chains may be independently synthesized and then joined together by a peptide bond, perhaps by a specific enzyme[9,10]. One may generalize these and other theories by saying that two fragments of (*a*) DNA, (*b*) RNA or (*c*) protein are joined together to provide information for, or to make, the complete polypeptide chains. In addition, most of the mechanisms imply that many different genes will ultimately control the variable part of the antibody molecule.

In this article we resurrect the hypermutation theory by suggesting a mechanism which allows the mutational acts to be restricted to specific regions of a few genes. In general terms, we propose that part of the nucleotide sequence of the gene specifying the invariable region acts as a recognition site for an enzyme which cuts the DNA. During repair of this local region errors are introduced at random at a high rate to generate a large number of different sequences. There are many variants of this general hypothesis, but it is useful to examine one in detail. This is shown in Fig. 1 where we consider a gene specifying one of the light chains. The messenger RNA carrying this information will be copied from one of the chains of DNA and will therefore have the same sequence as the complementary chain. The absolute polarity is given by the direction of translation of the messenger, that is, the 5′ end corresponds to the N-terminal end of the polypeptide chain[11–13]. We assume that a cleaving enzyme recognizes the nucleotide sequence on the DNA corresponding to part of the invariable sequence of the light chain near the variable region, and cuts one of the strands of the DNA, exposing a free 3′ end. An exonuclease then degrades the DNA, exposing a stretch of the complementary strand. The gap is then repaired by a polymerase and, during this repair, errors are introduced. These could be base pairing mistakes or additions or deletions. In this particular scheme, the trimming and repair are carried out by enzymes with the same mechanism of action as exonuclease III and DNA polymerase isolated from bacteria[14–16]. Enzymes with opposite polarity, trimming and repairing from the 5′ end, have not yet been found. In the proposed mechanism the right-hand end of the variable region is definitively fixed by the point of cleavage. We do not need to postulate a specific location for the left-hand end. For example, the polymerase may displace the exonuclease when enough single stranded DNA has been generated for it to bind sufficiently strongly to initiate repair. It is therefore possible that, on the average, the repaired length may only rarely include the extreme left-hand region, and this would be reflected in the clustering of errors towards the centre of the molecule.

It is obvious that repeated repair of the region cannot in general lead to the accumulation of errors. Furthermore, while the region of the gene subject to repair corresponds to the N-terminal half of the protein, the DNA strand repaired is not the one which is copied into messenger RNA. Therefore, in order to express and stabilize the changes the cell must divide and the DNA must be replicated in the normal manner. One of the cells produced is like the original type, the other contains a mutant sequence. Repetition of the process could therefore generate a very large number of different sequences, but it is interesting to note that every sequence produced, including the primordial one, is also conserved. Exactly how many division cycles are necessary to generate the deviations in sequence would depend on the mutagenic rate of the repair polymerase.

The mutation process depends in the first instance on the presence of the cleaving enzyme, and both it and the polymerase may only be expressed at specific stages of the differentiation of antibody precursor cells. Further steps of differentiation could stop the sequence of events by destroying or inhibiting the cleaving and/or repair

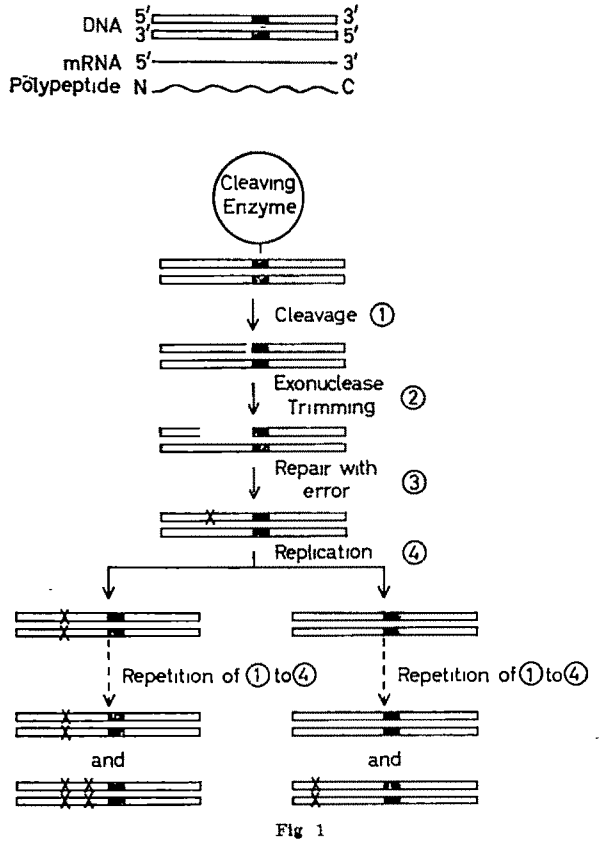


Fig. 1

enzymes. The subsequent clone produced from such a cell could therefore be genetically uniform and stable. As suggested by Lederberg[5], the immune response could involve selection of such clones by antigens.

The mechanism proposed has precedents in other biological systems. Excision and subsequent repair of DNA occurs after radiation damage in micro-organisms[17–19]. Specific recognition of DNA sequences for cleavage and chemical modification is the basis of host restriction and modification of bacterial and bacteriophage genomes[20]. Mutant DNA polymerases in bacteriophage *T4* produce high frequencies of errors[21] and an altered DNA polymerase may be responsible for the elevated mutation rate of one strain of *E. coli*[22].

We cannot propose a definitive test of this theory apart from the direct demonstration of a cleaving enzyme and a mutagenic polymerase in precursor cells, which may not be easy to do. One prediction is that the heavy chains of antibody molecules will have the same features as the L chains and, in particular, the junctional region should contain the same recognition site. We note, however, that this site must only have the same nucleotide sequence, and the amino-acid sequences of this region in H chains may not be the same as that in L chains but may be related to it by a phase shift. Unfortunately, this result, though striking, would only show that recognition occurs at the level of nucleic acid and would still be consistent with genetic incorporation models. Our main purpose in proposing this theory is to show that a mutational origin of antibody variation is consistent with the known facts and that we are not compelled to postulate more elaborate models.

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SYNTHESIS OF EXCESS LIGHT CHAINS OF GAMMA GLOBULIN BY RABBIT LYMPH NODE CELLS

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Low molecular weight proteins immunologically related to the light chains of gamma globulin have been detected in normal human serum[1,2] and urine[1–7]. In contrast to plasma cell tumours of mice and man which have been shown to produce excess L chains[7], it is not certain whether the free L chains[2] found in normal individuals result from *de novo* synthesis[6] or the degradation of whole gamma globulin[3,4]. This paper reports the synthesis and release of excess L chains by cells from the lymph nodes of hyperimmunized rabbits.

Popliteal lymph nodes were removed from rabbits 6–8 days after secondary stimulation with T_2 phage. Cells were teased from the nodes as previously described[8] in Eagle's medium[9] containing 1/50 the normal amounts of amino-acids (in order to deplete the cellular pool). After pre-incubation at 37° C for 15 min, the cells were exposed to carbon-14-labelled amino-acids (amino-acid-^{14}C, u.l. mixture (New England Nuclear)) for either 30 or 60 min. Incorporation was terminated by chilling. The centrifuged cells were disrupted by five cycles of freezing and thawing in 0·01 M *tris*-hydrochloric acid buffer (*p*H 8·1), and cellular debris was removed by centrifugation at 1,500 r.p.m. for 15 min. The cell lysates were treated at 37° C with 10 per cent acetic acid, 1 per cent sodium dodecyl sulphate (SDS), and 0·5 M urea[10]. Half of each sample was reduced with 1 per cent 2-mercaptoethanol (ME). Samples were dialysed overnight at room temperature against 0·1 per cent SDS and 0·5 M urea in 0·01 M phosphate buffer *p*H 7·1, and 0·1 per cent mercaptoethanol was added to the dialysis fluid of the previously reduced samples[10]. Electrophoresis of 0·3 ml. samples was carried out on 5 per cent acrylamide gels for 150 min at 4 V/cm. The gels were then extruded[10] on to planchets which were dried and counted in a low background gas flow counter (Nuclear Chicago model 1050).

The electrophoretic mobility of whole 7*S* gamma globulin, and of the component H and L chains, was determined by staining electropherograms of purified proteins treated in the same way as the cell lysates. Preliminary experiments confirmed that acetic acid, SDS, and urea did not dissociate purified 7*S* gamma globulin whereas the addition of mercaptoethanol resulted in the complete dissociation into H and L chains[11,12]. Purified H and L chains were prepared by 'Sephadex' chromatography of gamma globulin reduced with ME in the presence of SDS and urea.

An electropherogram of the intracellular proteins synthesized by rabbit lymph node cells during the first 30 min of exposure to carbon-14-labelled amino-acids is shown in Fig. 1. When the cell lysate was treated at low *p*H with SDS and urea but not mercaptoethanol (lower panel of Fig. 1), there was a large peak representing whole 7*S* gamma globulin and a smaller peak the migration of which was identical with that of L chains prepared by 'Sephadex' chromatography. No H chains are detectable above the background radioactivity due to cellular proteins. In the presence of mercaptoethanol (upper panel, Fig. 1) the peak representing whole gamma globulin disappeared, the amount of material in the L chain area increased approximately two-fold, and a new peak of intermediate migration appeared in the area where H chains were found on stained gels of 'Sephadex' purified

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reduced gamma globulin. Judged by the areas under the various peaks (above the background of cellular proteins), mercaptoethanol releases about twice as much radioactivity into the H chain area as into the L chain area. This result is consistent with the fact that H chains are

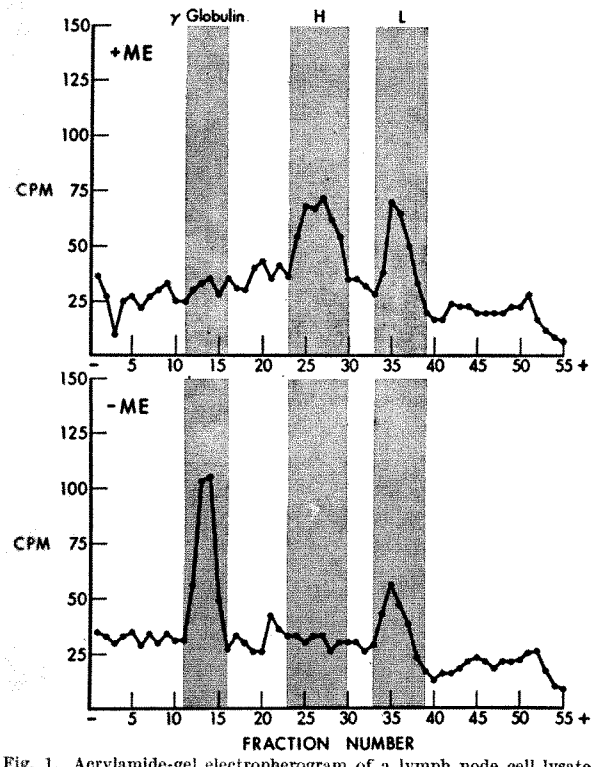


Fig. 1. Acrylamide-gel electropherogram of a lymph node cell lysate after 30 min exposure to carbon-14-labelled amino-acids. The lower panel presents the electrophoretic migration of the newly synthesized intracellular proteins before reduction with mercaptoethanol (−ME). In the upper panel, the same material has been treated with mercaptoethanol (+ME) to indicate where H and L chains migrate. The anode is on the right

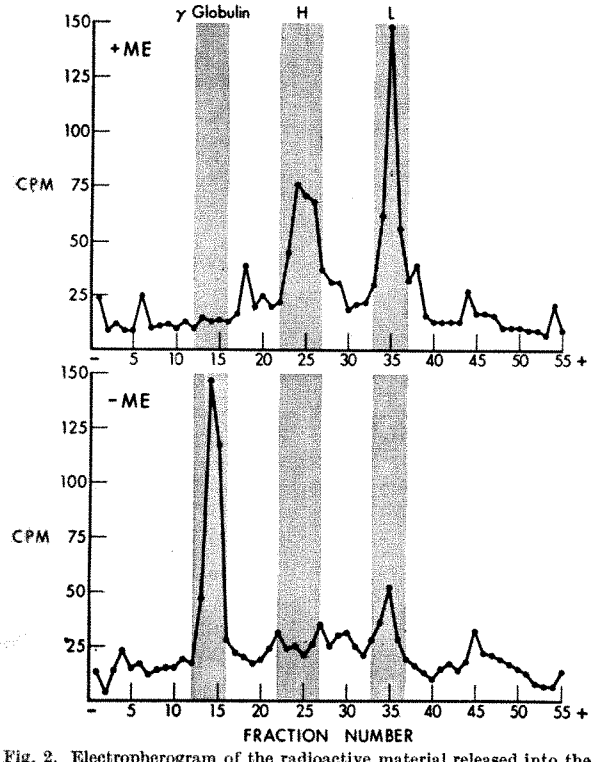


Fig. 2. Electropherogram of the radioactive material released into the culture medium after incubation for 60 min with label

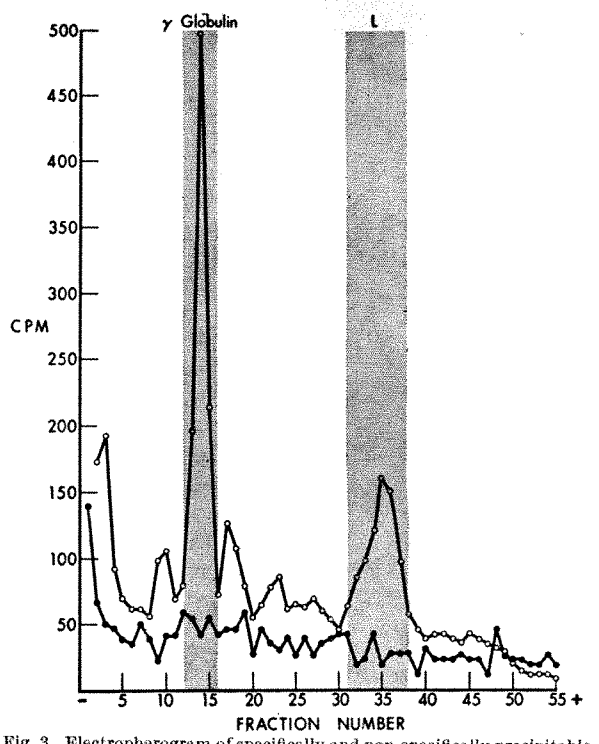


Fig. 3. Electropherogram of specifically and non-specifically precipitable material from radioactive cell lysates. The immune precipitates were dissociated with SDS, urea, and acetic acid but not with mercaptoethanol. ○—○, Specific; ●—●, non-specific

about twice as large as L chains. Further, preferential labelling of either chain was unlikely since a mixture of carbon-14-labelled amino-acids was used as precursor.

While, as shown by others[13], virtually no radioactivity was released from the cells into the medium in 30 min, after 60 min of incubation with carbon-14-labelled amino-acids both the whole gamma globulin and free L chains were found in the medium (Fig. 2).

The rapidly migrating material found in the cells at 30 min, and released into the medium by 60 min, had the electrophoretic mobility of L chains. In order to confirm the identity of this material, aliquots of the cell lysate were treated with either sheep anti-rabbit gamma globulin and unlabelled rabbit gamma globulin to coprecipitate newly synthesized gamma globulin, or with egg albumen and sheep anti-egg albumen as a control for non-specific coprecipitation[14]. The immune precipitates were treated with acetic acid, SDS, and urea, and analysed electrophoretically for radioactive material. As shown in Fig. 3, both whole gamma globulin, and the material which migrates as free L chains, were specifically precipitated.

The present experiments show that cells taken from lymph nodes of hyperimmunized rabbits 6–8 days after secondary stimulation synthesize and secrete excess free L chains. The absence of detectable amounts of H chains not associated with whole gamma globulin in solubilized whole cells suggests that the free L chains are synthesized *de novo* and are not the result of degradation of whole gamma globulin. Furthermore, under the immunization conditions of these experiments, the number of 7*S* gamma globulin molecules would seem to be limited by the number of H chains produced. Similar results have been obtained with cells from a limited number of animals 90 days after immunization. Preliminary experiments on cells from animals 3 days after secondary stimulation show a more complicated electropherogram perhaps related to the formation of 19*S* gamma globulin.

At a cellular level, the production of excess L chains could be due to either the synthesis of only L chains by some cells while others make equimolar amounts of both polypeptide chains or to the synthesis of more L than H chains by all cells. The latter possibility seems more

likely, since Bernier and Cebra have presented immunofluorescent evidence that in humans all gamma globulin producing cells contain both types of polypeptide chains[15].

It is not known whether the synthesis of excess L chains is determined at the level of the DNA, the synthesis of messenger RNA (transcription), or the reading of the messenger (translation). However, the observed synthesis of approximately twice as many of the smaller L chains as H chains would occur if there were equal numbers of H and L chain cistrons which were transcribed and translated at the same rate. In such circumstances, the synthesis of excess L chains might be more economical for the cell than the differential modulation of either the rate of synthesis or reading of the messenger RNAs for the two polypeptide chains.

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AN ESTIMATE OF THE NUMBER OF ADENOSINE DIPHOSPHATE BINDING SITES ON HUMAN PLATELETS

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THE surface charge on human platelets can be estimated from their electrophoretic mobility, and we have found that this mobility can be changed in two different ways. First, the electrophoretic mobility of platelets handled in siliconized containers is greater than it is after contact with a glass surface, and this change is probably due to the removal of adenosine diphosphate (ADP) from the platelet surface[1]. Secondly, charge can be changed by substances known to cause platelet aggregation, which in low concentrations increase mobility, and in higher concentrations cause a decrease in mobility[2]. We have suggested that the increase in mobility is due to the adsorption of ADP molecules by the platelet surface: because ADP is negatively charged like the platelet, the overall negative charge is increased. There are some differences between the actions of ADP and noradrenaline[3] and we have suggested that noradrenaline acts by causing the release of ADP from within the platelet on to its surface. We have also shown that in acute illnesses the sensitivity of the platelets to ADP and noradrenaline, as measured by the concentrations required to induce the maximum increase in mobility, is increased up to a hundred-fold[4]. In acute illnesses the sensitivity to ADP and noradrenaline changes in parallel, while in some patients with arterial disease, and in patients with hypercholesterolaemia there is a dissociation in sensitivity to these agents (unpublished observations).

In each of these situations there would seem to be a maximum platelet mobility: in the presence of higher concentrations of aggregating agents than induce this maximum there is a decrease in mobility. If the difference in platelet mobility between the basal post-contact state and the state of maximum mobility is due to the adsorption of ADP on to the platelet surface, then the difference in charge between the two states represents the charge carried by the adsorbed ADP molecules. An estimate of the number of molecules of ADP which each platelet can adsorb can therefore be made.

It is first necessary to show that there is a finite maximum platelet mobility:

(1) *The relationship between the glass-contact effect and the increase in mobility induced by aggregating agents.* The platelet mobility in thirteen subjects was measured before and after contact with a non-siliconized glass surface, and then the response to 0·005, 0·05 and 0·[illegible] of ADP was measured. The mean was 1·19 μ/sec/V/cm and the mean post-contact mobility was 1·08 μ/sec/V/cm. After 10 min incubation with 0·05 μg/ml. of ADP, which was the concentration inducing the maximum increase in mobility in each patient, the mean mobility was the same as in the pre-contact state (1·19 μ/sec/V/cm).

In twelve other subjects the pre- and post-contact mobilities were measured, and then the effect of three concentrations of noradrenaline was determined: in each patient the maximum increase in mobility was shown to be induced by 0·05 μg/ml. of noradrenaline. The mean pre-contact mobility was 1·15 and the mean post-contact mobility 1·05 μ/sec/V/cm. After incubation with noradrenaline 0·05 μg/ml., the mobility was the same as in the pre-contact state, 1·15 μ/sec/V/cm.

It would seem, therefore, that the pre-contact mobility is the same as the maximum mobility which can be induced by ADP or noradrenaline.

(2) *The maximum increase in mobility induced by ADP and noradrenaline:*

(*a*) *The normal response.* The response of platelet mobility to 0·05 μg/ml. of both ADP and noradrenaline was measured in seventeen normal subjects. The mean post-contact mobility was 1·07 μ/sec/V/cm; after incubation with 0·05 μg/ml. ADP or of noradrenaline the mean mobility was 1·15 μ/sec/V/cm with each agent. In normal subjects the maximum increase in mobility is therefore the same whether it is induced by ADP or by noradrenaline.

(*b*) *The abnormal response in acute illnesses.* Five patients admitted for elective surgery were investigated before operation and at intervals afterwards. Before the operation the platelet sensitivity was the same as that of the normal group of subjects, the maximum increase in mobility being induced by 0·05 μg/ml. of ADP or noradrenaline. In each patient the sensitivity to ADP and noradrenaline increased in parallel after the operation, and on the second day the maximum increase was induced by 0·005 μg/ml. of ADP or noradrenaline. Before operation the mean post-contact mobility was 1·05 μ/sec/V/cm; after incubation with either ADP or with noradrenaline the mean mobility was 1·14 μ/sec/V/cm. On the second post-operative day the mean post-contact mobility was [illegible]/sec/V/cm; the mean maximum mobility induced by [illegible] was 1·17 and the mean maximum induced by [illegible] was 1·16 μ/sec/V/cm. Thus, whatever [illegible] ADP or noradrenaline induces the

maximum increase in mobility, this final mobility is the same with both ADP and noradrenaline.

Four patients with acute myocardial infarctions were investigated serially: in each case, 24 h after the infarction the maximum increase in mobility was induced by 0·0005 μg/ml. of ADP. After a variable time each returned to normal sensitivity, the maximum increase being induced by 0·05 μg/ml. The post-contact mobilities in the abnormal and normal states of sensitivity were identical (1·05 μ/sec/V/cm) and the maximum mobility induced by ADP was 1·16 μ/sec/V/cm at the time of abnormal sensitivity, and 1·15 μ/sec/V/cm at the time of normal sensitivity. Whatever the sensitivity of the platelets the maximum mobility is not significantly changed.

(3) *Differential sensitivity to ADP and noradrenaline.* Twelve patients with either hypercholesterolaemia or obliterative arterial disease were found to have a dissociation of their dose-response curves to ADP and noradrenaline: the maximum increase in mobility was induced by 0·005 μg/ml. of ADP and 0·05 μg/ml. of noradrenaline. The mean post-contact mobility of these patients was 1·09 μ/sec/V/cm; with 0·005 μg/ml. of ADP the mobility was 1·17 μ/sec/V/cm, and with 0·05 μg/ml. of noradrenaline the mobility was also 1·17 μ/sec/V/cm. Here again, there is evidence that for a given group of patients, irrespective of the concentration of agents which induce the maximum mobility, this maximum mobility is the same.

Maximum increase in mobility. From these results we conclude that the state of maximum mobility is an entity: it can be produced in a variety of ways in different subjects and yet it has the same magnitude in any given group of subjects, however it is induced.

The platelets of twenty normal subjects were therefore tested with three concentrations of ADP and the maximum increase in mobility was shown to be induced by 0·05 μg/ml. The mean post-contact mobility was 1·06 μ/sec/V/cm and the mean maximum mobility was 1·16 μ/sec/V/cm, giving a difference of 0·10 μ/sec/V/cm. If this change in mobility were due to the adsorption of ADP molecules, it would represent a calculable change in surface charge, and could therefore give a quantitative estimate of the ADP binding.

Calculation of platelet surface charge. The surface charge density, σ, on the platelet can be calculated from its electrophoretic mobility using the Gouy-Chapman equation[5]:

$$\sigma = \left[1+(1-\alpha)^{\frac{1}{2}}\right] 2.\left[\frac{NDKT}{2000}\right]^{\frac{1}{2}}. I^{\frac{1}{2}} \sin h \left[\frac{eZ}{2KT}\right]$$

where α represents the fraction of space on the platelet surface not available for counter ions[6]. When $\alpha = 1$ the Gouy-Chapman equation is obeyed; when $\alpha = 0$ the actual surface charge density will be twice that calculated from the Gouy-Chapman equation. The value of α for platelets is not known, but because this correction should apply equally to the platelet in its post-contact state and in the state of maximum charge, it has been omitted. The remainder of the factors in the Gouy-Chapman equation are known or measurable constants: N is Avogadro's number; D is the dielectric constant of the medium; K is Boltzman's entropy constant; T is the absolute temperature; e is the electronic charge; I is the ionic strength of the medium and Z is the zeta potential of the platelet calculated by the Smoluchowski equation:

$$Z = \frac{4\pi\eta}{D} u$$

where η is the bulk phase viscosity, D the dielectric constant of the medium, and u the electrophoretic mobility of the platelet. As with Haydon's modification, any invalid assumption used in the estimation of any of these factors should apply equally to the platelet in both mobility states, and should not materially affect the calculation of the difference in charge density in the two states.

The surface charge density of the post-contact platelet as calculated from this formula is 4,620 E.S.U./cm² and of the maximally charged platelet 5,050 E.S.U./cm²: the difference in charge between the two states is thus 430 E.S.U./cm². If the mean platelet surface area is taken to be 28·3 μ² (ref. 7), then the mean charge per platelet can be determined; from this the number of "free electrons" per platelet is calculated by dividing by $4{\cdot}8 \times 10^{-10}$ (the charge per electron in E.S.U.).

If ADP molecules are attached to the platelet by their adenine rings[8] and if three negative charges of each molecule are available to influence the net negative charge on the platelet, then the number of ADP molecules attached is equal to the change in the number of free electrons on the platelet surface divided by 3. Using the results we have obtained, the number of ADP molecules which would change the mobility of a single platelet from the post-contact state to the maximally charged state is $0{\cdot}85 \times 10^5$; as no further increase in mobility can be produced this probably represents the maximum number of sites at which ADP molecules can be bound.

In these calculations we have made no correction for the electrical double layer and we have, of course, assumed that the change in mobility is entirely due to a change in surface charge. Although aggregating agents can change platelet shape[9] we are unable to account for the pattern of mobility changes which we have observed in terms of platelet shape or size, nor are the biphasic changes due to the aggregation process itself. Moreover, despite all the assumptions we have had to make, our estimate of $0{\cdot}85 \times 10^5$ ADP binding sites per platelet is in remarkable agreement with the value of 2×10^5 sites per platelet obtained by Born[10] using an entirely different approach.

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PRODUCTION OF HAPTEN-SPECIFIC DELAYED SENSITIVITY WITH LOW MOLECULAR WEIGHT CONJUGATES

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Two necessary conditions are required for the demonstration of hapten-specific delayed hypersensitivity: a carrier which will direct the ensuing response [illegible] hapten and a hapten which can by itself provide [illegible] sary interactive force with the delayed sensitivity moiety. Several properties of effective carriers have been investigated[1], and in general it has been found that they are [illegible] or antigens with respect to antibody production. The [illegible] characteristics of a hapten capable of inducing [illegible] delayed sensitivity have been under

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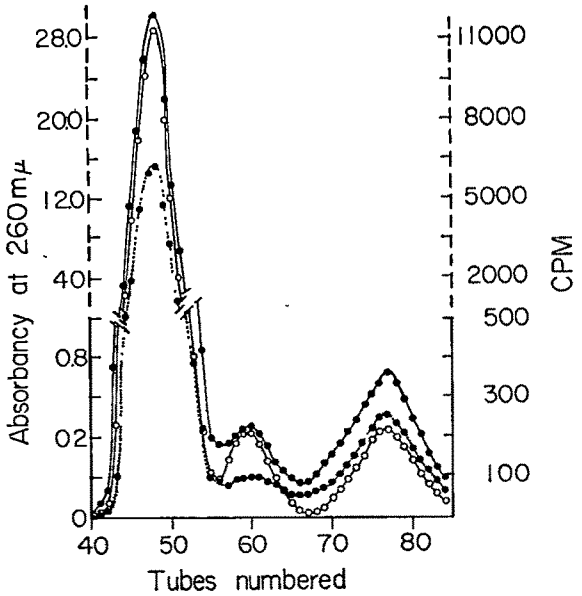


Fig. 2. Elution profile of microsomal mouse liver RNA after pulse labelling with carbon-14 labelled orotic acid. Open circles represent absorbancy, closed circles and dotted line the radioactivity after the 1-h pulse, closed circles and solid line after the 2-h pulse

To determine the quantity of 5*S* RNA on the ribosomes, ribosomal and microsomal rat liver RNA were fractionated on 'Sephadex *G*-100' and the total absorbancies of the three peaks at 260 mμ calculated. Two different concentrations of magnesium ions (0·001 M and 0·015 M magnesium chloride) were used in the sucrose media for isolation of ribosomes to ascertain whether the binding of 5*S* RNA to the ribosomes is dependent on magnesium ions. It was found that the 5*S* peak contained 1 per cent as much RNA as the high-molecular-weight RNA peak and that this relationship did not change with different concentrations of magnesium ions. Also, the relative amounts of 5*S* and ribosomal RNA were the same when RNA was extracted from ribosomes, microsomes, or whole cytoplasm. The amount of 4*S* RNA changed at different concentrations of magnesium ion from 1 per cent for ribosomes isolated in 0·001 M magnesium chloride to 2 per cent for ribosomes isolated in 0·015 M magnesium chloride, and was 3–4 per cent in the microsomal RNA. These results show that 5*S* RNA is a more stable component of the ribosomes than 4*S* RNA, and that 5*S* RNA is not contained in the membranes of the endoplasmic reticulum.

If we assume that the molecular weight of the RNA in one ribosome is $2·3–2·6 \times 10^6$ (ref. 12) and the molecular weights of 5*S* and 4*S* RNA are 35,000 and 28,000 respectively, then we find that each ribosome contains 1·1 (± 0·1) molecule of 5*S* RNA and from one to three molecules of 4*S* RNA depending on the experimental conditions. This is in agreement with the data of Rosset *et al.*[5] and supports the idea that 5*S* RNA may be a structural component of the ribosomes.

5*S* RNA is not produced by the degradation of RNA of higher molecular weight during extraction for the following reasons: (1) The amount of 5*S* RNA is independent of the experimental conditions of extraction or fractionation of RNA. (2) When ribosomes were dialysed against 0·01 M EDTA and then applied to a 'Sephadex *G*-100' column, some 5*S* RNA eluted separately from the ribosome peak; in this case no phenol extraction procedure was used.

To determine the rate of metabolism of 5*S* RNA, the labelling pattern of microsomal RNA 1 h, 2 h, and 4 h after injection of orotic acid labelled with carbon-14 in (6) position (30 mc./mmole) was investigated. Each mouse was injected intraperitoneally with 50 μc. for the 1-h pulse, and 30 μc. for the 2- and 4-h pulses. The extracted RNA was purified on 'Sephadex *G*-25', and fractionated on a 'Sephadex *G*-100' column. The specific activity of 5*S* RNA was similar to that of the high-molecular-weight RNA whereas the specific activity of 4*S* RNA was about twice as high at 1 and 2 h (Fig. 2). The same pattern of specific activity was found even at 4 h. This result rules out the possibility that 5*S* RNA is a precursor of 4*S* RNA, but suggests that 5*S* RNA is synthesized at the same rate as the two structural RNA components of the ribosomes.

The ability of 4*S* and 5*S* RNA to accept amino-acids was compared *in vitro* and *in vivo*. The procedure for *in vitro* acylation of Wettstein and Noll[13] was followed using carbon-14-labelled protein hydrolysate from *Chlorella*. Under conditions in which 4*S* RNA actively incorporated labelled amino-acids *in vitro*, 5*S* RNA accepted no label. 2 h after intraperitoneal injection of labelled amino-acids into mice, detectable radioactivity appeared in 4*S* RNA, but not in 5*S* RNA.

The nucleotide composition of RNA fractions from rat liver was determined after alkaline hydrolysis by the method of Katz and Comb[14]. The mole percentages uridine, guanine, cytosine and adenine in 5*S* RNA were 21·6, 33·2, 27·1, and 18·1 respectively, and in transfer RNA, 21·0, 30·8, 28 7, and 19·5. The quantity of methylated bases and pseudouridylic acid in 4 and 5*S* RNA was examined according to the method of Cantoni *et al.*[15]. 4*S* RNA contained about 3 per cent methylated nucleotides and 4 per cent pseudouridylic acid as reported by Cantoni *et al.*[15]. Only traces of methylcytidylic acid were found in 5*S* RNA and the pseudouridylic acid content was 0·3 per cent.

To confirm these findings 50 μc. of carbon-14-methyl methionine (29·5 mc./mmole) were injected intraperitoneally into each mouse and microsomal RNA from the livers was extracted 24 h later. As can be seen from Fig. 3, practically no incorporation of methyl groups was found in 5*S* RNA, whereas 4*S* RNA was labelled. To prove that the counts represented methylation of the nucleotides, carbon-14-methyl methionine-labelled high-molecular-weight RNA and 4*S* RNA were subjected to alkaline hydrolysis after the addition of carrier RNA, and the hydrolysates were examined for methylated nucleotides. The label in 4*S* RNA was recovered in the elution regions for methylated nucleotides whereas the label in high

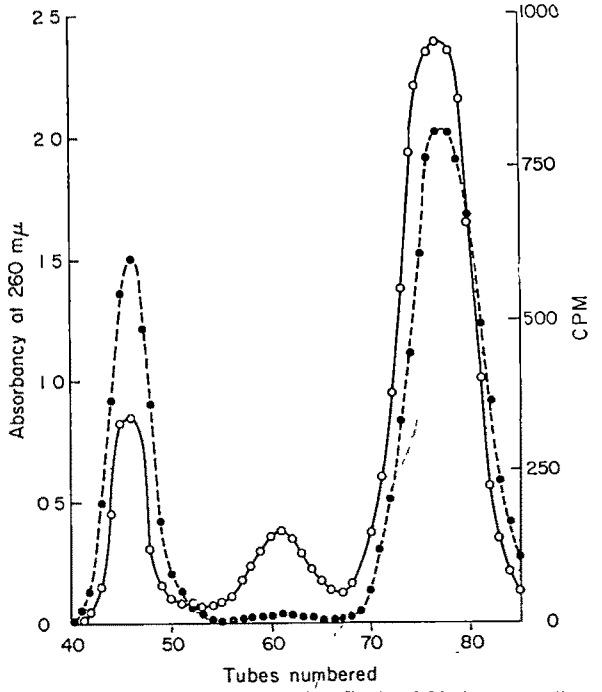


Fig. 3. Elution profile of mouse liver RNA soluble in 1 M sodium chloride after a 24-h pulse of carbon-14-methyl methionine. Open circles represent absorbancy and closed circles the c.p.m.

molecular-weight RNA did not represent methylation of nucleotides and was attributed to the poor deproteinization of this RNA.

Thus, although the base composition of 5*S* RNA was closely similar to that of 4*S* RNA, significant differences between the two RNAs were found so that 5*S* RNA cannot be a product of aggregation of 4*S* RNA.

The melting curve of 5*S* RNA was compared with that of 4*S* and high-molecular-weight ribosomal RNA (Fig. 4). In 0·15 M sodium chloride and 0·015 M sodium citrate buffer, *p*H 7·4, the total hyperchromicity of 4*S* and 5*S* RNA is similar. This suggests that they possess the same degree of ordered secondary structure. However, the T_m of 4*S* RNA is 49° C, compared with 55° C for 5*S* RNA, and at 37° C the 4*S* RNA has melted significantly more than the 5*S* RNA. These differences could result from the presence of fewer minor bases in 5*S* RNA and from variation in sequence of bases.

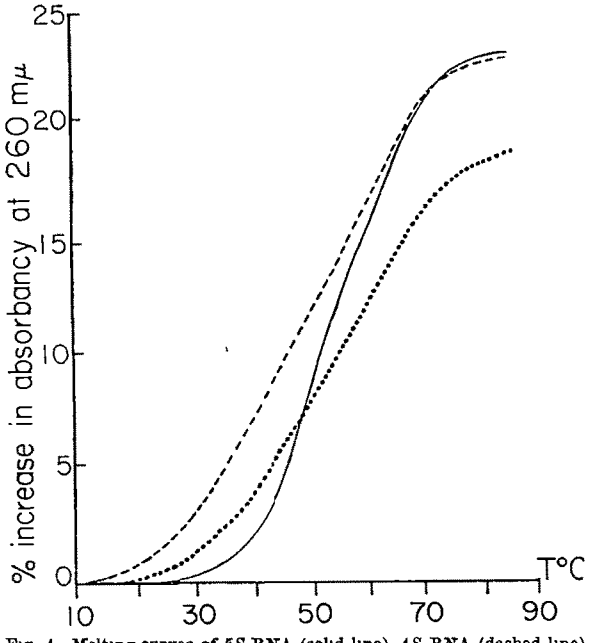


Fig. 4. Melting curves of 5*S* RNA (solid line), 4*S* RNA (dashed line), and high-molecular-weight ribosomal RNA (dotted line) in 0·15 M sodium chloride plus 0·015 M sodium citrate buffer, *p*H 7·4

The mammalian 5*S* RNA described here resembles the fraction found by Galibert *et al.*[7] in its presence on the ribosomes, its lack of acceptance of amino-acids, its gel-filtration properties on 'Sephadex *G*-100' and its chromatographic properties on MAK columns. Our data on its behaviour during agar electrophoresis, its *S* value, T_m, more stable binding to the ribosomes, and rate of synthesis, further distinguish 5*S* from 4*S* RNA. We suggest that 5*S* RNA may be a structural component of the ribosomes. Further experiments are in progress to elucidate the biological significance of 5*S* RNA.

We thank Dr. A. Morosko for performing the analytical ultracentrifugation and E. Horoshutina for her excellent assistance.

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INFECTION OF THYMIDINE KINASE-DEFICIENT *BHK* CELLS WITH POLYOMA VIRUS

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BIOCHEMICAL variants occur in heteroploid mammalian cell cultures, but have not been described in cultures of diploid human fibroblasts. They do occur in the *BHK* 21/13 line of hamster cells[1], which is also primarily diploid[2]. A *BHK* 21/13 variant deficient in thymidine kinase would be useful for various genetic studies, as have been kinase-deficient *L* cell variants[3,4]. In particular we wondered if infection of such cells with polyoma virus would cause: (1) a generalized transient increase in kinase activity—as occurs in mouse kidney[5,6] and mouse embryo[7] cultures; (2) occasionally the permanent acquisition of the enzyme in a transformed cell through integration of the viral genome; (3) derepression or reverse mutation in the host cell genome; (4) transduction.

Thymidine kinase-deficient cells are unable to phosphorylate 5-bromodeoxyuridine, and therefore are protected against the lethal effect of the incorporation of this analogue into DNA. The kinase-deficient *L* cell sub-lines were selected by virtue of their resistance to 5-bromodeoxyuridine; however, it was not possible to isolate resistant cells directly as clones and the selection took a considerable time[8,9]. For the present experiments *BHK* 21/13 cells, kindly supplied by Prof. M. Stoker, were grown in Eagle's medium containing four times the usual concentration of amino-acids and vitamins and supplemented with either 10 per cent calf serum for routine maintenance or 20 per cent foetal calf serum for cloning experiments. To determine the sensitivity of this line to 5-bromodeoxyuridine, 200 cells were plated in Petri dishes containing medium with various concentrations of the analogue. Compared with untreated cells, the survival was 97 per cent at 0·04 μg/ml., 43 per cent at 0·12 μg/ml., 9 per cent at 0·37 μg/ml., and 0 at 1·1 μg/ml. Then 100-mm dishes containing only 5×10^5 cells were exposed to concentrations of 5-bromodeoxyuridine somewhat greater than 1·1 μg/ml., as it was expected that a larger number of sensitive cells would inhibit the growth of any partially resistant variants[8]. After 10 days there were about twenty colonies in each dish containing 3·3 μg/ml. Cells from these colonies were exposed to 30 μg of 5-bromodeoxyuridine/ml., in order to select highly resistant variants. A similar number of colonies appeared, two of which were isolated and enlarged into sub-lines designated *B*1 and *B*2.

These sub-lines have remained highly resistant to 5-bromodeoxyuridine. For example, the plating efficiency (without feeder cells) of *B*1 cells was 32 per cent in the absence or presence of 30 μg of 5-bromodeoxyuridine/ml., and 29 per cent with somewhat smaller colonies in 300

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μg/ml. Cells of both sub-lines align themselves in parallel fashion when confluent like *BHK* 21/13 cells. They grow more slowly than *BHK* 21/13 (generation time about 15 h for *B*1). Preliminary studies suggest that each sub-line has lost two or three chromosomes, and detailed karyotype analyses are in progress. As expected, extracts of *B*1 and *B*2 cells contained very little thymidine kinase activity (Table 1). For these determinations it was important to use 2-day-old cultures containing not more than $1{\cdot}5 \times 10^6$ cells in each 100-mm Petri dish, as kinase activity decreases markedly in crowded cultures.

Table 1. THYMIDINE KINASE ACTIVITIES OF *BHK* SUB-LINES

Sub-line	μμmoles/10⁶ cells/10 min	Per cent
BHK 21/13	796	100
*B*1	24 0	3·0
*B*2	13·0	1·6
T × 1	11·3	1 4
T × 5	14·3	1 8
*B*1*R*1	491	62
*B*1*R*2	499	63

The values represent the μμmoles of thymidine-2-^{14}C phosphorylated in 10 min at 37° C by the extract of 10^6 cells, assayed in duplicate and corrected for a blank reaction mixture from which ATP was omitted, as previously described (ref. 9). *B*1 and *B*2 are the 5-bromodeoxyuridine-resistant sub-lines; *T* × 1 and *T* × 5 are polyoma virus-transformed sub-lines of *B*1; and *B*1*R*1 and *B*1*R*2 are aminopterin-resistant revertants isolated from the *B*1 sub-line.

The deficiency of kinase is reflected in the low rate of incorporation of thymidine-2-^{14}C into the DNA of growing *B*1 and *B*2 cells (Table 2). If the incorporation were increased during the hours following infection with polyoma virus, this would suggest an increase in kinase. *B*1 cells were infected with a small plaque type of polyoma virus (2×10^3 P.F.U./cell), and at the end of the virus adsorption period (1 h) medium containing thymidine-2-^{14}C was added. Twenty-four hours later the incorporation of thymidine-2-^{14}C into DNA was determined as previously described[10], and compared with that into uninfected control cultures. Likewise, *B*2 cells were infected with a large plaque virus (2×10^3 P.F.U./cell), and then exposed to thymidine-2-^{14}C for 48 h. In neither of these experiments was the incorporation of thymidine-2-^{14}C into DNA increased after infection (Table 2). The same preparation of small plaque virus (grown in mouse kidney cells) had been shown to induce a twelve-fold increase in kinase in confluent mouse kidney cultures 24 h after infection.

Table 2. THYMIDINE-2-^{14}C INCORPORATION INTO DNA OF *BHK* SUB-LINES

Sub-line	mμmoles/10⁶ cells/24 h	Per cent
BHK 21/13	14 8	100
*B*1	0·74	5·0
*B*2	1·0	6 8
*B*1 after infection	0 60	4·1
*B*2 after infection	0 82	5 5
T × 1	0 89	6 0
T × 2	0·85	5 7
T × 3	0·81	5 5
T × 5	0 65	4·4

Actively growing cultures containing not more than 3×10^5 cells/35 mm Petri dish were exposed for 24 or 48 h to medium containing thymidine-2-^{14}C (4×10^{-8} M, 2 5 μc /μmole), and then analysed as described previously (ref 10). The values represent the mμmoles of thymidine-2-^{14}C incorporated into DNA in 24 h by 10^6 cells, corrected for the incorporation into control cultures held at 4° C Certain cultures were infected with polyoma virus before the addition of thymidine-2-^{14}C, as described in the text *T* × 1, *T* × 2, *T* × 3, and *T* × 5 are polyoma virus-transformed sub-lines of *B*1.

It was important to know if the kinase-deficient cells could be transformed with polyoma virus-like *BHK* 21/13 cells. The ability of cells to grow in Eagle's medium containing 10 per cent calf serum, 2 per cent tryptose phosphate broth, and 0·34 per cent agar was used as the criterion of transformation[11]. None of $3{\cdot}3 \times 10^4$ *B*1 cells and 0·05 per cent of *B*2 cells was able to grow in this medium (Table 3). Under standard techniques of infection[12,13], *B*1 cells seemed normally susceptible to transformation, while *B*2 cells were less so. Thus in one experiment 4·4 per cent of *B*1 cells were transformed after infection, and in another experiment only 0·25 per cent of *B*2 cells were transformed (Table 3).

Four transformed *B*1 colonies (*T* × 1, *T* × 2, *T* × 3 and *T* × 5) were isolated during Experiment 1 of Table 3. These cells showed the random orientation in culture described for transformed cells[2]. They incorporated thymidine-2-^{14}C at the same low rate as untreated *B*1 cells (Table 2), and extracts of two of these transformed sub-lines contained no more kinase activity than extracts of *B*1 cells (Table 1). These experiments indicated that transformation was not regularly accompanied by permanent acquisition of the enzyme.

To determine if polyoma virus caused the acquisition of the kinase in an occasional cell, it was possible to take advantage of the fact that kinase-deficient cells, such as *B*1 and *B*2, cannot survive in medium containing 10^{-5} M aminopterin, 4×10^{-5} M thymidine, 10^{-4} M hypoxanthine, and 10^{-5} M glycine[9]. The endogenous synthesis of thymidylic acid is blocked by aminopterin, and thymidine in the medium can be utilized only by cells containing thymidine kinase. Normal or transformed *BHK* 21/13 cells show the usual plating efficiency in this medium (30–40 per cent), alone or in the presence of many killed *B*1 or *B*2 cells. Similarly transformed *BHK* 21/13 cells plate with the same efficiency in 0·34 per cent agar medium or in 0·34 per cent agar medium containing aminopterin, thymidine, hypoxanthine, and glycine. However, when *B*1 or *B*2 cells were infected with polyoma virus and 24 h later plated into this medium, no colonies appeared (Table 3). Thus none of the cells transformed had acquired the enzyme. It is likely that regain of one-half (see below), or perhaps less[9], of the usual thymidine kinase activity/cell would have permitted survival in this medium.

In other experiments (last 2 columns of Table 3) monolayer cultures containing even larger numbers of *B*1 or *B*2 cells were infected with polyoma virus, and aminopterin, thymidine, hypoxanthine, and glycine added to the medium after 24 h (Experiments 1, 2 and 4) or 48 h (Experiment 3). The cultures were re-fed with the selective medium once or twice, and colonies scored about 10 days after infection. In these experiments colonies occurred at about the same low frequency in both infected and control cultures (Table 3), indicating that infection had not led to the acquisition of the enzyme in even a rare cell.

None of these aminopterin-resistant colonies appeared morphologically to be transformed. Presumably they

Table 3. FREQUENCIES OF TRANSFORMATION AND REVERSION IN *BHK* SUB-LINES INFECTED WITH POLYOMA VIRUS

Expt.	Sub-line	Multiplicity of infection (P.F.U /cell)	Normal agar medium		Aminopterin agar medium		Aminopterin fluid medium	
			No. of cells	No. of colonies	No. of cells	No. of colonies	No. of cells	No of colonies
(1)	*B*1	0	$3{\cdot}3 \times 10^4$	0	—	—	—	—
		10^3	$1{\cdot}5 \times 10^4$	666	$1{\cdot}2 \times 10^6$	0	$1{\cdot}2 \times 10^6$	0
(2)	*B*1	0	—	—	—	—	$3{\cdot}4 \times 10^7$	3
		$3{\cdot}3 \times 10^3$	—	—	—	—	$4{\cdot}0 \times 10^7$	0
(3)	*B*1	0	—	—	—	—	$1{\cdot}7 \times 10^8$	12
		10^3	—	—	—	—	$1{\cdot}3 \times 10^8$	15
(4)	*B*2	0	7×10^4	35	—	—	$5{\cdot}6 \times 10^7$	1
		10^3	8×10^4	229	8×10^4	0	$3{\cdot}2 \times 10^7$	2
(5)	*T* × 1	—	—	—	—	—	10^7	2
	T × 5	—	—	—	—	—	10^7	2

See text for details For the experiments with agar medium, the number of cells indicated was plated into agar in several dishes 24 h after infection. For the experiments with fluid medium, monolayer cultures containing up to 10^6 cells/100-mm Petri dish were made 24 h before infection, and the number of cells given is an estimate of those present when aminopterin was added. Small plaque polyoma virus was used in experiments 1 and 2, and large plaque virus in experiments 3 and 4. The cells were infected in suspension in experiment 1, and otherwise in monolayer. In all cases virus adsorption was carried out for 1 h at 37° C.

represent revertant cells in the *B*1 and *B*2 sub-lines, such as occur in kinase-deficient *L* cells[9]. Extracts of cells grown from two of these colonies were found to contain 62 per cent and 63 per cent of the kinase activity of *BHK* 21/13 cells (Table 1).

The overall frequency of revertants in *B*1 cultures was 8×10^{-8}, and that in *B*2 cultures and two transformed *B*1 sub-lines was much the same (Table 3). To remove revertants, the cells used in Experiments 2 and 3 of Table 3 had been treated with 30 μg of 5-bromodeoxyuridine/ml. up to 5 and 15 days, respectively, before exposure to aminopterin during the experiments. From the reversion frequencies in these and two other experiments and the generation time of *B*1 cells it is possible to estimate[14] that the reversion rate is about 10^{-7}/cell/generation (range $0{\cdot}1–2{\cdot}0 \times 10^{-7}$).

The findings of these investigations indicate that variant cells markedly deficient in thymidine kinase activity can be isolated from the *BHK* 21/13 line of hamster fibroblasts. They were obtained by a stepwise procedure in which clones partially resistant to 5-bromodeoxyuridine were selected from the *BHK* 21/13 culture, and then markedly resistant clones selected from the partially resistant cultures. Infection of kinase-deficient cells with polyoma virus does not seem to cause an initial increase in kinase, as occurs during the lytic cycle in mouse embryo and mouse kidney cultures. The enzyme-deficient cells can be transformed with polyoma virus. Kinase is not permanently acquired by even a rare (10^{-7}) infected or transformed cell.

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MECHANISM OF ACTION OF BOVINE PANCREATIC RIBONUCLEASE

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RIBONUCLEASE has been shown to catalyse the hydrolysis of substrates of the type (I) in a two-step reaction involving the intermediate formation of a cyclic phosphate (II) (Fig. 1).

For substrates of low molecular weight and RNA, but apparently not for some homopolynucleotides, *B* must be a pyrimidine[1]. From an investigation of the kinetics of step 2 in a variety of solvents it was concluded that two histidine residues, one as an acid and the other as a base, form part of the active site of the enzyme[2,3]. Iodoacetate, but not iodoacetamide, inhibits the monomeric enzyme at *p*H 5·5 by reaction with either, but never both, of histidine residues 12 and 119[4]. The elegant hybridization experiments of Crestfield, Fruchter, Moore and Stein provide further evidence for the postulate that two histidine residues are involved at the catalytic site of the enzyme[5,6]. We have proposed that step 1 requires the interaction of one of the histidine residues (*a*), in the base form, with the 2′-hydroxyl, and the other (*b*), in the acid form, with the oxygen atom bridging *R* and *P* (ref. 3). In step 2, residue (*a*) in the acid form reacts with the 2′-oxygen and the base form of residue (*b*) binds the attacking water molecule. It was postulated that the proper positioning of the substrate with respect to the catalytic groups of the enzyme was achieved by specific interactions between the protein and the pyrimidine base, *B*. Spectrophotometric investigations in this laboratory and elsewhere lend support to this proposal[7]. Obviously there would be a tendency for interaction to occur between the negatively charged phosphate oxygen of (I) and the histidine cation (*b*) to give an abortive complex. Clearly this tendency would be counteracted by an interaction between a site on the protein and the group *R* restricting the rotational freedom about the P—OC_3 bond. The data of Witzel and Barnard (Table 1)[8] can be interpreted in these terms on the assumption that the strengths of the *R*-protein interactions are in the order adenine > guanine > cytidine > uridine. Interaction between the *R* group and the protein would be expected to affect the parameter k_{+2} to a much greater extent than K_m. This suggests that ribonuclease possesses two sites, one of which is specific for pyrimidines and the other of which prefers purines.

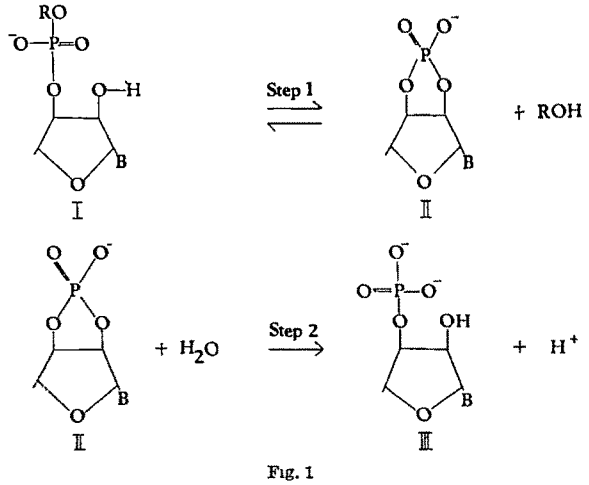


Fig. 1

Table 1. KINETIC CONSTANTS (ref. 8) AT *p*H 7·0 (IMIDAZOLE BUFFER), 26° C

Substrate	k^+_2 (sec^{-1})	K_m (mM)
CpA	3000	1·0
CpG	500	3·0
CpC	240	4·0
CpU	27	3·7

We have attempted to gain further information on the nature of these sites and the chemistry of their interactions with substrates by spectrophotometric and spectropolarimetric investigations of the binding of nucleotides, coupled with kinetic and protection experiments.

In this work, ribonuclease *A* was either prepared by the method of Taborsky[9], or was a commercial sample which had been rechromatographed to eliminate the appreciable

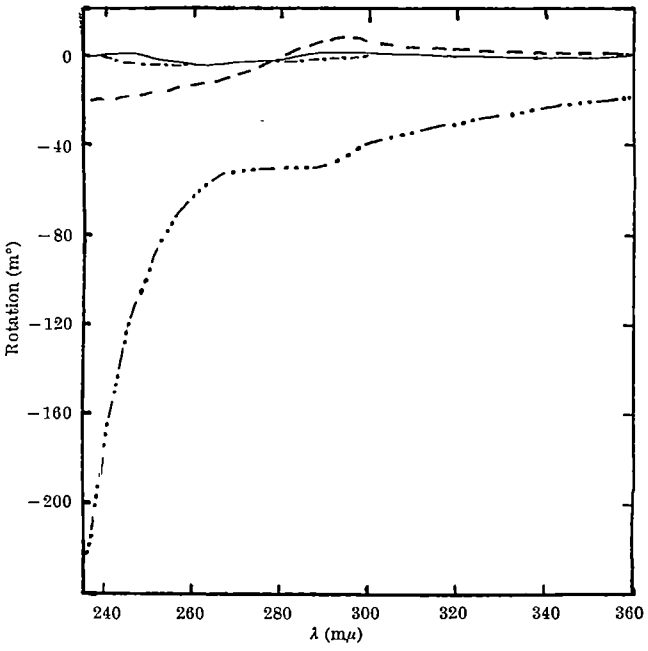


Fig. 2. Optical rotatory dispersion spectra of nucleotides ($1{\cdot}0 \times 10^{-4}$ M) and ribonuclease ($0{\cdot}5 \times 10^{-4}$ M) in a 1-cm cell. 0·02 M sodium acetate buffer *p*H 5·2, 25° C – – –, C3′P; ———, ATP; – · – ·, deoxythymidine 3′-phosphate benzyl ester, ·· –···–, RNase and CM–RNase

amounts of impurities which we have found more often than not in these preparations. Optical rotatory dispersion spectra were determined with a Bellingham and Stanley 'Polarmatic 62' spectropolarimeter fitted with a cell compartment maintained at a constant temperature of 25° C and equipped with an *X*–*Y* plotter. The instrument was flushed with nitrogen and the base-line determined in the same cell at the beginning and end of each run. Optical rotatory dispersion spectra of some nucleotides, ribonuclease and 119-carboxymethylated ribonuclease are shown in Fig. 2. Carboxymethyl RNase and native RNase have identical rotatory dispersion spectra within the limits of experimental error, making it unlikely that the loss of catalytic activity on carboxymethylation is a result of a conformational change in the protein. These spectra were determined at *p*H 5·2 and low ionic strength (0·02). Fig. 3 shows the difference in the optical rotatory dispersion spectra of enzyme–ligand mixtures and the computed sum of the separate components. Of particular note are the large differences, especially at low wave-lengths, observed with cytidine 3′-phosphate. We have recorded similar effects with a large number of other pyrimidine nucleotides, for example C2′P, C5′P, U2′ and 5′P, provided these possess a 2′-oxygen atom, with 2′-deoxynucleotides or their esters as illustrated for 2′-deoxythymidine 3′-phosphate benzyl ester in Fig. 3, no optical rotatory dispersion difference spectra are seen. For purine nucleotides including the 2′, 3′ and 5′ mononucleotides and the 5′ di- and tri-phosphates, only very small changes (if any) are detected. Similarly, no changes are found for pyrophosphate. It should be emphasized that all these compounds are powerful competitive inhibitors of the enzyme. Obviously they must bind to the protein and, indeed, with the exception of pyrophosphate, spectral changes can be observed on interaction with the ligand as illustrated in Fig. 4. These difference spectra were determined in split compartment cells type *T*/*E*8/1/5/41 manufactured by Thermal Syndicate Ltd., in a Cary 'model 14' spectrophotometer with a thermostatted cell compartment, on the 0–0·1 slide wire. We have found that split compartment cells manufactured to narrow tolerances have decisive advantages over tandem cell arrangements in that they yield much more reproducible results. They also enable artefacts to be eliminated because it is possible to recheck the base-line after mixing the component solutions in the reference compartment. The absence of any difference spectrum for pyrophosphate suggests that the difference spectra found with the nucleotides result from interactions involving the nucleoside. Spectrophotometric and spectropolarimetric titrations of the protein with nucleotides have been carried out for some

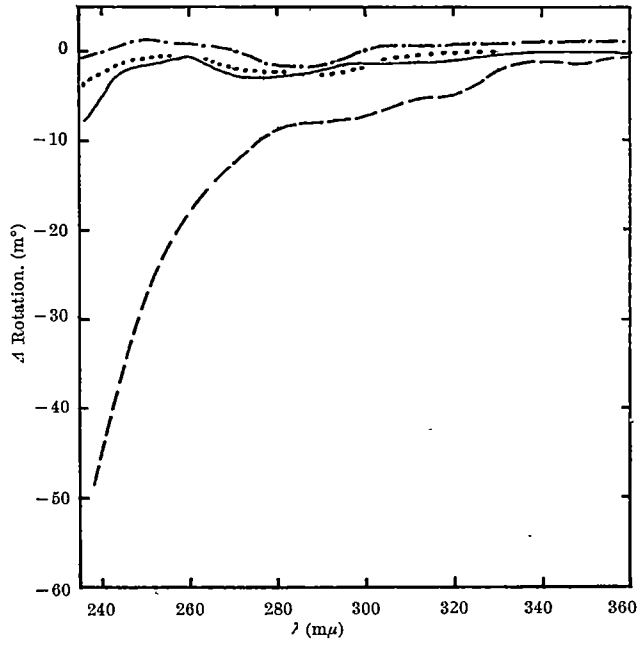


Fig. 3. Optical rotatory difference spectra of mixtures of ribonuclease and ligands measured under same conditions as in Fig. 2. – – –, C3′P, ———, ATP; –·–·–, deoxythymidine 3′-phosphate benzyl ester, ·····, pyrophosphate

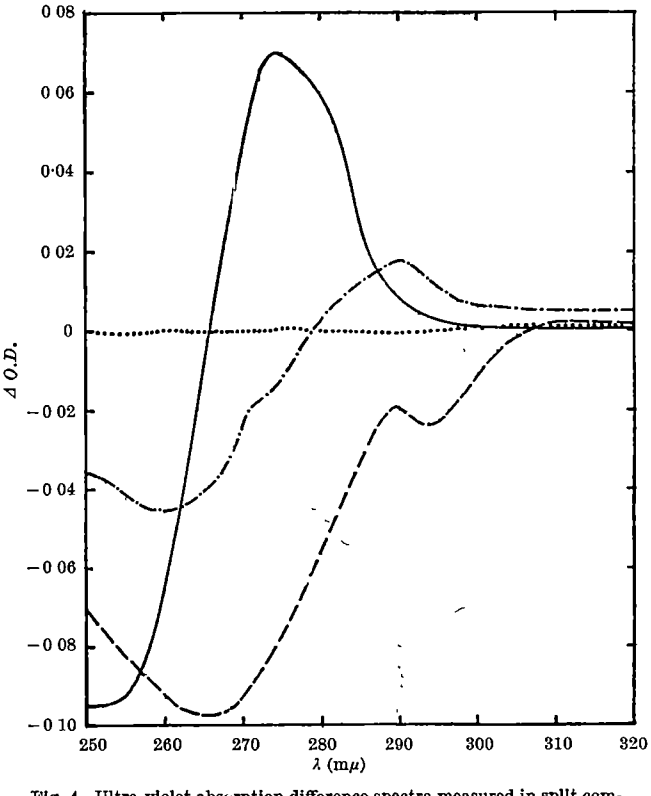


Fig. 4. Ultra-violet absorption difference spectra measured in split compartment cells (total path length 1 cm), in 0·02 M sodium acetate buffer *p*H 5·2, 25° C. All concentrations $0{\cdot}5 \times 10^{-4}$ M. – – –, C3′P; ———, ATP; ·–·–·, deoxythymidine 3′-phosphate benzyl ester; ·····, pyrophosphate

of the compounds and have demonstrated the formation of 1 : 1 complexes for ATP and C3′P. For C2′P the stepwise addition of two molecules of nucleotide has been observed by spectropolarimetry. No interactions were detected by either method between C2′P and 1-carboxymethyl histidine-119 RNase, 3-carboxymethyl histidine-12 RNase or 2 : 4-dinitrophenyl lysine-41 RNase. All these proteins had essentially the same optical rotatory dispersion spectra.

Dissociation constants of the enzyme ligand complexes have been calculated by measurements of their ability to protect the enzyme against alkylation by iodoacetate, and by determination of inhibition constants from the steady-state kinetics of hydrolysis of cytidine 2′ : 3′ phosphate. Some of the results are shown in Table 2. Spectrophotometric determinations of the binding constants of a commercial sample of ribonuclease with C3′P as a function of *p*H have been reported[10]. A maximum was observed at *p*H 5·5. We have found a similar maximum in our optical rotatory dispersion studies. At this *p*H there is also a minimum in the Michaelis constant for C2′ : 3′P. Unfortunately interpretations of mechanism from these data are impossible, in the absence of additional information, because the measured constants reflect the *pK* values of the free enzyme, free ligand and enzyme–ligand complexes. The decrease in binding which occurs on the acid side of *p*H 5·5 could well be associated with protonation of the cytosine ring, and in no way contravenes the mechanism that we have previously outlined[3].

Table 2 DISSOCIATION CONSTANTS OF ENZYME–INHIBITOR COMPLEXES. SODIUM ACETATE BUFFER *p*H 5·2, I = 0·02, TEMPERATURE 25° C

	$K_I \times 10^6$ (Ml.$^{-1}$)	
	Hydrolysis of cytidine 2′ : 3′ phosphate	Alkylation reaction
Cytidine 3′ phosphate	16	4·8
ATP	0·3	—
2′-Deoxythymidine-3′-phosphate benzyl ester	1·0	6·0
Pyrophosphate	7·6	4·9

Table 3. DISSOCIATION CONSTANTS (ref. 11) OF GROUPS AT THE ACTIVE SITE OF RIBONUCLEASE 25° C, I = 0·20

Free enzyme		Enzyme–substrate complex	
pK$_b$	5·22 ± 0·20	*pK*$_b$′	6·30 ± 0·09
pK$_a$	6·78 ± 0·20	*pK*$_a$′	8·10 ± 0·09

We wish to propose an extension of this model of the enzyme–substrate complex which encompasses the data presented in this article. The *pK* values for the functional groups at the active site are shown in Table 3. On interaction with the substrate C2′ : 3′P both *pK* values increase. It is to be expected that the group required in the acid form will have its *pK* raised if it interacts directly with the substrate. Similarly, the group required in the base form should have its *pK* lowered if it binds directly with the substrate. In fact, its *pK* is also shifted to higher values. It was suggested that this group functions by forming a hydrogen bond with the attacking water (or alcohol) molecule as shown in Fig. 5.

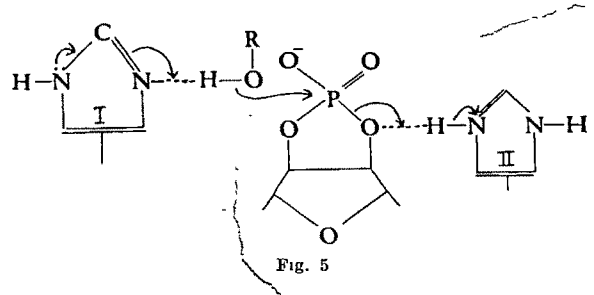


Fig. 5

This mechanism readily accounts for the raised *pK* of group (II) on formation of the enzyme substrate complex. In order to explain a similar shift in the *pK* of (I), it is necessary to postulate an acid-stabilizing inter-

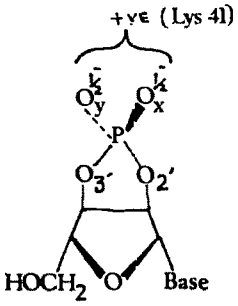


Fig. 6

action between this group and the substrate. If this interaction involves the pyrimidine ring it would give rise to shifts in the absorption spectrum of the type observed. To understand this problem in detail it is necessary to know the direction of approach of the attacking nucleophile to the phosphorus atom. The geometry around the phosphorus atom is shown in Fig. 6. Here the negative charges on the oxygen atoms *x* and *y* are shown as interacting with a positive charge in the protein, and this may well be the amino side chain of lysine 41. There are four possible directions along which a water molecule could approach. These may be defined by the trio of oxygen atoms surrounding the surface region of the phosphorus atom which makes contact with the attacking nucleophile. These four regions are defined by the following oxygen trios:

(1) O_2', O_3', O_x
(2) O_3', O_x, O_y
(3) O_2', O_x, O_y
(4) O_2', O_3', O_y

Classical nucleophilic displacement would involve (2). However, there is a considerable density of negative charge impeding attack in this region. If interaction occurs with lysine 41, access along this direction would be hampered by steric hindrance. The same objections apply to region 3. The approach to region 4 is prevented by the hydrogen atoms on $C_{2'}$ and $C_{3'}$. Therefore we suggest that the attacking water molecule approaches region 1. If a model is constructed from Cortauld space-filling atoms an interesting feature becomes obvious. The pyrimidine ring can be so positioned that its oxygen interacts, through a water bridge, with the hydrogen atom attached to imidazole (I), as illustrated in Fig. 7.

The geometry is difficult to illustrate in two dimensions, but the model shows that, in the active configuration, the imidazole and pyrimidine rings are co-planar and approximately at right angles to the plane of the sugar. The

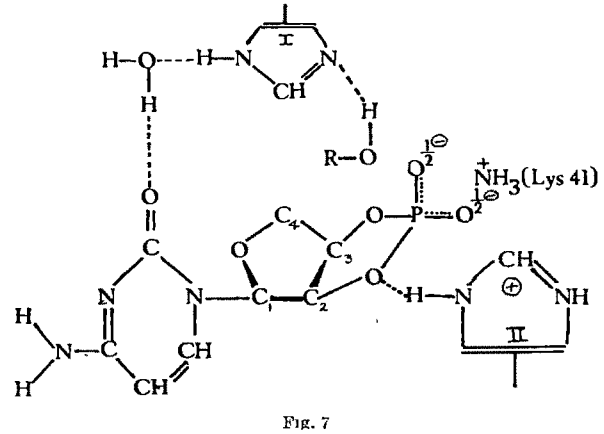


Fig. 7

hydrogen atom on C_2 of imidazole (I) is in Van der Waals contact with the hydrogen atom on $C_{1'}$ of the ribose. An analogous structure is not possible for the cyclic phosphate derivatives of purine nucleotides and this may account for the specificity of the enzyme. The interaction between the pyrimidine oxygen and the imidazole by way of the water bridge would stabilize this group in its acid form and hence raise its *pK*. Thus it would be expected that interaction of the enzyme with a cyclic phosphate substrate would result in the elevation of both *pK*s at the active site, as has been found experimentally. A further consequence of the interaction would be a perturbation of the pyrimidine spectrum similar to that found for enzyme–inhibitor complexes.

Alkylation of imidazole (I) in Fig. 7 should cause complete loss of activity. This suggests that this group is histidine 119. We propose that histidine 12, in the acid form, interacts with the 2′ oxygen of the substrate. Alkylation of this histidine would not necessarily cause complete loss of activity since the alkylated imidazole could still function as an acid catalyst[12]. The optical rotatory dispersion studies reported in this article indicate that conformational changes on binding require the presence of an oxygen atom on $C_{2'}$ of the sugar. It is possible that these conformational changes arise during the formation of a hydrogen bond between $O_{2'}$ and histidine 12.

We thank the Science Research Council for a grant for the purchase of the spectropolarimeter and a personal grant to one of us (A. D.). We also thank the Wellcome Trust for a grant for the purchase of a Cary model 14 spectrophotometer and Miss Angela Hardy for skilled technical assistance. We are grateful to Prof. Lord Todd for samples of deoxyribonucleotides.

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SWARMING OF *Proteus* — A SOLUTION TO AN OLD PROBLEM?

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THE swarming of *Proteus* has intrigued bacteriologists ever since Hauser first isolated and described this organism in 1885 (ref. 1). Anyone who has attempted to isolate pathogens from faeces must have known the irritation of finding a thin veil of *Proteus* covering the whole surface of his plate culture including the colonies of possible pathogens which he had hoped to isolate and identify.

This phenomenon of swarming raises a host of questions to which a clear answer has not yet been given. Why do many other highly motile bacteria fail to swarm over the surface of plate cultures? Why does a period of stationary growth (in which transference to liquid media indicates the presence of motile cells) always precede the phase of swarming[2–4]? Why do many chemically unrelated compounds inhibit swarming[5]? Why does the absence of sodium chloride and certain sugars from the medium prevent swarming[5]? Why does the production of a negative chemostatic substance[4] make certain zones of a plate culture "forbidden zones" to the swarming cells? Why is swarming associated with the production of giant cells up to twenty times longer than the non-swarming, but still motile, *Proteus* cells[2,3,6,7]?

The work described here has a limited purpose, namely, to see whether the behaviour of the individual cells during swarming could be related to the fact that *Proteus* cells can swarm, whereas other equally motile and similarly peritrichously flagellate cells do not normally do so. The technique used was simple if tedious.

Meat extract agar plates were inoculated at the centre of the plate with a strain of *Proteus mirabilis* and incubated at 37° C or at room temperature. After 3–4 h, in the case of plates incubated at 37° C, and a longer period for plates kept at the lower temperature, the cultures were placed on a microscope stage and the surface growth observed with the 4-mm objective and a 10 × eyepiece using bright field illumination. Sometimes continuous observation was made for several hours with the plate resting on a heated stage at 37° C. Sometimes the surface growth was examined at regular intervals with the plate returned to the 37° C incubator between observations. With plates incubated at a higher temperature, microscopic observation was frequently continued until swarming had covered the whole surface of the plate. With plates incubated at lower temperatures the need for an interval for sleep prevented continuous observation.

Over a period of time a more or less clear understanding of the sequence of events from the first multiplication of the cells of the inoculum to the arrival of swarming cells at the edge of the plate was established. The conclusions of a series of observations can be summarized as follows:

(1) When the cells of the inoculum begin to multiply without swarming the development of a normal type of colony occurs and the cells produced are motile and a few microns in length

(2) Somewhere between 3 and 4 h after inoculation and subsequent incubation at 37° C, cell division diminishes and longer cells become visible.

(3) Motion of these long cells without emergence from the edge of the colony becomes apparent and they extend in length.

(4) Eventually, cells of between 20μ and 80μ in length protrude from the edge of the colony and move towards the edge of the plate, losing contact with the main mass of short bacteria. Cells often move outwards in the form of large rafts.

(5) The process intensifies and within 30 min cells can be seen journeying outwards, with some (but only a minority) circling in their tracks and returning towards the central mass of growth.

(6) This outward motion of long cells occurs in groups of cells and not by single-cell movement.

(7) The group may appear to move as one unit or may obviously consist of rapid movement of one or more cells along other cells forming part of the group.

(8) Sometimes a cell is left behind by the group; when it has parted company with the other cells in the group, movement only continues for a short space of time.

(9) Sometimes a moving group comes into contact with an immobilized cell and moves alongside the single cell, which is then reactivated into movement.

(10) Eventually, large groups of cells showing outward movement tend to break down into smaller groups, each

showing intermittent movement until finally two cells part company and each component cell ceases to move.

(11) In the early stages the main movement is outward until an annulus of thin growth of about 2-cm diameter is formed which surrounds the central and initially stationary, colonial, type of growth. Towards the end of the first swarming stage the moving cells tend to curve back towards their starting point and form eventually a continuous entanglement of long cells with no further swarming movement at the periphery.

(12) The long cells break up into normal-sized *Proteus* cells 2–4μ in length, which undergo multiplication, giving a mass of stationary growth consisting of short cells in which some long cells which have failed to sub-divide remain entangled.

(13) After 0·5–1 h, the whole sequence of events repeats itself until at the end of 8–10 h incubation at 37° C we find ourselves with a plate culture covered with cells arranged in concentric rings of thin and thick growth.

It would be tedious and space-consuming to illustrate photographically the whole of the foregoing series of events and, in any case, only part of the description is relevant to the problem of why *Proteus* cells swarm and other motile bacteria do not. Fig. 1 contains a sequence of photographs taken at about 4-sec intervals. In these can be seen a number of the foregoing features, namely, the movement of a raft of cells in which the position of the component cells relative to each other differs from one photograph to the next; the reversal of the direction of movement of a group of cells, the movement of cells along the surface of other cells, and the cessation of movement when cells become isolated.

Bright-field illumination was chosen as a matter of convenience though phase-contrast observation was also used. The use of bright-field illumination with the condenser adjusted to a suitable distance from the stage and with the brightness adjusted to give the maximum of contrast between cells and medium with the minimum of eye-strain, and with the medium interposed between the light and the camera, made the achievement of aesthetically satisfying photographs difficult. Fig. 1 does, however, show the manner in which the cells move even if the

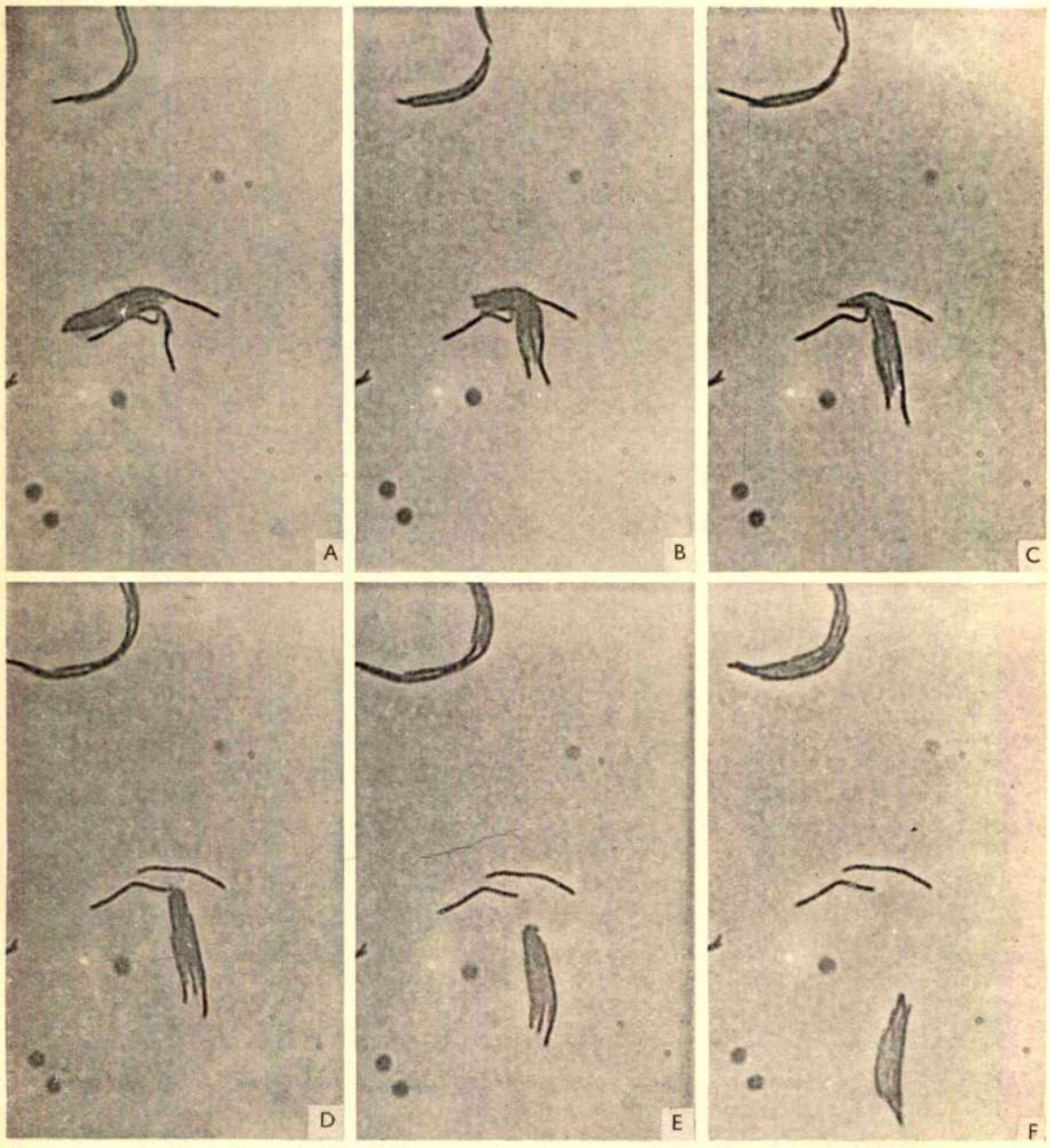


Fig. 1. A sequence of photomicrographs, lettered *A-F*, of swarming cells of *Proteus mirabilis* taken at intervals of about 4 sec. (× *c.* 350)

background is full of blemishes from the photographer's point of view.

To summarize the conclusions drawn from the observations, it might be stated that the swarming of *Proteus* is a consequence of the movement of long cells and that this movement is only continuous when one cell is in contact with another. The long, swarmer, cells of *Proteus* move (and sometimes with startling rapidity) by climbing or sliding along each other. When contact between cells is broken, movement soon stops. When contact is re-established, a motionless cell may once more begin to move. The cells appear to slide along each other and usually the group movement is towards the periphery of the plate, but sometimes retrogression of the group towards the stationary growth occurs. Often when a raft of cells appears to move as a whole, careful study shows that the movement is a kind of shuffling or shuttling of the individual cells upon each other.

One might frame the hypothesis that the flagella of these very long, heavily flagellate, cells are able to obtain a thrust on neighbouring cells which gives them sufficient impetus to propel themselves over the surface of a solid medium. To use an analogy, when the cell is isolated it is like a beached galley with the oars beating uselessly on air. When it is in contact with other cells it can be likened to a galley with scores of oars and oarsmen which, even on dry land, can move forward by leverage on an adjacent galley if the friction between galley and ground should differ in each case. The small, less heavily flagellate cells[7] are like boats with a few oarsmen who have not the force at their disposal to overcome the resistance to movement.

One must not, of course, mistake an analogy for an explanation and much remains to be explained. What is the role of negative chemotactic substance[4], what is the role of electrolytes and sugars[5], and why do all sorts of chemically unrelated compounds inhibit swarming[5]? Why do the cells not climb on top of each other? How is the movement of the large number of flagella on a swarm cell co-ordinated? What is the signal which sometimes leads to a cell reversing direction like a shuttle which has reached the limit of its travel? This long-observed phenomenon still presents many questions intriguing to the curious mind. The work described here makes no attempt to answer them and is, in fact, a description of the behaviour of swarming cells which must have been observed many times in the past even if it has not been described in these terms. It is interesting to note in this connexion that the excellent photographs of swarming cells presented by Hoeniger[6] show a number, of features illustrated in Fig. 1; she did not, however interpret them in the foregoing way.

It is perhaps of some consolation to the older bacteriologist that the application of simple, classical, techniques with the long-used tools of the bacteriologist's trade can still give rewarding information about a very old and intriguing problem.

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INTERACTION OF BUBBLES AND BACTERIA IN THE FORMATION OF ORGANIC AGGREGATES IN SEA-WATER

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MEASUREMENTS of particulate organic matter in the ocean have shown that below the top 200 m the amount of particulate material does not consistently decrease with increasing depth[1–5]. This conflicts with the observation that organic particles sink and are consumed by organisms as they do so[4]. The contradiction may be resolved by supposing that much of the particulate material in sea-water is formed by the aggregation of dissolved organic carbon[6–8] in a physical process by which organic molecules come out of solution to form an organic skin around a rising bubble. At the surface of the sea the bubble is ejected into the air, leaving behind its organic skin as a collapsed monomolecular film which then may sink through the water column, providing a seed on to which additional dissolved material may aggregate.

This physical process, promoting exchange between the dissolved and particulate organic pools of carbon in the ocean, has been used by ecologists to explain the common gap in the amount of food required by filter feeders and the amount of particulate food available to them in the form of phytoplankton cells and debris[9–13]. From this ecological point of view, it is important to be sure that the aggregation of dissolved material is a simple physical process and not the result of a complex interaction involving bacteria. The experiments described in this report were therefore designed to determine whether organic molecules will aggregate into discrete particles under conditions of complete sterility.

Carbon-14 incorporated into organic molecules was used to follow the distribution of the organic material. The labelled molecules were prepared by growing the diatom *Nitzschia closterium* in the presence of carbon-14 sodium bicarbonate. The labelled diatoms were concentrated and washed to remove inorganic carbon-14 and then ruptured by sonication. The particulate debris was spun down and discarded, and the supernatant containing the soluble cell material was passed through a 'Bio-Gel *P*-100' polyacrylamide gel column. This molecular gel, which retards molecules with a molecular weight usually less than 100,000, was hydrated and eluted with fresh '*HA* Millipore'-filtered sea-water. The retarded fraction was collected and added to fresh '*HA* Millipore'-filtered sea-water to make several litres of solution with about 120 c.p.m./ml. carbon-14 activity after correction for background and self-absorption. The sea-water used in these experiments was bucket-dipped as needed from Monterey Bay (California) at the site of the Hopkins Marine Station sea-water intake.

The foam tower was a modification of that described by Baylor and Sutcliffe[8]. Air bubbled through the solution was thoroughly heated to ensure combustion of any organic contaminants and was then cooled. Sterile barriers made it possible to draw the solution through a 'Millipore' filter into a 2-l. 'Plexiglas' foam tower, to bubble the solution, and to draw the solution out of the foam tower through a 'Millipore' filter without introducing contamination. All the components of this system were autoclaved before use and assembled aseptically. During the experiments, sterility was assayed by adding 1 ml. inocula from the foam tower to 'Difco *AC*' medium rehydrated with autoclaved sea-water.

The experimental assay is based on the assumption that if the labelled organic material in the bubbled sea-water forms aggregates, they will be retained on a 'Millipore'

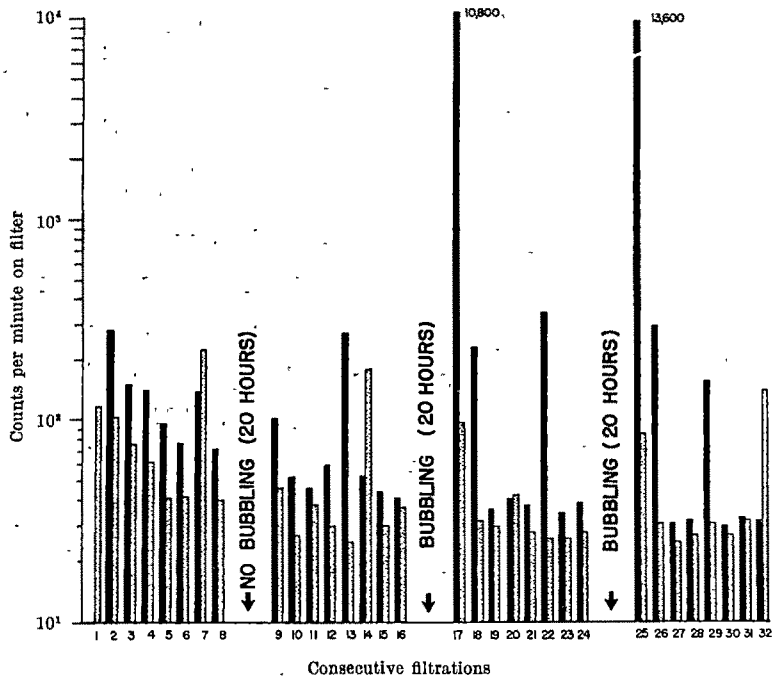


Fig. 1. The effect of bubbling a sea-water solution containing labelled organic molecules of molecular weight less than 100,000. Each bar represents the c.p.m. on a particular 'Millipore' filter through which the entire 1 l of solution has been passed. The solid bars show the results of experiment *A*, using '*HA* Millipore' (0·45-μ) filters; the stippled bars show the results of an identical experiment, experiment *B*, done with '*GS* Millipore' (0·22-μ) filters

filter when the solution is filtered. The amount of carbon-14 activity on the filter is assumed to reflect the amount of material aggregated. The 'Millipore' filters were mounted on planchets and counted by a Nuclear Chicago gas-flow counter, model *D*-47. Preliminary experiments showed that when a solution of labelled organic molecules is repeatedly filtered, the amount of organic material retained on the filters occasionally varies rather markedly. To establish the average amount of material retained on the filter, the solution was filtered eight times before and after all experimental and control treatments.

The data from two experiments using this procedure are shown in Fig. 1. The abscissa shows the sequence in which the solution was filtered and bubbled; the ordinate is a three-cycle log scale of counts per min (c.p.m.) on the filter. Each bar represents the c.p.m. on a particular 'Millipore' filter through which the entire solution passed. The solid bars show the results of experiment *A*, using '*HA* Millipore' (0·45-μ) filters; the stippled bars show the results of experiment *B*, identical with *A*, except that it was carried out with '*GS* Millipore' (0·22-μ) filters.

In experiment *A*, c.p.m. on the filter increased greatly following bubbling. After both the 20-h bubbling periods c.p.m. increased more than 200-fold, indicating that a significant amount of organic material came out of solution in the form of particles large enough to be retained on the filter. These data are shown in Fig. 1 by the solid bars of filtrations Nos. 17 and 25. However, the sterility assay showed that bacteria were present throughout. Anderson and Heffernan's[14] isolation of several marine microbes that readily pass through 0·45-μ filters suggested that it would be necessary to use a 0·22-μ filter to remove all the micro-organisms from the sea-water.

In experiment *B*, 0·22-μ rather than 0·45-μ filters were used for all thirty-two filtrations; the 20-h bubbling periods did not cause a significant increase in c.p.m., and the sterility assay showed that the sea-water solution was free from bacteria throughout the experiment. After the control treatment in experiment *A* there was no increase in c.p.m., indicating that when the filterable bacterium is present but not bubbled there is no increase in the amount of organic material retained on the filter. When an inoculum of the microbe isolated from experiment *A* was added to a sterile solution, a solution that had produced no aggregates, the c.p.m. on the filter increased after bubbling. These data suggest that neither bacteria alone nor bubbling alone will cause a significant increase in the amount of particulate material produced in this system.

When the 'Millipore' filters were cleared with immersion oil and examined under a phase microscope, the filters with increased c.p.m., that is, those from the filtrations following bubbling, were found to contain large amounts of detrital material. Much of the material on these filters appears to be identical to the organic aggregates described by Riley[9] and Wangersky[13]. Some are plate-like with definite, markedly refringent edges; others have very fuzzy edges and many small refringent inclusions. The relatively amorphous appearance of the aggregates on the filter and their similarity to the detrital material found in the sea indicate that the increased amount of organic material on the filters after bubbling does not represent merely an increase in the bacterial biomass.

Sieburth[16] found that without bubbling, natural bacterial populations formed aggregates when proteinaceous substrates were decomposed producing ammonia. He suggested that the microzonal alkalinization that must occur during ammonia liberation could initiate the precipitation of inorganic nuclei around which organic aggregates could form. Such a mechanism could be operating in the experiments described here.

Carlucci and Williams[15] showed that bubbling concentrates the bacteria in sea-water in the surface film and froth. In our experiments, such partitioning of the bacterial flora would not have been detected because the entire solution, water and foam, was filtered in all thirty-two filtrations. The increased amount of labelled organic material retained on the filter after bubbling represents a net increase in particulate matter in the complete system.

While more work is needed to clarify the interaction of bubbles and bacteria in the formation of aggregates from dissolved organic matter, it can be concluded from the experiments reported here that bubbling will not aggregate organic molecules of molecular weight less than 100,000 into discrete particles under sterile conditions.

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ORGANOCHLORINE PESTICIDES IN THE ATMOSPHERE

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THE problems introduced by the pollution of our atmospheric environment have been concerned largely with those pollutants the effects and presence of which are readily demonstrated, for example, smog, sulphur dioxide, sooty materials and dusts. More recently, attention has been directed to the polynuclear hydrocarbons[1–3], although their presence in air is by no means solely of modern origin.

Recent publications have given evidence for the presence of some organochlorine pesticides in rainwater[4,5]. It has been suggested[5] that the minute traces found arose from the 'scrubbing' action of the falling rain on the air through which it passed, in a manner analogous to the collection of the inorganic constituents of rainwater[6]. The present work describes the experiments which have been carried out to ascertain the extent to which these organochlorine compounds are present in the atmosphere.

Pesticidal compounds may enter the atmospheric environment in several ways and in various physical states. Direct drift from spraying operations will contribute particulate or globular matter at concentrations which are likely to vary inversely as the distance from the site of application, such effects being mainly local but possibly appreciable in extent. It has been shown[7,8] that several organochlorine insecticides volatilize from treated soils, thus adding a slow, long-term contribution. Effluents and vapours from industrial processes, such as pesticide manufacture or moth-proofing of garments, may also contribute, while further quantities may accrue from the use of domestic aerosol insecticides and thermal vaporizers, and the dust from treated clothing and carpets.

Wheatley and Hardman[4] examined rainwater for traces of pesticides in order to try to account for residues found in soil from untreated plots of land. The amounts which they found were insufficient to account for more than a very small proportion of the pesticides present in such soil. Although the concentration of these compounds in air is lower by a factor in the range of 10–100 than that in rainwater, the vastly greater volumes of air may render this contribution of greater significance. As well as the normal, constantly changing contact between air and soil, the mass of air in surface soil may alter by up to 5 per cent owing to diurnal variations in temperature. Pesticides may thus be absorbed by the soil acting as a gas-solid chromatographic 'column' as well as by direct surface adsorption mechanisms. Assuming a tendency to equilibrium, treated soils will lose pesticides while untreated plots will gain them.

Preliminary experiments to illustrate the presence of pesticides in air were carried out by using a small portable constant-rate air-sampler which was used to suck air through a hexane trap. Although this method gave a total sample of only about 180 l. of air per power-canister, gas-liquid chromatography showed the presence of traces of BHC, DDT, DDE, TDE and dieldrin in samples taken in central London and its suburbs. Dieldrin alone was observed in a sample taken in Norfolk, while Aberystwyth air gave no detectable amounts of pesticide at this sample size. It was clear from these preliminary observations that an analytical problem existed and that larger samples would be required before reliable data could be obtained. Accordingly, an electrically operated vacuum pump capable of drawing 15 l. of air per min for long periods of time was brought into use. At this rate of flow hexane was too volatile, but dimethylformamide proved to be a suitable, non-volatile extractant. Examination of the separate contents of a sequential train of four solvent traps showed that nearly all the pesticide content was retained in the first vessel. Attempts have also been made to ascertain whether the pesticides present were in the vapour state or in association with the particulate matter of the air. Interposing bacteriological membrane filters (pore size 0·22 μ) between the air inlet and the solvent trap showed that variable proportions of the pesticides were retained on the filter. The amount retained, however, tended to reach an equilibrium value, indicating that the compounds were volatilizing again from the filter into the air stream. Similar effects have been observed with some polynuclear hydrocarbons[9]. Thus, although the use of membrane filters offers a simple qualitative test for pesticides in air, quantitative results can only be obtained by solvent scrubbing procedures.

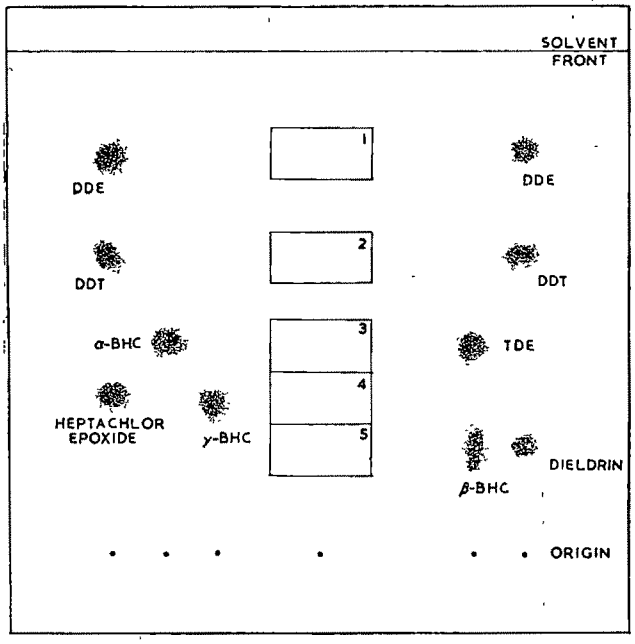


Fig. 1. Thin-layer chromatoplate

Metropolitan air was sampled by suction at about 15 l./min through 100 ml. of dimethylformamide contained in a Drechsel bottle fitted with a coarse sinter at the inlet to the solvent to ensure adequate diffusion. Dispersion of the solvent in 500 ml. of 5 per cent sodium sulphate solution followed by hexane extraction yielded a clean solution which, after evaporation to small bulk, was applied to a 250 μ silica-gel chromatoplate. This was developed with hexane : acetone, 99 + 1, for 35 min and appropriate fractions eluted with hexane[10] for examination by gas-liquid chromatography on both silicone and 'Apiezon' columns with electron-capture detection[11].

Fig. 1 shows the positions on the thin-layer plate of the more important organochlorine pesticides and indicates the areas containing the residues which were re-

moved for examination. Figs. 2–4 illustrate gas-chromatograph traces obtained from some of these areas; peaks corresponding to known pesticidal materials are marked—the other peaks have not been positively identified as yet. The appearance of a peak at the appropriate retention time on two chromatographic columns of differing polarity on injecting the eluate from the chromatoplate area of correct R_F value for the compound concerned has been taken as evidence of the identity of the compounds. Table 1 lists the quantities of pesticides found on examination of 8,000 l. of air taken on the roof of Cornwall House in August, 1965. The presence of alpha-BHC, gamma-BHC, dieldrin (HEOD), *pp'*-DDE, *pp'*-TDE and *pp'*-DDT was confirmed as already described; substantial agreement between quantitative results on both columns confirmed the absence of any appreciable amount of interfering non-pesticidal material of similar retention time.

Table 1. ORGANOCHLORINE PESTICIDES FOUND IN LONDON AIR

Compound	R_F range of chromatoplate area	Concentration found (parts per 10^{12} w/w) by gas-liquid chromatography on (a) Silicone *E*.301	(b) 'Apiezon *L*'
Alpha-BHC	0·37–0·48	1	1
Gamma-BHC	0 27–0·36	5	11
Dieldrin (HEOD)	0 17–0 26	18	21
pp'-DDE	0·75–0 84	4	7
pp'-DDT	0·54–0 64	3	3
pp'-TDE	0·37–0 48	3	8

Silica-gel chromatoplate developed with hexane : acetone, 99 + 1.
Silicone column: 1·2 per cent silicone *E*.301 plus 0·12 per cent 'Epikote 1001' on 'Silanized Chromosorb *G*' (70–80 mesh) at 158° C; nitrogen carrier gas, 100 ml./min.
'Apiezon' column: 3 per cent 'Apiezon *L*' plus 0·3 per cent 'Epikote 1001' on 'Celite 545' (100–120 mesh) at 186° C; nitrogen carrier gas, 100 ml./min.

Great care must be taken in the interpretation of the gas-liquid chromatograms obtained from air samples. Even after the thin-layer separation process used, each fraction from the plate contained several peaks of unidentified materials showing electron-capturing properties. Some of the short-retention time peaks (Fig. 3) corresponding to section 2 of the chromatoplate (R_F 0·54–0·66) have been tentatively identified as the polynuclear hydrocarbons acenaphthylene (R_F 0·57), anthracene (0·67), fluoranthene (0·57) and fluorene (0·70). Retention times on both columns confirmed these identities; these substances are known to be among the polynuclear hydrocarbon ingredients of atmospheric dusts[2,12].

The three peaks of longest retention times obtained from section 1 of the chromatoplate (Fig. 2) would seem to result from compounds the behaviour of which on the plate and on each column is identical with that shown by the compounds giving the first three of a series of unidentified peaks often found in chromatograms of extracts from

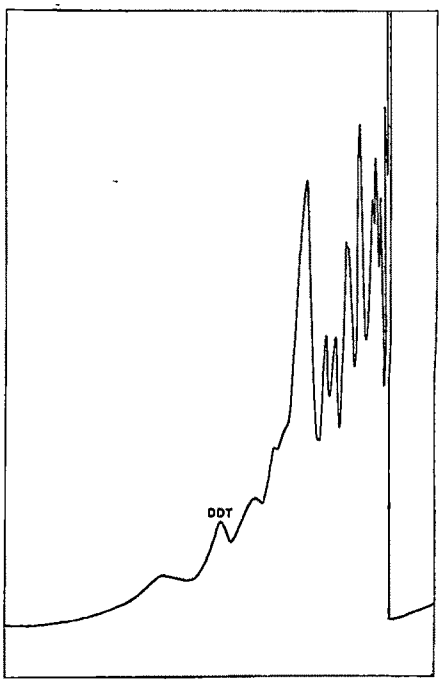


Fig. 3. Gas-liquid chromatogram from section 2 of the thin-layer chromatoplate

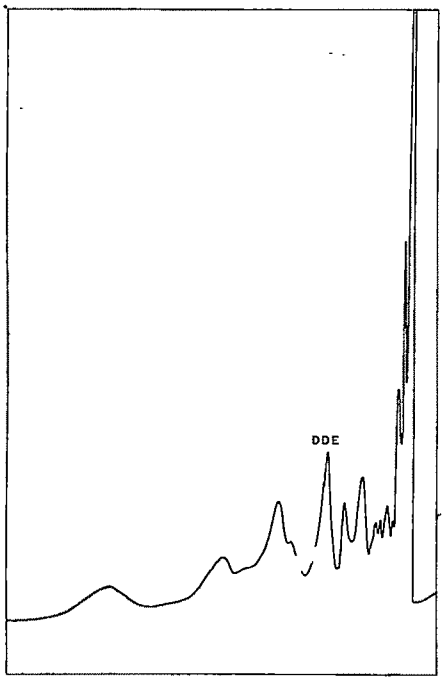


Fig. 2. Gas-liquid chromatogram from section 1 of the thin-layer chromatoplate

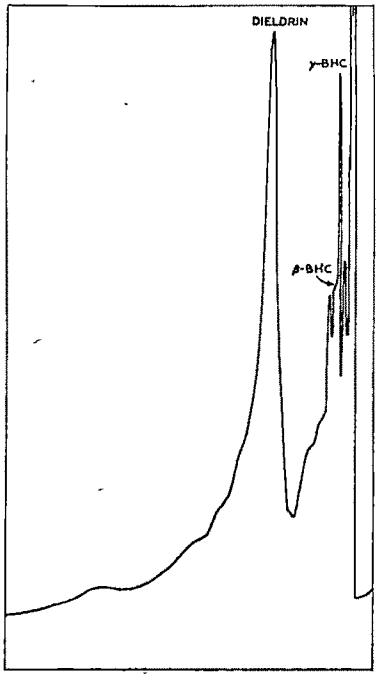


Fig. 4. Gas-liquid chromatogram from section 5 of the thin-layer chromatoplate

the tissue and eggs of some predatory birds. Their concentrations relative to each other in these two sample sources are also closely similar. Total chlorine determinations on avian tissue samples have indicated that these compounds contain chlorine, and because they are only found in samples containing appreciable residues they are probably breakdown products of organochlorine pesticides.

The evidence presented here and from rainwater examination[4,5] indicates the general occurrence in the atmosphere of minute traces of some of the persistent organochlorine pesticides. Such compounds have also been found in samples of air taken during the spraying season in American peach-orchard districts[13]. The concentrations of these residues are so low that it is necessary to work almost at the limit of sensitivity of the detection systems at present in use. For this reason, scrupulous care is necessary in order to avoid contamination at each manipulative stage, from the initial apparatus preparation to the final injection on to the gas chromatograph. Blank experiments must be performed at each stage and on all materials used. Particular care is required to exclude laboratory dust, for it is found that it is frequently a carrier of interfering electron-capturing materials.

Although the levels concerned are extremely low, these indications of the presence in air of organochlorine pesticides call for further and more detailed investigation. Confirmation of the identity of these residues by other methods such as infra-red spectroscopy or mass spectrometry would also be desirable, although far larger samples would be required in order to obtain sufficient material in a suitably pure state. It would also be of interest to compare the levels found in London with those found in other parts of the country; seasonal variation and correlation with other indices of atmospheric pollution would also be worth investigation. In these ways it may be possible to obtain some indication as to whether the pesticides derive from within the shores of the British Isles or from further afield.

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HORMONES OF THE VERTEBRATE HOST CONTROLLING OVARIAN REGRESSION AND COPULATION OF THE RABBIT FLEA

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In Nature, the rabbit flea (*Spilopsyllus cuniculi* Dale) only undergoes maturation on the healthy host during the last 10 days of the doe's pregnancy, or on nestlings 1–7 days old. Soon after the young rabbits are born, the fleas transfer to the nestlings, on which they feed avidly and pair: egg-laying on the body of the young and in the nest follows[1,2]. Between 8 and 22 (average 11) days later, the fleas abandon the young rabbits and return to the lactating doe. Any gravid fleas which remain on the doe after parturition, or are still gravid when they return to the lactating doe, regress rapidly and the developing oocytes are resorbed[1]. If, at a later date, the doe has a second pregnancy, these fleas are capable of undergoing maturation for a second time[3].

It was shown previously[3] that one of the principal groups of hormones governing maturation was the corticosteroids under the control of adrenocorticotrophic hormone (ACTH), while the oestrogens (and, to a lesser degree, thyroxine) played an important contributory part in development and egg-laying. It is probable that prolactin is a subsidiary factor in maintaining and promoting maturation on the nestling rabbits. The experiments described here indicate that luteinizing hormone (LH) and the progestins are two of the principal factors governing ovarian regression and that somatotropin (growth hormone), also secreted by the anterior lobe of the pituitary gland, constitutes one of the major "copulation factors".

Earlier, it was claimed[3–5] that not only fleas feeding on buck rabbits injected with cortisone, corticosterone or hydrocortisone, but also fleas sprayed externally with these hormones, matured their eggs and displayed the various characteristics associated with maturation. It was thus shown that the hormones acted directly on the flea and not indirectly by way of the host. During the early stages of the investigation, it was thought that the rise and fall of the corticosteroid-levels in the blood of the host, and, to a lesser degree, in the levels of oestrogens and thyroxine during pregnancy, were sufficient to account for both maturation and regression. Thus the increased defaecation rate, which indicated an increased flow of blood through the body of the flea in response to the rise in hormone-level, automatically ensured that larger amounts of both hormone and food were available to the parasite. Conversely, a fall in the hormone levels cut down the defaecation rate (Table 2) and the amount of blood passing through the body of the flea, thus further reducing the amount of available hormones and nutrient material. Reduction in food intake alone does not account for the rapid regression of the ovaries of fleas feeding on the postpartum or oestrous doe; gravid fleas removed from the host and completely starved do not

Table 1. Movement of Fleas between Doe and Nestlings

Total No. of litters observed	66
Successful litters in which at least some young survived 10 days or longer	51
Unsuccessful litters: (*a*) all dead	15
(*b*) some dead (16 of these litters born during winter months)	18
No. of litters in which some or all fleas transferred from postpartum doe to nest or nestlings (9 litters in which fleas were removed by hand are not included)	38
No. of litters in which no fleas transferred (14 were winter litters)	19
No. of litters in which all fleas transferred to nestlings or nest	
Mean No. of days in nest before returning to doe (range 8–22)	11
Average time (approx) for transfer from doe to young after parturition (range 10 min–26 h)	7–8 h
Day on which fleas of F^1 generation first emerged from pupa: Range 21–56 days Mean 30 days	

regress nearly as rapidly as they do when feeding on the non-pregnant host, and no resorption or degeneration is observed for 7 days after they have ceased feeding altogether[1].

Experiments were performed (Table 3) to test the possibility that other hormones, among them luteinizing hormone and progestins, are involved in the phenomenon of ovarian regression. Buck rabbits (New Zealand Whites), infested with fleas (bred in an artificial warren or collected from wild rabbits on the myxomatosis-free island of Herm, Channel Islands), were injected with 0·32–0·75 mg of hydrocortisone ('Hydrocortisyl', Roussel, London, *B.*6572/9G8T) until maturation of the female fleas was clearly visible externally and the defaecation

Table 2. DEFAECATION RATE OF FLEAS ON NORMAL AND INJECTED RABBITS

	Fleas feeding on oestrous does		Fleas feeding on pregnant does 3 days prepartum	Fleas feeding on pregnant does one day prepartum		Fleas maturing on does injected with cortisol	Fleas regressing after the does were injected with cortisol and progestin
Time between defaecations of fleas (min)	29 67 ± 12·24		6 28 ± 3·09	3·8 ± 1·375		6 42 ± 1·78	24·42 ± 7·82
No. of longitudinal sections (cut at 8μ) per flea	♂ 35–53	♀ 38–59 No yolk in oocytes	Not known	♂ 51–65	♀ 88–104 Ovaries with yolk in oocytes	♀ 78·34 ± 8 85 Yolk present in 23 out of 25 specimens examined	♀ 56·64 ± 8·41 Yolk absent in 24 out of 25 specimens examined

Table 3. OVARIAN DEVELOPMENT AND DEFAECATION RATE OF FLEAS UNDER THE INFLUENCE OF MAMMALIAN HORMONES (SELECTED EXPERIMENTS)

Injection into the rabbit (mg daily)	Spray on to the fleas	Duration of the experiment (days)	Condition of fleas' ovaries	Defaecation rate of fleas (No. per min)
(Doe and Buck Rabbits) (Controls)				
Before commencement of experiment	Nil		Not matured	Mean 29·67 ± 12 24
First period: hydrocortisone, 0 25–0·75	Nil	4, 5; 7; 9; 10; 12, 14, 18; 24	Mostly matured All matured	1·3–10 min (approx.) Mean 6·42 ± 1·78
(Doe Rabbit) (Control)				
First period: hydrocortisone, 0 30–0·50	Nil	6	Matured	1·12–16
Second period: hydrocortisone, 0·30–0·50	Sprayed with inert spray (excipient only) at 5-day intervals	6	Matured	1·9–16
Third period: hydrocortisone, 0 30–0·50		4	Matured	1·8–9
Buck Rabbit				
First period: hydrocortisone, 0 32–0 75	Nil	7	Matured	1:6–8, last day before progesterone 1.7–9
Second period: hydrocortisone, 0 32–0·50 plus 20 mg progesterone daily	Nil	7	Regressing	1:20–40
Third period: hydrocortisone, 0·32–0·50 plus 20 mg progesterone daily	Nil	6	Regression complete	1:20–40
Fourth period: hydrocortisone, 0 32	Nil	14	Maturing Chorionated eggs present	1:6–8
Buck Rabbit				
First period: hydrocortisone, 0 32	Nil	11	Matured	6th–11th day 1:4–12 Last day before spray 1:5–8
Second period: hydrocortisone, 0·32	Progesterone spray	7	Regressing	1.6–20
Third period: hydrocortisone, 0·32	Progesterone spray: second dose	7	Regressing further	1:6–31
Fourth period: hydrocortisone, 0·32	Nil	10	Redeveloping yolk present in oocytes	1 4–7
Doe Rabbit				
Before commencement of experiment	Nil		Not matured	1:20–29
First period: hydrocortisone, 0·32–0·50	Nil	9	Matured	1:2–6
Second period: hydrocortisone, 0 32	20α-OH (0·8 mg)	14: 4	Regression very marked	1 13–26
Third period: hydrocortisone, 0 32	20α-OH (0·2 mg)	14: 10	Regression very marked	1:16–28
Fourth period: hydrocortisone, 0·32–0 50		12	Matured (yolk present)	1·10–14
Doe Rabbit				
First period: hydrocortisone, 0 75	Nil	15	Matured	1:9–14
Second period: hydrocortisone, 0·75 + 10 mg chlormadinone sprayed on to host's flanks over a period of 20 days	Nil	20	Regression noticeable by 5th day Total regression by 12th day	1:17–21 1.17–26
Third period: hydrocortisone, 0 75	Nil	2 after last spray	Total regression	1:26–29
Fourth period: hydrocortisone, 0 75	Nil	21 after last spray	Matured Chorionated eggs present	1 15–22

Table 3 (*continued*). OVARIAN DEVELOPMENT AND DEFAECATION RATE OF FLEAS UNDER THE INFLUENCE OF MAMMALIAN HORMONES (SELECTED EXPERIMENTS)

Injection into the rabbit (mg daily)	Spray on to the fleas	Duration of the experiment (days)	Condition of fleas' ovaries	Defaecation rate of fleas (No. per min)
Doe Rabbit				
First period: hydrocortisone, 0·30–0·50	Nil	10	Matured	1 . 5–8
Second period: hydrocortisone, 0·30–0·50, plus 2 mg LH (ovine) daily	Nil	10	Regression on 4th day Completely regressed by 9th day	1 : 17–27
Third period: hydrocortisone, 0·30	Nil	3	Fully matured Chorionated eggs present	1 : 23–26
Ovariectomized Doe Rabbit				
Before commencement of experiment	Nil	5	Not matured	1 : 23–50
First period: hydrocortisone, 1·0	Nil	15	Matured	1 : 7–10
Second period: hydrocortisone, 1·0, plus 2 mg LH (bovine) daily	Nil	10	Regression on 6th day Completely regressed 10th day (when sectioned 10th and 11th day)	1 : 16–30 1 : 20–26
Third period: hydrocortisone, 1·0	Nil	15	Maturation	1 : 13–28
Doe Rabbit				
Before commencement of experiment			Not matured	1 · 35–41
First period: hydrocortisone, 0·75	Nil	8	Matured	1 : 6–8
Second period: hydrocortisone, 0·75	Testosterone spray in jar and replaced on doe	14	Egg-laying 6th day	1 . 2–7
Third period: hydrocortisone, 0·75	Testosterone spray	7	Regression complete 6 days later	1 · 16–20
Doe Rabbit				
Before commencement of experiment			Not matured	1 . 27–30
Nil	Sprayed with testosterone twice at 4-day intervals	11	No maturation	1 : 18–37
Buck Rabbit				
First period: hydrocortisone, 0·32	Nil	9	Matured Chorionated eggs present	1 . 7–9
Second period: hydrocortisone, 0·32 daily, plus prolactin (ovine) 5	Nil	5	Matured Chorionated eggs present	1 . 7–9
Buck Rabbit				
First period: hydrocortisone, 0·32	Nil	20	Matured Chorionated eggs present	1 . 6–8
Second period: hydrocortisone, 0·32, plus 2 mg oestradiol daily	Nil	9	Matured Chorionated eggs present	1 · 6–8
Buck Rabbit				
Before commencement of experiment	Nil		Not matured	1 : 18–37
First period: hydrocortisone, 0·32	Nil	12	Matured Chorionated eggs present	1 : 7–9
Second period: hydrocortisone, 0·32 plus 10 units oxytocin daily	Nil	5	Matured Chorionated eggs present	1 . 7–9
Doe Rabbit				
Before commencement of experiment	Nil		Not matured	1 : 18–26
First period: prolactin (ovine) 5·0	Nil	5	Slight development visible Yolk in larger oocytes	1 . 30–38 (decreased rate)
Second period: prolactin (ovine) 5·0	Nil	5	Partial maturation but uneven	1 18–24
Third period: prolactin (ovine) 5·0	Nil	8	Full maturation of some fleas only with chorionated eggs	1 · 10–14

rate had risen (from one pellet passed every 15–30 min) and stabilized at about one pellet passed every 6–8 min. This defaecation rate is characteristic of female fleas at about 3 days before parturition of the host (see Table 2) and before egg-laying commences. Following stabilization of the defaecation rate, 20 mg progesterone ('Progestin', B.D.H., London, 33326/13/2501, 40437/15/2506 and 24717/13/2408) was injected daily into the rabbits for a further period in addition to 0·32–0·50 mg of hydrocortisone. By the seventh day defaecation rates of the fleas had fallen to 1 : 20–40 min, resorption of the largest oocytes was occurring and empty follicles were present in the pedicels of the ovarioles. Little if any yolk could be seen in the developing oocytes. At the end of a further 6 days, resorption of developing oocytes was complete, yolk had been totally resorbed and the ovaries had regressed to a resting stage; the internal organs generally resembled those of spent fleas. The process of regression was then reversed by continuing daily injections of 0·32 mg of hydrocortisone only (without progesterone), for a further 14 days until maturation was again clearly visible externally; yolk was present in the developing oocytes and chorionated eggs were present in the oviducts of a proportion of the fleas sectioned. Regression and resorption could also be obtained not only by injecting the host, but also by spraying matured gravid fleas externally with progesterone or 20α-hydroxy-pregn-4-en-3-one (Roussel metered spray; 100 mg of progesterone dissolved in 30 ml. of fluid carrier; 20 mg of 20α-hydroxy-pregn-4-en-3-one dissolved in 12 ml. of fluid carrier) and then replacing them on doe or buck rabbits receiving 0·32 mg of hydrocortisone daily.

Rabbit fleas mature their ovaries if they are transferred on to castrated buck rabbits which have previously received 0·40 mg of hydrocortisone sprayed (externally) on to the tip of the ear with the Roussel spray[3]. It was possible to induce reduction of defaecation rate and ovarian regression of gravid fleas by feeding them on a female rabbit which had been sprayed externally with (i) 0·20 mg 20α-hydroxy-pregn-4-en-3-one (20α-OH) on the ear, and (ii) 2 mg chlormadinone (6-chloro 6-dehydro 17-acetoxy progesterone) on the flanks for 10 alternative successive days, although daily injections of 0·30–0·75 mg of hydrocortisone were continued throughout the experiments.

Luteinizing hormone was available for four experiments only. In the first experiment 2 mg of LH (*NIH/LH/S/7* ovine) were injected into an ovariectomized New Zealand White doe in three daily doses for a period of 6 days. No changes were observed in the fleas feeding on this rabbit. In the second experiment, fleas were matured on 0·30–0·50 mg of hydrocortisone injected into a buck New Zealand White rabbit, daily, for 20 days. On the tenth day, 2 mg of LH (*NIH/LH/S/6*, ovine, lot 1334) was also injected into the rabbit, in three daily doses, and continued for 10 days. The defaecation rate of the fleas began to fall on the fourth day and regression and resorption were complete by the ninth day. Three days only after the LH injections were stopped the fleas had fully matured and once again had chorionated eggs in their ovaries.

This experiment suggested that LH itself was probably exerting a direct effect on the fleas and not merely stimulating a high secretion of progestins and testosterone in the rabbit. Fleas which have regressed completely, owing to high blood levels (or prolonged effects of smaller levels) of these steroids, require a period of 9–14 days, or longer, in which to return to the chorionated egg stage of maturation.

In the third and fourth experiments a castrated buck and an ovariectomized doe were chosen as hosts, as it is unlikely that injections of LH stimulate the release of steroid hormones in these rabbits. The fleas were matured on daily injections of 0·75–1 mg of hydrocortisone (into the rabbit) for 15–18 days, a higher dosage being generally required to mature the parasites on the castrated host. Afterwards, for 10 days, 2 mg of LH (*NIH/LH/B/3* bovine) was injected in three daily doses in addition to hydrocortisone. The defaecation rate of the fleas fell by the fourth day and regression appeared complete by the eighth day. The fleas on the ovariectomized doe redeveloped on the seventh day after injections of LH had ceased; probably owing to the prolonged high dosage of cortisol required on the castrated host (see Table 3) redevelopment was slower than in the previous experiment.

No regression or resorption of the yolk or of the chorionated eggs was found in gravid matured fleas transferred to buck and doe rabbits which were later injected with 0·25–0·75 mg of hydrocortisone for periods ranging from 4 to 24 days, or injected with 0·32 mg of hydrocortisone together with (i) 5 mg of prolactin (National Institutes of Health, Bethesda, ovine prolactin 23116) for 5 days; (ii) oxytocin (10 units 'Pitocin', Parke Davis, Ltd., London, 3/160/12/16) for 5 days; and (iii) 2 mg oestradiol ('Oestroform', B.D.H., 33753/124/2501) for 9 days. No regression was observed if matured fleas were sprayed with the Roussel spray excipient only. Among the control samples there were a few exceptions in which fleas after 7 days (or longer) feeding on rabbits injected with 0·75 mg of hydrocortisone had either failed to respond or had responded and then afterwards regressed. It must be remembered that many of the fleas had previously been used in other experiments involving maturation and it has been noted that such fleas can fail to respond to further hormone stimulation. On the other hand, it is possible that with large pharmacological doses of either corticosteroids or oestrogens the fleas become exhausted and regression occurs spontaneously without the influence of either luteinizing hormone or progestins. It is therefore important, when LH or progesterone regression experiments are involved, to ascertain that, after the cessation of the experiment with the regression factor, the fleas can be brought back immediately to egg-laying when stimulated with corticosteroids.

Our experiments suggest that luteinizing hormone, progestins (and to a certain limited extent, testosterone) can play an active part in regression and resorption in the rabbit flea and, moreover, that these particular hormones exert a direct effect on the flea and do not act only indirectly by way of the host. In Nature, maturation followed by bouts of egg-laying in the nest may predispose the female flea to rapid regression on the postpartum host.

Insufficient 20α-OH was available to compare its activity with that of progesterone on the fleas' ovaries, as we were able to do in the case of corticosterone and hydrocortisone. In the rabbit[6], 20α-OH is two thirds less active weight for weight than progesterone and when direct applications were made to fleas a correspondingly low activity was anticipated. However, results suggested that 20α-OH is the more active of the two hormones in fleas when sprayed on externally—a somewhat surprising conclusion because the Roussel spray used contained about half as much 20α-OH per measured dose as the progesterone spray. Furthermore, the defaecation rate of fleas sprayed with the latter hormone returned to the pre-spraying rate (1 : 6–8) after about 9 days on a rabbit treated with hydrocortisyl, whereas those sprayed with 20α-OH remained below the pre-spraying rate for 21 days.

It is evident that the reduced intake of blood (estimated by the reduced defaecation rate), which is characteristic of fleas feeding on rabbits with a high blood-level of LH or progestins, plays an important part in regression. Apart from any other consideration, such as the reduction of nutrient material ingested, it follows that the intake of corticosteroids is thus drastically curtailed. It was noted earlier[3] that on thyroidectomized pregnant does maturation occurred without accelerated defaecation rates suggesting that maturation and yolk formation, although linked to faster feeding rates in Nature, were not dependent on it. Similarly, by raising the corticosteroid-levels of the blood very slightly (0·1 mg of hydrocortisone into the 10 lb. rabbit) yolk formation could be engendered in the fleas without raising their defaecation rate. A similar effect could be produced by injecting somewhat larger doses of corticosterone simultaneously with progesterone. Conversely, relatively large doses of progesterone injected into the rabbit, in conjunction with smaller doses of hydrocortisone, could produce the reverse effect, namely regression of the ovaries without a reduction in the feeding rate.

It would seem, therefore, that maturation, egg production, ovarian regression and the resting stages of the rabbit flea (feeding on both the doe and buck rabbit) depend on the relative blood-levels of various groups of hormones under the control of the anterior lobe of the pituitary gland. Maturation is probably delayed by the high levels of luteinizing hormone and progestins present during the early stages of the rabbits' pregnancy, even if the corticosteroid blood-levels have already begun to rise before the twentieth day, when maturation of the flea first becomes evident. Regression of fleas on the postpartum doe seems to indicate a higher level of progestin (particularly 20α-OH) circulating in the peripheral blood than has hitherto been suspected[7], because the corticosteroid levels are still somewhat elevated in the lactating doe[8]. It is of interest that regression of the fleas on the postpartum doe is more rapid if the rabbit is re-mated immediately following parturition when, in consequence, the level of luteinizing hormone and progestins in the blood is raised[9]. Nevertheless, at this stage of the life-cycle other factors may influence regression. It has been noted, for example, that fleas

which have been matured by spraying with oestradiol show, after 12 days, features reminiscent of the spent flea, which are rarely seen in fleas sprayed with corticosteroids, even after such fleas have been maintained for periods of several weeks in the matured condition. In particular, the secretions in the oviducts of oestradiol-sprayed fleas coalesce to form thin sheets of tissue[10]. If these fleas thus sprayed with oestradiol, are replaced on rabbits with high corticosteroid blood-levels, the characteristics of the spent flea become grossly exaggerated. It would appear, therefore, that oestrogen blood-levels may speed up egg-laying and the process of maturation and play some preparatory part in the regression of fleas which invariably occurs on the postpartum doe, in which the corticosteroid levels are still elevated.

In Nature, fleas do not mature on the healthy buck rabbit over the age of 10 days, although they may do so feeding on diseased rabbits[4]. The results of the experiments with testosterone were somewhat perplexing and, although the general picture suggests that this hormone acts as a long-term inhibitory factor, further experiments are required. If fleas are sprayed externally with testosterone and placed on a normal oestrus doe, no maturation occurs and no change in the defaecation rate and the state of the ovaries can be observed. If, on the other hand, fleas are first matured on a doe receiving daily injections of 0·75 mg of hydrocortisone, then sprayed with testosterone and replaced on the doe, the fleas temporarily increase their defaecation rate and lay eggs by the sixth day, and then regress, despite the continued injections into the host of 0·75 mg of cortisol daily, and are fully regressed by the fifteenth day, the defaecation rate dropping from 1 : 2–7 to 1 : 21–30. If fleas are fed on either oestrus does or ovariectomized does injected with 50 mg of testosterone every fourth day for 20 days, a somewhat similar picture is presented. There is uneven maturation of the fleas during the first week, but here without increase in the defaecation rate, although yolk is present in the oocytes, followed later by regression.

It was pointed out earlier[3] that the relationship between the hormone-levels, maturation and feeding behaviour of the fleas required further investigation, and this still remains true. There are indications that certain factors in the blood, apart from the various hormones mentioned above, play an important part in both initiating and maintaining maturation. This is clearly the case in other species of fleas because we have obtained maturation, pairing and egg-laying of *Xenopsylla cheopis* Roths. feeding for 5 days on the hypophysectomized male white rat, and maturation of *Nosopsyllus fasciatus* Bosc. and *Ceratophyllus gallinae* Schrank feeding for 5 and 12 days, respectively, on the castrated adrenalectomized male white rat. Furthermore, the rabbit flea does not undergo maturation on the pregnant cat—one of the few animals on which this host-specific flea will feed steadily. Certain cultures of the rabbit flea collected on Herm Island are relatively very resistant to cortisol and oestrogens injected into the host[11]. Furthermore, it is not at all clear how many of the various changes seen during maturation and regression arise directly from the larger or reduced intake of blood engendered by the influence of the hormone in question.

Copulation of the rabbit flea does not occur in Nature on the adult host but only on the new-born young from one to seven days of age[1,3]. The copulation factors (like the maturation factors) are most active in nestlings during the first day of life and thereafter wane fairly rapidly until at the seventh day few, if any, fleas are stimulated to copulate. The copulation factors were assumed to be present in the prepartum doe at low levels because, after feeding on certain individual pregnant does, mature fleas occasionally copulate if removed from the rabbit and placed in test-tubes. It was also noticed that sometimes males were stimulated to attempt copulation in the test-tubes but that they were not accepted by the females. Just as there is variation in the speed and degree of maturation of fleas feeding on individual does, so there is variation in the 'ripeness' for copulation of fleas feeding on individual prepartum female rabbits, and also in the time interval between parturition and their transfer to the nestlings from the postpartum doe (see Table 1).

If individual new-born young rabbits were placed in observation boxes and matured female fleas off a prepartum doe were removed from the doe's ears and introduced on to them, pairing first occurred after 40–50 min, whether the males present had fed and matured previously on the pregnant doe, or merely fed on a non-pregnant doe or buck. If, on the other hand, unmatured female fleas with ovaries at the resting stage were introduced, even together with males matured for 30 days on the prepartum doe, pairing did not occur until after these female fleas had fed for a minimum of 10 h on the day-old young. Copulation, and in some cases fertilization, then occurred, although the ovaries of these fleas had not yet matured and no yolk was as yet present in the oocytes. Again, if female fleas matured on the prepartum doe were transferred to the day-old young and allowed to feed on them for 3·5 h and male fleas then introduced, pairing occurred within 10–12 min, sometimes before the males had fed.

These observations indicate that it is the female which responds to the copulation factors—the male copulating directly she is ripe for pairing. Furthermore, copulation can precede maturation providing sufficient of the required factors are present in the blood of the host.

The act of leaving the doe for the young and possibly the change of temperature in the nest (which is lower than the ear (skin) temperature of the doe) may predispose the gravid female flea to copulate.

Preliminary trials with beef growth hormone (supplied by the Department of Biochemistry, Cambridge) in which 3-week-old nestlings were injected with 1·80 mg and a few pairs of gravid and matured fleas observed, afterwards, pairing on them, suggested that this hormone might provide one of the necessary factors for copulation. No rabbit growth hormone is available for experiments and therefore three trials were later carried out using human growth hormone (somatotropin, also supplied by the Department of Biochemistry, Cambridge). Two age-groups of rabbits were selected for these experiments: (*a*) 10-day-old rabbits, on which age-group we have not previously seen fleas pairing and from whose nest the fleas returned to the doe the same day on which the experiment was performed (indicating that these particular nestlings had ceased to be attractive to fleas). (*b*) Eight-week-old rabbits, an age-group which is still comparatively unattractive to fleas and on which copulation of fleas has not hitherto been observed.

Two 10-day-old nestlings from the same litter were placed in observation boxes and forty matured fleas from a doe, immediately prepartum, were introduced on to each of them. One of the nestlings was injected with 1·80 mg of human growth hormone dissolved in normal saline at 12.30 p.m. Two hours later, five pairs of fleas were copulating on this 10-day-old nestling, nine pairs at 3.15 p.m. and a further eight pairs at 3.45 p.m. No copulation had occurred up to this time on the control rabbit. Therefore at 4 p.m., 3·5 h after the commencement of the experiment, the control was in turn injected with 1·80 mg of human growth hormone. At 6 p.m. two pairs of fleas were seen in copulation on this rabbit, three further pairs at 7 p.m., and two pairs at 9 p.m. No further pairing was seen on either 10-day-old rabbit after 10 p.m. This experiment was repeated with two 10-day-old rabbits from another litter except that in this case the experimental rabbit was injected with 1·67 mg of human growth hormone dissolved in normal saline (at 3.40 p.m.) and the control rabbit received at the same time an injection of normal saline only. One hundred matured fleas were introduced on to each rabbit from a prepartum doe. One pair of fleas was seen in copulation before they were introduced to either

nestling. Pairing was first seen on the rabbit injected with growth hormone at 4.2 p.m., 22 min after transfer from the doe, when four pairs were seen to be copulating. A total of thirty pairs were counted up to 11.5 p.m. One pair of fleas was noted copulating on the control 35 min after their transfer from the doe, but no further pairing occurred on the control for the following 4 h. Therefore at 7 p.m. it was injected with 1·67 mg of growth hormone. One hour later three pairs were seen in copulation, and, in all, twenty-five pairs were counted on the control rabbit following the injection. Pairing was not observed on the experimental nestling after 11.5 p.m. but continued on the injected control until 2 a.m. when three pairs were still in copulation.

Gravid fleas do not behave in the same manner on the 10-day-old nestling as on the day-old nestling. Possibly the relatively high level of corticosteroids in the blood of the day-old rabbit[8] accounts for the fact that the fleas tend to scatter over the body without fixing to any specific area, feeding chiefly on the sacral regions and the flanks; the naked skin of the new-born rabbit may also play a part in determining their behaviour. The defaecation rate of fleas on the day-old nestling is at a maximum of 1 : 1–4 but drops steadily until on the 10-day-old it is about 1 : 30. Unmatured fleas, fed for 26 h on the 9–10-day-old rabbits and later sectioned, are found to show no trace of development, whereas those fed for similar periods on 1–2-day-old nestlings invariably display signs of maturation. On the 10-day-old rabbit the fleas, possibly in response to the longer hair and relative lack of certain hormones, tend to congregate on the head and fix in this position.

The experiment on the 8-week-old rabbit was far less satisfactory. One of these was injected with 4·50 mg of human growth hormone and forty fleas matured on a prepartum doe were introduced on to it. Observations were difficult in the longer hair of this age-group and were not considered altogether satisfactory. Three pairs of fleas were seen in copulation 4·5 h after the injection and none were observed on the control. All fleas on the injected rabbit were later sectioned, but only one specimen (despite the three observed pairing) had been successfully fertilized.

Although it seems clear that growth hormone is a powerful copulation factor if injected into young rabbits about the time when the fleas normally abandon them for the doe, yet it is insufficient to stimulate much pairing on the 8-week-old rabbit. Some other factors are probably required or else some inhibitory elements are present in the 8-week-old rabbit which counteract the effect of the growth hormone.

Fleas matured and feeding on adult rabbits were not stimulated to copulate by intramuscular and subcutaneous injections into the host of ACTH, hydrocortisone, corticosterone, cortisone, prolactin, oxytocin, adrenaline, oestradiol, luteinizing hormone, progesterone, 5-hydroxytryptamine, and fructose (intravenous injections) and 'Metopirone' (Ciba Laboratories, Ltd., Horsham, Sussex), nor by cholesterol administered orally to the host, or farnesol sprayed on to the fleas externally. No matured fleas were observed to pair on 10- or 12-day-old nestlings injected with hydrocortisone or normal saline, but two pairs were seen in copulation 14 h after an injection of 2·5 mg of prolactin (*NIH/P/S/6*, ovine), and one pair 40 min after an injection of 10 mg of testosterone.

There is considerable variation in the behaviour of copulating fleas. Bird fleas of the genus *Ceratophyllus* will pair immediately on emerging from their pupae without a blood meal[12], but the female (not the male) of *Xenopsylla* and *Echidnophaga* requires a blood meal before she will pair[13]. The specialized behaviour of the rabbit flea, in which the insect's copulation appears to be partly under the control of a protein hormone secreted by the anterior lobe of the pituitary gland of the host, seems to be unique.

The various changes associated with maturation can all be observed, with minor variations, during the maturation of *Xenopsylla*, *Echidnophaga*, *Nosopsyllus* and *Ceratophyllus*. Owing to the fact that maturation, pairing and egg-laying in the rabbit flea are compressed into a very short breeding period (10 days on the prepartum doe and 10 days in the nest), these changes appear with dramatic suddenness but, apart from the time element and certain specializations, such as the simultaneous maturation of large batches of eggs, and sperm bundles (which can also be observed in *Echidnophaga gallinacea*), the process of maturation is fundamentally similar in the four genera which have been observed and sectioned during their development from the unfed imago to fertilization and egg-laying. It is not, of course, known how the hormones of the rabbit act on the flea, but it is unlikely that they each exert a direct effect on the reproductive or other organs of the insect; it would seem more probable that they influence the release of the fleas' own hormones. It is possible to envisage a progressive increase in the release of the juvenile hormone, and a specific response in the various target organs in the flea to these different levels of juvenile hormone. The gradual increase in defaecation rate seen in both sexes of the flea during the last 10 days of the host's pregnancy could, for example, reflect a rise in the level of juvenile hormones released in response to the rise in blood levels of certain hormones in the host. It is also possible that a hormone resembling that recorded[14–16] from the corpus cardiacum of *Periplanata americana*[14,15] is concerned with the accelerated defaecation rate. This hormone, which is released by feeding, stimulates an increase in the rate and amplitude of the muscles of the heart, gut and Malpighian tubes. It is also claimed[16] that a factor (P_2), which is more or less specific for the hind gut, can be separated from extracts of the corpus cardiacum. On the other hand, it is also possible that a much lower level of juvenile hormone is sufficient to stimulate maturation without affecting the defaecation rate. Nevertheless, these observations suggest that the rabbit flea may have a specific hormone (distinct from the juvenile hormone controlling maturation[18,19]) which is concerned with the regulation of ovarian regression and resorption.

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A FAST-NEUTRON SOURCE FOR RADIOTHERAPY

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As part of a comprehensive study of the effects of radiations of differing linear energy transfer (LET), dose-response curves for the survival of reproductive capacity of murine *P*-388 leukaemia cells were obtained *in vivo* after irradiation with fission-spectrum and 14-MeV monoenergetic fast neutrons[1] and after irradiation with cyclotron-produced fast neutrons with an average energy of 6 MeV (ref. 2). Comparison between the slopes of the dose-response curves indicated that there was a rather large difference in the radiosensitivity of these cells over a narrow range of difference in LET between the monoenergetic 14-MeV neutrons and those produced in the Medical Research Council's cyclotron at Hammersmith Hospital. On the basis of this difference, it was suggested that there might be similar gross differences in the sensitivity of anoxic tumour cells *in situ* over this narrow range of LET, and this would have important implications and limitations in the application of fast neutrons of these energies to clinical radiotherapy[3–5].

The present experiments were designed to repeat and extend Berry and Andrews's original observations[1] using 14-MeV monoenergetic neutrons, and to compare the response of tumour cells irradiated in a mouse in air with that of tumour cells in a mouse confined at 10 cm depth within a tissue-equivalent phantom to simulate the response of a deep-seated human tumour.

The *P*-388 lymphocytic leukaemia of the *DBA/2JN* mouse and the assay of the reproductive capacity of its cells by serial dilution techniques have been described previously[6]; the tumour does not provoke any detectable immune response from the brother–sister mated recipient animals of the $DBA/2JN_{oxf.}$ sub-line[2,6]. The statistical handling of the data has also been described[7]; the 'surviving fraction' (the fraction of cells retaining reproductive capacity after irradiation) was determined by the expression:

$$S.F. = \frac{E \text{ (irradiated)}}{E \text{ (control)}}$$

where E is a fraction analogous to the 'plating efficiency' of cells grown in clonal culture *in vitro*, and represents the percentage of morphologically intact tumour cells injected into recipient mice which are capable of producing tumours in those mice.

Tumour-bearing donor animals were irradiated 7 days after the intraperitoneal injection of 10^6 cells; at the time of irradiation their ascites fluid volume was 5–7 ml. and the majority of tumour cells were anoxic when the mouse itself was breathing air[6], so that the radiation survival curves obtained were those for anoxic tumour cells. The 14-MeV neutrons were produced in a *SAMES* neutron generator. Deuterons were accelerated by an electrostatic generator operating at 150 kV. At a beam current of 400–500 μamp, the deuterons were used to bombard a thin tritiated (titanium) target (5·5–9·5 c., initial activity) supplied by the Radiochemical Centre, Amersham. The radiation exposure was measured with a tissue-equivalent type '*B*' Rossi chamber[8], which had been calibrated against radium. This chamber was placed in the positions occupied by the tumour-bearing donor mice, and simultaneous readings were obtained with a remotely placed tissue-equivalent protection chamber which was connected to a vibrating reed electrometer. Instantaneous measurements of dose-rate were obtained on the remotely placed chamber at regular (5 min) intervals throughout the irradiation period. These were then integrated to give an estimate of the final total dose received by each individual mouse. Because of the rapid depletion of the tritium target at these high beam currents, the dose-rate varied during the exposure. At the position in which the tumour-bearing donor mice were irradiated, the maximum dose-rate was 11 rads/min and the minimum was 2·2 rads/min.

The polythene containers used to confine the mice during irradiation were identical with those used by Berry, Bewley and Parnell[2], as was the tank of tissue-equivalent solution used for irradiation of one tumour-bearing donor animal at a depth of 10 cm. The containers were placed 5 cm from the cooling water jacket around the tritium target and were rotated during the exposure to produce the most uniform distribution of radiation dose within the ascites fluid in the abdomen.

The results obtained in two separate experiments are shown in Table 1, and plotted graphically in Fig. 1. In addition, the earlier data obtained by Berry and Andrews[1] have been subjected to the improved statistical analysis[7] used in the present experiments, and are shown in Table 2 and plotted against the current results in Fig. 1. For purposes of comparison, the dose-response of anoxic *P*-388 leukaemia cells to X-rays, cyclotron-produced fast neutrons[2] and fission-spectrum fast neutrons[1] is also shown.

The present results extend the survival curve for 14-MeV neutrons below one cell in a thousand, and form a quite homogeneous set of observations. The response of tumour cells in the donor animal irradiated at a depth of 10 cm in tissue-equivalent material to simulate a deep-seated human tumour did not differ from the optimal regression line drawn through the data for survival of tumour cells irradiated in donor animals in air. This indicates that there is no significant change in the relative biological efficiency of these 14-MeV neutrons due to degradation of energy caused by passing through tissue to a depth of 10 cm.

Table 1. SURVIVAL OF REPRODUCTIVE CAPACITY OF *P*-388 LEUKAEMIA CELLS *in vivo* AFTER IRRADIATION WITH 14-MeV MONOENERGETIC NEUTRONS

Neutron dose (rads)	E ('plating efficiency')	$\text{Log}_{10} E \pm S.E.$	Fraction of surviving cells
Experiment I.			
Control (0)	0·28	−0·552 ± 0·134	(1·0)
710	0·0090	−2·046 ± 0·196	0·032
1,240	0·00020	−3·693 ± 0·40	0·00072
Experiment II			
540	0·011	−1·964 ± 0·184	0·039
740	0·0051	−2·296 ± 0·175	0·018
860	0·0012	−2·928 ± 0·190	0·0042
290 (at 10 cm depth in phantom, see text)	0·057	−1·244 ± 0·184	0·20

Table 2. DATA OF BERRY AND ANDREWS[1] FOR SURVIVAL OF *P*-388 LEUKAEMIA CELLS AFTER IRRADIATION WITH 14-MeV MONOENERGETIC NEUTRONS. RE-CALCULATED USING THE METHOD OF PORTER AND BERRY[7]

Neutron dose (rads)	E ('plating efficiency')	$\text{Log}_{10} E \pm S.E.$	Fraction of surviving cells
Control (0)	0·28	−0·557 ± 0·026	(1·0)
125	0·30	−0·520 ± 0·138	1·08
250	0·10	−0·989 ± 0·094	0·37
500	0·027	−1·565 ± 0·126	0·097
750	0·015	−1·821 ± 0·176	0·054
1,000	0·010	−1·988 ± 0·204	0·037

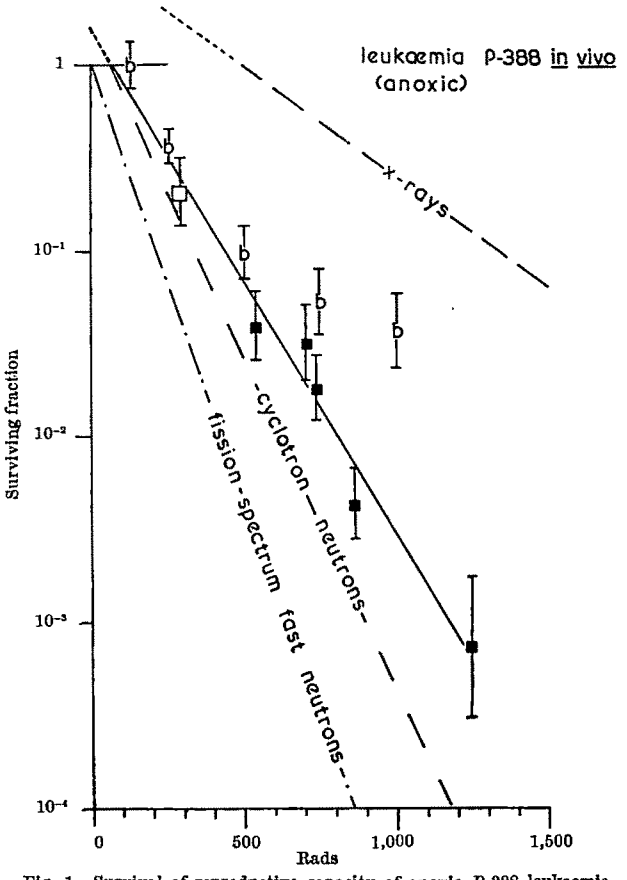


Fig. 1. Survival of reproductive capacity of anoxic *P*-388 leukaemia cells *in vivo*. ■, Present series of experiments, irradiation with 14-MeV monoenergetic neutrons, donor mouse irradiated in air; □, present experiment, irradiation with 14-MeV monoenergetic neutrons, donor mouse confined 10 cm deep in tissue-equivalent phantom; *b*, data re-calculated from Berry and Andrews[1], Irradiation with 14-MeV monoenergetic neutrons at Brookhaven National Laboratory. "X-rays, Cyclotron, Neutrons", data from Berry, Bewley and Parnell (ref. 2). "Fission-spectrum fast neutrons", data re-calculated from Berry and Andrews (ref. 1). The vertical bars indicate the standard error of each determination of fraction of surviving cells

The present results, however, differ somewhat from those obtained by Berry and Andrews in the earlier experiments[1] in which *P*-388 leukaemia cells were irradiated with 14-MeV neutrons produced in the Van de Graaf generator at the Brookhaven National Laboratory. The physical parameters of the radiation exposure, however, differed in that at Brookhaven a low, but relatively constant, dose-rate was achieved. It has been shown that *P*-388 cells are capable of recovery between fractionated acute X-ray doses spaced over 4 h apart[6,9], and that recovery occurs in these cells during protracted γ-irradiation at low dose-rates[10]. Recovery has also been shown to occur between fractionated acute doses of 14-MeV fast neutrons[1]. The initial agreement between the two sets of results at low radiation doses, followed by divergence between them at doses where the Brookhaven exposure times were excessively long (more than 8 h) owing to interruptions caused by temporary machine failures, etc., suggests that intra-cellular recovery during the radiation exposure may be an explanation of the differences observed; in the present series of experiments, none of the exposures exceeded 6 h in overall duration and the high initial dose-rate meant that a majority of the dose was delivered even more rapidly (presumably before recovery could occur).

The present results indicate that there is relatively little difference in the biological response of mammalian tumour cells to 14-MeV monoenergetic neutrons, and to the fast neutrons of quite similar average LET produced in the Medical Research Council's cyclotron. If the generators of 14-MeV neutrons can produce sufficiently high outputs[11], and the problems of collimation of a beam of these neutrons can be overcome[12] (as now seems practicable[13]), there now seems little doubt that irradiation with 14-MeV neutrons in clinical radiotherapy could produce a useful decrease in the protection normally afforded to tumour cells by anoxia[14] when they are irradiated with low-LET X- or gamma-radiation.

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INFRA-RED DETERMINATION OF THE PERCENTAGE OF CRYSTALLINITY IN APATITIC CALCIUM PHOSPHATES

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MANY solid systems contain an amorphous phase admixed with a crystalline phase. Because the exact proportion of the mixture contributes to the chemical and physical properties of the system, the measurement of the percentage of crystallinity (that is, the percentage by weight of the material found in the crystalline form) is of fundamental importance. Various methods such as X-ray diffraction, infra-red spectroscopy, specific volume and density measurements have been used to determine the ratio of the crystalline phase to the amorphous phase in polymers[1]. This report presents an infra-red spectrophotometric technique designed to measure the percentage of crystallinity on a series of apatitic calcium phosphates.

The first solid formed during the precipitation of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, from a carbonate-free alkaline medium is a non-crystalline, or amorphous, calcium phosphate with a calcium to phosphorus molar ratio (Ca/P) of 1·50 (ref. 2). Afterwards, this solid in contact with the precipitating solution converts slowly to crystalline apatite (calcium : phosphorus = 1·67) by an autocatalytic mechanism[2]. By sampling the reaction at intermediate stages it is possible to obtain a series of

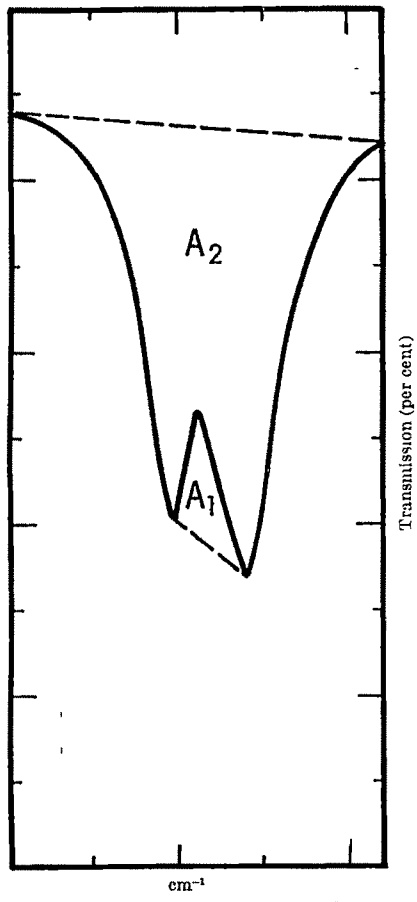


Fig. 1. Method of obtaining splitting function

calcium phosphates with varying amorphous to crystalline compositions with the crystalline content remaining at a constant crystallite size[2]. Infra-red spectra of the solids formed in the conversion of amorphous calcium phosphate to crystalline hydroxyapatite show a gradual splitting of the 600 cm^{-1} phosphate ion antisymmetric bending frequency (ν_4) from a broad singlet for amorphous calcium phosphate to a well-defined doublet for the fully crystalline solid[3]. Because the X-ray diffraction assay of the percentage of crystallinity has certain experimental limitations (for example, large sample size), we decided to use this infra-red splitting effect to develop a quantitative measure of the percentage of crystallinity of these mixtures of amorphous and crystalline calcium phosphates.

All infra-red absorption spectra were obtained on a Beckman *IR*-9 spectrophotometer by the potassium bromide pelleting technique; 700-mg disks of potassium bromide 22 mm in diameter, which contained 2·0 mg of sample (ground to 325 mesh) pressed at 52,000 lb./in.^{2} under vacuum, were used.

Standard samples of varying percentages of crystallinities were obtained by physically admixing, in a dental amalgamator, different proportions (by weight) of pure amorphous calcium phosphate and a pure crystalline hydroxyapatite prepared as described elsewhere[2]. Identification and purity of the phases used as standards were determined by X-ray diffraction. The Ca/P ratios for these standard mixtures were obtained by weight averaging the analyses obtained from the amorphous and crystalline phases in the proper proportion. These prepared mixtures, labelled as series 1, 2 and 3, ranged from 0 to 100 per cent crystalline. The methods of chemical and X-ray diffraction analyses used throughout provided a precision of ± 1 per cent for the Ca/P ratios and ± 10 per cent for the percentage of crystallinities.

A second specimen series, labelled VI, VII, VIII and XIII, was obtained by sampling the preparation of hydroxyapatite[2] at various times in the conversion of the precursor amorphous calcium phosphate to the final crystalline phase. The samples obtained in this manner ranged from 0 to 100 per cent in crystallinity as determined by X-ray diffraction.

X-ray diffraction line broadening showed the apatite crystals in both sample series examined by infra-red to have about the same crystal size. To ascertain the effect of crystal growth in the infra-red data, a preliminary examination was made of two synthetic hydroxyapatites (100 per cent crystalline) of differing crystal size ($\sim$ 125 Å and $\sim$450 Å in average *c*-axis crystal diameter).

The method of measuring the splitting of the phosphate ion antisymmetric bending mode at about 600 cm^{-1} is depicted in Fig. 1. As shown, a base line was drawn, by visual estimation, at the high transmission (top) end of the absorption band to mark the area limits, A_2, of the band. The two minimum points of transmission on the band were connected to form the splitting area, A_1. Both areas were measured by planimetry and the amount of splitting is given by the ratio of the two areas, A_1/A_2, and is defined as the splitting function (SF). For a completely amorphous calcium phosphate where a single broad peak exists, a value for the splitting function of zero is obtained as A_1 is equal to zero. The precision of the measurement of the splitting function was found to be ± 3 per cent.

A plot of splitting function values against X-ray diffraction-assayed percentage of crystallinity (per cent *C*) for both series of calcium phosphates is shown in Fig. 2. A statistical analysis of variance showed no significant differences in either splitting function or per cent *C* between standard mixture sample points (1, 2, 3) and experimentally obtained points (VI, VII, VIII, XIII). Using all the data, mixtures as well as experimental samples, a regression analysis yielded a line with a slope of 549·5 per cent *C*/SF units and an intercept on the abscissa of 6·4 per cent *C*. The correlation coefficient for this line is 0·799 and a statistical test for linearity was positive ($P < 0{\cdot}001$).

This plot shows that the infra-red splitting function measurement can serve as a quantitative measure of the percentage of crystallinity for the calcium phosphate system. Despite the low value of per cent *C* intercept on the abscissa, the practical lower limit of detection of the percentage of crystallinity by this infra-red method was found to be about 15 per cent. The precision of splitting function analysis above this lower limit is, as already stated, ± 3 per cent.

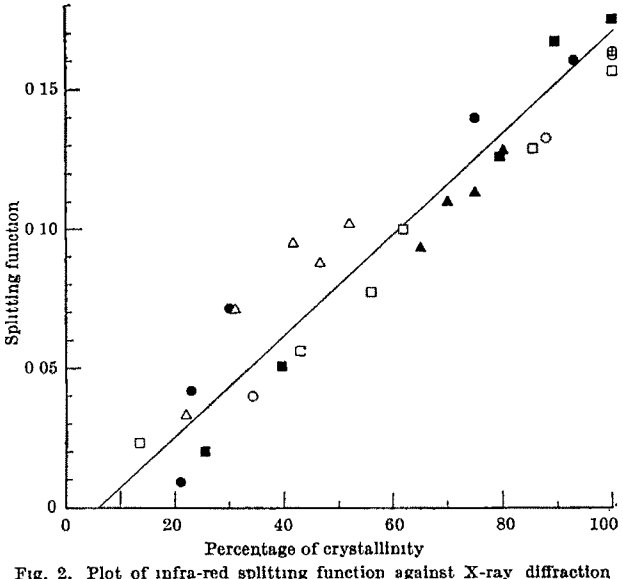


Fig. 2. Plot of infra-red splitting function against X-ray diffraction percentage of crystallinity of a series of calcium phosphate. ⊕, VI; ○, VII, ●, VIII, □, XIII, △, 1-, ■, 2-; ▲, 3-

Fig. 3 shows a plot of the splitting function against the Ca/P ratio for all the samples. Again, no significant differences could be observed between the standard mixtures and the samples obtained at various time intervals from the preparation of hydroxyapatite. Regression analysis yielded a line of slope 0·52 $\frac{\text{Ca/P}}{\text{SF}}$ units with an intercept on the abscissa of 1·51 Ca/P units. This line gave a correlation coefficient of 0·961 and a high level of linearity significance ($P < 0·001$). Thus, within the limits of the experiment, the splitting function values can be used as a means of obtaining the Ca/P ratio of a given calcium phosphate mixture of amorphous (Ca/P = 1·50) and crystalline hydroxyapatite (Ca/P = 1·67). Because the Ca/P and the per cent C of a given sample in both series are related, it is reasonable that both these values can be obtained from a measure of the splitting function.

The crystal size was shown to have an effect on the value of the splitting function of a fully crystalline hydroxyapatite. The values of the splitting function observed for the two extreme samples with sizes of about 125 Å and 450 Å were 0·155 and 0·250, respectively. This preliminary result points up the necessity for further investigation of the effect of crystal size on the infra-red splitting function factor. It should be emphasized, however, that all the samples examined in the foregoing study contained hydroxyapatite with the same crystal size.

This method of determining the percentage of crystallinity in a mixture of amorphous and apatitic calcium phosphates has a few simple advantages over the present X-ray diffraction method. The infra-red study uses only a few milligrams of sample, while the X-ray method requires at least twenty-five to fifty times as much. This could be particularly important in the analysis of such biological calcium phosphates as bone and tooth minerals, if organic matrix effects are taken into account. In addition, the time for the infra-red analysis is much

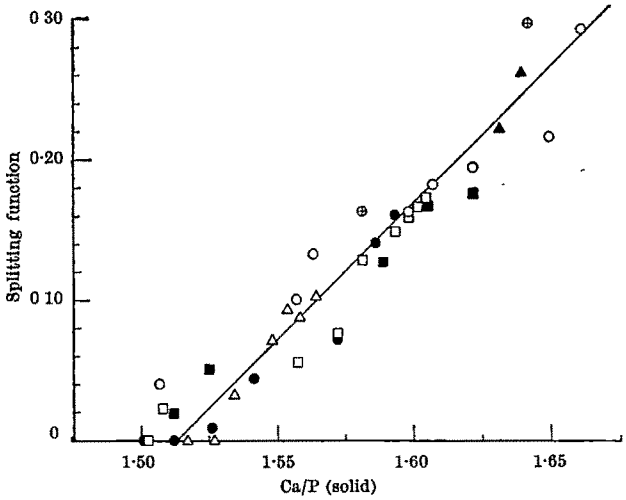


Fig. 3. Plot of infra-red splitting function against molar Ca/P of a calcium phosphate series. Symbols as in Fig 2

shorter than that required for X-ray diffraction analysis. The method should be immediately applicable to studies on bone and tooth minerals if the effect of crystal size is taken into account, where necessary. Application of the splitting function technique to other chemical systems is possible, but as this is an empirical method, experimental factors must be found suitable for the system under study.

The chemical analyses, X-ray diffraction studies, and certain calcium phosphate samples were provided by Drs. I. H. Gillessen and E. D. Eanes of this laboratory. This work was supported in part by a grant from the National Institute of Dental Research.

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AGE OF ALLUVIAL CLAYS IN THE WESTERN GEZIRA, REPUBLIC OF THE SUDAN

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THE Gezira plains occupy a triangle of about 5 million acres between the Blue Nile, the White Nile and the Kosti-Sennar railway-line. Khartoum forms the northern apex.

Tothill[1] noted that freshwater snail shells occur down to depths of 2 m in vertisolic clays in a belt of land up to 30 km wide on either side of the White Nile. He believed that the shells were laid down under swampy or lacustrine conditions. The climate would have resembled that now prevailing in the *toich*-lands north of Malakal (mean annual rainfall 820 mm; compare Kosti, 406 mm, Khartoum, 164 mm).

Sub-fossil shells of the land snail *Limicolaria flammata* (Cail.) are common in the upper few feet of Gezira clays in areas now too dry for it to survive[2]. *Limicolaria* is present in occupation sites near Khartoum dated by Arkell at between 5,000 B.C. and 3,000 B.C.[1,2]. The invasion of *Limicolaria* probably occurred when conditions became too dry for the aquatic mollusca, but still not as dry as now.

During the course of a recent reconnaissance soil survey along the White Nile, undertaken on behalf of the Sudan Ministry of Agriculture by Messrs. Hunting Technical Services, Ltd., I collected unbroken freshwater mollusc shells from soils of White Nile origin at distances up to 15 km from the present river.

One sample came from a pit 11 km east of Esh Shawal, in latitude 13° 34′ N., at a ground elevation of 381·5 m (3·75 m above local high river level). *Lanistes carinatus* (Oliv.) and *Cleopatra bulimoides* (Oliv.) occurred in a matrix of fine sandy loam at a depth of 145–170 cm, and were overlain by slightly calcareous clays. From carbon-14 measurements, it would seem that this shell layer was deposited 11,300 ± 400 years B.P. (Date by Isotopes, Inc., by courtesy of Prof. A. J. Whiteman, Department of Geology, University of Khartoum.)

Another sample came from a pit between Kawa and Hashaba, 2 km east of the river, in latitude 13° 56′ N., at a ground elevation of 381 m (*c.* 4 m above local high river level). Large shells of *Ampullaria wernei* (Phil.) were set in a silty calcareous matrix at a depth of 160–190 cm, and were overlain by a calcic horizon 10 cm thick. By carbon-14 dating it was found that these shells were laid down 8,370 ± 350 years B.P. (date by Isotopes Inc.). Above the carbonate pan, the calcareous clay contained a further shell band (depth 140–160 cm) for which no date is available, and this band is in turn overlain by dark grey cracking clay.

Tothill[3] regarded the 382 m strand-line at Hashaba, with its associated water-worn shells of *Cleopatra* and *Melanoides*, as the highest level attained by the White Nile lake at the time of its greatest development. Between 15 cm and 200 cm of clay overlie locally fossiliferous calcic layers in the Kawa-Hashaba-Esh Shawal area. In the first pit, more than 140 cm of clay were laid down some time after 10,900–11,700 B.P.; in the second pit more than 150 cm of clay were laid down after about 8,000–8,700 B.P. Deposition at the latter site was intermittent, with impeded drainage and calcium carbonate precipitation sufficient for the development of a calcrete pan, followed by a phase of perennially wet conditions during which aquatic mollusca flourished, and finally succeeded by a period characterized by seasonal swamps.

From dated shell-beds in silt terraces between the First and Third Cataracts[4], it would seem that aggradation on the Main Nile began between 30,000 and 15,000 years ago, and reached its peak about 10,000 years B.P. At Singa on the Blue Nile, Levallois-type implements were found in alluvial sands beneath snail-bearing alluvial clays which were in turn overlain by Neolithic artefacts[5]. These clays must therefore have been laid down at some stage after the formation of the lower Palaeolithic terrace and before about 6,000 B.P. The evidence from the Blue Nile and the Main Nile suggests that in Mesolithic times, deposition was roughly synchronous. At some time, after about 8,000–12,000 B.P., some 150 cm of clay were laid down over snail-bearing deposits of White Nile origin. It is still uncertain whether these later clays are of Blue Nile origin, as the abundance of gypsum and mica in them suggests, or whether they represent purely local aggradation in swamps bordering the receding White Nile.

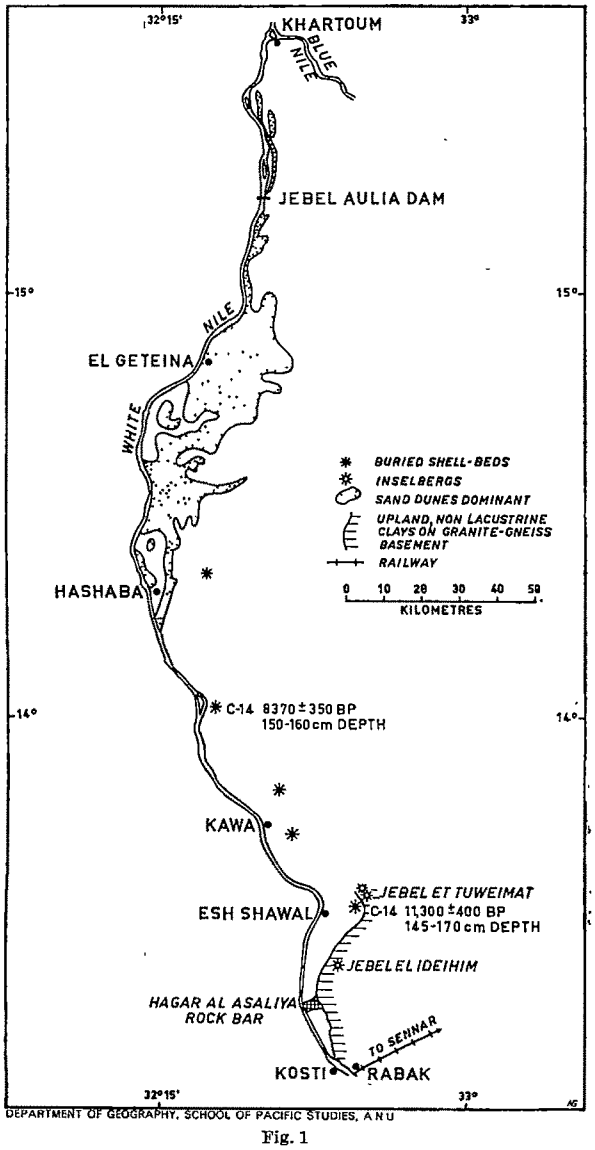


Fig. 1

To the north of Hashaba, winding sand dunes and former meander channels follow a sinuous course from south-south-east to north-north-west. They occupy perhaps 3–400,000 acres, and may be traced on air photo mosaics from near Sennar on the Blue Nile, through the scattered *qozes* of the Central Gezira, to the country around El Geteina, on the right bank of the White Nile, where they attain fullest development (Fig. 1). The dune sands overlie calcareous clays of unknown derivation, and Mesolithic and Neolithic artefacts are common on some of the dunes. It seems that as the White Nile dwindled to its present size, sandy distributaries of the proto-Blue Nile meandered across the alluvial clay plains left exposed by the receding White Nile lake and a series of sinuous channels with sandy bedloads came into being.

As discharge diminished, perhaps due to climatic desiccation, the channels were abandoned and their bedloads resorted by wind action. Inversion of relief occurred as channel deposits were raised into dunes by aeolian activity, levees were removed by deflation, and the formerly channelled localities became filled in and levelled off.

Conditions on the clay plains and inter-dune swales remained sufficiently unattractive (perhaps owing to swamps or to dense woodland) to discourage Mesolithic and Neolithic settlement other than on the dunes. Evidence[6] at Esh Shaheinab near Khartoum points to an annual rainfall of 800 mm in Mesolithic times, diminishing by Neolithic times (*c.* 4,000 B.C.). This accords with the inferred drainage chronology in the sand dune region around El Geteina.

The recent depositional history of the land bordering the right bank of the White Nile between Jebel Aulia and Rabak may be summarized as follows: Until about 8,000–12,000 years ago, the White Nile formed a lake up to 50 km wide which reached a height of 382 m. There is some evidence of a higher lake level at 386 m (ref. 7), and I found shell-beds in pits up to an elevation of 384 m. These lakes probably had similar surface gradients to the present river when the reservoir at Jebel Aulia is full (377·8 m elevation), at which time the gradient between the dam and Rabak is 0·1 cm/km.

Fine sands and silts were laid down in the 382 m lake as littoral deposits. They contain mica and gypsum, and are far too coarse in texture to be authigenic White Nile deposits. They are probably re-worked deposits of Blue Nile origin. As the lake shrank, up to 150 cm of clay were laid down under swampy conditions over the snail-bearing silts between Hashaba and Esh Shawal. North of Hashaba, the proto-Blue Nile transported medium to coarse sands in braided or meandering channels, and fine-textured sediments accumulated in back-plain situations. By about 6,000 B.P., annual rainfall had diminished to about 600 mm near Khartoum, the level of the White Nile had fallen to 0·5–1 m above its present mean high level, and the Blue Nile was either in the final stages of meandering across the clay plain north of El Geteina, or had already been diverted northwards to its present confluence with the White Nile at Khartoum.

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ORIGIN OF TRITIUM IN HYDROTHERMAL SOLUTIONS

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THE difficulty of deciding whether the tritium content of a natural water is a function only of the age of the water, or whether it arises from admixture of old water with new tritium, is particularly marked in the case of hydrothermal water. The results of forty-three determinations of the tritium content of the water tapped by drillholes at the Wairakei Geothermal Power Development have now been made available by Taylor *et al.*[1].

Wilson[2] recently discussed the tritium determinations in the light of the chloride contents of the bore waters and the enthalpies of the bore discharges, and concluded that even tritium concentrations of 0·1 T.U. could not be explained by dilution with surface water. (1 T.U. is a concentration of 1 tritium atom in 10^{18} hydrogen atoms.) The alternative explanation is that the tritium concentration indicates the lapse of time since pre-thermonuclear rainwater, of average tritium concentration 2 T.U., left the surface, was heated, and ascended again to be tapped by the bores. It is not only that the times indicated (the shortest is 40 years) do not seem long enough for the formation of a large body of heated water of volume at least 3 km^3, with fairly uniform chloride and heat content, but also that the variation in tritium concentrations from 0·05 to 2·3 T.U. for water from bores of good output does not seem likely if age is the explanation.

A possible cause of the occurrence of tritium seems to be the radioactivity of rock. Morrison and Pine[3], in giving evidence for the radiogenic origin of helium-3, estimate that a million alpha particles give a yield of two thermal neutrons, of which 5 per cent are captured by lithium-6 to produce tritium. Calculations, using estimates of the uranium, thorium and lithium contents and the water content of deep rock at Wairakei, show that the concentration of radiogenic tritium would be under 0·01 T.U.

My first new suggestion is that consideration must be given to the transport of radioactive elements by the deep geothermal water, and their deposition in more concentrated form. It is well known that uranium minerals may be deposited by low-temperature hydrothermal solutions, and the assumption is that the Wairakei underground water can also act as such an ore-forming fluid. Leonova[4] recently suggested that thorium can also be transported by low-temperature hydrothermal solutions. Further, the concentration of radium and radium-*D* can also be considered, along with the evidence at Wairakei[5] for the transportation and concentration of lithium.

If certain reasonable assumptions are made about the water content at great depth (4 per cent of the mass of the rock), and the flow of the water (0·5 cm/h), and it is assumed that uranium or radium is concentrated to 100 times the content normally present in rock, then a thickness of 100 m of such a deposit would give a concentration of 0·1 T.U. to the water.

There are enough consistent results on drillholes of high output and average enthalpy to give a good average result for the tritium concentration of the underground water in 1960. The value for seven samples was $0{\cdot}11 \pm 0{\cdot}01$ T.U. The possibility that this small concentration is due to radioactivity is of no great significance, but of fifty Wairakei samples collected from 1956 to 1963, twenty-eight were found to have tritium concentrations from 0·3 to 2·3 T.U.[1]. Many more determinations on rain and river waters have given values in agreement with reasonable hypotheses, and contamination or gross error in the above values is most unlikely. For most of the results, dilution can be excluded[2], and an age effect is unlikely so that it is particularly for these values that a more satisfactory explanation is required.

An additional hypothesis to explain these high values was suggested by a rough correlation with the enthalpy of the bore discharge. This suggested that high tritium concentrations were due to mixture near the bore intake of the underground water with steam held in pockets in rock with some concentration of radioactivity. If steam replaces water, and tritium production is the same, the T.U. values will be thirty times as great because of the lower density of steam. If the combined effect of increased radioactivity and lithium content of the rock is assumed to be fifty times that which is normal to the rock, it can be calculated that after 40 years (three times the half-life of tritium), the tritium concentration will be about 10 T.U. Of course the actual concentration will be less because some of the HTO will diffuse out of the steam and there may be some flow of steam (example below of reduction to 0·4 T.U.). In two cases given by Wilson[2], the enthalpy was known to be 340 cal/g instead of the normal 270, indicating a steam dilution of 20 per cent. The tritium concentration of the steam would have to be 2·6 T.U. to give the concentration found in the samples, 0·6 T.U.

The best evidence for a steam horizon in New Zealand is the blow-out at Hole 204, which is described by Bolton[6]. However, around the large fumarole, Karapiti Blowhole, there is a steaming area of 350,000 m^2 with a heat output of 100,000 kcal/sec. The average tritium concentration of four samples from the fumarole was $0{\cdot}8 \pm 0{\cdot}3$ T.U. This steam is coming from hot water about 600 m below and from this source it would bring 0·1 T.U. The ground water to cool the steam from 250° to 120° C could add only 0·15 T.U., a total of 0·25 T.U. The upward velocity of the steam is estimated as 8 cm/h, and allowance for this gives a calculated tritium concentration due to radioactivity of 0·4 T.U., giving a total in the steam of 0·7 T.U.

There is not yet much direct evidence in favour of this hypothesis. It is hard to prove the presence of excess steam in the discharges of high tritium bores, for the enthalpy is not measured at the time of sampling, and in many cases the excess steam will be steam flashed recently from underground water, and hence of low tritium concentration. If the steam is indeed from steam horizons in the country, then these should gradually be exhausted. From determinations on five bores at different times, it has been found that for an initial concentration of 1 T.U. the average decrease is 0·5 T.U./yr.

Well-logging equipment has been constructed by this Institute, but unfortunately it cannot be used above 180° C. Considerable differences in radioactivity were found by Ministry of Works engineers in three rather cold bores[7]. An eight-fold difference in gamma-ray counts was found at one bore at different levels in the same size casing, but this does not go far in supporting the assumptions made.

Investigations are required to check the different possibilities in the assumed production of tritium by alpha-rays. For instance, if lithium is absorbed on the surface of grains in porous rock, its local concentration may be so high that a much greater proportion than 5 per cent of the neutrons may be captured. The effect of radon in the steam would have to be considered, although in water it is ineffective in the production of tritium.

It may be objected that by juggling with assumptions one can make a case for the production of tritium by radioactivity. However, there is a good reason for not waiting for actual measurements before drawing attention to the

possibility of this origin for tritium. Soon, owing to the difficulty with high tritium levels in rain-water from bomb tests, there will be no interest in the use of tritium determinations on thermal waters for determining the age of the water. Dilution by surface water can be measured in other ways. It is difficult to apply any conclusions as to the age or the effect of radioactivity to thermal areas other than Wairakei. Only in this area has drilling made it possible to get enough data on the chloride and enthalpy of the underground water to exclude the possibility of dilution by surface water. However, in the case of steam from fumaroles or drillholes, in hot spring areas or volcanic craters, it may be possible from the temperature of the steam to set an upper limit to the dilution by surface water. However, this will be useful in the Southern Hemisphere only for samples collected before the autumn of 1962, and in the Northern Hemisphere before 1954, for after these dates tritium from bomb tests brought concentrations in rain to values over 10 T.U.

In New Zealand the only other samples of interest are from two drillholes at Kawerau (1·6 and 0·3 T.U.), and two from White Island crater[1] with tritium about 0·6 T.U. Results for other places are given in a list of the International Atomic Energy Agency[8]. Concentrations at hot springs in the United States are obviously due to surface water, except for one case of 0·6 T.U. at Lassen Park. The only values for steam are four samples from deep wells at Lardarello, Tuscany, and the tritium concentrations were 0·3–2·7 T.U. The impression has been given from the New Zealand results that the circulation time of geothermal water may be only 50 years[9], and the same deduction may have been made for Lardarello steam. It should be pointed out that a radioactive origin for most of the tritium would surmount the difficulty of accepting such a short age for geothermal waters.

The suggested origin of tritium will not apply to deep cold water, as this is unlikely to transport and concentrate uranium and radium. However, it would be of interest before it is too late to have determinations of tritium in water vapour saturating natural gas in underground reservoirs. Unless the outward diffusion of HTO was rapid, the concentration of tritium in this vapour due to natural radioactivity should be appreciable.

I thank Mr. C. B. Taylor of this Institute for drawing my attention to the article by Morrison and Pine, and for helpful discussions.

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POLARITY OF THE EARTHQUAKE SOURCE

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THE direction in which the ground first moves at the onset of an earthquake can give important information about the nature of the earthquake source. In a given earthquake, some seismograph stations record an initial compression (motion away from the source), and others an initial dilatation (motion towards the source). By projecting these observations back to the earthquake focus it is possible, in principle, to determine the pattern of first motions about the source. In practice only part of the pattern is ever well defined, the main reason being that all radiation from the upper half of the source reaches the surface within so small an area (of radius comparable with the focal depth) that few stations are in a position to observe this radiation.

Without any presuppositions about the mechanism of earthquakes it would be natural to analyse an observed pattern of first motion on the focal sphere into a series of components; for example, it might be possible to find a spherical component (either of compressions or of dilatations), a second component with compressions in one focal hemisphere and dilatations in the other, a third component with compressions and dilatations in alternate quadrants, and so on.

It is widely supposed, however, that earthquakes are generated by sudden fault movement, and that this movement produces an essentially quadrantal pattern of *P*-wave motion about the source—no other components being generated. In accord with this supposition, the analysis of recorded first motions has customarily taken the form of finding the orientation of that quadrantal pattern which best fits the observations, and thus arriving at a "fault-plane solution" for the earthquake. This does not mean that the observations definitely prescribe a quadrantal pattern; moreover, different authors have often given widely different quadrantal solutions for the same earthquake[1].

It is debatable whether or not fault movement really is the cause of earthquakes. It has been suggested[2] that sudden faulting may be but a gross kind of earthquake damage. Indeed, it is difficult to see how shear fracture, as usually understood, could occur at depths greater than a few kilometres, because the expected frictional forces become prohibitively large.

Thus there is scope for an investigation of first-motion patterns from points of view other than that of the fault hypothesis. A full analysis of the various components would require more accurate information about the amplitude of the initial wave than is at present available. If the simplest of these components—the spherical one—is important in the earthquake source, it will make the pattern of first motions for any given earthquake either dominantly compressional or dominantly dilatational; though in practice, because coverage of the focal sphere is limited, an observed dominance of one direction of first motion over the other is not conclusive evidence for a spherical component.

Polarity

Observations of first motions for a large number of earthquakes have been collected and tabulated by the Dominion Observatory, Ottawa, Canada. For sixty-five earthquakes in the period 1955–62 the number of observations per earthquake is a hundred or more. Data have been published[3–5] for the years 1955–58, and Dr. J. H. Hodgson has kindly provided me with data for 1959–62. The sixty-five earthquakes are well distributed over the major seismic regions of the world, and also in depth of focus; the magnitudes vary from 6·5 to 8·5.

The numbers of compressions (N_C) and dilatations (N_D) observed for these earthquakes are shown in Fig. 1, in which N_C is plotted against N_D for each earthquake. There is a clear tendency for one direction of first motion to be dominant over the other: in fifty-two earthquakes (80 per cent) the ratio exceeds 2 : 1; in thirty-eight earthquakes (58 per cent) the ratio exceeds 3:1. This is, in fact, what would be expected if the first motion at the

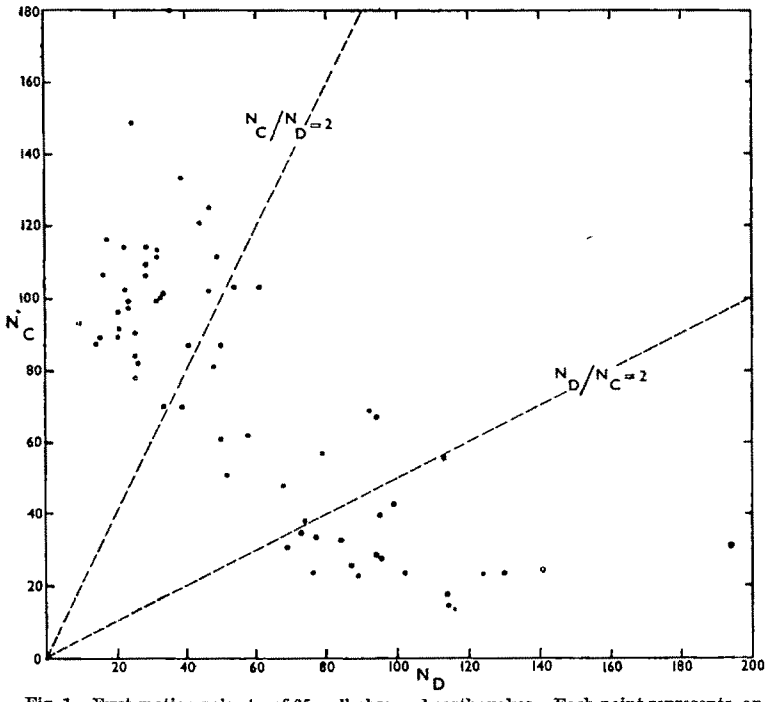


Fig. 1. First-motion polarity of 65 well-observed earthquakes. Each point represents an earthquake and shows the observed number of compressions (Nc) plotted against the observed number of dilatations (ND)

earthquake source had a strong spherical component; however, for reasons already mentioned, an apparent dominance could still arise from sources that were essentially hemispherical, or even of higher order.

Polarity and Environment

Of the sixty-five earthquakes investigated, forty gave a majority of compressional and twenty-five a majority of dilatational first motions. The polarity of an earthquake seems to be related to focal depth and geographical position. Adopting the earthquake parameters published by the U.S. Coast and Geodetic Survey, and bathymetry from the 1961 World Map published by the U.S. Navy Hydrographic Office, the following correlations are found. In most deep-focus earthquakes the first motions are dominantly dilatational: twelve out of fifteen earthquakes with a focal depth (h) of 200 km or more were dilatational. Most of the shallow-focus earthquakes which occur beneath deep ocean trenches are also dominantly dilatational: five out of six earthquakes with epicentres coinciding with a water depth (d) of 2,500 fathoms or more were dilatational. Most other earthquakes are dominantly compressional: thirty-six out of forty-four earthquakes with focal depths less than 200 km and epicentres on land or in water shallower than 2,500 fathoms were compressional. These statistics are summarized in Table 1.

The forty-four earthquakes in category (ii$b$) of Table 1 can be further classified according to geographical location, as in Table 2. Here it can be seen that compressions were dominant in twelve out of thirteen earthquakes located in island chains, in eighteen out of twenty-four on coasts (continental margins), and in six out of seven located in continental hinterlands.

Considering Table 1 as the formulation of a global rule about the occurrence of dominantly dilatational and dominantly compressional earthquakes, it is seen that the number of exceptions is 20 per cent or less in each of the three categories. This seems a remarkable degree of correlation, especially as some apparent exceptions to the proposed rule would result from uncertainties in the calculated epicentres and focal depths. For example, a strongly dilatational earthquake situated near the Chile–Argentina border was given a focal depth of 71 km by the U.S. Coast and Geodetic Survey, and appears as one of the exceptions in Tables 1 and 2; but the focal depth according to the U.S.S.R. Central Station, Moscow, was 250 km, which would make this earthquake conform to the rule. The possibility remains, though it seems remote, that the correlations are a fortuitous effect of such factors as the distribution of existing seismograph stations relative to the main seismic regions. Detailed work is in progress to examine such possibilities.

What physical significance can be ascribed to these apparent correlations between the polarity of the earthquake source and its geographical environment?

Geophysical Implications

Any interpretation of the observed polarity as arising from a strong spherical component in the earthquake source leads to a consideration of the source as (in part) a sudden volume change: an implosion for deep sources and for those shallow sources that are situated beneath deep ocean trenches; and an explosion for all other shallow sources.

Volume changes accompany many of the polymorphic transitions that occur in minerals within the range of pressure and temperature occurring in the upper mantle and crust. The idea that these solid–solid phase changes might be the immediate cause of earthquakes has been discussed by several authors[2,6–8], though it is not yet known whether such reactions could run sufficiently rapidly through the requisite quantity of material. Some support for this hypothesis of earthquake causation is implied by the unexpected similarity found between the seismic radiation from underground nuclear explosions and from natural earthquakes. The present study of radiation from a variety of well-observed earthquakes provides a more direct kind of evidence that earthquakes in general may be caused by sudden polymorphic transitions.

On this hypothesis, the opposite polarities of deep and shallow earthquakes are what one might expect. In those disturbed regions where most of the world's seismic, volcanic and diastrophic activity occurs one can envisage that the outer mantle is at present undergoing gravitative differentiation. As the heavier fractions sink they become concentrated under conditions of higher pressures; some minerals become unstable and implode to a denser polymorph, giving deep-focus earthquakes. At the same time the lighter fractions rise and become concentrated under conditions of lower pressure; some of these minerals become unstable and explode to a less dense polymorph, giving shallow-focus earthquakes.

Thus most earthquakes originating at shallow levels should be explosive in character, as in the present study. These earthquakes may be regarded as part of the process of diastrophic uplift, to which they contribute sporadic sudden increments. On the other hand, in certain limited

Table 1. NUMBERS OF EARTHQUAKES WITH DILATATIONS AND WITH COMPRESSIONS DOMINANT IN THE OBSERVED FIRST MOTIONS

	Dilatational	Compressional
(i) $h \geq 200$ km	12	3
(ii) $h < 200$ km		
(a) $d \geq 2{,}500$ fm	5	1
(b) $d < 2{,}500$ fm	8	36

Table 2 NUMBERS OF EARTHQUAKES ($h < 200$ KM, $d < 2{,}500$ FM) WITH DILATATIONS AND WITH COMPRESSIONS DOMINANT IN THE OBSERVED FIRST MOTIONS

	Dilatational	Compressional
Insular	1	12
Coastal	6	18
Continental	1	6

regions, as exemplified here by deep offshore trenches, the diastrophic tendency is one of subsidence, and implosive earthquakes prevail.

Because the disturbed regions of the world are by no means all alike, it is to be expected that the boundary between deep and shallow earthquakes (defined as h = 200 km in Table 1) and the boundary of ocean trenches (defined as d = 2,500 fm in Table 1) will actually differ somewhat from place to place. Furthermore, in the range of focal depth 200–700 km it is likely that a number of different polymorphic transitions would occur, and it is also conceivable that some might be explosive rather than implosive. Such variations may well account for some of the observed exceptions to the global rule given in Table 1.

The observed polarities could well be interpreted as arising not from a spherical component but from a hemispherical or higher-order one. On this supposition, I am unable to suggest how it would be possible to account for the correlations with focal depth and geographical location.

Earthquake Source

I propose that earthquakes can be classified as dominantly explosive or implosive, and that the observed polarity is in accord with the hypothesis that earthquakes are caused by sudden polymorphic transitions.

In the present investigation of the first motions observed in sixty-five well-recorded earthquakes the ratio of dilatations to compressions, or vice versa, is typically about 3 : 1. It is not suggested, however, that the minority observations are in error and that the pattern of first motions at the source is simply spherical. Higher-order components are presumably present. These in turn may perhaps be accounted for by the phase-change hypothesis; for example, a quadrantal distribution of compressions and dilatations is produced if a spherical body changes shape to an ellipsoid[9].

The conclusions arrived at in this study can be tested as observations of first motion of further earthquakes become available.

I thank Dr. R. M. Hamilton and other colleagues for their advice.

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ORIGIN OF HIGH-ENERGY ELECTRONS BEYOND THE MAGNETOSPHERE

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EVIDENCE of the existence of high-energy electrons with energy $E \geq 30$ keV in the transition region beyond the magnetosphere was reported recently by Fan, Gloeckler and Simpson[1] and also by Anderson, Harris and Paoli[2]. As there can be no possibility of prolonged geomagnetic trapping in this region, the origin of these high-energy electrons is a problem of considerable interest. We suggest that, because of the characteristic geometry and distorted structure of the interplanetary magnetic field near the magnetopause in the transition region, these electrons are locally accelerated by means of a Parker–Wentzel version of the Fermi mechanism[3–5]. (Parker considered the acceleration of charged particles trapped between two shocks which cross each other. Wentzel considered the case that charged particles trapped ahead of a single shock wave which moves into a stronger magnetic field.) The general picture is illustrated in Fig. 1*a*, a modification of Ness *et al.*'s Fig. 40 (ref. 6). In the transition region II, which starts at the shock front, the solar wind and magnetic field can be considered as frozen-in and both are influenced by the downstream magnetosphere. The solar wind compresses interplanetary magnetic field against the magnetosphere. The field strength increases with distance. According to Ness *et al.*[6], it was observed to be turbulent and unsteady with finite amplitude. The unsteadiness can be considered as magnetohydrodynamic waves[7] propagating toward the downstream magnetopause. Because of the low density, the waves suffer no dissipation until they meet each other and build up a large amplitude pulse. The total field strength along the field lines inside the transition region may be expressed as:

$$B(Z,t) = B_0(Z,t) + \sum_i H_i(Z)\,\delta\,(Z - Z_i - U_i - t) \qquad (1)$$

where Z is the distance measured downstream from shock front along the line of force, $B_0(Z,t)$ is the background magnetic field, the strength of which in general increases as Z increases and depends weakly on the time t, that is, the interplanetary magnetic field is compressed against the magnetopause (compare Fig. 1*a* and Fig. 1*b*). Z_i is the position of the ith magnetic pulse with strength $H_i(Z)$ at $t=0$, and U_i is the parallel component of Alfven wave velocity of the travelling magnetic pulse along the field line based approximately on B_0. In such a collisionless region, the thickness of these large amplitude hydromagnetic waves is expected to be of the order of the Larmor radius of a thermal ion[7], which is much larger than the electron's Larmor radius. Thus the local electrons with pitch angles larger than

$$\tan^{-1}\left[\frac{B_0(Z)}{H_i(Z)}\right]^{\frac{1}{2}}$$

will be trapped by the advancing pulse and be pushed toward the stronger magnetic field. The equation of motion for a charge particle in an adiabatically varying magnetic field is:

$$\frac{\mathrm{d}}{\mathrm{d}t}(mV_{\parallel}) = -\mu\,\frac{\partial B(Z,t)}{\partial Z} \qquad (2)$$

Here, $V_{\parallel}$ is the parallel (to the field line) component velocity of the particle and

$$\mu = \frac{W_{\perp}}{B}$$

is an adiabatic invariant, where $W_{\perp}$ is the transverse component of the particle's kinetic energy.

Equation (2) indicates, as is well known, that the longitudinal motion of a particle in an adiabatically varying field is mathematically equivalent to that of the motion of a particle under the force of a one-dimensional potential $\mu B(Z,t)$. In our case, μ being an invariant,

the potential is related to two parts of the magnetic field. One part is a "hill", that is, the background magnetic field B_0, and the other part is a series of barriers, that is, the magnetic pulses $H_i(Z)$, which move up the hill with velocity U_i. Note that the transverse kinetic energy of the particle is identical with the potential μB. Therefore the kinetic energy of the particle in this one-dimensional model is the actual longitudinal kinetic energy $W_\parallel$ in the three-dimensional space, while its potential energy is the actual transverse kinetic energy, $W_\perp$.

The collision of the particle with the advancing pulse pushes the particle up the hill until all its kinetic energy is converted into the potential energy, then the particle starts to move backward and meets with the pulse again. Each collision increases the particle's energy by an amount of $4V_\parallel U + 4U^2$. Because the pulse is moving up the hill toward a stronger field, a part of this energy is transformed to potential energy.

The collision process repeats itself until the kinetic energy of the particle becomes larger than the height of the pulse or "barrier", at the point Z_2, $W_\parallel(Z_2) \geq \mu H(Z_2)$ then the particle will roll over the crest of the pulse and go down the hill. (Some of the particles with large longitudinal kinetic energy may overcome the "hill" and leak to the dark side of the Earth.) Let Z_1 be the point where the particle was first trapped by the pulse. We have:

$$W_\perp(Z_2) = \mu B_0(Z_2) = \frac{B_0(Z_2,t_2)}{B_0(Z_1,t_1)} W_\perp(Z_1) \qquad (3)$$

$$W_\parallel(Z_2) \geq \mu H(Z_2) = \frac{H(Z_2,t_2)}{B_0(Z_1,t_1)} W_\perp(Z_1) \qquad (4)$$

On the other hand, the particle can be trapped by the pulse at Z_1, only if:

$$\frac{W_\perp(Z_1)}{W_\parallel(Z_1)} \geq \frac{B_0(Z_1,t_1)}{H(Z_1,t_1)} \qquad (5)$$

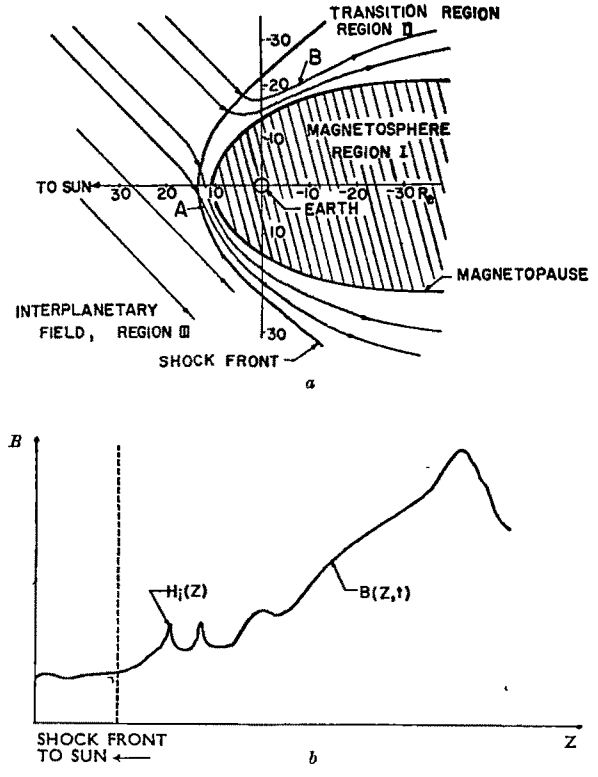


Fig. 1. *a*, The structure of magnetic field outside the magnetosphere on the ecliptic plane. Charged particles move along the field line where the line drifts towards the east because of rotation of the Sun. *A* and *B* are bottlenecks of magnetic field. *b*, Total magnetic field stength $B(Z,t)$ along the magnetic line. $H_i(Z)$ refers to the position of the pulses

Combining (3), (4) and (5) we obtain:

$$W(Z_2) \geq \frac{B_0(Z_2,t_2) + H(Z_2,t_2)}{B_0(Z_1,t_1) + H(Z_1,t_1)} W(Z_1) \qquad (6)$$

Equation (6) gives the energy gain of the particle in a single trip.

Consider Z_1 to be some point in the outer transition region and Z_2 to be some point along the background magnetic lines where the magnetic field increases in strength near the shoulder of the magnetopause. We may assume some values of B_0 and H consistent with *Explorer* 18 measurements: $B_0(Z_1) \sim 7\gamma, H(Z_1) \sim 7\gamma, B_0(Z_2) \sim 30\gamma$ and $H(Z_2) \sim 7\gamma$.

This gives $W(Z_2) \approx 3W(Z_1)$.

Clearly it is unlikely that electrons trapped only once can be accelerated to 30 keV energy.

Now consider the situation after the particle rolls over the crest of the pulse; as it moves down the hill, its transverse kinetic energy is converted into longitudinal kinetic energy. Without some kind of effective redistribution mechanism of the longitudinal energy, the particle just rolls backward over successive pulses and can scarcely be trapped again. We propose that superimposed on B_0 are small-scale magnetic turbulence or irregularities due to local current loops. These irregularities scatter electrons and randomize their energy distribution[8]. The transfer of energy by scattering from one component to another is proportional to the degree of anisotropicity. Parker[8] also found out that scattering efficiency diminishes as the scale of irregularity becomes either large or small compared to the Larmor radius of the particle and is maximum when the scale is comparable with the Larmor radius. Thus, whether the particle can be retrapped or not depends on the density of irregularities or the average scale of the magnetic eddies. If the condition

$$\frac{W_\perp}{W_\parallel} > \frac{B}{H}$$

can be re-established before the particle escapes from the transition region (that is, across the shock boundary), the particle will again be trapped by the wave train of advancing pulses and be pushed up to the stronger field. Under ideal conditions, this process of acceleration and redistribution will repeat itself and boost up the kinetic energy of the electron until its Larmor radius becomes comparable with the thickness of the magnetic pulse, which is estimated to be of the order of the Larmor radius of a thermal ion ($W \sim 1$ keV). This gives the electron energy an upper limit of 2×10^3 keV.

The time required to boost the energy of an electron to 30 keV can also be estimated. The electrons are trapped by the magnetic pulse in the outer portion of the transition region and penetrate the pulse near the magnetopause. Thus the distance travelled by the pulse is 10,000 km. Assume the propagation velocity of the pulse is ~ 100 km/sec (the Alfvén velocity in the transition region), then it takes 100 sec to triple the energy of the electron. Therefore, for an electron with initial energy X in eV, $10^2 \log_3 \frac{(3 \times 10^4)}{X}$ sec are needed to increase its energy to 30 keV. For example, to reach 30 keV energy a 10 eV electron has to be trapped seven times and the time required is ~ 10 min.

In this calculation we have assumed μ to be a constant as the particle was trapped ahead of the magnetic pulse and advancing toward a stronger field. The scattering of the small-scale field with the particle will change the value of μ and, in general, will increase the energy gain per trip. An elaborate calculation included this effect, and the effect of attenuation of large amplitude waves was carried out and will be published elsewhere[9]. However, the inclusion of these factors does not affect the basic assumptions and the major consequences of the proposed acceleration process. They may be listed as follows.

Basic assumptions. (1) There are large amplitude waves propagating toward the magnetopause causing the trapping of electrons. (2) There are small-scale magnetic irregularities or turbulences causing the redistribution of the energy of the electron. (3) The interplanetary magnetic field in the transition region is, in general, stronger near the magnetopause through compression than in the outer portion of the region.

Major consequences. (1) Only electrons are accelerated. (2) The accelerated electrons tend to bunch in the magnetic valleys because the trapping regions are ahead of the wave. (3) The velocity distribution of the accelerated electrons is anisotropic. (4) The intensity of the high-energy electrons is closely correlated with the degree of disturbance of the magnetic field in the transition region (the evidence of the presence of large amplitude waves). After the transition region is hit by a magnetic storm, there should be a time lag of the order of a few minutes before any increase in the intensity of high-energy electrons. (5) The accelerated electrons may leak out of the shock front to interplanetary space immediately after a magnetic storm. They may also be pushed over the "hill" (the bottleneck part of the field shown in Fig. 1*a*) and leak to the dark side of the Earth.

Comparison with the existing experimental measurement[1,2] indicates good qualitative agreement for consequences (1), (2), (4) and (5). The angular distribution of the high-energy electrons, so far as we know, has yet to be measured. So there is no comparison for consequence (3) now. However, we think this is the most interesting consequence. According to the proposed mechanism, after the particle has been pushed up the "hill" and is about to penetrate the magnetic pulse at Z' (compare with Fig. 2)

$$W_{\parallel} \approx \frac{H(Z')}{B(Z')} W_{\perp} \text{ and } \tan^2\theta \approx \frac{B(Z')}{H(Z')} > 1$$

But after it penetrates the pulse and moves down the hill, most of its energy is transformed to the longitudinal component. Conservation of energy gives:

$$W_{\parallel}(Z'') \approx \frac{H(Z') + B(Z') - B(Z'')}{H(Z') + B(Z')} W$$

$$\text{and } \tan^2\theta \approx \frac{B(Z'')}{H(Z') + B(Z') - B(Z'')} < 1$$

As it moves further away from the magnetopause toward the outer transition region the scattering with small-scale irregularities gradually smears out the asymmetry and and $\tan^2\theta$ approaches unit.

A measurement of the pitch angle along the field line would strongly indicate the correctness of our conjecture.

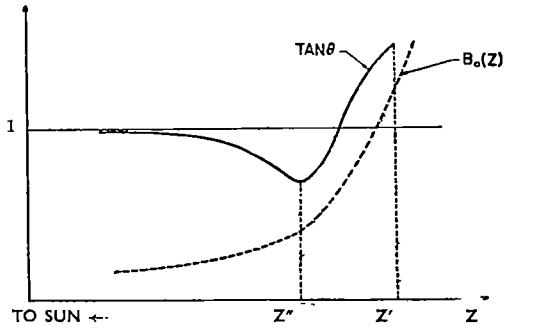


Fig. 2. Estimated distribution of pitch angle θ and the background $B_o(Z)$ along the magnetic line

Jokipii and Davis have suggested[10] that these high-energy electrons are accelerated outside the shock front (that is, in the region III) by a Fermi mechanism similar to that discussed in our model. As they pointed out, it takes 200 reflexions to accelerate a 1-keV electron to 50 keV in their model. Because the strength of the background magnetic field in the interplanetary region is more or less flat, reflexions between magnetic fluctuation and shock front increase only the longitudinal energy of electrons. Thus it requires extremely efficient randomization to keep the electrons trapped for 200 reflexions. In the model we discussed, the acceleration occurs inside the transition region near the shoulder of the magnetopause. Because of the gradual increase of the field strength there, the pitch angle of the electrons can be kept from decreasing even without efficient randomization. This is required only when the electrons are not trapped by but roll over the advancing magnetic pulse. The distance travelled then is many times that in a single reflexion, and a 1-keV electron needs to be trapped only three or four times to increase its energy to 30 keV. Thus comparatively the requirement on randomization efficiency is more relaxed in our model than in that of Jokipii and Davis.

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ON THE ORIGIN OF THE MICROWAVE BACKGROUND RADIATION

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THE recently discovered microwave background radiation[1–3] is far more intense than was expected, and its existence has led to many speculations. Between 3·2 cm and 20·7 cm its spectrum is approximately thermal with an effective temperature $\sim 3°$ K. Dicke *et al.*[4] have suggested that it is in fact black-body radiation at $\sim 3°$ K, and that this radiation is a residue from the "hot big bang" origin of the universe (this residue would be expected to consist of black-body radiation, except possibly in the neighbourhood of 300μ and 30 cm[5]). In support of this suggestion, Field and Hitchcock[6] have argued that the observed[7,8] excitation of interstellar CN molecules indicates a radiation temperature $\sim 3°$ K at a wave-length of 2·6 mm, which again is far more intense than that due to any known sources.

The demonstration that the microwave background consists of black-body radiation would be of the utmost importance for cosmology. In the first place it would almost certainly enable the steady-state model to be ruled out. Secondly, it would give considerable information about the early stages of the universe which would be of interest in itself and of importance for a number of problems, such as the formation of galaxies[9,10] and of helium[11–13], and the role of quarks[14] (if they exist) in the early stages. Thirdly, the resulting universal radiation density of ~ 1 eV cm^{-3} would be large enough to have

several important direct effects, such as inverse Compton collisions with relativistic electrons in galaxies, radio galaxies and intergalactic space[15–18], absorption of high-energy cosmic γ-rays[19,20], and the slowing-down of the highest energy cosmic ray particles[21]. In view of all these consequences of the universal existence of a 3° K blackbody radiation field it is important to be sure that there is no alternative explanation of the observations. My aim in this article is to show that an alternative explanation does in fact exist.

In the first place the CN excitation could arise from collisions with protons and electrons, as Thaddeus and Clauser[8] have shown. They find that the ionized gas density required ~ 1 cm^{-3} (not 100 cm^{-3} as Field and Hitchcock state), which is reasonable[22] if the CN molecules are formed in the vicinity of an HI/HII boundary, as Münch[23] had previously suggested. A similar suggestion has recently been made[24] for the place of origin of OH molecules. Admittedly two different regions in the galaxy show[6] a similar degree of CN excitation as would be expected if the excitation mechanism were universal, but such studies will have to be extended before this evidence becomes conclusive. Now the maximum of the blackbody spectrum at 3° K occurs near 1 mm, so if the radiation temperature at 2·6 mm is far less than 3° K, the total energy density of the universal microwave radiation field will be far less than 1 eV cm^{-3}. If, for example, the spectrum of the radiation drops sharply at wavelengths less than, say, $\sim 1{\cdot}5$ cm, the energy density will only be $\sim 3\times 10^{-4}$ eV cm^{-3} and all the scattering phenomena already described will become negligible. In this connexion, note should be made of Greisen's[21] remark that the fact that even one cosmic ray event with an energy of 10^{20} eV has been observed[25] is surprising if the blackbody radiation is present.

There remains to consider the radiation in the wavelength range 3·2–20·7 cm. The natural suggestion is that it represents the integrated radiation from a population of radio sources the spectra of which peak at centimetre wavelengths. This possibility led Shapiro and Weinreb[26] to observe at wavelengths of 2 cm and 3·75 cm the blue stellar objects with large red-shifts discovered by Sandage[27]. Their results were negative, the upper limits at the two wavelengths being 2 and 0·5 flux units, respectively (1 flux unit is 10^{-26} Wm^{-2} (c/s)$^{-1}$). In addition Howell and Shakeshaft[3] assert that it is "most unlikely to be the explanation of the 20·7 cm excess since Kellermann and Read[28] have carried out a survey of radio sources at 21 cm wavelength in an area of 1 steradian and found no such population. The integrated emission from all the radio sources at this wavelength is thought to be less than 0·1° K".

This statement of Howell and Shakeshaft's overlooks the fact that the sources which contribute most to surveys which have a limiting flux density may not be those which contribute most to the integrated background. For example, if we have two populations with concentration ρ_1, ρ_2 per unit volume and intrinsic radio luminosity P_1, P_2, their contributions to a source survey depend on their relative values of $\rho P^{3/2}$, and their contributions to the background on their relative values of ρP. We might then have $\rho_1 P_1^{3/2} \gg \rho_2 P_2^{3/2}$ and $\rho_2 P_2 \gg \rho_1 P_1$. If also the intrinsically weaker sources have a different spectrum from the intrinsically stronger ones, then the background will have a different spectrum from most of the discrete sources observed in a particular survey.

Let us see if we can construct a plausible model by exploiting this fact. In the first place we note that it is not unreasonable to postulate a population of sources the flux density of which increases with frequency at high frequencies. Sources with such spectra are already known[29–31] (the Seyfert galaxy *NGC* 1275(3*C*84), the quasar 3*C*279, 2145+06 and 0430+05). These sources actually have a minimum in their spectrum in the range 1,000–3,000 Mc/s, and it is probable that they are composite, the low-frequency radiation coming mainly from an extended envelope and the high-frequency radiation coming mainly from a compact quasi-stellar region. The compact components are probably[32,33] suffering from synchrotron self-absorption at all frequencies up to $\sim 10{,}000$ Mc/s (if these sources were thermal or were undergoing absorption by ionized gas the associated optical emission would be far greater than is observed). The sources we shall introduce must not have a minimum, however, or they will make too large a contribution to the background temperature at low frequencies. We shall therefore suppose that a quasi-stellar source which is self-absorbed out to high frequencies can form without its envelope radiating an appreciable amount of low-frequency radiation.

Secondly, we must consider the spectrum of the integrated radiation from such self-absorbed sources. An individual source will have a special index of $\sim +2{\cdot}5$ up to some maximum frequency ν_0. We shall assume for simplicity that at higher frequencies the spectral index is -1, but our results will not be sensitive to this choice. In the steady-state model the spectrum of the integrated radiation is then:

$$\nu^{2\cdot5}\left(1-\tfrac{7}{8}\left(\frac{\nu}{\nu_0}\right)^{\frac{1}{2}}\right). \qquad \nu \leqslant \nu_0$$

(In other models we would have to specify the intrinsic evolution of the sources.)

This spectrum peaks at $0{\cdot}9\ \nu_0$ and differs insensibly from the observed spectrum only in the decade of frequencies from $0{\cdot}07\ \nu_0$ to $0{\cdot}7\ \nu_0$. (A modest dispersion in ν_0 would not affect these results significantly.) We must therefore assume that the CN excitation is collisional in origin, and take $\nu_0 \sim 20{,}000$ Mc/s. Accordingly, the background radiation should peak at $\sim 18{,}000$ Mc/s (1·7 cm). In view of the uncertainty in the spectral index of the sources for $\nu > \nu_0$, and the effect of a dispersion in ν_0 and intrinsic luminosity, we may round these numbers off and predict that the background and sources will peak in the general vicinity of 20,000 Mc/s (1·5 cm).

The next question to consider is whether there are enough of these self-absorbed sources to account for the observed intensity of the background. In view of the illustrative nature of our model we shall assume that all these sources have the same intrinsic radio luminosity $P_2(\nu)$, and number density ρ_2. Similarly we shall represent the bulk of the radio sources observed down to a given limiting flux density by the quantities $P_1(\nu)$ and ρ_1. The Kellermann–Read[28] (K–R) survey at 1,420 Mc/s is consistent with the choice.

$$P_1(1{,}420) \sim 10^{25}\ \text{W (c/s)}^{-1}\ \text{ster}^{-1}$$
$$\rho_1 \sim 4\times 10^{-25}\ \text{pc.}^{-3}$$

This choice is also consistent with the median radio luminosity at 178 Mc/s of Longair and Scott[34] (based on optical identifications) and with the spectra[28,35] of these sources, and gives an integrated background temperature at 1,420 Mc/s of $\sim 0{\cdot}05°$ K, in agreement with the estimate of Howell and Shakeshaft.

Our main problem is now to estimate ρ_2 and P_2. We do this by determining whether any K–R sources which have a flux density $S \geqslant 2$ flux units are not in the 4*C* catalogue (the first part of which to be published[36] covers the regions of sky studied by Kellermann and Read). Since the 4*C* survey is complete down to a limiting flux density of 2 flux units at 178 Mc/s, such sources would have a positive spectral index between 178 Mc/s and 1,420 Mc/s, and we might assume that they are of type 2. In determining their number we excluded K–R sources in the region between 19h 35m and 20h 20m because of the confusing effects of Cyg *A*. We find then that there are no sources of type 2 out of a total of 30 K–R sources with $S \geqslant 2$ flux units. Thus at most ~ 3 per cent of the sources at 1,420 Mc/s can be of type 2. We provisionally adopt this upper limit and set:

$$\rho_2 P_2^{3/2} \sim 0{\cdot}03\ \rho_1 P_1^{3/2}$$

To account for the microwave background we need:

$$\rho_2 P_2 \sim 60\ \rho_1 P_1$$

Thus

$$P_2 \sim 3 \times 10^{-7}\ P_1$$

and

$$\rho_2 \sim 2 \times 10^{8}\ P_1$$

We thus find

$$P_2(1{,}420) \sim 3 \times 10^{18}\ \text{W (c/s)}^{-1}\ \text{ster}^{-1}$$

and

$$\rho_2 \sim 8 \times 10^{-17}\ \text{pc.}^{-3}$$

If the fraction of type 2 sources is actually $3x$ per cent, then P_2 and ρ_2 must be multiplied by the factors x^2 and x^{-2}, respectively.

A population of sources with these properties would show self-absorption out to $\sim 20{,}000$ Mc/s if they have a radius $\sim 4 \times 10^{16}$ cm, and a magnetic field strength $\sim 0{\cdot}1$ gauss. As their concentration is rather high (see later), we assume that their lifetime is long, say 10^{17} sec. Then they must be stabilized by gravitation and we would expect equipartition of energy to be set up between the relativistic electrons and the magnetic field. The total energy content at any time in these forms $\sim 10^{48}$ ergs (the radiation rate being $\sim 10^{39}$ ergs sec^{-1}). The total energy converted into radiation in 10^{17} sec is the mass-equivalent of $\sim 100\ M_\odot$. The energy content of 10^{48} ergs could be confined if the source consisted of a central mass of, say, $10^7\ M_\odot$ surrounded by a gas filling the radio-emitting region with a density of $\sim 10^4$ atoms cm^{-3}. Such sources would contribute only 5×10^{-32} g cm^{-3} to the mean density of matter in space.

The individual sources thus appear to have a reasonable structure which results in their possessing a radio luminosity at 1,420 Mc/s which is rather less than that of a normal spiral galaxy. What is more striking is that they are required to be about 3,000 times as numerous as normal galaxies. Is this a reasonable requirement? In this connexion we note that the radio sources must be very compact if they are to be self-absorbed out to 20,000 Mc/s (radius $\sim 4 \times 10^{16}$ cm). Moreover, because at low frequencies they have to be very weak radio sources, they are unlikely to be surrounded by an extensive envelope (in contrast to *NGC* 1275). We might thus expect the associated optical object to be also rather compact. Such compact optical sources are indeed known[37–39]. Arp[37], for example, has discovered a galaxy which is only 70 parsecs across. As he emphasizes, we do not know the lower limit of the amount of matter that can form an isolated galaxy. Moreover, the number of galaxies per unit volume increases rapidly as fainter galaxies are considered. The problem involved here is exactly the same as for the radio sources; surveys of galaxies down to a limiting apparent magnitude are quite inadequate for determining the contents of a unit volume of space. Furthermore, with the parameters we have adopted here, our sources would look like stars rather than galaxies. I therefore do not regard it as an impossible requirement that the faint compact sources must be 3,000 times as numerous as galaxies, although I recognize, of course, that my proposal is entirely speculative, and must be tested by direct observation.

I shall finally consider the question of the optical appearance of the sources. It does not seem possible to estimate their absolute magnitude with any reliability, although a lower limit would be provided by the likely[32,40] operation of the inverse Compton scattering of the radio photons by relativistic electrons in the source. Arguing by analogy with quasi-stellar radio sources and quasi-stellar galaxies[27] (some of which have recently been found[41,42] to be weak radio sources) we might expect our sources too to have the characteristic ultra-violet excess and to be numbered among the blue stellar objects. This would be consistent with the negative result of Shapiro and Weinreb[26] as they observed blue stellar objects with large red-shifts. At the corresponding large distances our sources would be much too faint to have been detected by them.

This discussion may be summarized as follows. If the CN excitation is collisional in origin it is possible to account for the background microwave radiation field as the integrated effect of a population of radio sources. These sources are required to have the following properties: (i) They must show self-absorption out to their peak at $\sim 20{,}000$ Mc/s. (ii) Their intrinsic radio luminosity at high frequencies must be somewhat less than that of a normal galaxy. (iii) They must be at least $\sim 3{,}000$ times as numerous as normal galaxies. (iv) Optically they may be blue stellar objects. Their integrated radiation would then have the following properties: (i) Its spectrum would be "thermal" from 1,400 Mc/s (~ 2 cm) to 14,000 Mc/s (2 cm). (ii) Its spectrum would peak at $\sim 20{,}000$ Mc/s (1·5 cm). (iii) Its energy density would be $\sim 3 \times 10^{-4}$ eV cm^{-3}, so that its scattering and absorptive power would be negligible.

Finally, I would like to point out that if my explanation is correct the existence of the microwave background would be consistent with the steady-state model of the universe.

I thank G. B. Field and J. L. Hitchcock, P. Thaddeus and J. E. Clauser, and T. Howell and J. R. Shakeshaft for informing me of their results in advance of publication. This work was begun at the Laboratorio di Astrofisica in Frascati, and I thank Prof. L. Gratton for his hospitality. I would also like to thank M. J. Rees, W. C. Saslaw, and J. R. Shakeshaft for their helpful advice.

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LETTERS TO THE EDITOR

PHYSICS

A Dense Packing of Hard Spheres with Five-fold Symmetry

MR. B. G. BAGLEY'S interesting letter[1] on this topic, by making contrasts with quintuple twins, seems continually to argue that his structure should be regarded as one pentasymmetric crystal. I suggest that this notion is invalid on two distinct grounds.

By definition, a crystal is an aggregate of particles such that each identical or 'equivalent' particle has an identical spatial environment, which includes not only co-ordination number—here always 10—but also co-ordination angles and distances. Also, by definition, the symmetry elements of a single, continuous crystal lattice can be found at 'any' place within such a structure. In the assemblage proposed by Bagley: (*a*) all the identical particles (his 'hard spheres') do not have identical spatial environments, and (*b*) the symmetry elements of one 'place' in the assemblage differ from those at other 'places'. His assemblage has, in fact, three distinct types of 'place': (1) a 'general' place; (2) a planar place; (3) an axial place—the last two being normal to the plane of the diagram (Fig. 1). Place (1) has orthorhombic symmetry; place (2) has monoclinic (*m*, 'Domatic'); place (3) pentagonal symmetry.

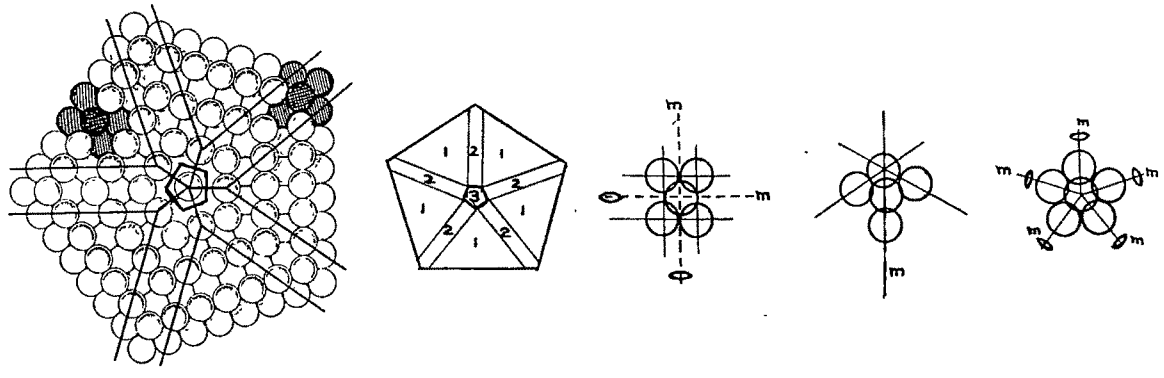


Fig. 1. *A*, "A layer of hard spheres based on a packing sequence of concentric pentagons." *B*, The three symmetry places. *C*, Orthorhombic unit from place (1). *D*, Monoclinic (*m*) unit from place (2). *E*, Pentagonal unit from place (3)

Because only place (1) is 'general', in being three-dimensional, this seems to show quite unequivocally that the assemblage is, and must be regarded as, a perfect quintuple orthorhombic twin, the five distinct portions joining each other at place (2) and place (3). Even Bagley does not seem fully convinced, as he makes a point of remarking (end of second paragraph) that the assemblage "has a *unique* axis, the *single* 5-fold rotation axis" (my italics).

Any notion that the structure is one crystal because a particular tetrahedron (Bagley's 5·15 per cent distorted one) can be found *throughout* the structure is quite invalid as (*a*) this is also true of any twinned crystal, and (*b*) the conjunctions of identical tetrahedron are different in the three regions of the assemblage. Finally, as in this structure the co-planar 'concentric' pentagons do not touch each other, no known packing mechanism would account for the continual planar growth of these concentric pentagons—a fact strongly supporting the purely five-fold axis propagation direction of extremely 'narrow' quintuple-twin 'whiskers', from some initial (and fortuitous ?) five-sphered planar ring.

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PROF. J. D. BERNAL * writes:

"I agree with Mr. Clarke's criticism of Mr. Bagley's communication. The arrangement he illustrates is definitely a twin in terms of classical crystallography for the reasons which he gives. However, this seems to me more a matter of amending precise definitions of the laws of crystallography than of insisting on them. If precise equality of interatomic distances is not required, arrangements forbidden by these laws can occur. They do, in fact, occur, not only in the cases mentioned by Mr. Bagley but also in structures of many polyhedral viruses, as discussed by Dr. A. Klug and Dr. D. L. D. Caspar[1]. Furthermore, the arrangement Mr. Bagley shows is quite a special one of pentagonal symmetry in a plane. Far more general is the arrangement in an icosahedral shell which has been described by Dr. A. L. Mackay (see Fig. 7, ref. 2). This possesses not a single five-fold axis but six of them. In other words, it has symmetry 532 but is nevertheless strictly a polysynthetic twin with orthorhombic elements. The origin of twins is not, in my opinion, at all difficult to explain. Ideally, a crystal lattice consists of an infinite number of particles all similarly situated. When the number is finite, it is impossible to satisfy the condition for each particle. It can never be similarly surrounded by others. Surface effects are bound to influence the arrangement of the particles when they are only of the order of a few hundreds of them. I have discussed the existence of such nuclei which I have referred to as pseudonuclei[3] which can never grow extensively and all crystallographers will have noticed that precipitated nuclei are often twinned from the start."

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Space-group Representation in Condensed Models of Inorganic Close-packed Structures

EARLIER I described some condensed models of inorganic close-packed structures[1]. These were based on standard transparent sheets of film, representing layers of the main types of close packing, in which the occupied holes could be marked out. An approximate three-dimensional representation was obtained by placing these sheets at equal distances in an appropriate supporting rack. It became apparent that the conventional symbols for the elements of symmetry of space groups could also be marked out on these sheets, completing the information contained in the models. The top layers of two of these models, olivine and spinel, are represented in Fig. 1*a* and *b*. When the layers are placed in the appropriate supporting rack, screw axes, inversion axes, glide planes, etc., can be easily visualized and related to the actual structure.

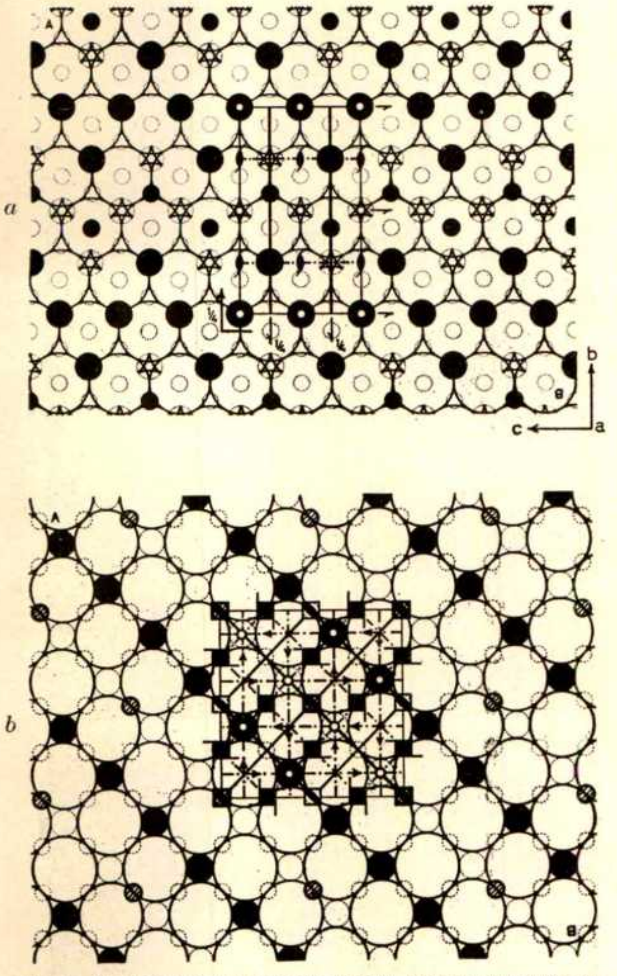


Fig. 1. Top layers of two examples of space-group representation in condensed models of close-packed structures. *a*, Olivine, Mg_2SiO_4, space group *Pbnm*; oxygen (large open circles) in hexagonal closest packing; magnesium (large filled circles) in octahedral holes; silicon (small filled circles) in tetrahedral holes. *b*, Spinel, Al_2MgO_4, space group *Fd3m*; oxygen (large open circles) in cubic closest packing ((001) face); aluminium (large filled circles) in octahedral holes; magnesium (small shaded circles) in tetrahedral holes. In the case of spinel, to facilitate the reading of the picture, only a partial representation of the space group is given (for example, triad axes, diad axes, etc., are not represented). The centres of symmetry that are marked out are at 7/8; those at 5/8 are omitted because they belong to the layer immediately underneath. The inversion tetrads that are at 3/4 are also omitted—they also belong to the layer immediately underneath

A complete representation of the orthorhombic space group of olivine is easily achieved, but in the complex example of the cubic space group of spinel a partial representation is more satisfactory. Such a partial representation enables the location in this structure of the complete representation which appears in the work of Buerger[2].

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Ice Nucleation by Proteins and Surface Films

DURING an investigation of the icing characteristics of solid surfaces, Schaefer[1] observed that certain protein films induced supercooled water drops to freeze at temperatures near zero. The possible importance of proteins as a source of natural ice nuclei cannot be over-emphasized because proteins are able to concentrate irreversibly at water surfaces and thereafter find their way into the atmosphere in particles large enough to act as ice nuclei. The ocean surface, in particular, would be an immense source of air-borne protein[2]. Furthermore, protein films are of great fundamental interest because they possess the unique property that their state of compression, and therefore their geometric similarity to an ice lattice, can be varied continuously, thus providing a means of testing the relationship between 'fit' and nucleation temperature. It is therefore surprising that while several authors[3–5] have accepted proteins as ice nucleators, no confirmation or extension of Schaefer's work has been reported.

Schaefer observed ice formation on films of egg albumen and pepsin when these films were prepared in the form of 'dipped out' layers on a chromium plated glass slide. In the present work this experiment has been repeated, the proteins (twice recrystallized, Mann Laboratories) being spread from solution on to iso-electric substrates as described by Langmuir, Schaefer and Wrinch[6]. Films of the types *PRA*, *PRB* and *PRAB* (terminology of Langmuir *et al.*) were prepared, and tested as ice nuclei by placing the film-coated slides in a cold stage and passing moist air over them until condensation appeared on the slide. On no occasion was any ice detected on the protein film unless the temperature was below $-10°$ C, this behaviour not being different from that of slides without the protein coating.

A possible reason for the failure of these protein films to nucleate ice was the lack of order resulting from the complex arrangement of amino-acids in the peptide chains. Therefore, a number of poly-amino-acids were tested as nucleators in the form of "dipped out" layers, the expectation being that the uniformity of the peptide chains might confer on the film enough two-dimensional crystallinity to promote nucleation. However, no ice nucleation was detected on either *PRA* or *PRB* films of the following polymers—poly-*l*-leucine (molecular weight of 12,000), poly-*dl*-leucine (mol. wt., 8,000), poly-*l*-alanine (mol. wt., 3,000), poly-*dl*-alanine (mol. wt., 2,500), and poly-*l*-tyrosine (mol. wt., 130,000) (supplied by Yeda Research and Development, Israel).

As cholesterol was known to be a good ice nucleator with an onset temperature of $-5°$ C (ref. 7), it was of interest to investigate its nucleating properties when deposited on chrome-plated slides using the technique of Langmuir, Schaefer and Sobotka[8]. Again no ice nucleation was obtained, even on 'built-up' layers of cholesterol up to 100 monolayers in thickness. This was perhaps to be expected because, although the packing of the interlayer spacing of the layer is almost identical with that of the cholesterol crystal[8], the molecules of the outermost monolayer are all oriented with their hydrocarbon tails facing outward, whereas the equivalent plane of a cholesterol crystal has half the molecules oriented with the hydroxyl group outward[9]. Even a 'built-up' layer which had been

scratched, split or etched showed no nucleation ability, but after the layer had been recrystallized by melting, the normal onset temperature of $-5°$ C was obtained.

To study the nucleation behaviour of some monolayers at various surface pressures, a different technique was used which enabled these monolayers to be examined *in situ*. Small gold cups, 1 mm in diameter, were rendered hydrophobic by treatment in hot chromic acid followed by ignition to red heat. After lowering the cups into a surface trough, the monolayer was spread on the water surface and compressed to the appropriate pressure. Cups were then withdrawn through the surface layer so that they were brimming with water covered by the monolayer at the pressure chosen. The freezing point of the water in each cup was determined on a cold stage. The following films were tested:

(*a*) Poly-*l*-leucine, poly-*dl*-leucine, poly-*l*-alanine, poly-*dl*-alanine and poly-*l*-tyrosine.

(*b*) Mixed films of cholesterol and lanostane in proportions ranging from 1 : 2 to 2 : 1. (The non-polar lanostane reduces the surface density of hydroxyl groups in the monolayer.)

(*c*) Atactic copolymers of vinyl benzene with methacrylic acid, the composition ranging from 5 to 20 per cent methacrylic acid (mol. wt., 30,000).

(*d*) Polyvinyl acetate, hydrolysed to various extents (80–98 per cent), thus introducing hydroxyl groups into the polyester chain.

None of the foregoing monolayers induced freezing at a temperature higher than the freezing point of the film-free water ($-20°$ C). Thus in the whole survey no surface film showed any ability to nucleate ice even though the surface density of hydrogen-bonding groups was varied over a wide range. Indeed, if polymer films were good ice nucleators they would constitute an anomaly owing to the fact that all the recognized ice nucleators, with the possible exception of the colloidal silicas[10], are crystalline.

At first sight, the present finding that pepsin and egg albumen do not nucleate ice would seem to contradict the finding of Schaefer[1]. However, Schaefer actually observed that these materials formed films which ". . . would develop a thin frost coating which would instantly seed condensed water drops as they cooled below 0° C". This observation has been confirmed, but the 'frost coating', which is a sheet of non-oriented fine-grained ice crystals, is not the result of numerous nucleation events 'on the film', but arises from spontaneous nucleation elsewhere on the slide, after which the ice front creeps slowly (0·5 mm/sec) through the fog condensed on the protein film, leaving in its wake myriads of tiny crystals. The advance of the ice front is detected only by careful observation under the microscope. Thus Schaefer's statement is true but should not be interpreted as 'ice nucleation' in the present-day sense.

The conclusion is, therefore, that pepsin, egg albumen, and a whole wide range of long-chain surface films do not promote ice nucleation, but this statement does not preclude the possibility that a condensed surface film with the correct two-dimensional geometry may yet be found to nucleate ice.

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Holographic Microscopy

THE use of holography[1] on a restricted class of objects in the visible spectrum was recognized by Gabor[2] and Parrent *et al.*[3], but a problem occurred in the contrast of the reconstructed image owing to the presence of zero-order light, as no angular interference of the object

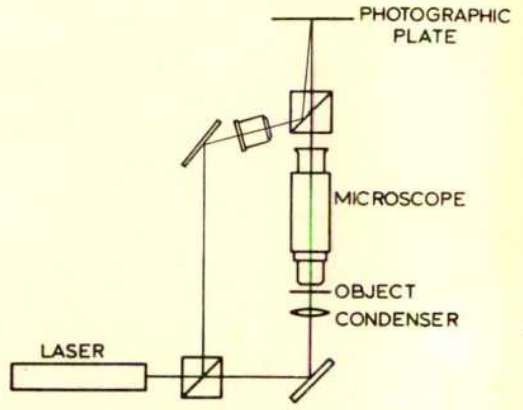
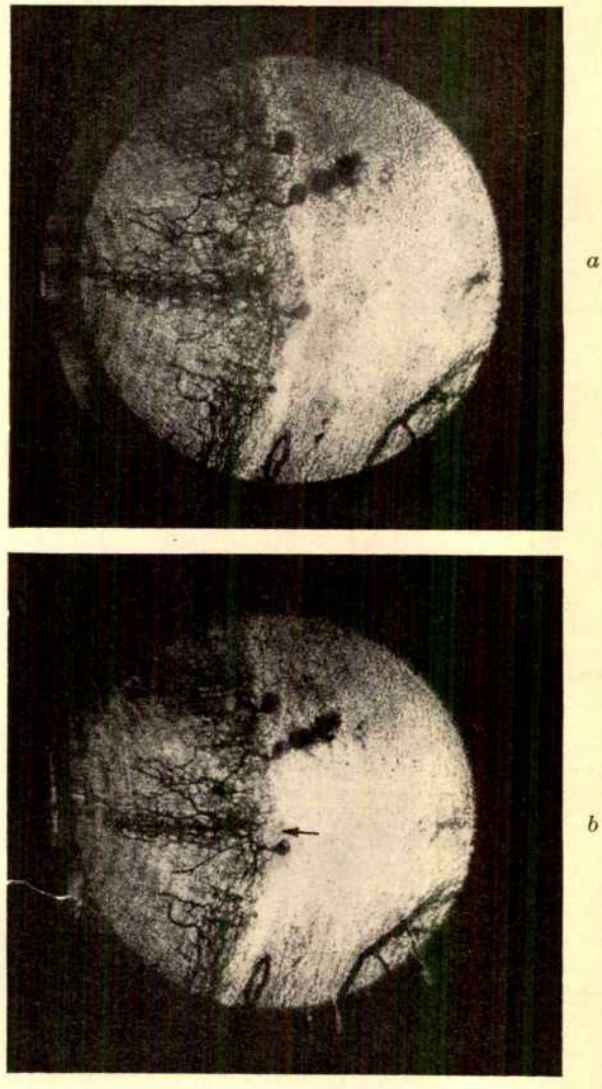


Fig. 1. Schematic diagram showing the holographic microscope



a

b

Fig. 2. *a*, Reconstruction from a hologram containing the three-dimensional record of a microscopic specimen. *b*, Reconstruction from the same hologram as in *a*, but now 40μ away in depth. The arrow indicates a fibre of about 1μ

and reference beams was used. Leith and Upatnieks[4] as well as Lohman[5] have described a technique which makes possible holography on more general and non-microscopic objects. This same technique was used to perform holography without the use of a lens on small objects by Leith and Upatnieks[6]. In experiments which we have carried out on more general objects, the optimum lower level of detectability was about 12μ when holography was performed without a lens. However, the experimental difficulties of obtaining a resolution of about 5μ are very serious. We have used a microscope (objective lens) to record holographically microscopic images of a general character. Although the attractive feature of 'lens-less' imagery was not used, the ability to record a substantial depth, as well as phase information in the object, has been preserved.

Fig. 1 shows a schematic diagram of the experimental arrangement. Fig. 2 shows a reconstruction of a stained specimen of neurones. The difference between Fig. 2*a* and 2*b* is that they are derived from the same hologram, but in planes at different depths. In the specimen this difference in depth corresponds to a de-focusing of 40μ. The arrow in Fig. 2*b* points to two fibres that have a diameter of about 1μ. In both reconstructions a set of concentric ring sections appear which result from multiple reflexions in the optics producing the reference beam.

The hologram was produced on Eastman Kodak '649-*F*' spectroscopic plate. The exposure time was 20 sec with a 4 mW output from a helium-neon laser.

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Half-lives of Barium-137*m*, Silver-109*m* and Rhodium-106 measured with Calculable Overall Accuracy

A FEW years ago, Wasserman, Twardock and Comar[1] investigated the metabolic dissociation of caesium-137–barium-137*m* in animals. A value for the half-life of barium-137*m* could be estimated from their data; it appeared to be about 2·52 min, compared with the 2·60 min previously measured by Mitchell and Peacock[2].

By separating daughter from parent by cation-exchange elution and using the ratio-method of measurement[3], an accurate value for the half-life of barium-137*m* has now been determined with a known degree of contaminational uncertainty. In this respect, any long-lived caesium-137 in the sample introduces virtually no bias into the measured half-life, despite the fact that it increases the background counting rate. Using a crystal of sodium iodide, several mutually consistent series, each comprising fifteen to thirty single measurements, gave the average value $t_{\frac{1}{2}} = 2{\cdot}557 \pm 0{\cdot}005$ min, where the stated standard deviation covers the over-all contaminational uncertainty involved. The standard deviation excluding contaminational uncertainty is lower by a factor of three, as shown in Table 1.

The presence of a contaminant with a half-life, $t_{\frac{1}{2}}^{*}$ (differing from $t_{\frac{1}{2}}$), must induce a bias on the measured value. When the ratio-method is used and the measurements are duplicated with samples 2^n times stronger at the moment of production, but $n \times t_{\frac{1}{2}}$ min older at the start of the counting period, the bias, b, on the mean, $T_{\frac{1}{2}}$, of fresh and aged samples is given by:

$$b = \frac{2^{n-n^*} + 1}{2^{n-n^*} - 1} \tfrac{1}{2}\Delta \qquad (1)$$

where Δ denotes the apparent increase in half-life due to ageing the sample for $n \times t_{\frac{1}{2}}$ min, and $n^* = (n \times t_{\frac{1}{2}})/t_{\frac{1}{2}}^{*}$.

Denoting the standard deviation by σ, we have $\sigma_{T_{\frac{1}{2}}} = \frac{1}{2}\sigma_{\Delta}$, whence, according to equation (1):

$$\sigma_b = 3\sigma_{T_{\frac{1}{2}}} \text{ for } \left| n - n^* \right| = 1 \qquad (2)$$

For barium-137*m*, Δ was found to be nil. I have made the arbitrary assumption that a null value of Δ indicates that $T_{\frac{1}{2}}$ is unbiased by any contaminant(s) for which $\left| n - n^* \right| \geqslant 1$. As can be seen from equation (2), this criterion of radio-purity only increases the over-all uncertainty of $t_{\frac{1}{2}}$ (through σ_b) by a limited factor of three. Concerning the possible contaminants for which $\left| n - n^* \right| < 1$, a thorough search through all known nuclides[4] disclosed only a few that were of sufficiently long-lived parentage to survive the year-long ageing of the caesium-137 used. Samples of barium-137*m* were carefully examined for these possible contaminants, using a γ-spectrometer, and in each case the contaminants were found to be absent, that is, so small an upper limit to their amounts could be set that the contribution to the uncertainty of $t_{\frac{1}{2}}$ was negligible.

A chain of genetically related contaminants might, conceivably, make $\Delta = 0$, and yet induce a bias on $T_{\frac{1}{2}}$. However, all significant chains would include α-emitters, and careful scannings of nuclear emulsions, into part of which samples of barium-137*m* were absorbed, revealed no α-tracks above background. After checking the method with uranium-treated emulsions, an upper limit to chain-contamination was established and was found to be negligibly small.

In the case of silver-109*m*, which was separated from cadmium-109 by a modification of Sunderman's method of isotopic exchange[5], the possibility of chain-contamination was disregarded since the parent was not a fission-product like caesium-137. Otherwise, the investigation of radio-purity was quite analogous to that already described.

In the case of rhodium-106, ruthenium-106 material purified by Glendenin's distillation procedure[6] was re-distilled, and it was found feasible to trap ruthenium tetroxide in cold 70 per cent perchloric acid, which meant that a sequence of rhodium-106 samples could be prepared by successively re-cycling parental material through the still (taking up the distillational residue in a counting container resembling a slightly V-shaped pipette with capillary stems). Some ruthenium-103 was probably present in the parental material, but rhodium-103*m* could not be detected by the stilbene crystal used. No trend was seen in thirty non-stop measurements of half-life based on thirty redistillations of a single portion of parental material and, consequently, contaminational uncertainty was assumed to be negligible in comparison with the uncertainty involved in extrapolating the observed values of $t_{\frac{1}{2}}$ to the point of zero dead-time loss of counts.

Table 1

Nuclide	Present value $t_{\frac{1}{2}}$ (sec)	Standard deviation excluding contaminational uncertainty (sec)	Standard deviation including over-all contaminational uncertainty (sec)	Previous value (ref. 7) $t_{\frac{1}{2}}$ (sec)
Barium-137*m*	153·4	0·1	0·3	156
Silver-109*m*	39·80	0·06	0·19	40
Rhodium-106	30·36	0·15	0·15	30

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Flux Relationships for Diffusion in Microcapillaries

IT is well known that flux ratios in small capillaries, that is in capillaries where the Knudsen number (N_k, defined as the ratio of capillary diameter to the mean free path of the diffusing molecule) is much less than one, vary as the square root of the inverse molecular weight ratio for binary systems. It has been shown[1] that for counter-diffusion in an 'open' system, corresponding to the physical situation in most transport experiments of the Wicke–Kallenbach type[2], flux ratio may vary from dependence on the square root to the first power of the inverse molecular weight ratio for the bulk diffusion mechanism alone. For capillaries of a fixed length, the progression from square root to first power dependence is associated with increasing Knudsen numbers, the latter mechanism corresponding to $N_k \gg 1$. It is clear, then, that at $N_k \ll 1$ the predominant mechanism of transport is that of Knudsen diffusion, and flux ratios are equal to the square root of inverse molecular weight ratios, and at $N_k \gg 1$ the predominant mechanism of transport is that of bulk diffusion. Flux ratios for sufficiently large N_k will then be equal to the first power of the inverse molecular weight ratios (provided capillary length–diameter ratios do not approach the order of unity).

In the transition region, $0{\cdot}01 < N_k < 100$, where both types of transport are of importance, it is not known whether a range of capillaries of larger diameter might be included in which under some circumstances the flux ratio for Knudsen transport (always the square root proportionality) would differ from that for the bulk mechanism (tending toward the first power proportionality for sufficiently large diameters). The overall flux ratio would depend on the relative contribution of each mechanism to the overall transport process.

We resolve this problem by determining, under specified conditions, the fraction of total momentum transfer within the capillary due to molecule-wall interactions. This fraction represents the probability, ψ, that a molecule in transit will interact with the wall. In pure Knudsen diffusion all momentum transport is due to molecule wall collisions, and this probability is unity. Conversely, for the purely bulk transport mechanism this probability is essentially zero, and in the transition region $0 < \psi < 1$. A simple momentum balance for binary diffusion under isothermal, isobaric conditions in the capillary yields:

$$N_A = \left[\frac{1}{D_A} + \frac{1 - y\alpha}{\mathcal{D}_{AB}}\right]^{-1} \nabla C_A \qquad (1)$$

in which N_A is the flux of component A in moles/time-area, D_A the Knudsen diffusion coefficient for A within the capillary, $\mathcal{D}_{AB}$ the bulk diffusivity of the A–B pair, y the mole fraction of A, C_A the concentration of A in moles/volume and α defined as $(1 + N_B/N_A)$. The bracketed term in this equation is proportional to total momentum transfer. On integration over total capillary length for unity Δy, it may be shown that ψ as obtained from the ratio of the Knudsen contribution to the total is:

$$\psi = \left(\frac{1}{N_k \alpha}\right) \ln \left\{\frac{N_k + 1}{N_k (1 - \alpha) + 1}\right\} \qquad (2)$$

If it is desired to define the transition region in terms of set limits on ψ, as above, then equation (2) provides the means for computing corresponding capillary dimensions under specified conditions of temperature and pressure. For a given value of ψ, the capillary diameter corresponding is directly proportional to temperature and inversely proportional to pressure. Capillary dimensions corresponding to the upper bound of transition zone diffusion (at $\psi = 0{\cdot}1$, for example) are of the same order of magnitude as those reported as characteristic of many porous structures, particularly those used in catalysis (with the exception of very high pressure conditions). These diameters range generally from 1000 Å to 10,000 Å, and transition region diffusion is thus probably the predominating transport mechanism in such structures.

Flux ratios for counter-diffusion in the transition region can be determined as a linear combination of flux ratio for pure Knudsen diffusion, R_k, and that for the bulk process, R_B, under the conditions of operation. The probability determined from equation (2) is utilized in this computation:

$$R_T = \psi R_k + (1 - \psi) R_B = 1/(\alpha - 1) \qquad (3)$$

in which R_T is the observed flux relationship for the combined process. R_k is, of course, always equal to the square root of inverse molecular weight ratio, and the proportionality of R_B to the inverse molecular weight ratio is determined by methods outlined previously[1]. This is a trial-and-error solution, since ψ depends on the flux ratio, as specified by equation (2).

Figs. 1 and 2 show the results of such computations for the helium–argon (I) and hydrogen–argon (II) systems, which are typical of those used in the study of diffusion through porous structures. The computations are based on an argon mean free path of 1100 Å at 300° K and one atmosphere, and, in Fig. 2, a capillary diameter of 2000 Å, and $L/D = 50$, which is an appropriate average value for catalyst support structures as discussed below. Fig. 1 demonstrates the form of ψ in terms of the Knudsen number as a function of the overall flux ratio, R_T (equation (2)). In Fig. 2 it is seen that as diameter is varied the deviation of α (or R_T) from the square root of inverse molecular weight ratio becomes apparent only for low values of (L/D). Our investigations of porous structures[3,4], however, indicate that characteristic values of (L/D) are normally of the order of 50–100, and one would expect to observe, in general, no deviations. For (L/D) of these magnitudes, indeed, further computation indicates that flux ratio deviations would never become apparent, other

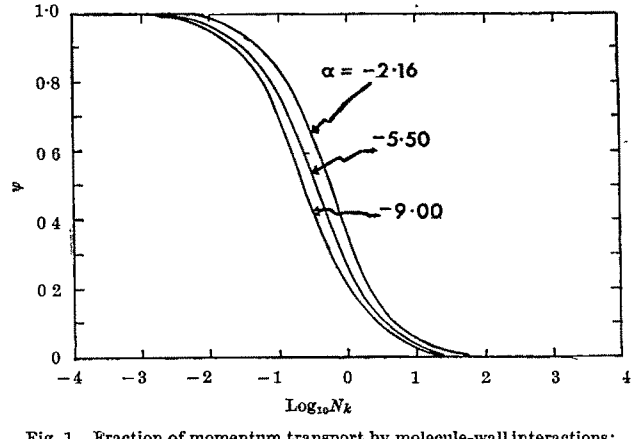


Fig. 1. Fraction of momentum transport by molecule-wall interactions: helium–argon system (equation 2)

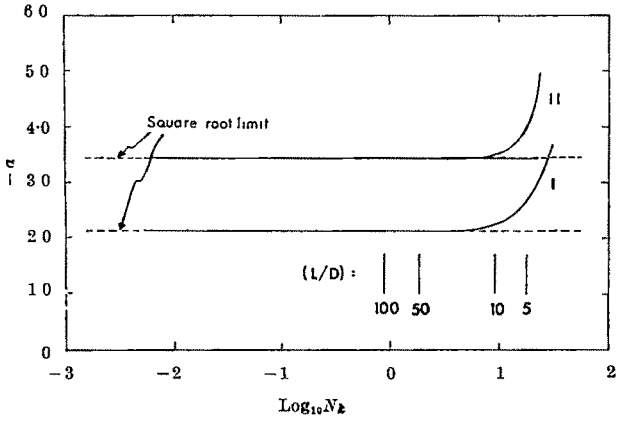


Fig 2. Overall flux ratio for capillary of typical dimensions; helium–argon (I) and hydrogen–argon (II) at 1 atm., 300° K

than at extreme conditions of pressure or temperature—in which case selective surface migration would probably be an important transport mechanism and flux ratios would be determined by other considerations.

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ASTRONOMY

Evidence of an Ephemeral Earth Satellite

The possibility that the Earth could acquire natural satellites from the debris of large meteoritic impacts on the Moon has been discussed by O'Keefe[1]. A possible capture mechanism for bodies into temporary Earth orbit has been investigated by Baker[2]. On February 9, 1913, a widely observed, long duration procession of meteors and fireballs gave rise to the suggestion by Chant, Mebane, and others[3] that they actually were such ephemeral Earth satellites which were finally consumed at perigee. More recently, a long, extremely narrow field of both craters and meteorites in Argentina was investigated by Kohman *et al.*[4] and they suggested that these were the end-result of a captured Earth satellite finally spiralling into the denser atmosphere.

During the mid-fifties I analysed evidence of two swarms of such objects in retrograde orbits[5]. However, it has never been conclusively proved that such an ephemeral satellite, with a perigee in the upper atmosphere, has been observed while in orbit and before its final revolutions. I now suggest that the Earth has had such a satellite and that it was observed at least eight times during the period 1956–65. Fortunately, these eight known observations were recorded with sufficient accuracy to permit the determination of an orbit. These observations are listed in Table 1.

The first three observations were accidentally made by Metcalfe and forwarded to me[6] as possible evidence of an inner planet. The brilliance of the objects to the naked eye caused most persons contacted to doubt that they were astronomical despite the fact that this was confirmed with optical aids. However, when Kayser and I made the fourth observation (also with optical aid) I computed an approximate orbit, assuming all four observations to be of an Earth satellite. Continued surveillance over the next 60 days during every possible evening twilight period resulted in the fifth and sixth observations being made.

Table 1

Long.	Lat.	Date	Time	Azimuth	Elevation	Magnitude	Observer
87·7 W.	39 6 N	Nov. 17 1956	01 : 20	248	20		E. Metcalfe
			01 · 33	242	0		E. Metcalfe
87.7 W.	39.6 N.	May 2 1957	01 : 20	291	20		E. Metcalfe
			01 : 37	291	0		E. Metcalfe
87.7 W.	39 6 N.	Nov 13 1957	23 : 30	287	40		E. Metcalfe
			23 · 58	287	0		E. Metcalfe
118.5 W.	34.0 N.	Dec. 9 1964	03 · 32	233	19		B. Kayser
			03 . 35 · 40	260	3·5	3	J. P. Bagby
118.5 W.	34 0 N.	Dec. 29 1964	02 . 20 : 39	250	12·5	2	J. P. Bagby
			02 · 22 . 24	280	5	2	J. P. Bagby
118 5 W.	34.0 N.	Jan. 13 1965	02 : 16 : 48	278·5	5 5	3	J. P. Bagby
			02 : 16 : 56	280	5	3	J. P. Bagby
118.5 W.	34 0 N.	Oct. 25 1965	02 · 08	231	23	2	R. M. Hartmann
			02 : 11	281	4 5	2	J. P Bagby
118 5 W.	34 0 N.	Dec. 14 1965	02 · 57 . 09	303·5	30	3·5	J. P. Bagby
			03 : 00 · 10	296	13	3 5	J. P. Bagby

Table 2

	Nov. 17.0, 1956–Dec. 29.0, 1964	Dec. 29 0, 1964–Oct. 25 0, 1965	Oct. 25 0, 1965–Dec. 14.0, 1965
a	$14{,}600-0\cdot109\,t$	$14{,}300-0\cdot109\,t-1\cdot52\times10^{-3}\,t^2$	$12{,}900-9\cdot24\,t-1\cdot03\,t^2$
e	$1\cdot00-(6{,}600/a)$	$1\cdot00-(6{,}600/a)$	$1\cdot00-(6{,}600/a)$
i	137·4°	137·4°	137·4°
R.A. Node	$86°+0\cdot820\,t+6\cdot38\times10^{-6}\,t^2$	$52°+0\cdot858\,t+3\cdot56\times10^{-4}\,t^2$	$342°+1\cdot071\,t+9\cdot40\times10^{-3}\,t^2$
π	$27-0\cdot130\,t-1\cdot52\times10^{-6}\,t$	$347-0\cdot139\,t-5\cdot50\times10^{-6}\,t^2$	$300-0\cdot172\,t-1\cdot52\times10^{-3}\,t^2$
Period	$294-3\cdot03\times10^{-3}\,t$	$285-3\cdot03\times10^{-3}\,t-4\cdot45\times10^{-4}\,t^2$	$244-0\cdot270\,t-2\cdot70\times10^{-2}\,t^2$
Epoch	Nov. 17 0, 1956	Dec. 29 0, 1964	Oct. 25.0, 1965

In the equations: the semimajor axis (a) is in km; the eccentricity (e) would be 0 00 for a circular orbit and 1 00 for a parabolic orbit; the inclination (i) exceeds 90° due to the retrograde sense of the orbit; the longitude of perigee (π) is the algebraic sum of the right ascension (R A.) of the node and the argument of perigee: the anomalistic period is in mean solar minutes; and the symbol t is the number of mean solar days elapsed since the epoch.

The fifth observation was made with optical aid, but the sixth was by naked eye only. These further observations made it possible to improve the determination of the orbits, and osculating orbits were distributed to about twenty individuals and astronomical agencies.

During most of 1965, no one else reported to me that they had seen the object. Then Hartmann and I made the seventh observation, and this further extended the orbit description. It also led to another period of evening twilight surveillance, which resulted in an eighth observation being made and further orbital refinement. These new results on orbits were recently distributed to thirty individuals and astronomical agencies, but so far no one else has reported an observation to me. It is probable that the object decayed in the Earth's atmosphere during January 1966. The orbital elements are given in Table 2.

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GEOPHYSICS

Temperature and Fractional Melting in the Upper Mantle

The velocities of compressional (V_P) and distortional (V_S) seismic waves, through the Earth's upper mantle, were computed with much detail by Gutenberg[1], who found the existence of a low-velocity layer at depths greater than about 70 km.

At least in the lower mantle, where the thermal gradient is expected to be nearly constant, Bullen[2] found that the bulk modulus ($\varkappa$) and the rigidity (μ) have a reasonably linear variation with depth, or with pressure which may be considered proportional to depth. Therefore, it was

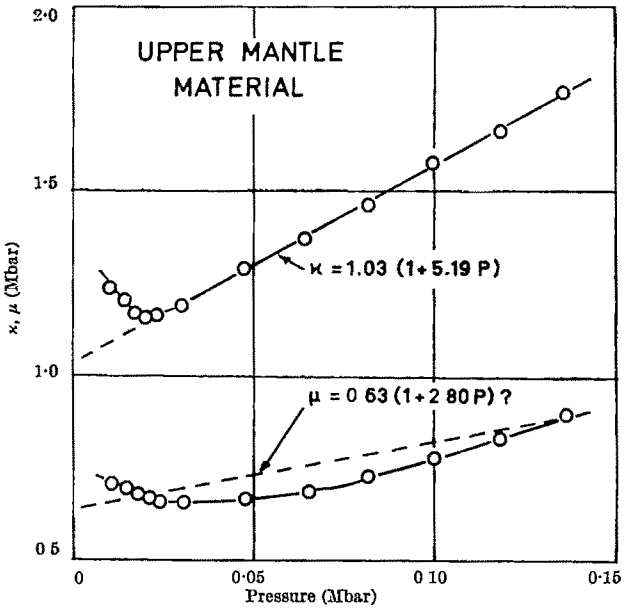


Fig 1. Variation of the bulk modulus (κ) and of the rigidity (μ) in the Earth's upper mantle

found convenient to derive these elastic moduli from Gutenberg's velocities, by the usual equations:

$$V_P = \sqrt{[(\kappa + 4\mu/3)/\rho]}$$
$$V_S = \sqrt{(\mu/\rho)}$$

ρ being the density, for which Bullen's values were adopted; equivalence of depth and pressure was also taken from the same author[3]. The results are shown in Fig. 1.

We see that at depths greater than 70 km the bulk modulus (plotted against pressure P) gives a good fit to a straight line; but no similar fit can be obtained for the rigidity. These facts were interpreted as being due to the presence of a molten interstitial fraction, in accordance with a suggestion of Wager[4]. I have considered various aspects of this elsewhere[5].

Assuming that the material is completely solid at depths of 70 km and of 400 km (which is in reasonable agreement with the relative frequency of earthquakes at the various depths[6]), the values of the rigidity of the mantle material, without partial melting, will probably obey the linear equation $\mu = 0{\cdot}63\ (1 + 2{\cdot}80P)$, with μ and P in megabars.

Knowledge of the law connecting the rigidity of a mixture with the rigidities and the proportions of the components would permit the determination of the amount of interstitial liquid at every depth. Using Eshelby's[7] equation for small spherical inclusions, the maximum liquid fraction (with $\mu = 0$) is estimated at 4·5 per cent at a 200 km depth, where $P = 0{\cdot}065$ Mbars. If, alternatively, the rigidity could be averaged proportionally to the volume of each component, the estimated liquid fraction reaches about 9 per cent. It is most desirable that some laboratory should determine the exact form of this law, as it has considerable implications for various upper-mantle models.

Concerning the thermal effects, the variation of κ and μ with the temperature T is described by the equations:

$$d\kappa/dz = (\partial\kappa/\partial P)_T \rho g + (\partial\kappa/\partial T)_P dT/dz$$
$$d\mu/dz = (\partial\mu/\partial P)_T \rho g + (\partial\mu/\partial T)_P dT/dz$$

where z is depth, g is gravity and, as usual, the subscripts T or P denote that the derivatives were carried out at constant temperature or at constant pressure.

As the upper-mantle material is at present assumed to be dunite (or some closely related peridotite), the partial derivatives can be determined in laboratory experiments on that rock, under the pressure and temperature conditions prevailing in the upper mantle. Unfortunately, not much work of this kind has been done; the most complete measurements on dunite appear still to be those of Hughes and Cross[8], who determined V_P and V_S under pressures up to 5 kbars and temperatures up to 300° C (subsequent data by Hughes and Maurette[9] confirmed the previous results). With their values for pressures greater than 1·5 kbars and temperatures of 100° C or above, the moduli κ and μ were computed and plotted on Fig. 2 (the values at pressures lower than 1·5 kbars would have been still more erratic, probably because they are affected by residual porosity). A least-squares adjustment gave

$(\partial\kappa/\partial P)_T = 16{\cdot}5 \pm 2{\cdot}8$, $(\partial\kappa/\partial T)_P = -(33{\cdot}4 \pm 4{\cdot}9) \times 10^{-5}$ Mbars/deg, $(\partial\mu/\partial P)_T = 5{\cdot}5 \pm 0{\cdot}9$, and $(\partial\mu/\partial T)_P = -(12{\cdot}7 \pm 1{\cdot}6) \times 10^{-5}$ Mbars/deg

The values of ρ and g are given by Bullen[10], and $d\kappa/dz$ and $d\mu/dz$ can be derived from the curves of Fig. 1 (it should be noted that $dz = dP/\rho g$).

Using all these values (for $z > 70$ km, $d\mu/dz$ was obtained from the aforementioned linear equation), the thermal gradient shown in Fig. 3 was computed. By a graphical integration, the temperature can also be derived and this is shown on the same figure.

The temperature obtained by this means appears to be rather high; in fact, it would imply complete melting of the mantle material below a depth of about 90 km. This is contradicted by the transmission of distortional waves, which confirms a non-zero rigidity through the whole mantle. It would seem, then, that the thermal gradient is generally high by some 7 or 8 deg/km (whereas the standard error, for most values, is only 3–5 deg/km).

It is concluded that the extrapolation of the partial derivatives of κ and μ, from a few kilobars and 100°–300° C up to 20 kbars or more, and to temperatures close to 1,000° C, is probably not valid, and laboratory work under higher pressures and temperatures is certainly desirable.

Of course, there are at least two other possibilities: one is that Gutenberg's velocities are affected by some systematic error, and the other is that dunite and its elastic properties are not representative of the solid material in

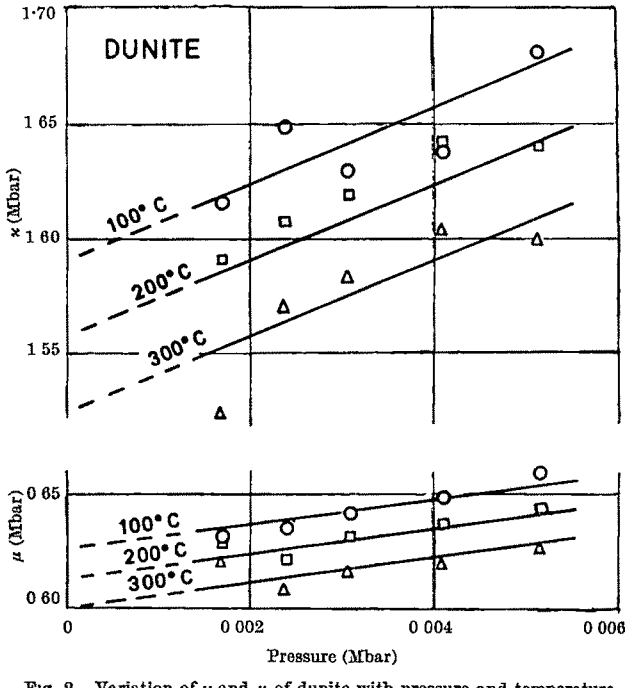


Fig 2 Variation of κ and μ of dunite with pressure and temperature (circles, squares, and triangles refer, respectively, to temperatures of 100, 200, and 300° C)

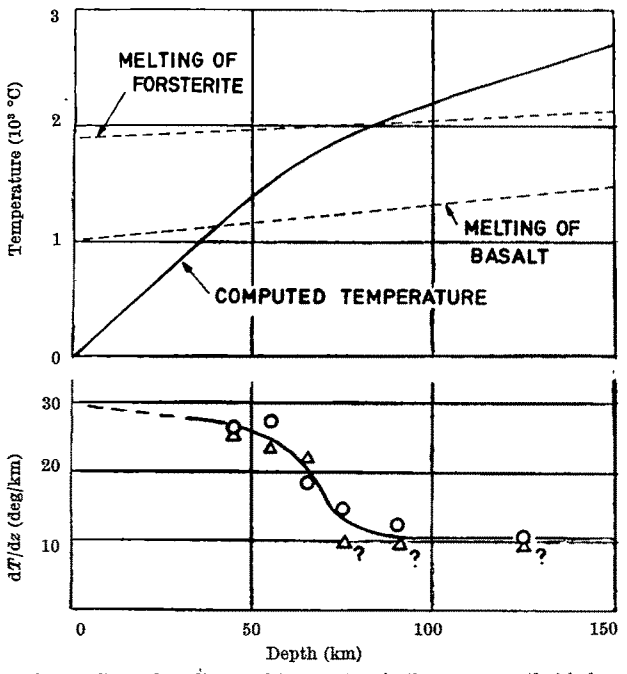


Fig. 3. Thermal gradient and temperature in the upper mantle (circles are values derived from $d\kappa/dz$; triangles are values derived from $d\mu/dz$)

the upper mantle. At present, however, both these possibilities seem to be unlikely.

This report was prepared during a visit to the Department of Geology and Mineralogy of the University of Oxford.

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Southern Storm Centres and Ocean Wave Spectra

COLLECTION of tide and wave data at Macquarie Island (54·30 S., 158·50 E.) was resumed in March 1964, 50 years after Leslie Blake operated his tidal gauge on the Island, when a simple pressure recorder was installed near the permanent Australian National Antarctic Research Expedition station. It was destroyed 6 weeks later by a severe storm after recording the Tsunami of the Alaskan earthquake of March 28, 1964[1]. An improved type of pressure recorder with wider frequency response was installed early in 1965 and began to yield satisfactory records a few days before the relief ship returned to Australia. The present communication refers to the analysis of these preliminary data and their interpretation.

Power spectra[2] computed from 20 min of sea surface records yielding approximately 800 ordinates are shown in Fig. 1 for 0000, 1200, 2400 h E.A.S.T. (Eastern Australian standard time) on March 13, 1965. After removal of the tidal trend, the autocorrelations involved lags up to 80 ordinates. Fig. 2 shows the verification of the location of the peaks in the power spectra using the high pass filter

$$Y_t = -(\tfrac{1}{4})X_{t-1} + (\tfrac{1}{2})X_t - (\tfrac{1}{4})X_{t+1}$$

applied to the original data X_t.

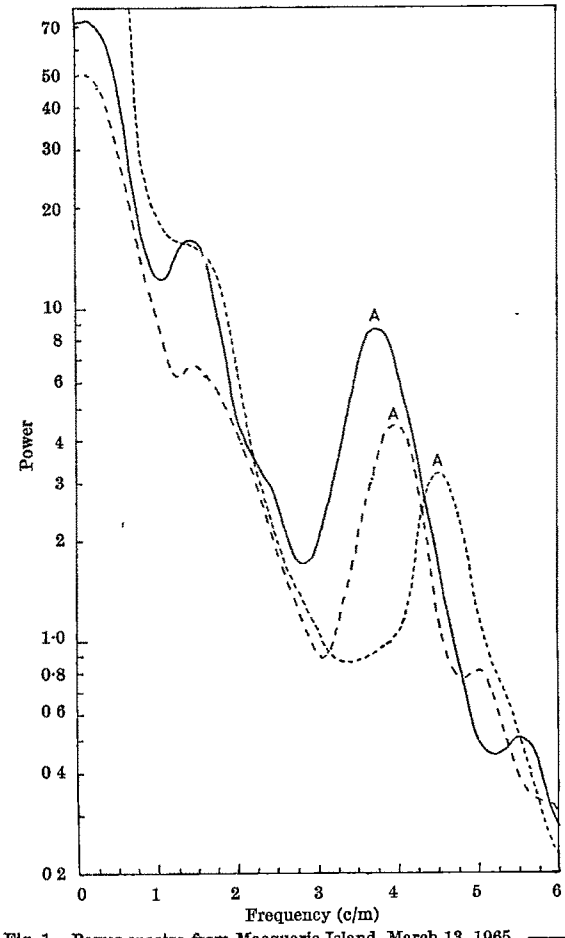


Fig. 1. Power spectra from Macquarie Island, March 13, 1965. ———, 0000 h E.A.S.T.; — · — · —, 1200 h E.A.S.T.; – – –, 2400 h E.A.S.T.

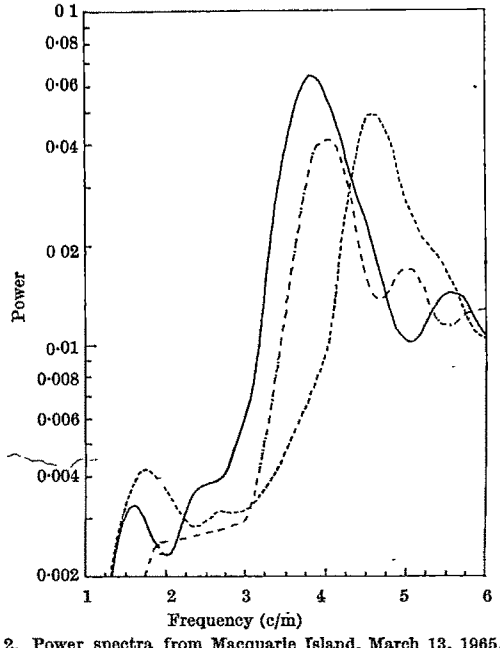


Fig. 2. Power spectra from Macquarie Island, March 13, 1965, after high-pass filtering. ———, 0000 h E.A.S.T.; — — —, 1200 h E.A.S.T., – – –, 2400 h E.A.S.T.

The peak marked A in Figs. 1 and 2 has moved during the 24 h covered by the records from a frequency $f_1 = 3{\cdot}75$ to $f_2 = 4{\cdot}5$, indicating that the corresponding waves may have originated from a storm a distance

$$X = (g/4\pi)/(\mathrm{d}f/\mathrm{d}t) \approx 2{,}500(t_2 - t_1)/(f_2 - f_1) \qquad (1)$$

from Macquarie Island, where it has been assumed that the motion of the storm centre is negligible, the depth of the ocean is constant, and the times t_i are given in days. Hence the distance of the storm centre from Macquarie Island is 3,400 miles.

Since the group velocity C_g equals half the phase velocity

$$C_g = (gT)/4\pi \simeq 2{,}500/f \qquad (2)$$

where T sec is the period corresponding to the frequency of f c/m, and C_g is given in statute miles per day, the swell of frequency $f_1 = 3{\cdot}75$ travelled approximately 670 miles/day, that is, it took 5 days to reach Macquarie Island and must have been generated on March 7.

Isobaric charts of the Commonwealth Weather Bureau in Melbourne showed a depression of 975 mbar near Kerguelen Island at 1800 E.A.S.T. on March 6, with recorded winds of 30 knots, which subsequently subdivided into two depressions of 962 and 968 mbar on March 7 (see Fig. 3). It is suggested here that this was the source of the swell recorded at Macquarie Island on March 13.

M_2 and S_2 Tidal Components at Hyderabad

As reported earlier[1] the 'Askania *GS*.11' gravimeter has been in continuous operation at Hyderabad as part of the extended programme of earth tide investigations. In its present location the instrument rests on a separate pier with a shallow concrete foundation. The 'Askania' tide recording equipment is now being used instead of the photographic registration described earlier[2]. The results of harmonic analysis of tidal data by Pertzev's method for 29 days of observation[3] (after elimination of drift by the running mean method[4]) are presented. Considering the latitude of this station (17° 26′ N.) it is expected that only the semidiurnal components would yield reliable results. So far only the two important semidiurnal components, namely, M_2 and S_2, have been evaluated and the corresponding gravimetric factor G has been calculated. In the case of the S_2 wave it should be noted that the results are only a first approximation. Using the values of G obtained, the tidal elastic constants have been calculated and are tabulated in Table 1, where h and k are love numbers, l is the Shida number, D is the diminishing factor, L is Lambert's constant and μ is the rigidity of the earth in 10^{12} dynes/cm^2.

The mean gravimetric factor is 1·17 for M_2 and 1·19 for S_2. The value of G equal to 1·17 for M_2 is in very good agreement with the results reported by Harrison *et al.*[5]

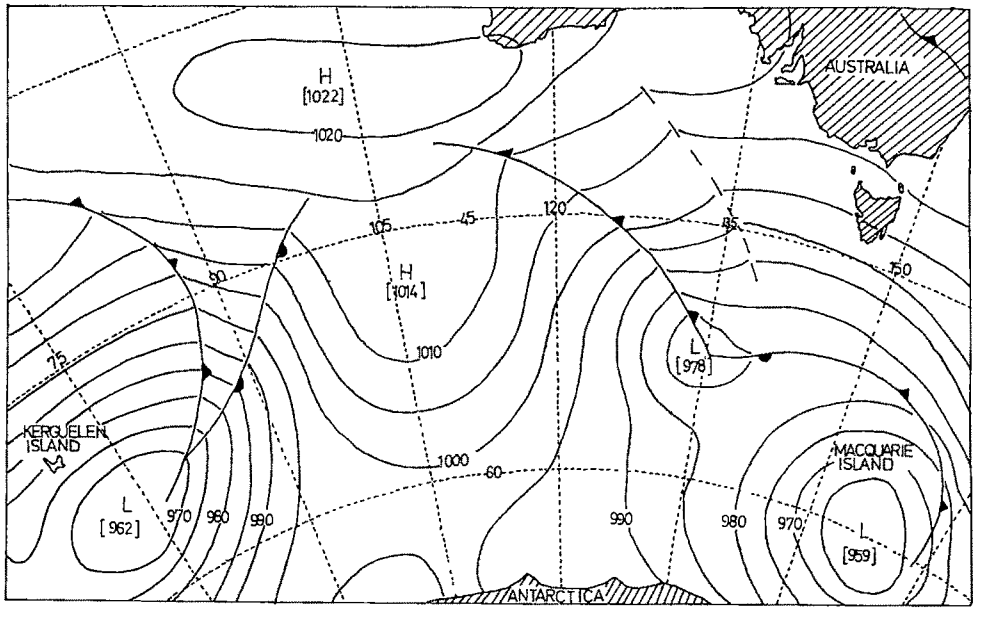


Fig 3. March 7, 1965

In the near future, similar wave recorders are to be installed at Cape Sorrell (Tasmania), Cape du Couedic, Kangaroo Island (South Australia), and a number of other locations along the Australian south coast and on Kerguelen Island. Eventually, it is hoped to separate local and global effects by auto- and cross-correlations of these records and thus to achieve a firmer basis for this method of focusing of storm centres.

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for continental stations occupied during the International Geophysical Year. For the term (S_2 K_2) they have reported a value for G of 1·20. The first approximation of G equal to 1·19 for S_2 would thus appear to be consistent with expectations. The fluctuations in the value of G

Table 1. TIDAL ELASTIC CONSTANTS

1965	Component	G	h	k	l	D	L	μ
Jan.	M_2	1·15	0·54	0 26	0 07	0·72	1·19	
	S_2	1·18	0 65	0 31	0 09	0 67	1·23	
Feb.	M_2	1 17	0 61	0·29	0 08	0 68	1 21	
	S_2	1·20	0·71	0 34	0·09	0 63	1·25	
March	M_2	1 17	0·61	0 29	0·08	0·68	1 21	
	S_2	1·20	0 71	0·34	0 09	0 63	1 25	
April	M_2	1·18	0 65	0 31	0 09	0·67	1 23	
	S_2	1·22	0·79	0 38	0·10	0·59	1·20	
May	M_2	1·18	0·65	0·31	0 09	0·67	1 23	
	S_2	1·21	0·75	0·36	0 10	0·61	1·23	
June	M_2	1·17	0·61	0·29	0 08	0·68	1 21	
	S_2	1·18	0 65	0·31	0 09	0·67	1 23	
July	M_2	1·16	0·57	0·27	0·07	0·70	1·20	
	S_2	1·18	0 65	0 31	0 09	0·67	1·23	
Mean	M_2	1·17	0 61	0·29	0 08	0 68	1·21	1·13
	S_2	1·19	0·68	0·33	0 09	0·65	1·24	

for different months in Table 1 are presumably similar to those suggested by Kramer[6] and indicate that the value of G obtained by Pertzev's method fluctuates periodically depending on the zero hour to which the amplitude corresponds. The rigidity value at this station, as calculated from the gravimetric factor G of 1·17 is $1·13 \times 10^{12}$ dynes/cm^2.

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GEOCHEMISTRY

Identifying the Geographical Source of Artefact Obsidian

DURING an avocational search for means of identifying the geographical source of artefact obsidian, what appears to be a functional tool has been found. It was postulated that, because the concentration ratio

$$\frac{\Sigma \text{ strontium}}{\Sigma \text{ rubidium}}$$

in rocks is determined by the origin and age of the rock, it would also be diagnostic of material from a given locality. This communication reports the results of preliminary experiments designed to test the feasibility of the hypothesis.

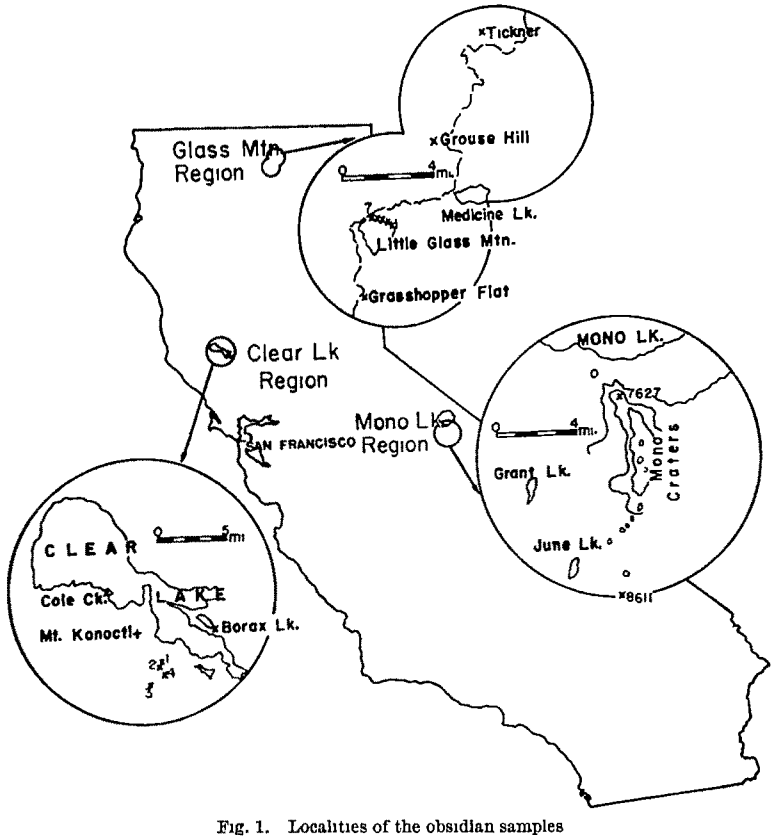


Fig. 1. Localities of the obsidian samples

Table 1. $(Sr/Rb)_a$ FOR SEVERAL OBSIDIAN SOURCES IN CALIFORNIA

Site		$(Sr/Rb)_a$	Range*	No. of samples
A. Glass Mountain Area				
(*a*) Little Glass Mountain† local site	1	0·72		1
	3	—	0·69–0·76	2
	4	0·73	0·65–0·79	2
	5	0·75		1
	7	0·69		1
Average	—	0·72	0·65–0·79	7
(*b*) Grasshopper Flat		0·50	0·49–0·53	2
(*c*) Grouse Hill		0·84		1
(*d*) Tickner Road		0·89		1
B. Mono Lake Area				
Local site	7627	$0·06_5$	$0·05_8$–$0·06_3$	1
	8611	$0·02_9$	$0·03_4$–$0·02_3$	1
Average		0·05	0·03–0·07	2
C. Clear Lake Area				
Mount Konocti‡				
Local site 1, surface		0·37		1
1·5 ft. below surface		0·36		1
8 ft. below surface		0·41		1
26 ft. ,, ,,		0·36		1
32 ft. ,, ,,		0·38		1
Local site 2		0·39		
,, ,, 3		0·39		
,, ,, 4		0·37		
Mount Konocti average		0·38	0·36–0·41	8
Cole Creek (pebbles)		0·38	0·31–0·52	2
Borax Lake		0·12	0·08–0·17	2

* Ranges include either replicate analyses on the same sample or analyses on more than one sample from the same site, or both.

† Little Glass Mountain comprises at least two flows. Samples 1–5 were taken at least ¼ mile apart around the outer perimeter of one flow. Sample 7 is from what would seem to be a second flow. The total distance from site 1 to site 7 is about 1 mile.

‡ The samples from Mount Konocti were taken from several road cuts in a distance of about 2·5 miles. The series representing several depths were taken from the side wall of a borrow pit.

Obsidian samples were collected from three regions in California, each including several local outcrops, as illustrated in Fig. 1. X-ray spectroscopy was used to estimate the strontium/rubidium ratio on samples prepared simply by sawing or grinding appropriately shaped pieces from hand specimens. The sawn or ground surface was 'X-rayed' without further treatment. A Norelco X-ray spectrograph, equipped with a tungsten target fluorescence tube, was used under the following conditions: X-ray tube excitation, 45 kV, 45 m.amp; crystal, lithium fluoride; detector, scintillation, operated at 0·95 kV; pulse height analyser, 9-V base, 16-V window; air path, 0·005 in. beam collimator, 0·040 in. detector collimator; scanning rate, 1° per min.

Fluorescent X-ray intensity was continuously recorded as 2 θ was scanned from 24·50° to 27·50° to include the strontium $K\alpha$ and rubidium $K\alpha$ emission lines. The ratio of the heights of the recorded X-ray intensity peaks was used as a measure of the strontium/rubidium concentration ratio in the sample. No attempt was made to calibrate in terms of actual amounts of strontium and rubidium present, and the ratios are labelled $(Sr/Rb)_a$ as a reminder of this fact. Our results are summarized in Table 1.

In the Clear Lake region, Cole Creek drains Mount Konocti. The obsidian from Cole Creek, which occurs as rounded pebbles in contrast to the sharp angular pieces found on Mount Konocti, apparently originated on Mount Konocti. This conclusion is supported by the close agreement in $(Sr/Rb)_a$. It is clearly possible to decide whether a sample of material known to be from the Clear Lake region came from Borax Lake or Mount Konocti. Similarly, it is easily possible to distinguish within the Medicine Lake area among samples originating from Grasshopper Flat, Little Glass Mountain or Tickner Road, but probably

not between Tickner Road and Grouse Hill. It would also seem possible to distinguish one crater from another in the Mono Crater complex, although confirmation is needed in view of the small number of samples considered.

The three regions investigated were among the most important sources of obsidian for the Indians of Northern California. Before the geographical source of artefact obsidian can be identified with confidence, however, it will be necessary to survey more completely all quarries of known or suspected significance.

When a more complete compilation of strontium/rubidium ratios is attempted, it will probably be found that greater precision in determining strontium/rubidium is needed. To permit interchange among investigators, the analyses should be calibrated so that the actual concentration ratio is reported. Refinement is possible in geological sampling, in X-ray sample preparation, and in statistical treatment of data.

Careful geological sampling is necessary to prove that variations in the strontium/rubidium ratios within a locality or outcrop are smaller than differences among localities. Our preliminary results (Table 1) indicate that this is so.

Improved sample preparation will reduce the range of values observed in replicate analyses on the same sample[2]. It is conceivable that an X-ray spectrographic technique could be developed to provide non-destructive analyses of artefacts. If this is attempted, however, attention should be paid to possible alteration in the strontium/rubidium ratio by weathering of exposed surfaces. Powdered samples prepared by grinding several small chips taken from inconspicuous places on a single artefact should prove entirely adequate.

With careful refinement of technique, it should prove possible to locate the geographical origin of obsidian artefacts with confidence and precision.

We thank the School of Earth Sciences at Stanford for the opportunity to use school facilities on an extra-curricular project. We also thank Dr. H. J. Moore for his help in collecting the samples from the Mono Lake region.

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METALLURGY

Ageing and the Bauschinger Effect in Mild Steel

As part of a larger programme of study of anisotropy and the Bauschinger effect in cold-worked metals, I conducted a series of tests on deformed and age-hardened mild steel. Only a limited series of tests were carried out and so the generality of the results has not been confirmed. However, it was considered worthwhile to report on these with the view of establishing whether such a phenomenon had been observed previously.

The experiments were carried out on annealed semi-killed 0·2 per cent carbon steel having the following composition (per cent): carbon, 0·21; manganese, 0·53; silicon, 0·23; sulphur, 0·032; phosphorus, 0·032; chromium, 0·20. Specimens in the form of strips 0·2 in. thick, 3/4 in. wide, with a 1 in. gauge length were deformed under tension to a strain of about 0·35 per cent, corresponding to a yield strength of about 50,000 lb./in.^{2}. After this advance deformation, the test pieces were subjected to ageing for 1 h at temperatures of 100° C, 200° C, and 300° C, respectively. Some test pieces were then tested again under tension while others were tested in compression. The results of these tests are shown in Fig. 1, which also shows the results of a compression test on a specimen which had not been aged. The time between deformation and subsequent testing was made as short as possible in order to avoid additional age-hardening.

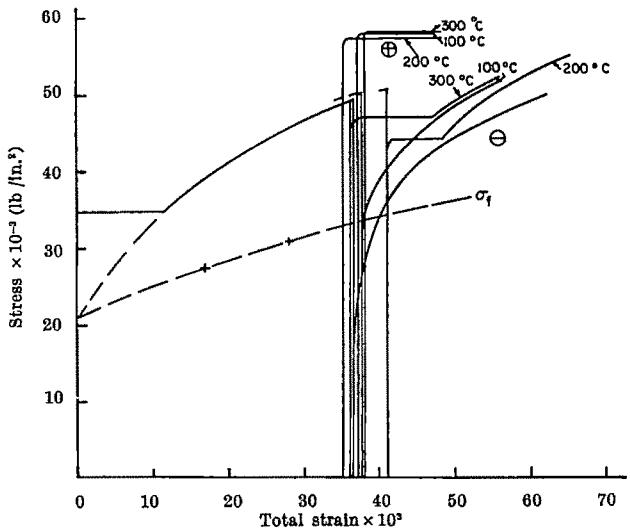


Fig. 1

If the flow stress required to maintain plastic deformation is the sum of a "frictional" stress σ_f required to overcome local stress fields and a back stress σ_b due to piled-up dislocation arrays, then:

$$\sigma = \sigma_f + \sigma_b$$

On reversal of deformation, then we may write:

$$\sigma_r = \sigma_f - \sigma_b$$

as the back stress will assist the deformation. In addition to these, ageing of body-centred cubic metals leads to locking of dislocations by solute atoms contributing a locking stress σ_l. Thus after ageing at temperature T:

$$\sigma_T = \sigma_f + \sigma_{bT} + \sigma_{lT}$$

and:

$$\sigma_{rT} = \sigma_f - \sigma_{bT} + \sigma_{lT}$$

where it is assumed that the locking stress and the back stress both depend on the ageing temperature.

Since σ_f will depend on the mean density of dislocations, it will be assumed that it is independent of ageing temperature. On the other hand, it will be further assumed that, in the limited time allowed, no dislocation locking will occur at room temperature and, therefore, $\sigma_{l_0} = 0$. As a consequence, the various components of the stress can be evaluated from the equations:

$$\sigma_f = \frac{\sigma_0 + \sigma_{r_0}}{2} \qquad \sigma_{b_0} = \frac{\sigma_0 - \sigma_{r_0}}{2}$$

$$\sigma_{lT} = \left(\frac{\sigma_r + \sigma_{rT}}{2}\right) - \sigma_f \qquad \sigma_{bT} = \frac{\sigma_T - \sigma_{rT}}{2}$$

where σ_0 is the stress at the end of pre-strain and σ_{r_0} the reversal flow stress in the absence of age-hardening. The variations of σ_{bT} and σ_{lT} have been determined from the results already presented and demonstrated in Fig. 2.

These results would seem to demonstrate an interaction between the presence of piled-up arrays and the diffusion of solute atoms. Thus, the higher interaction potential field surrounding the piled-up arrays will lead to a preferred diffusion to the head of the array. One effect of this is to relax the strain energy field leading to a reduction in σ_b, and the other is to reduce the rate of build-up of the locking stress. However, as the ageing temperature is raised, the piled-up arrays relax further and a greater proportion of solute atoms becomes effective in locking dislocation sources. Thus, σ_b decreases and σ_l increases.

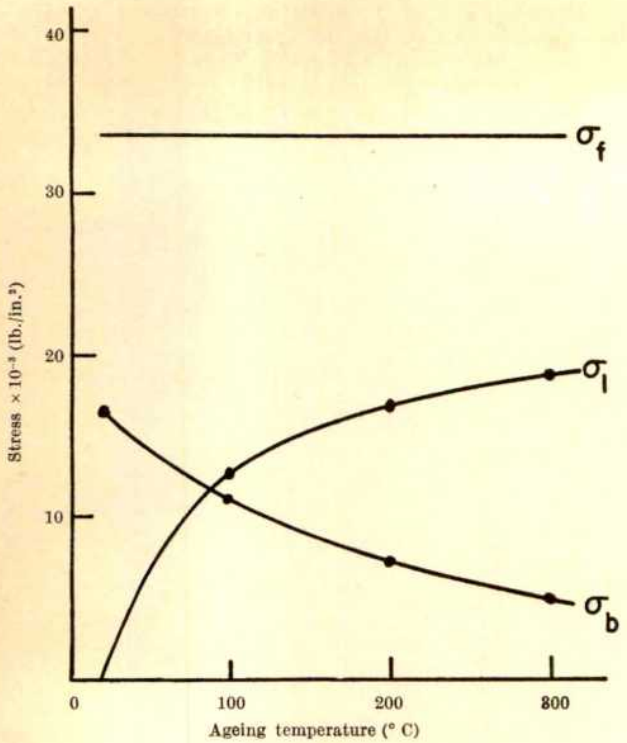


Fig. 2

Further tests indicate that the recovery temperature for this material was about 500° C. A reasonable extrapolation of the σ_b curve gives a value of zero at this temperature, that is, the Bauschinger effect would then be completely eliminated.

It may be observed that the value of σ_f so calculated is close to the value of the initial yield stress. On further investigation of other tests, however, it is seen that this agreement is a coincidence because of the pre-strain chosen. However, if the same procedure of calculating a frictional stress σ_f and a back stress σ_b is applied to these results, the variation of σ_f is shown by the dotted line in Fig. 1. As it may be observed, this curve may be extrapolated back to the zero strain axis where it meets the flow stress curve extrapolated below the flat yield plateau.

The form of the analysis then suggests that the flow stress is the sum of three components ($\sigma = \sigma_f + \sigma_b + \sigma_i$) where the locking stress has been eliminated. It is then of a higher magnitude than normally considered (for example, it is normally taken as $(\sigma_T - \sigma)$). I am planning to carry out further experiments to verify the validity of this approach.

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High-temperature Embrittlement of Stainless Steel irradiated in Fast Fluxes

IT has been widely demonstrated that irradiation in thermal fluxes results in the loss of high-temperature tensile ductility in stainless steels[1–9], and strong evidence has been advanced to support the contention that helium produced from the boron-10 ($n\alpha$) reaction with thermal neutrons is responsible for this loss in ductility[9,10]. Development of bubbles in irradiated stainless steels has been observed by thin foil electron microscopy (Fig. 1), and consideration of mechanical property, metallographic and electron microscope data led to the formulation of a theory of high-temperature embrittlement[11–13]. This was based on the weakening of grain boundaries by the migration to these areas, under stress, of helium to form bubbles larger than a critical size, failure occurring as the bubbles grew and linked together.

In predominantly fast fluxes, such as those found in the Dounreay fast reactor, capture cross-sections are, in general, much lower than in thermal fluxes, so that the production rate of helium from boron-10 is very much slower (the fast reactor rate is about 1 per cent of the thermal reactor rate). However, at high neutron energies (greater than about 3 MeV) ($n\alpha$) reactions occur in nearly all materials[14], so that helium production in a fast flux environment is not reduced by as much as might be expected. Furthermore, whereas the production of helium in a thermal flux is critically dependent on the boron content and virtually ceases when all the boron-10 has been burnt up, that is, after a thermal dose of about 10^{21} neutrons/cm², helium production in the fast flux is not so dependent on the boron content and increases approximately linearly with time—this is because every atom is a potential helium source (Fig. 2).

Table 1

C	Si	Mn	Cr	Ni	Fe	Mo	S	P	B	Co	As	N
0·036	0·44	1·67	16·84	13·25	Bal.	2·25	0·01	0·013	0·0002	0·022	0·012	0·017

Irradiation in the Dounreay fast reactor, at reactor coolant temperatures (250°–350° C) of tensile test pieces of a modified A.I.S.I. type 316 stainless steel (Table 1),

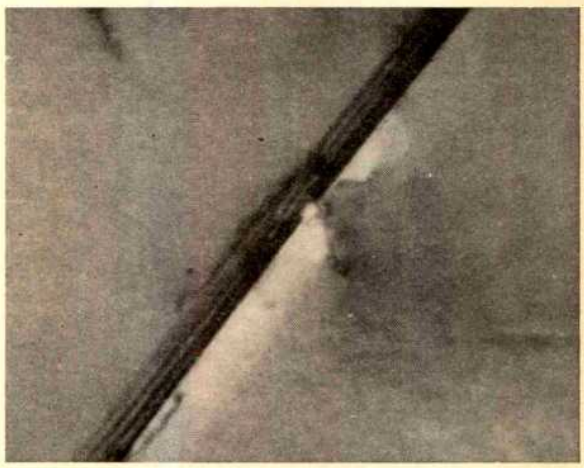


Fig. 1. Grain boundary bubble formation in irradiated type 316 stainless steel. (× c. 80,000)

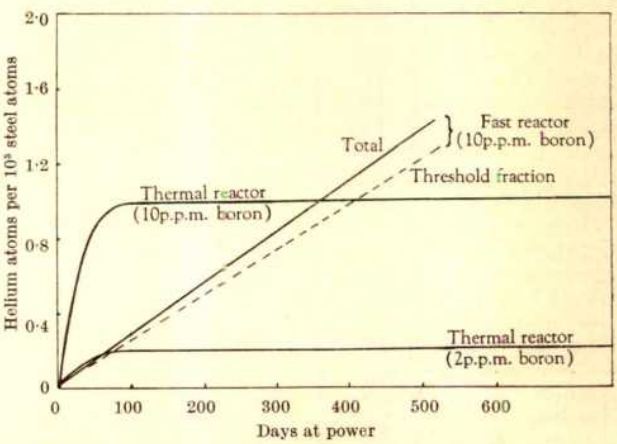


Fig. 2. Helium production in fast and thermal fluxes. Flux in the fast reactor, $2{\cdot}5 \times 10^{15}$ n/cm²sec; flux in the thermal reactor, $1{\cdot}6 \times 10^{14}$ n/cm²/sec

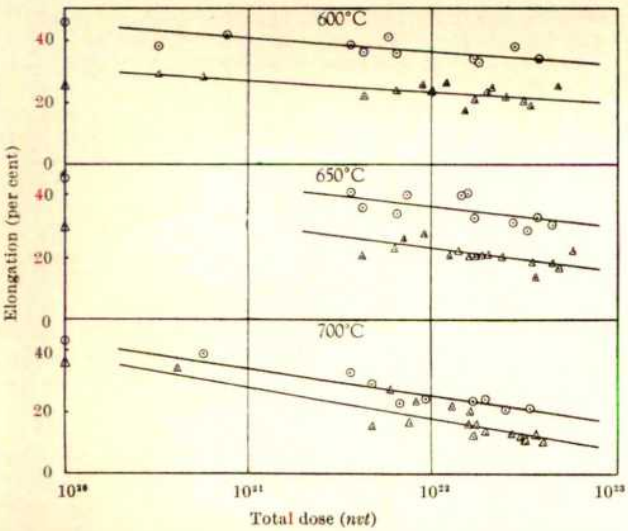


Fig. 3. Ductility of solution treated and 17 per cent prestrained 316 stainless steel at test temperatures of 600° C, 650° C and 700° C after irradiation in the fast reactor at 250°–350° C. ○, Solution treated; △, 17 per cent prestrained

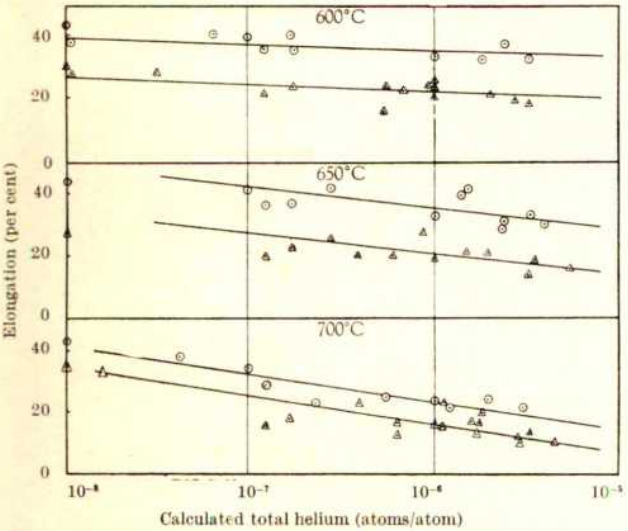


Fig. 4. Ductility/helium plot for solution-treated and 17 per cent prestrained 316 stainless steel. Tested at 600° C, 650° C and 700° C after irradiation in the fast reactor at 250°–350° C. ○, Solution treated; △, 17 per cent prestrained

has been carried out to neutron doses within the range 10^{21}–5×10^{22} *nvt*. At test temperatures >600° C (the temperature range in which high-temperature loss in ductility was observed in stainless steels irradiated in thermal fluxes), little degradation in ductility occurred with doses less than 10^{21} *nvt*. Further increase in dose above 10^{21} *nvt* resulted in losses in ductility which, over the dose range examined, were linear with log total dose (Fig. 3). Strength properties were in general little affected by increasing dose; there was, however, an increase in yield stress at 600° C, due to irradiation hardening.

It has also been shown that ductility decreases linearly with the log calculated total helium content (Fig. 4); helium contents determined in irradiated specimens by means of mass-spectrographic techniques correlated well with values calculated from reactor physics data[15].

Helium produced in thermal reactors during low-temperature irradiation does not seem to exert its full damage potential. In specimens of the same material, tested at temperatures above 600° C, the helium content required to produce the same effect on elongation in fast irradiations as that observed in thermal flux irradiations was less. One possible explanation of this is the segregation of boron in boron-rich segregates, or oxide or carbide inclusions[16], leading to localized hold-up of helium in thermal irradiations, in contrast to predominantly fast flux irradiations where the helium is formed homogeneously throughout the matrix.

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Electron Microscopic Observation of Coatings on Titanium Dioxide Pigments

IT is common practice in the final stages of the manufacture of titanium dioxide pigments to apply coatings to the crystal surfaces, in order to reduce their photochemical activity and improve their dispersibility in various media. These coatings often take the form of 1–3 per cent of oxides or hydrated oxides precipitated in an aqueous dispersion of the pigment. It is important to know whether the products are truly coated pigments, merely mixtures of the pigment and coating material, or something between the two extremes. For a uniform coating,

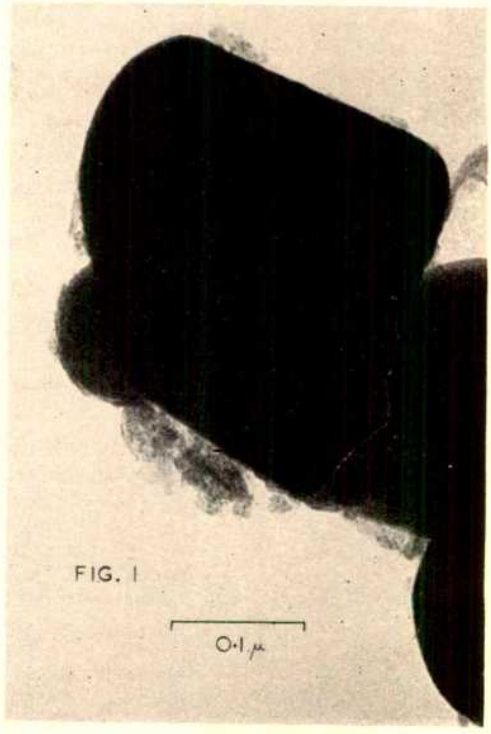


Fig. 1



Fig. 2

the thickness to be expected is about 50 Å, or one-fiftieth of the crystal diameter.

The coatings may be observed directly in the electron microscope by sprinkling the pigment on to a carbon membrane, and taking the usual precautions to minimize build-up of contamination layers. Fig. 1 shows a very uneven deposit on rutile, contrasting with the considerable degree of uniformity on pigment (Fig. 2) which is in agreement with the improved technological performance of the second pigment. Replacement of the coating by a carbon–platinum technique[1] to give enhanced contrast does not appear to be necessary, and could lead to erroneous estimations of the coating thickness.

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CHEMISTRY

Pulse Radiolysis Investigations of Unimolecular Elimination Reactions in Free Radicals produced by OH Addition to Hydroxybenzenes

During the course of the experimental research programmes in pulse radiolysis carried out independently at the Mount Vernon Hospital and the Paterson Laboratories, a new series of unimolecular free radical decomposition reactions has been observed. Some of the separate findings have been combined and are summarized in this introductory communication.

In investigations of the kinetics of the formation of quinonoid free radicals from quinones and hydroquinones[1] a reaction has been characterized which involves the unimolecular elimination of a water molecule from the OH adduct of hydroquinone (trihydroxy*cyclo*hexadienyl radical). The product of this reaction is the benzsemiquinone radical. A similar water elimination reaction has been found for the dihydroxy*cyclo*hexadienyl radical obtained by OH addition to phenols[2].

These reactions are analogous to the well-known dehydration reactions of aromatic dihydrols to form phenols in that the facility of these reactions is related to the increase in resonance energy gained when the molecules change to the completely aromatic form. Similar reactions have been observed for the OH adducts of *p*-cresol, tyrosine, β-naphthol and resorcinol. The general phenomenon is illustrated by specific reference to investigations with *p*-benzhydroquinone and phenol. The pulse radiolysis techniques used in this work have been fully described in earlier communications[3,4].

p-Benzhydroquinone. Previous investigations have shown that OH radicals react rapidly with *p*-benzhydroquinone (QH_2)[5] ($k = 1{\cdot}2 \times 10^{10}$ M^{-1} sec^{-1}). Pulse radiolysis studies, at various concentrations of neutral solutions of QH_2 saturated in nitrous oxide, however, showed the formation of two transient absorbing species. One of these species was formed during a 0·2-μsec pulse of 1·8-MeV electrons, whereas the second developed more slowly (Fig. 1). Under the conditions where all hydrated electrons react with nitrous oxide, the yield of oxidizing radicals was increased about two-fold and, because similar results were obtained when nitrous oxide was replaced by hydrogen peroxide which also converts e^-_{aq} to OH radicals, it was tentatively concluded that the first transient was formed by addition of OH radicals to the aromatic ring.

The transition to the species with an absorption maximum at 4250 Å was kinetically first-order under all conditions and, since the reaction order was unaffected by the absolute value of dose/pulse, doses of between 1 and 2 krads were generally used. The experimental rate constant of $4{\cdot}6 \times 10^4$ sec^{-1} at 20° C was independent of solute concentration but showed a small temperature coefficient. An activation energy of 4·2 kcal M^{-1} was obtained. The reaction became very rapid at values of *p*H on either side of neutrality indicating strong acid-base catalysis.

The second transient species disappeared by simple second-order kinetics, but the absolute rate was slower in neutral solution compared with that at *p*H 2. Salt effect studies demonstrated that the species decaying in neutral solution carried a unit charge consistent with its assignment to the anion of the benzsemiquinone radical.

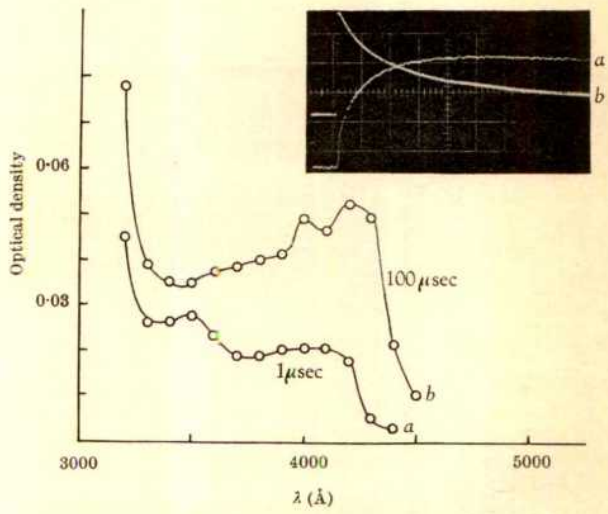
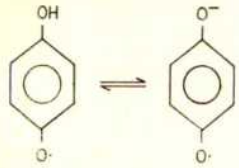


Fig. 1. Transient absorption spectra after pulse radiolysis of 5 mM hydroquinone saturated with nitrous oxide. Curve *a*, Absorption immediately after the pulse; curve *b*, absorption after 100 μsec delay. *Inset*, Oscillogram showing: *a*, transition from the OH adduct to the semiquinone ion ($\lambda = 4180$ Å); *b*, decay of the semiquinone ion. Ordinate, percentage absorption, 2·6 per cent per large division; abscissa, (*a*) build-up, 20 μsec per large division; (*b*) decay, 1 msec per large division

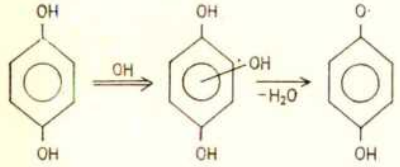
The absorption spectrum was similar to that previously attributed to semiquinone anions[6,7,11].

Investigations of the effect of *p*H on the absorption at 4300 Å have shown that the *p*K of the equilibrium (1) is 4·0.



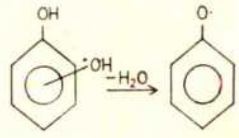
(1)

The foregoing evidence supports the view that the oxidation of hydroquinone by OH radicals proceeds according to equation (2).



(2)

Phenol. Previous investigations[8] have shown that OH radicals react very rapidly with phenol to form dihydroxy*cyclo*hexadienyl radicals, λ_{max} 3200 Å. Pulse radiolysis of aqueous solutions of phenol (10^{-3} M) saturated with nitrous oxide showed that in addition to the adduct radical formed immediately with λ_{max} at ~3300 Å a secondary transient species with maxima at 4000 Å and 2950 Å built up during the first few hundred μsec after the pulse (Fig. 2). The spectrum of the secondary species was consistent with it being due to the phenoxyl radical Φ0· (ref. 9). In this case, a low pulse dose (13 rads) was necessary in order to reduce second order radical-radical reactions to an insignificant level, Φ0 being relatively unstable compared with the benzsemiquinone anion. The rate of build-up of Φ0· at 4000 Å was found to follow first-order kinetics ($k \sim 5 \times 10^3$ sec^{-1} at 20° C), and was about equal to the rate of decay of the 3300 Å absorption, when these low doses were used (Fig. 3). The reaction was temperature-dependent and acid-base catalysed, as was the formation of $\dot{Q}^-$ from trihydroxy*cyclo*hexadienyl radicals. These results suggest that elimination of water accounts for the decay of most if not all of the OH adduct radicals formed (3).



(3)

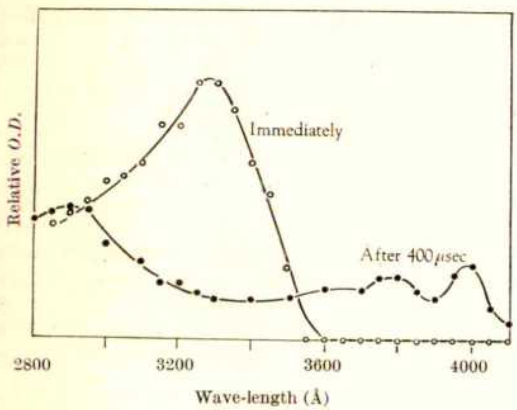


Fig. 2. Transient absorptions after pulse radiolysis of 10^{-3} M phenol in water, saturated with nitrous oxide (dose ~140 rads)

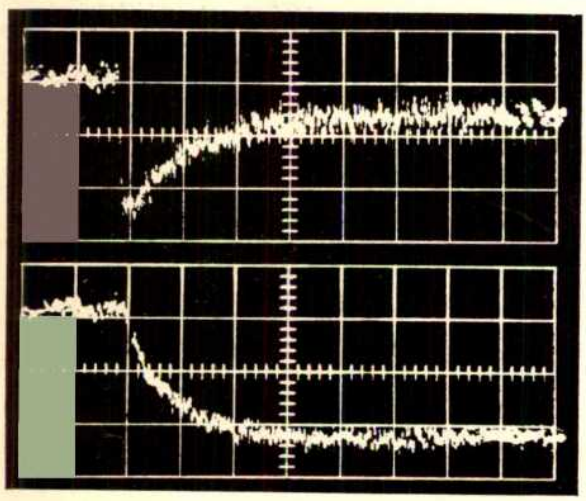


Fig. 3. Oscilloscope traces of decay of initial transient absorption at 3300 Å (top) and formation of secondary transient absorption at 4000 Å (bottom). Dose ~13 rads. Vertically upward displacements, increasing transmission. Horizontal displacements, time (1 large division = 200 μsec)

These investigations are being extended to aqueous solutions of aromatic amines, and preliminary pulse radiolysis experiments on solutions of aniline and *N*,*N* dimethyl aniline show the eventual analogous formation of highly resonance stabilized anilino radicals[10].

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Ionization Detector for Gas-phase Titration

GAS-PHASE titrations are rarely used because of the lack of suitable and sensitive titration end-point detectors. We have found that ionization detectors[1] are eminently suitable to follow the course of a gas–gas reaction in a continuous flow system. The response of such detectors is, in general, unspecific. A number of detectors were investigated; the following discussion deals with ionization cross-section detectors.

These detectors respond to all gases and their output current is directly proportional to gas concentrations from 0 to 100 per cent and to the ionization cross-section of the gas molecules. Changes in the flow rate and the polarizing voltage have negligible effect on the output when the detector is operated at the plateau of its current-voltage characteristic.

Fig. 1 shows a schematic diagram of the titration system. A carrier gas flowing with constant velocity is continuously mixed, in a reaction chamber (*A*), with a sample gas, also flowing with constant velocity, and with a titrant gas. The gas mixture then enters the cross-section detector (*B*). The detector is polarized by the voltage supply (*D*) connected in series with the current meter (*C*). The flow rate of the titrant is varied from zero to a value significantly larger than that corresponding to the end-point; the current values are plotted against the flow rate of the titrant introduced into the carrier flow.

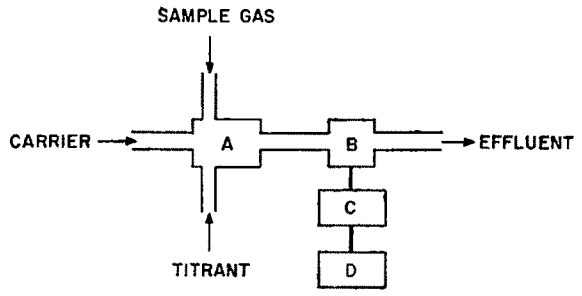


Fig 1. Schematic arrangement of gas–gas titration system with ionization cross-section detector *A*, Mixing and reaction chamber; *B*, ionization detector; *C*, current meter; *D*, voltage supply

Assuming perfect mixing of all gases introduced in the system, 100 per cent yield of reaction, and collection and production of ions in the detector without losses, the detector current is:

$$I = K \frac{PV}{RT} \sum_i Q_i x_i \qquad (1)$$

where P is the pressure in the detector, V its active volume, R the gas constant, T the absolute temperature and K a constant determined by the dimensions of the detector and the radiation intensity. Q_i and x_i represent the ionization cross-section for the type of ionization present in the detector and the molar fraction of the constituents of the gas mixture entering the detector.

The process for the calculation of the detector current is shown in Fig. 2 for a reaction:

$$A + B \rightarrow C \qquad (2)$$

where A is the unknown sample, B is the titrant and C is the product of the reaction. Fig. 2*a* shows how the molar concentration of each of the components of the reaction changes during the titration. The concentrations of the carrier and of the product decrease after the end-point because of an increase of the total flow rate.

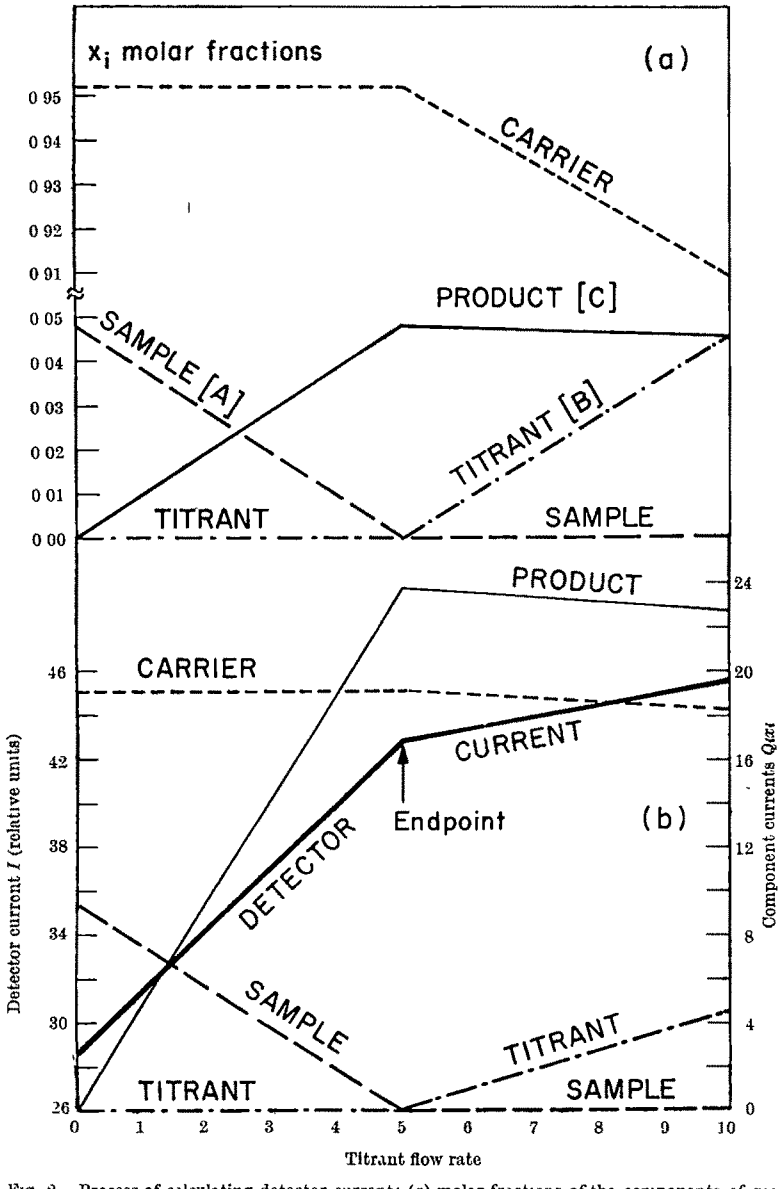


Fig 2 Process of calculating detector current: (*a*) molar fractions of the components of gas flow systems, (*b*) component and detector currents

In Fig. 2*b* the independent current contributions of each of the components are plotted according to equation (1), and assuming the following values of relative ionization cross-sections:

$$Q_A = 2,\ Q_B = 1,\ Q_C = 5 \text{ and } Q_{\text{carrier}} = 0{\cdot}2$$

These values are closely related to possible experimental conditions. The ionization cross-section detector current I is the sum of all the component currents at each value of titrant flow rate. The titration end-point appears as a change in the slope of the plot of current against titrant flow rate.

The slope of the titration curve is directly related to the cross-section, concentration and ratio of the components of the titration and to the rate of the reaction. The greater the difference between the cross-sections of the reacting species and the product, the sharper the break will be at the end-point. A carrier with much lower cross-section than the other components of the reaction will decrease the background current.

The method provides a new technique for the analysis of gases. Appropriate selection of the titrant can provide specific determination of a reacting gas in a mixture. Furthermore, the direct relation between the detector current and the concentrations and cross-sections of gases present during a reaction can be used in kinetic studies of gas-phase reactions.

Several gas-phase titrations have been measured. An example, the result of the gas–gas titration, measured with an ionization cross-section detector, of oxygen with nitric oxide represented by the reaction:

$$O_2 + 2NO \rightarrow 2NO_2 \qquad (3)$$

is shown in Fig. 3. Additional research is being conducted.

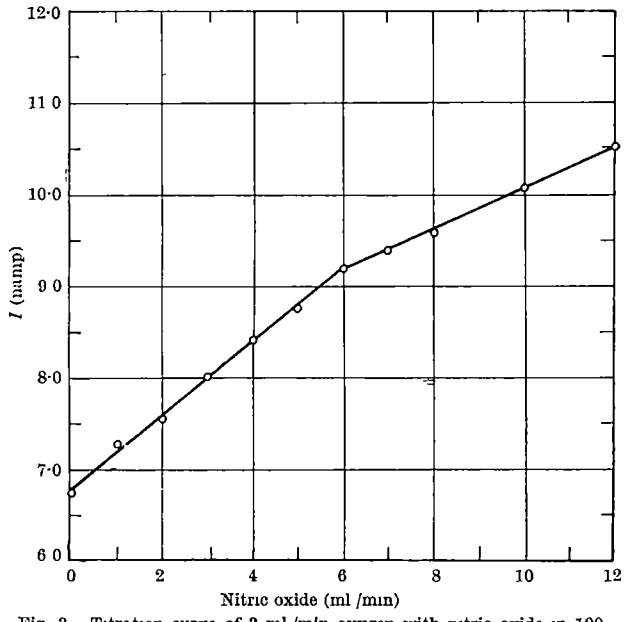


Fig. 3. Titration curve of 3 ml./min oxygen with nitric oxide in 100 ml./min helium carrier (*T*, 150° C)

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Isotope Fractionation of Methyl Esters by Gas–Liquid Chromatography

THE radiohomogeneity of tritium- and carbon-14-labelled methyl linoleate *in vivo* and the stability of the labelled methyl esters during gas–liquid chromatography has recently been investigated[1]. It was shown that the tritium : carbon-14 ratio of the entire peak was unchanged by gas–liquid chromatography. However, when portions of the effluent peak were collected the tritium : carbon-14 ratio varied. In view of the separation potentialities of gas chromatography, it seemed worthwhile to investigate whether or not any difference in the mobilities of the two isotopic molecules during chromatography caused these variations. Accordingly, several columns containing commonly used polar and non-polar phases were used and a number of methyl esters labelled with both tritium and carbon-14 were chromatographed. The tritium : carbon-14 ratios obtained for successive fractions were examined for evidence of any difference in the mobilities of the two compounds.

Linoleic acid labelled with tritium at the 9, 10, 12 and 13 positions was prepared as reported earlier[2]. Linoleic-1-^{14}C acid was purchased from California Corporation of Biochemical Research and it was purified on a silica–silver nitrate column[3]. Stearic and oleic acid doubly labelled with both isotopes were prepared as described[3]. Methyl esters of all fatty acids were formed by reaction with diazomethane. Gas–liquid chromatography was performed by means of an Aerograph model *A*-90-*C* (Wilkens Instrument and Research, Inc.) equipped with a thermal conductivity detector. Copper columns 3·5 in. × 6 ft. were packed with either 20 per cent diethylene glycol succinate polyester (DEGS), 14 per cent ethylene glycol succinate or 1 per cent *SE*-30 on 'Gas Chrom *P*' operated at temperatures ranging between 160° and 190° C. The column effluents were collected on cartridges packed with *p*-terphenyl impregnated with 5 per cent *DC*-550 silicon oil[5]. An automatic fraction collector (Packard 'Tri-Carb' model 830) was used and fractions were collected every 20 sec throughout the fatty ester peak. At the completion of the analysis each cartridge was removed from the fraction collector and was placed in a scintillation vial[4]. The background radioactivity of the column was checked before analysis, and whenever it was found to be higher than the normal the column was replaced.

Scintillation counting was carried out in a Packard 'Tri-Carb' model 314 *EX*. Channel settings were: lower channel 400–100 division, gain 500: upper channel 76–400, gain 1,000: coincidence monitor *A'* setting 7·6, gain 1,000 : voltage setting 1,150. Computation of the number of disintegrations (d.p.m.) tritium, d.p.m. carbon-14 and the isotope ratio tritium : carbon-14 from the counts in each channel was made according to the method of Kabara *et al.*[5]. In addition, three forms of computation were applied, that is, probit analysis based on cumulative percentage, fraction by fraction, of each isotope eluted, ln φ (φ = ratio of tritium : carbon-14) and MODI-φ based on the isotope ratio of successive fractions by using an established programme in the IBM/620 computer[6].

The tritium : carbon-14 ratios among the fractions throughout the fatty ester peak in a number of chromatographic runs are presented in Table 1. A progressive decrease in the tritium : carbon-14 values indicated that fractionation occurred during the chromatographic process and that the tritium-labelled methyl ester moved ahead of the carbon-14-labelled methyl ester. The presence of one or two double bonds in the fatty acid chain did not seem to be critical as the effects were similar for methyl stearate, oleate and linoleate. These effects were more apparent in non-polar than in polar columns, becoming almost negligible when an aged polar column was used. Frequently, the tritium : carbon-14 ratio increased in the tail of the peak.

Table 1. TRITIUM : CARBON-14 RATIO OF FRACTIONS COLLECTED EVERY 20 SEC AFTER THE INJECTION OF METHYL ESTERS OF FATTY ACIDS DOUBLY LABELLED ON DEGS AND *SE*-30 COLUMNS IN THE GAS CHROMATOGRAPH

Column	Methyl ester: Stearate New	Stearate After 3 wk.	Oleate New	Oleate After 3 wk.	Linoleate New	Linoleate After 3 wk.
SE-30	2 30	2·40	2 78	2·96	1·82	2·35
	1·98	2·34	2·72	3·20	1·91	2·37
	1 83	1·91	2 62	2·81	1·77	2·05
	1·72	1·82	2 34	2·50	1 65	2·10
	1·69	1·73	2·50	2·47	1 69	1·95
	1 52	1·62	1 97	2·36	1·53	1·92
	1·48	1·51	1·73	2·31	1·50	1·46
	1·53	1·80	3·06	1·34	1·48	1·26
	1 65	1·85	—	1·69	1 44	—
	—	—	—	3·45	1 58	—
	—	—	—	—	1·65	—

Column	Methyl ester: Stearate New	Stearate After 3 wk	Oleate New	Oleate After 3 wk.*	Linoleate New	Linoleate After 3 wk
DEGS	1·87	2·70	2·66	—	2·73	2·42
	1·62	2·66	2 47	—	2·68	2·76
	1·66	2·64	2·50	—	2·83	2·56
	1·73	2·71	2·22	—	2 86	2·66
	1·63	2·56	2·29	—	2·77	2·79
	1·46	2·69	2·25	—	2·74	2 61
	1·42	2·67	2·01	—	2·76	2·55
	—	2·62	2·07	—	2·61	2·61
	—	2·67	2 34	—	2·58	2·63
	—	2·59	2·28	—	2·54	2 60
	—	2·63	2·40	—	2·67	3 02
	—	2 68	—	—	—	—
	—	2·72	—	—	—	—

* Not determined.

The results of the runs recorded here were carefully examined for artefacts. Radiochemical artefacts were excluded when the methyl esters were subjected to several criteria for radiochemical purity[3]. Instrumental artefacts were examined and were found to be absent. For this purpose, radioactivity of either type was added to each fraction and it was fully recovered. On the other hand, the same mixture at several count rates gave the same tritium : carbon-14 ratio excluding any dependence

of the isotope ratio on count rate. The possibility of chromatographic artefacts was given special consideration. Chromatographic fractions from all regions of the peak were rechromatographed on a 'Hy-Fi' Aerograph model 600 *B* equipped with a flame detector. In all cases, no other peak than that anticipated appeared. Memory effects due to a binding of the labelled methyl ester on the column and subsequent elution in the following chromatographic run were ruled out when cold methyl arachidate (Applied Science Corporation State College, Pennsylvania) was included in the injected sample and its entire peak was collected and measured for radioactivity. Only traces of tritium and carbon-14 were present in the methyl arachidate eluate.

It seemed reasonable that fractionation of the two differently labelled methyl esters occurred during the chromatographic process. Calculation of the displacement of either isotope from the other was made by the three methods indicated and the results using data from chromatographic runs of doubly labelled methyl linoleate are recorded in Table 2. It is evident that the tritium-rich molecule displaced the carbon-14-labelled molecule (M_1 M_2) in all cases where displacement was observed.

Table 2. COMPARISON OF RESULTS FROM PROBIT ANALYSIS BASED ON CUMULATIVE PERCENTAGE, FRACTION BY FRACTION OF EACH ISOTOPE ELUTED

Ln-φ and MODI-φ were based on the isotope ratio of successive fractions obtained from an established programme in an IBM/620 computer (ref. 6)

	SE-30 New column	*SE*-30 Aged column	DEGS New column	DEGS Aged column
M_1	12·129 ± 0·073	13·338 ± 0·065	11·849 ± 0·107	9·123 ± 0 119
M_2	12·927 ± 0·078	12·923 ± 0·053	11·739 ± 0·102	9·158 ± 0·114
σ_1	3·380 ± 0·086	2·360 ± 0·085	4 513 ± 0 110	2·990 ± 0·116
σ_2	3·490 ± 0 093	2·256 ± 0·067	4·470 ± 0·105	2·992 ± 0·113
M% Probit	1·536 ± 0·821	3·108 ± 0·631	0·923 ± 1·253	0·386 ± 1 814
Ln-φ	2·155 ± 0·434	2·713 ± 0·604	0·730 ± 0·288	0·239 ± 0 331
MODI-φ	2·141 ± 0·338	2·802 ± 0·496	0·697 ± 0·189	0·249 ± 0·334
Fractionation	Present	Present	Present	Absent

M_1, Retention volume and σ_1 dispersion of tritium-labelled fatty acid methyl ester

M_2, Retention volume and σ_2 dispersion of carbon-14 labelled fatty acid methyl ester.

These results were in accord with those obtained in previous investigations and demonstrated that gas–liquid chromatography, utilizing commonly used columns, has sufficient resolving power to segregate isotopic molecules that differ less than 2 per cent in mass. Although the existence of isotopic fractionations in many chromatographic processes[3,6–12] may be possible, the mechanism by which these effects occur is unknown in most cases.

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Osmotic Emergency Purifier of Sea Water

WHEN a hypertonic solution is separated from a hypotonic one by a semipermeable membrane, solvent will flow from the hypotonic to the hypertonic system until equilibrium is established. Sea water is essentially a 0·6 N solution of polar solutes in water. A membrane which would, under certain conditions, reject sodium and larger ions and allow migration of water free of solutes can be obtained[1]. Cellulose acetate, for example, fulfils the condition of rejection of sodium chloride[2]. A semipermeable membrane system for concentrating beverages was conceived some 30 years ago[3], but practical development has awaited the advent of appropriate membranes.

When a concentrated solution of hexoses and organic acids such as a fruit juice concentrate is placed on one side of a cellulose acetate membrane and immersed in sea water, water will enter the system and continue flowing across the membrane until equilibrium is reached. The pressure drop across the membrane appears to be largely a function of membrane thickness (Table 1).

Dialysis bags for a preliminary test were fashioned by heat-sealing cellulose acetate, cellulose acetate butyrate, and cellulose triacetate. A small sample of invert sugar was placed in each bag and the bags were immersed in sea water collected at the south-western tip of the San Francisco Golden Gate Bridge. The sea water sample was 0·6 N as the sodium ion. This normality corresponds to 10·8 per cent invert sugar. Tables 1 and 2, however, show a flattening out of the dilution curve at values closer to 20 per cent sugar than to the theoretically correct one. A possible explanation of this reaching of a "point of diminishing return" is that energy losses from the passage of water across the membrane gain in prominence with decrease of the driving force, that is, decrease in the osmotic pressure difference between the solutions on the *cis* and *trans* sides.

Absence of the sodium and chloride ions from the dilute sugar in the dialysis bag shows that the membrane excludes soluble salts. It is clear that the membrane would also exclude the relatively long-lived radioactive debris such as calcium, strontium, iodine, etc. This means that water contaminated by radioactivity can be a source of a potable beverage.

The rejection of strongly polar solutes indicates that the membrane would even more readily reject organic substances unless they attack the membrane. It is likely to remove organic toxins from both sea water and fresh water. Such a device might prove useful where water is contaminated by either man or Nature.

The rejection of organic matter by a cellulose acetate membrane was tested by placing a dialysis bag with concentrated sugar into fresh water to which phenolphthalein was added. The water uptake followed the pattern of acetate in Table 2. No phenolphthalein passed through the membrane. This means that compounds with

Table 1. COMPARISON OF CELLULOSE ACETATE MEMBRANES 88 AND 120 MILS THICK

Time (h)	88 '*CA* 143' ° Brix of grape concentrate	120 '*CA* 148' ° Brix of grape concentrate
0	54·5	54·5
16	43 6	47·5
18	42·3	46·7
20	40·9	45·5
104	16·5	21·0
120	15 4	19 3
Sodium concentration at 105 h	0·015 N	0 0075 N

Table 2. DILUTION OF SUGAR BY WATER PASSING THROUGH DIFFERENT MEMBRANES

Contact time (h)	Membrane type: Acetate	Acetate butyrate	Triacetate
1	76·4° Brix	76·5° Brix	76·4° Brix
2	70·9	75	—
20	45·4	65·3	64·0
44	33·8	65	—
48	26·4	55	54

The starting material is invert sugar with citric acid at 78° Brix and *p*H 3·8.

fairly high molecular weights and of such a nature as not to dissolve cellulose acetate will tend to be rejected.

Table 2 shows clearly that the fastest rate of water transfer occurred across the cellulose acetate membrane, and further work was done with that type of membrane only. 5 ml. of commercial grape juice concentrate at 46·8° Brix was placed in each of three dialysis bags, the immersed area of which was 115·2, 230·76 and 576 cm², respectively. Water uptake was found to be an area function. A thirst-quenching dilution was obtained in 30 h.

Other experiments dealt with more concentrated juice, necessary for preservation without a can. The results are given in Table 1.

It is possible to obtain by dialysis of sea water or contaminated water a sustaining beverage which is potable and, unlike fish juices, is culturally acceptable. Cellulose acetate (88 mils thick) appears to be an acceptable dialysis membrane.

The method might also be useful for hydration of, for example, "Incaparina", edible jellies, and similar foods in areas with unsafe water.

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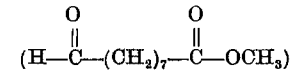
Identification of Methyl Octanoate Derivatives from Autoxidized Methyl Linoleate by Mass Spectrometry, Nuclear Magnetic Resonance and Infra-red Spectroscopy

From our investigations on the volatiles from the mild autoxidation of methyl linoleate, we have already reported the isolation and identification of methyl octanoate[1]. Frankel *et al.*[2] postulated a mechanism for the decomposition of unsaturated hydroperoxy esters that predicts the formation not only of methyl octanoate but also of 8-formyl methyl octanoate, and 8-hydroxy methyl octanoate from 9-hydroperoxy methyl linoleate. Ellis[3] also postulated the formation of semi-aldehydes from oxidation of unsaturated fatty acids. This communication reports further results on an extract of the autoxidized oil which show the presence of 8-formyl methyl octanoate and the formate of 8-hydroxy methyl octanoate (8-formoxy methyl octanoate).

Frankel *et al.*[2] identified 8-formyl methyl octanoate from oxidized methyl oleate, methyl linoleate, methyl linolenate, and linseed oil methyl esters, establishing their identity by measuring their retention times on several gas-liquid chromatographic columns. Nonaka[4] identified some semialdehydes from oxidized fish oil, oleic acid and linolenic acids, but not from linoleic acid.

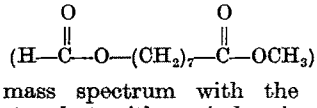
20 g of methyl linoleate (97 per cent as analysed by gas-liquid chromatography) on purified glass wool was oxidized by passing purified oxygen through it at the rate of 10 ml./min at room temperature in laboratory light. The ester had a peroxide number after oxidation of approximately 1,000. The methyl linoleate from five oxidations was combined and extracted with 500 ml. of isooctane by stirring for 2 h at room temperature. The isooctane solution was then concentrated to a volume of about 25 ml. with a rotary evaporator under water aspirator vacuum. Appropriate volumes, 25–40 μl., of the concentrated isooctane solution were chromatographed on a 4-ft. × 1/4-in. stainless-steel column packed with 20 per cent 'Apiezon *M*' on 'Chromosorb *P*' at 172° C. Two components which had not been present in our investigations of the volatile fraction[1] were separately collected for mass spectrometry, infra-red spectroscopy, and nuclear magnetic resonance analysis. The component with the shorter retention time was designated as *A* and the other as *B*.

Compound *A*, 8-formyl methyl octanoate:



gave a mass spectrum with all the important characteristics of a methyl ester[5], except that the molecular ion was not sufficient for certain identification. In addition, other peaks at mass 171, 178, 158, and 136 indicated an additional functional group. Nuclear magnetic resonance data indicated the presence of an aldehyde, a finding which proved consistent with the observed mass spectrum. Prominent bands in the infra-red spectrum at 2,720 cm^{-1}, 2,820 cm^{-1} and 1,735 cm^{-1} indicated the presence of ester and aldehyde groups in compound *A*. Assuming a molecular ion of mass 186, then the loss of the CH_3 group gives mass 171; loss of water and C_2H_4 (aldehyde characteristics[6]) gives mass 168 and mass 158. Presumably 136 arises from a loss of 18 (water) followed by a loss of 32 (formaldehyde). 8-Formyl methyl octanoate was thus indicated. An authentic sample of this compound gave an identical mass spectrum to that of the unknown compound. The infra-red spectrum of the authentic compound was also identical to that of compound *A*.

Compound *B*, 8-formoxy methyl octanoate:



also gave a mass spectrum with the characteristics of a methyl ester, but with no indication of the parent ion. Prominent ion currents were observed as masses 171, 124, and 96 which were assumed to result from the presence of an additional functional group. Nuclear magnetic resonance data revealed a formate group, but traces of impurities made the estimation of chain-length unreliable. The infra-red spectrum of compound *B* showed prominent bands at 1,735 cm^{-1} and 1,180 cm^{-1} indicating the presence of ester and formate groups. The mass peak at 171 indicated one ether-type oxygen, but whether the fragment lost was CH_3O— or HCO_2— could not be decided without comparison with authentic samples.

A sample of 8-formoxy methyl octanoate was not readily available, but samples of 9-formoxy methyl nonanoate, 10-formoxy methyl decanoate, and 10-formoxy ethyl decanoate were synthesized and analysed in the mass spectrometer. The mass spectrum of the nonanoate showed a mass peak at 185 due to the loss of CH_3O, and both the decanoate spectra showed a mass peak at 199 due to the loss of CH_3O and C_2H_5O, respectively. These corresponded to the mass peak at 171 in the unknown compound and established the compound as an octanoate. The other characteristic peaks (masses 124 and 96) were similarly shifted by 14 and 28 mass units in the nonanoate and decanoate, thus confirming the unknown compound as 8-formoxy methyl octanoate.

8-Formyl methyl octanoate was synthesized by cleavage of threo-9,10-dihydroxystearic acid by periodate[7] and treatment of the half-aldehyde acid with diazomethane. 9-Formoxy methyl nonanoate was prepared by the following series of reactions: 8-formyl methyl octanoate was reduced with sodium borohydride in methanol at room temperature, then formylized in a mixture of formic acid and acetic anhydride[8]. 10-Formoxy methyl decanoate and 10-formoxy ethyl decanoate were synthesized from a sample of authentic 10-hydroxydecanoic acid. The methyl ester was prepared by treating the hydroxy acid

with diazomethane. The ethyl ester was synthesized by refluxing the hydroxy acid with excess absolute ethanol and a catalytic amount of concentrated sulphuric acid for 2 h. Both the methyl and ethyl esters of 10-hydroxydecanoic acid were converted into formates by Reber's technique[8].

All intermediate compounds used in the synthesis, as well as the final products, were purified by means of preparative gas-liquid chromatography with the 4 ft. × 1/4 in. 20 per cent 'Apiezon *M*' column at 172° C.

The three related compounds we have identified, methyl octanoate, 8-formyl methyl octanoate, and 8-formoxy methyl octanoate, appear to form by decomposition of the 9-hydroperoxy ester of linoleic acid. This experimental evidence supports the hydroperoxide theory of oxidation of unsaturated fatty acid esters and lipids that contain unsaturated fatty acids. The isomeric 13-hydroperoxide of methyl linoleate should produce two unsaturated semialdehydes and an unsaturated methyl ester, but our investigation did not detect these compounds. These unsaturated semialdehydes and the unsaturated ester should be unstable under the conditions used and they probably decomposed during isolation The formate may form by reaction of the ω-hydroxy ester with formic acid; however, no free 8-hydroxy methyl octanoate was found. Therefore, it seems more probable that the formate was formed by a concerted mechanism, that is, from 8-hydroxy methyl octanoate and a hydroperoxide[10].

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Yields are presented in Table 1; for each the ketone (1 mole) was heated with a representative borane, *tris*-butylaminoborane (1·1 mole) under reflux in benzene.

Table 1

Ketone	Time	Yield (per cent)*
Acetophenone†	20 h	78
*Cyclo*hexanone	8 min	61
Diethyl ketone	8 h	77 5
Methyl isopropyl ketone	24 h	76

* The yield given is of isolated, characterized product
† Pentane solvent.

Excellent yields of amide were produced by heating the reagent (1·5 mole) with a monocarboxylic acid (1·0 mole) in benzene, as shown in Table 2.

Table 2

Acid	Time (h)	Yield (per cent)*
Hexanoic	3·5	98
Pivalic	21·5	90
Phenylacetic	4·0	90
Benzoic	5 0	94

*The yield given is of isolated, characterized product.

Using dicarboxylic acids (Table 3), 5 mole equivalents of the reagent to 1 mole of the acid were used, the solvent being tetrahydrofuran. In this case the salts separated out initially and could be examined. It is noteworthy that no succinic anhydride was produced, and that the reaction time for complete reaction (diamide produced) was shortened. This latter factor may arrive from the somewhat larger excess of reagent used.

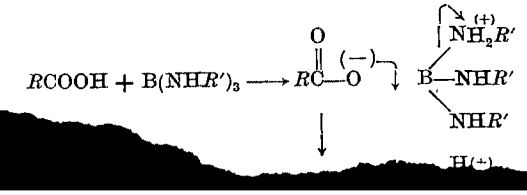
Table 3

Acid	Time (min)	Yield (per cent)*
Succinic	30	83
Adipic	45	74

*The yield given is of isolated, characterized product.

That mechanism of amide formation involved initial salt formation was ascertained by examination of the infra-red spectrum immediately after mixing. The carbonyl stretching frequency at 1,710 cm^{-1} was absent; it had been replaced by a broad band at ~1,540 cm^{-1}, which was associated with the carboxylate anion. However, addition of mineral acid regenerated the carboxylic acid; little amide had been formed at this stage.

The mechanism may be formulated as in Fig. 1.



carbonyl group, leaving it highly susceptible to nucleophilic attack. With suitable substituents X and Y (obviously $X=Y=NHR$ cannot be generally useful), many uses for such mixed anhydrides present themselves. Reports on the systematic variation of X and Y, together with applications of the compounds produced, will be presented shortly.

N-Butylphenylacetamide. Phenylacetic acid (0·51 g) in dry benzene (6 ml.) was added to *tris*butylaminoborane (1·4 g) and the solution heated under reflux for 4 h. The reaction mixture was diluted with ether (5 ml.) and extracted with 3 N hydrochloric acid (2 × 5 ml.), water (5 ml.), saturated aqueous potassium carbonate (2 × 5 ml.) and water (2 × 5 ml.). The acid extract was brought to *p*H 9, saturated with sodium chloride and re-extracted with ether. The organic phases were combined, dried with magnesium sulphate, filtered and the solvent removed to yield 0·65 g (90 per cent) of a pure product with a melting point of 54°–55° C and which was identical in all ways with an authentic sample.

N-Butylisopropylidene imine. Diethyl ketone (0·518 g), *tris*butylaminoborane (1·5 g) and benzene (10 ml.) were heated under reflux for 8 h. The benzene was removed at water-pump vacuum, and the remaining solution brought over at 0·1 mm into a cooled (−75° C) receiver. Fractionation gave 0·554 g (77·5 per cent) of a product with a boiling point of 86°/42 mm; its molecular weight was 141·1515 (and that required for $C_9H_{19}N$ is 141·1517).

One of us (T. L.) thanks the Science Research Council for a research grant.

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Effect of Hydrogen-containing Impurities on Ionization Times in Shock-heated Argon

THE mechanism by which impurities influence the time for shock-treated argon to reach equilibrium ionization has been considered on numerous occasions[1–4]. No results have as yet been published, however, describing the effect of specific impurities. To remedy this situation, I am now making measurements of the total ionization times (see Fig. 1) in primary shocks through argon to which controlled amounts of various gaseous impurities have been added. The degree of ionization is mon[illegible] by following the time dependence of th[illegible] continuum emission at 900 Å. Pr[illegible]

Previously it has been assumed that the sole effect of an impurity I is to increase the rather slow ionization rate in the atom–atom region by a catalytic mechanism:

$$I + Ar \rightarrow I^* + Ar$$
$$I^* + Ar \rightarrow I^+ + \varepsilon^- + Ar$$
$$I^+ + Ar \rightleftharpoons I + Ar^+$$

While hydrogen can participate in such a scheme, it seems unlikely that the overall rate of ionization would be increased to an extent large enough to explain the observed results.

In the electron–atom region, the rate of ionization is limited by the rate at which energy can be transferred from the translationally hot argon atoms and ions to the electrons, which lose a considerable amount of energy in the ionization process:

$$Ar + \varepsilon^- \rightarrow Ar^* + \varepsilon^-$$
$$Ar^* + \varepsilon^- \rightarrow Ar^+ + 2\varepsilon^-$$

The dominant term in the rate of energy transfer at temperatures about 10,000° K is that between the electrons and argon ions, and can be written:

$$Q_{Ar^+} = \frac{N_{Ar^+}N_e e^4}{m_{Ar^+}}\left(\frac{8\pi m_e}{kT_e}\right)^{\frac{1}{2}}\left(\frac{T_{Ar^+}}{T_e} - 1\right)\ln\left[\frac{9(kT_e)^3}{8\pi N_e e^6}\right]$$

where m_e, N_e and T_e are the electron mass, number density and temperature respectively, and $m_{Ar}+$ and $T_{Ar}+$ can be taken equal to the corresponding atomic values. Because the hydrogen ion H^- is about forty times lighter than the argon ion, this term will be increased in the presence of hydrogen to:

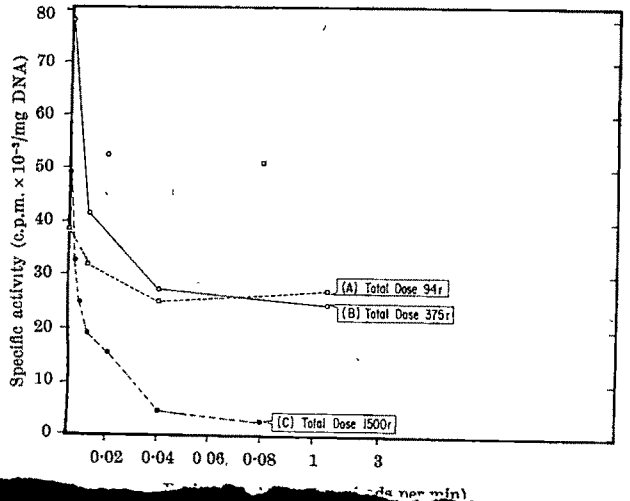
$$Q_L = \left(1 + \frac{N_{H^+}m_{Ar}}{N_{Ar+}m_{H^+}}\right)Q_{Ar^+}$$

At 10,000° K, the mechanism changes[1] from atom–atom to electron–atom at a degree of ionization, α, of about 5×10^{-4}. The presence of one part in 10^5 of H^+ will not only nearly double the rate of ionization at this point, but will extend the range over which the electron–atom mechanism is dominant to a lower value of α. It should also be pointed out that, while the commonest hydrogen-containing impurities in a shock tube are molecular hydrogen and water, molecules with many atoms of hydrogen, for example pump oil, may well play an important part, especially since their presence may not be detected.

At concentrations above 5 parts in 10^4, this effect can lead, in addition to a considerable reduction in the ionization time, to an increase in the ionization rate over [illegible]

on DNA synthesis in 21 h regenerating rat liver: (*a*) A small dose of radiation (94 rads) produces a significant depression in DNA synthesis; (*b*) for a specific total dose, the slower the dose rates of irradiation, the greater is the depression in DNA synthesis within certain limits; and (*c*) the time period of irradiation is a major factor in producing this change, while the effect of total dose is secondary within the range of the experiments.

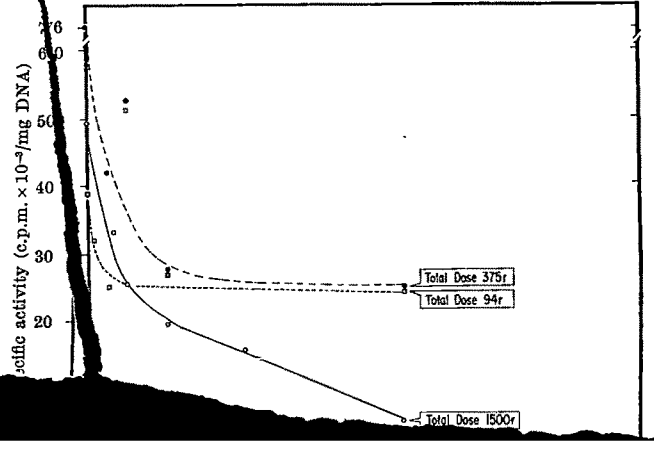
Experimental design and analytical procedures have been described in detail in an earlier publication[4]. Lewis strain male rats, weighing about 200 g, were used. The irradiation was given locally to the liver by using a 200 kVp. X-ray machine of half value layer, filtered with 1 mm copper and positioned by a 3-cm cone. The experiments were so designed that all periods of radiation, with variations in dose rate, ended at 21 h after partial hepatectomy. Total doses of 94, 375 and 1,500 rads, at the dose rates of 300, 200, 100, 50, 25, 12·5, 6·2, 3·1 and 1·6 rads/min, have been used. Fifty μc. of tritiated 5-methyl thymidine (3 c./mM) were given at the end of the period of irradiation. The animals were killed 1 h after administration of labelled thymidine. A modified Schneider method[5] was used for the extraction of DNA, and the DNA content was determined by the diphenylamine method[6]. Radioactivity was assayed by liquid scintillation counting.



the asynchronous cells of the intestines of the rat[7,8]. A total radiation dose of 1,500 rads was given to four groups of animals at rates of 300, 50, 25 and 12·5 rads/min. There was a consistent lowering of the rate of DNA synthesis with increasing slower rates of radiation delivery. However, these data are not in agreement with the results obtained in regenerating liver under different sets of experimental conditions[9], in which it was found that a dose of 300 rads at 40 rads/min gave a greater depression of incorporation of 6-(¹⁴C) orotic acid into DNA than at 19 and 10 rads/min. The 6 h time period between irradiation and labelled-precursor administration and the 12 h period between irradiation and killing make the two experiments difficult or impossible to compare. Changes in the cell population of both irradiated and control animals, as well as such factors as recovery, would obviate any direct comparison between two sets of data.

Total radiation dose, within the range investigated here (Table 1), seems of secondary importance to dose rate in the depression of DNA synthesis. Total doses of 94 rads at 25 rads/min, 375 rads at 50 rads/min, and 1,500 rads at 300 rads/min resulted in the comparable depression of DNA synthesis to approximately one-third of control values.

An alternative view is that the time period of irradiation seems to be most important in the inhibition of DNA synthesis (Fig. 2). The primary depression of DNA synthesis occurred in the first 5 min. After that, little difference is noted between total doses of 94 rads and 275 rads; however, the depression at the total dose of 1,500 rads continues downward.



in the depression of DNA synthesis in *in vivo* studies of local radiation over a dose range of 94–1,500 rads and a dose rate range of 1·6–300 rads/min. The most pronounced effects of radiation occur during the first few minutes (3·75). Depression of DNA synthesis occurs at total dose (94 rads) equivalent to the D_{37} dose for cell survival for mammalian cells. The process of DNA synthesis is not radio-resistant when the three parameters, (*a*) total dose, (*b*) dose rate and (*c*) duration of radiation, are taken into consideration.

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Toxic Effects of Irradiated Foods

THE report by Holsten *et al.*[1] on the direct and indirect effects of radiation on plant cells has produced many comments in both popular and specialist scientific journals. These comments, however, were not brought about by the results reported, as similar data were published earlier (see work referred to in ref. 1). Instead, they arose owing to the statement "The work has other and obvious implications for the radiation sterilization of food . . . .".

Cook and Berry[2], and also Goldblith[3], recently rejected the suggestions that the findings by Holsten *et al.* have such implications for the radiation preservation of food. Cook and Berry first imply that even produced toxic substances

food can cause a slight, age-dependent lymphopenia in rats and also possibly an effect on the thymus[7,8], thus indicating that reactive, radiation-induced compounds can perhaps by-pass the detoxification process in animals.

It has repeatedly been stated that no other method for food preservation by additives (the 'additives' are formed *in situ* in the case of irradiation) has and is being scrutinized as thoroughly as that by radiation. However, there are good reasons for this, as (*a*) for irradiation the 'additives' are largely unknown and are likely to remain so for a long time; (*b*) the additives are not the same in any two different foods owing to the different radiation chemical reactions; (*c*) the amount of additives given in a feeding test cannot be increased beyond a certain value, limited by the ingested amount of food and the non-linear dose dependence of most radiation chemical yields at higher doses.

Most chemical food additives can be tested at concentrations 100 or 1,000 times greater than those technically necessary and thus an acceptable safety factor can be attained. There are two possible ways of avoiding the dilemma regarding the safety factor for irradiated food

(1) Each food could be prepared exactly as it would be for human consumption and then tested on a large number of animals for a long time. If, for example, none of a test group is found to be affected in any harmful way by the treatment, this only tells that within a 90 per cent confidence limit 2·3 cases could have been caused by the treatment[9]. In a hypothetical test with 100,000 animals the true frequency will be $2{\cdot}3 \times 10^{-5}$ with the chosen confidence limit[9]. So far, not more than 1,000 animals have been investigated thoroughly after feeding with any single, tested and cleared, irradiated food[10]. This means that the true frequency of possibly deleterious effects could be as high as $2{\cdot}3 \times 10^{-3}$ at a 90 per cent confidence limit.

(2) More easily scored systems, such as cytotoxic investigations of plant cells and cell cultures *in vitro*. could be resorted to as a primary step towards the evaluation of the eventual wholesomeness of an irradiated material[4,11]. Foods passing these primary tests can then be investigated on 'large' numbers of animals—'large' as the available resources for each food might increase with the decrease in the number of foods likely to be thought worthwhile to test on animals. For example, genetical and teratogenic effects require the development of more elabor

BIOLOGY

Influence of Electrostatic Fields on Seed Germination

RECENT investigations have stimulated wider interest in the possible biological significance of naturally occurring electric fields and related phenomena. Murr[1-3] has shown that high intensity electric fields appear to inhibit development in grass seedlings; he points out, however, that earlier workers, notably Jorgensen and Priestley[4], and Shibusawa and Shibata[5], observed increases in plant growth under electric field conditions, although the frequent occurrence of contradictory results in much of the early work on this subject has been emphasized by Lund[6]. The physiological effects of air ionization have been studied by a number of workers[7]. Krueger *et al.*[8] report that exposure to positively or negatively ionized air produced more rapid germination in *Avena sativa* seeds with subsequent increases in growth and dry weight; slight increases in growth were also noted with seedlings placed in positive or negative electric fields of 955 V over 30 cm; in these experiments, however, the seed containers were grounded to prevent the accumulation of surface charge. The present communication directs attention to an apparent dependence on polarity of the response of germinating seeds to induced electrostatic charges.

Four batches of 100 seeds of lettuce (*Lactuca sativa*), variety 'Cluseed Borough Wonder', were sown on filter paper laid on each of four 6 cm square sections of aluminium foil attached by a thin film of 'Vaseline' to the upper surface of a sheet of glass 45 cm × 7·5 cm. Foil sections were similarly attached to the under-surface of a second sheet of glass resting on spacers so arranged as to separate the two sets of 'field plates' by a vertical distance of 0·5 cm. Two pairs of plates were connected to 90 V batteries arranged in series to give a potential difference of 180 V, one pair positive over negative and the other pair negative over positive; the remaining pairs were left uncharged as controls; the relative positions of charged and uncharged plates were changed after each replication. After sowing, each batch of seeds was moistened with 1·5 c.c. of water, the apparatus being then enclosed in a polythene container and placed in a constant-temperature room for 24 h. Most of the experiments were conducted either in total darkness or with 12/12 photoperiod, but no attempt has been made to discriminate between the two conditions in the results given in Table 1. A comparison is given in Table 2 between twenty paired replications carried out in light and dark rooms simultaneously.

The criterion of germination in these experiments was taken as the emergence of the radicle when viewed under a × 15 lens. The margin of error inherent in purely visual determinations of this type must obviously be so great as to preclude the possibility of obtaining any precise estimate of the magnitude of the electrostatic response. Sufficient results have, however, been obtained to provide some indication of the general pattern of response, although it must be emphasized that considerable variation occurs in the germination level within any one treatment.

The results of 150 replications (Table 1) are in agreement with the work of Murr[1], in that a positive field

Table 1. DIFFERENCE IN PERCENTAGE GERMINATION AVERAGED OVER 150 REPLICATIONS

Difference from control		Difference between polarities
+ / −	− / +	
−4·9468	+0·9863	5·9331

Variance-ratio = 3·95 (5 per cent significance point = 3·00).

Table 2. COMPARISON OF GERMINATION BETWEEN TWENTY REPLICATIONS EACH IN LIGHT AND DARK

	Difference from control		Difference between polarities
	+ / −	− / +	
Dark	−12·2	+4·65	16·85
Light	+0·85	+6·4	5·55

appears to inhibit plant development, but from the present work it is further apparent that the nature of the plant response is dictated by the sign of the electrostatic field, and not merely by the presence of the field *per se*. The possible influence of light in reducing the magnitude of this polarity effect may be significant (Table 2). A comparison with the work of Krueger *et al.*[8] is not possible in the absence of ion-density and current measurements, but Edwards[9] found that a positive field slowed down the rate of development of *Nepytia* pupae; he also suggested that contact-induced electrostatic charges might influence insect behaviour[10,11].

The present experiments were initiated in an attempt to provide a possible basis for correlation between geoelectric phenomena and the manifestation of psychiatric disturbance symptoms, assuming such a relationship to be mediated through variations in neuro-hormone activity resulting from electrostatically induced changes in tissue respiration levels. Preliminary gas-analysis studies appear to indicate a possible polarity-dependent electrostatic influence on the rate of production of carbon dioxide in germinating seeds. Further work along these lines is in progress.

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Some Responses of *Drosophila* to Weak Magnetic and Electrostatic Fields

A VARIETY of reactions of organisms to natural levels of electromagnetic fields has been reported in recent years. This communication reports an investigation of the responses of *Drosophila melanogaster* to magnetic and electrostatic fields of low intensity. Previously, Barnwell and Brown[1,2] cited unpublished work showing that *Drosophila* responded to weak magnetic fields. Edwards[3] reported that electrostatic fields depressed the activity of *Drosophila*.

An orientational–statistical approach was used in this investigation because it was thought that the response to these fields would be weak. Oregon *S* wild type flies were allowed to emerge from a 'Plexiglas' and 'Masonite' corral, through a narrow corridor, into an expanded field. The amount of turning from a straight line was determined by noting the 10° sector crossed by the insect 3 cm from the corridor exit. The flies were returned to the corral by shaking after they had all emerged. About fifty flies were allowed to orient for each sample. The orientation chamber was inclined 30° and centred under an illumination of 2 lux in a black compartment. The flies were raised under an 8 a.m.–8 p.m. light–dark cycle. Experimental runs were conducted between April 4 and August 25, 1962, from 9.30 to 11.30 a.m. and 2.30 to 4.30 p.m. C.S.T.

The amount of turning was noted in the measured 2-G horizontal field of an 18 cm alnico magnet centred under the experimental chamber in a rotatable standard. The flies emerged headed toward true 'Earth' magnetic north under the following configurations of the experimental

magnet: (1) Aligned with the Earth's field. (2) The reverse of the Earth's field. (3) "Compass north" to the east. (4) "Compass north" to the west.

In another series, a horizontal 3 V/cm electrostatic gradient was established by aluminium plates sandwiched in black painted glass, located to the right and left of the orientation chamber. When not in use the plates were switched off, interconnected and grounded.

Additional runs were made with the orientation device in a grounded Faraday cage of brass shim stock and 1-mm copper screening.

The experimental series were arranged in randomized order preceded and followed by a control. A total of 24,825 fly orientations were recorded during 618 replicate runs.

Analysis indicated a statistically significant response to all the magnetic configurations (Table 1). No significant differences were found between the morning and afternoon samples. Daily variations in the response of mud snails to magnetic fields have been reported[4].

The distribution of the control sample means about zero during semi-monthly periods indicated that the flies turned most strongly to the left during the new moon and first quarter (Table 2). All the magnetic configurations showed a reverse pattern of response with the major difference being an increased left deviation from the control during the full moon–third quarter period. This suggests that the effect of a superimposed magnetic field is to prolong the normal left turning response through the full moon–third quarter period. Lunar rhythms in similar responses have been found in snails[5,6], *Paramecium* and planarians[7].

A summary of the results from a group of twenty replicates run in an electrostatic field with the flies headed north as they emerged from the corridor is presented in Table 3. An increased left turning was noted. Brown[8] reported that planarian worms headed north in an electrostatic field also tend to turn counterclockwise.

Table 1. ORIENTATIONAL RESPONSES OF *D. melanogaster* TO VARIOUS MAGNETIC FIELD CONFIGURATIONS AS INDICATED BY ANALYSIS OF PAIRED SAMPLES OF FIFTY FLY ORIENTATIONS

Configuration	Mean difference*	T_D†	P	$S.E.$	N
Combin[illegible]	10°			1·16	62
With Ea[illegible]	1·00°	3·88	< 0·001	2·04	62
"Compa[illegible] E	−1·22°	5·00	< 0·001	1·92	62
Opposir[illegible]h	−2·13°	16·77	< 0·001	1·60	62
"Compa[illegible]N" to W	−1·45°	5·82	< 0·001	1·96	62

* +, Right; −, left.
† The mean difference between the control and experimental means is given except in the case of the controls.

Table 2. CHI SQUARE ANALYSIS OF THE DISTRIBUTION OF THE EXPERIMENTAL MEANS ABOUT THE CONTROL VALUES WHEN CLASSIFIED BY SEMI-MONTHLY PERIODS

	Semi-monthly period: New moon—first quarter*				Full moon—third quarter			
	Right	Left	Zero	χ^2	Right	Left	Zero	χ^2
Control†	6	29	1	13·86‡	11	15	—	0·62
With Earth	16	18	—	0·12	8	20	—	7·78‡
"Compass N" to E	18	15	1	0·11	8	20	—	7·78‡
Opposing Earth	16	18	—	0·12	5	23	—	11·58‡
"Compass N" to W	12	20	2	2·00	5	23	—	11·58‡

* Included the days of the lunar phase ± 3 days.
† Distribution of the control means about zero.
‡ $P < 0·01$.

Table 3. RESPONSES TO A 3 V/CM HORIZONTAL ELECTROSTATIC FIELD AS INDICATED BY POOLED DATA FOR THE ENTIRE SERIES

Configuration	Mean*	$S.E.$	t	P	N
Control	−0·40°	2·24°			1,966
+ to right	−2·00°	2·20°	1·839	< 0·10	976
+ to left	−2·80°	2·18°	2·759	< 0·01	979
+ to right and Faraday cage	−2·45°	2·23°	2·356	< 0·01	1,002
Faraday cage in natural fields	−3·27°	2·15°	3·337	< 0·01	1,000

* +, Right; −, left.

Table 4. CHI SQUARE ANALYSIS OF THE DISTRIBUTION BY SEMI-MONTHLY PERIODS OF CONTROL REPLICATE MEANS COMPARED WITH THOSE RUN IN A FARADAY CAGE WITH NATURAL FIELDS

	Semi-monthly period: New moon—first quarter				Full moon—third quarter			
	Right	Left	χ^2	P	Right	Left	χ^2	P
Control	9	22	4·65	< 0·05	3	21	13·44	< 0·01
Faraday cage	9	23	6·10	< 0·05	13	10	0·17	—

Classification of the control and Faraday-cage means (Table 4) indicates that the lunar variation is the reverse of that mentioned for the controls in the magnetic series (Table 2). This is interpreted as a result of the control means being obtained in August while the control means of the magnetic series were obtained in April–June. This implies that there is some sort of variation with periods longer than a month. This interpretation is supported by a least squares regression line using all the magnetic series control samples. This line indicated that the average value of the control mean tended to move to the left by 0·01° per day. When this is extrapolated to the end of August, an expected mean control value of −2·3° (+, right; −, left) is given. The actual value of the August controls was −3·2°. The shift would be enough to obscure the semi-monthly effect. The Faraday-cage experiments showed a reverse pattern of orientation (Table 4) during the full moon–third quarter period.

The effectiveness of the Faraday cage was checked by using the cage in an imposed electrostatic field. The response in the Faraday cage with an imposed field did not differ significantly from the response to the cage without the field.

A limited amount of data collected concerning orientation in a Faraday cage in a 2-G magnetic field did not reveal significant differences between the samples within the cage as compared to the magnetic series outside the cage.

During a series of ten replicates, the apparatus was turned so that the flies emerged from the corral in each of the four cardinal directions. No significant differences were found.

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Propylene Phenoxetol as a "Preservative" for Living Holothurians

A WELL-KNOWN disadvantage of the use of the holothurian *Stichopus moebii* for the preparation of isolated organs for physiological experimentation is the process of local degeneration[1] initiated in the body wall by the trauma of dissection. If the dissection is at all prolonged, the connective tissue begins to 'deliquesce' and the body wall flows out in an ooze, much as it does when subjected to heat. It seems useful as well as of some interest to know that an agent used for narcotizing invertebrates and fish can also retard the local degeneration reaction.

Because the anti-oxidant[2] propylene phenoxetol is so effective in relaxing living molluscs[3] it was chosen for relaxing *Stichopus moebii* while making preparations of the isolated cloaca. If the severed hind end of a "sea pudding" is allowed to remain an hour or more in a container of sea-water to which propylene phenoxetol has been added to form a globule from which it gradually diffuses, the specimen will become unreactive and expanded, although the body wall retains normal tone. It is then possible to proceed leisurely with dissection without encountering the local degeneration response. Viscera and longitudinal retractors appear normal after

the treatment with propylene phenoxetol; for example, rhythmicity persists for as long as 48 h in isolated cloacas used for a student laboratory project. After dissection, the severed hind end left moist with propylene phenoxetol remained as firm to the touch as a normal intact animal on the sea bottom. After 5 days, oozing had only occurred immediately under the integument of such a specimen whereas one moistened with clear sea-water degenerated to ooze in minutes.

Propylene phenoxetol is useful as a preservative only for fixed specimens[4,5] and it is thus surprising that it can also be used to retard deterioration of a living animal.

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Successful Transplantation of a Frog Bladder-fluke from One Host Species to Another

GOODCHILD[1] described experiments in which adult bladder-flukes, *Gorgodera amplicava* and *Gorgoderina attenuata*, were successfully transplanted from one species of *Rana* to another. Healthy transplanted flukes were capable of attaching themselves to the bladder of their new host, and they survived for periods of up to 3 weeks, which was as long as the experiment lasted. Furthermore, Goodchild[2] successfully transplanted individuals of the same two species into the bladders of painted turtles, *Chrysemys picta*, and newts, *Triturus viridescens*; these parasites survived in their new habitats for periods of up to a week. In contrast to these results, Goodchild found that the same adult trematodes were incapable of survival when transplanted into the rectum or into the abdominal coelom of *Rana pipiens*. The flukes in the rectum were unable to grip the mucosa and were soon eliminated with the faeces. Those in the abdominal coelom were encapsulated by host cells and died within 24–72 h.

The experiments described here were carried out using *Gorgoderina vitelliloba* from the common frog, *R. temporaria*.

In the first series of experiments the donor host, which had been fed with infective metacercariae more than a month previously, was pithed and the bladder opened under 65 per cent amphibian Ringer's solution. Careful teasing of the bladder caused the flukes to release their grip and they were then drawn into a smooth-ended Pasteur pipette, the tip of which had been curved slightly. It was found possible to transfer the parasites into a lightly anaesthetized recipient host by sliding the tip of the pipette along the mid-ventral line of the cloaca until it entered the bladder.

When *R. temporaria*, *Bufo bufo* and *B. viridis* were used as recipient hosts, the adult parasites survived and developed normally for periods of up to 8 months, which probably approximates to the normal life-span of the adult fluke[3].

At the time of transplantation, certain of the flukes were small and contained few eggs in the uterus. When such individuals were recovered from the recipient host several weeks later they had increased considerably in size and their uteri had become packed with embryonated eggs.

Within the definitive host, the juvenile form of *G. vitelliloba* spends some time in the kidney before entering the bladder[4]. A second series of experiments was therefore performed in which juvenile flukes teased from the kidneys of a recently infected common frog were used. Such juveniles were transferred to the bladder of the recipient hosts, as described for bladder to bladder transplantations, and their subsequent development was observed. The young flukes were found to leave the bladder and migrate up the ureters to enter the kidneys. They eventually left the kidneys and returned to the bladder at intervals of time corresponding to the normal movements in the donor hosts. Within the bladder of their recipient hosts the parasites completed their normal growth and commenced egg production.

A further series of experiments has been carried out using the European tree frog, *Hyla arborea*, as the recipient host. In this frog the kidney behaves as a physiological barrier to the normal course of experimental infection[5]. Individuals which had been transplanted bladder to bladder succeeded in establishing themselves and remained healthy for at least a week. On the other hand, kidney to bladder transplanted parasites migrated to the kidneys where they were destroyed.

Several attempts have been made to transplant adult specimens of *G. vitelliloba* into the rectum of various amphibians. Although most of the parasites were eliminated from the hosts on defaecation, a few managed to establish themselves in the recta of *R. temporaria*, *R. pipiens* and *Bufo viridis*. They were found to be grasping the rectal mucosa firmly and appeared quite normal at the time when the hosts were killed, 4 days after transplantation. The main problem confronting the parasite seems to have been the initial grasping of the mucosa. Once the parasite has established its grip on the rectal mucosa it seems capable of maintaining its position there successfully.

The final experiment consisted of transplanting juvenile flukes from the kidneys of one specimen of *R. temporaria* into the abdominal coelom of another. This was accomplished by injecting the juvenile flukes through a small incision in the latero-ventral body wall of the recipient frog. When the recipient hosts were examined after 24–84 h no live flukes were recovered. Recognizable remains of dead flukes were found encapsulated in host tissue and firmly adherent to various organs and mesenteries of the body cavity.

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Susceptibility of Pigs to Infection with *Schistosoma haematobium* from Egypt

PIGS have been incriminated as natural hosts for *Schistosoma japonicum*, *S. incognitum* and *S. bovis*[1–3]. Various attempts to infect pigs with *S. mansoni* attained limited success[4–7]. Hill and Onabamiro[8] reported natural infections of two pigs with *S. haematobium* in Nigeria. The present experiment was carried out to investigate the susceptibility of pigs to infection with *S. haematobium* from Egypt.

Two pigs about 8 weeks old obtained from a local breeder in St. Albans were selected for the experiment. Each pig was exposed by tail immersion to about 1,000 cercariae shed by experimentally infected *Bulinus* (*B.*) *truncatus* for about 20 min. One week later, each of the two pigs was re-exposed to about 500 cercariae, this time by applying the cercarial suspension to the shallow groins of the abdomen and the mucous membranes of the buccal cavity. The pigs were re-exposed for the second time 1 week later by the subcutaneous injection of about 500 cercariae per pig. By that time each pig had been exposed to a total number of about 2,000 cercariae. When the pigs were exposed to infection, control albino mice were also exposed to cercariae shed by the same group of infected snails.

After the first cercarial exposure, the pigs were observed to twitch their tails vigorously. After the second exposure there was more evidence of cercarial penetration in the form of minute red papules on the skin of the groins. Urine and faecal samples were negative when examined twice weekly from the tenth to about the twenty-seventh week after exposure. One of the pigs was killed 28 weeks after exposure and a thorough search for eggs or adults of the parasite in various internal organs was undertaken. However, no macroscopic lesions which could be attributed to *Schistosoma* infection were observed in any of the visceral organs. The liver, lungs and spleen were normal in size and appearance. Examination of small pieces of these organs between two slides under the microscope was negative for worms, eggs and bilharzial pigment. The small and large intestines were searched carefully for eggs but none was found. The mesenteric veins and the hepatic portal vein were dissected carefully, but no worms were observed. The urinary bladder was also negative. The control mice exposed to infection were killed and dissected 13 weeks after exposure. All showed evidence of successful infection. Eggs of the parasite were demonstrated in press preparations of the liver and small intestine. Coupled worms were also collected from the liver and the superior mesenteric veins.

The results of the present experiment show that *S. haematobium* from Egypt failed to develop in pigs. On the other hand, albino mice became infected when exposed to the same batches of cercariae used for infecting the pigs. *S. haematobium* has a marked host specificity when compared with other human schistosomes and only a few mammals can easily be infected with this species[9]. For this reason, it was of great importance to verify experimentally the report of Hill and Onabamiro. A critical review of their report has revealed that there are various questions which have to be answered before accepting that report as final. They reported that one of the pigs had haematuria and passed eggs of *S. haematobium* type in the urine. This pig continued to have haematuria for 47 days and was then slaughtered. No gross lesions attributable to bilharziasis had been observed in any of the internal organs and no worms were found in the blood vessels. In my opinion, it seems rather improbable that the haematuria was caused by *S. haematobium* with no deposition of eggs in any of the internal organs, particularly the urinary bladder. The second pig with haematuria was treated with 'Nilodin' and when it was killed they observed pin-point haemorrhagic foci in the mucosa of the urinary bladder. However, all the organs were free from adult worms and eggs and the histopathological examination of the urinary bladder was also negative. It is well known that after treatment the *Schistosoma* worms are usually swept from the mesenteric veins into the liver, where they may die or revive and return to their normal habitat in the mesenteric or vesical veins. If they die in the liver, they usually stimulate a strong tissue reaction there. However, Hill and Onabamiro maintained that all the organs were negative for worms or any pathological reaction. This seems rather improbable for any genuine schistosome infection. Moreover, Hill and Onabamiro attempted unsuccessfully to infect two young pigs experimentally with *S. haematobium* from Nigeria. The presence of *S. haematobium* eggs in the urine of pigs having haematuria but no adult worms or eggs in any of the internal organs cannot be explained, although contamination of the urine of the pigs cannot be excluded.

It should be noted here that if the authenticity of Hill and Onabamiro's report can be convincingly verified, then the failure of the Egyptian strain of *S. haematobium* to establish itself in the pigs might be due to differences in the strain of the parasite. There is considerable evidence from work on *S. mansoni* to demonstrate the importance of the strain of the parasite in the determination of the degree of association between it and the definitive host[10,11].

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BIOCHEMISTRY

Characteristics of α-Amylase Formation by *Bacillus subtilis*

ALTHOUGH there are a large number of reports dealing with the formation of α-amylase by *Bacillus subtilis*, the nature of the mechanism which controls its synthesis has not been subjected to a detailed examination.

Fukumoto, Yamamoto and Tsuru[1] showed that α-amylase is produced in the culture medium in the presence of a wide variety of carbon sources, the total amount of enzyme formed after prolonged incubation showing considerable variation with the different carbon compounds. These workers also studied the progress of α-amylase formation in relation to cell growth and found that enzyme production lagged slightly behind cell growth, maximum accumulation occurring when cell autolysis was just beginning[2]. This was supported by the work of Coleman and Elliott[3], who found the enzyme to be produced maximally in the post-logarithmic phase of growth. However, Nomura, Maruo and Akabori[4] reported experiments which showed a distinct separation of several hours' duration between the end of the logarithmic phase of growth of *B. subtilis*, during which no α-amylase production was observed, and the phase in which the enzyme was secreted in significant amounts. They concluded that α-amylase formation occurs only in old cells in which normal cellular multiplication no longer occurs.

A more critical investigation has now been made of the relationship between increase in cell mass and α-amylase formation in the presence of different carbon sources. This work was carried out in order to answer two questions. First of all, is α-amylase formed in appreciable amounts during the logarithmic phase of growth? Secondly, is α-amylase synthesized by an inducible system?

The organism was grown aerobically at 30° C in a medium containing a mixture of L-amino-acids, vitamins and salts with either starch, maltose, glucose or glycerol, at a concentration of 1 per cent, as the carbon source (see legend to Fig. 1). In each case increase in cell mass and α-amylase secreted into the medium were determined over a 14 h period. It was found that, irrespective of the carbon source used, the cells grew logarithmically until a cell density of 0·3 mg dry weight/ml. was achieved and after this time growth occurred at a reduced rate until the end of the experimental period. The differential rate of α-amylase formation, that is, the relationship between enzyme formation and increase in cell mass, in the presence of each of the carbon sources is shown in Fig. 1. It can be seen that a low linear differential rate of enzyme synthesis occurs with all the carbon sources tested until the end of the logarithmic phase. During this period the rate is higher in the presence of starch and maltose

Table 1. MAXIMUM AND MINIMUM DIFFERENTIAL RATES OF α-AMYLASE FORMATION IN THE PRESENCE OF VARIOUS CARBON SOURCES

Carbon source	Differential rate of enzyme formation (Amylase units formed/mg dry wt. increase in cell mass) Minimum (during log phase)	Maximum (post-log phase)
Starch	43	375
Maltose	43	374
Glucose	17	345
Glycerol	20	354

(doubling time 2·2 h) than with either glucose or glycerol (doubling time 1·6 h and 1·7 h, respectively). However, after the end of the logarithmic phase the rate increases to very nearly the same high level in all cases (Table 1).

In the absence of the carbon source cell growth and α-amylase formation occurred, but the results are not directly comparable as amino-acids were utilized as a source of energy and logarithmic phase growth ended when a cell density of 0·18 mg dry wt./ml. was reached. However, the general characteristics, of a low rate of enzyme secretion during the logarithmic phase and a much higher rate after logarithmic growth had ceased, were observed.

So far as the question of inducibility of the enzyme is concerned it is not possible to give a clear-cut answer. The higher rate of enzyme synthesis during the logarithmic phase in the presence of the substrate, starch and maltose, an end-product of the enzyme action, than with glucose or glycerol might be regarded as evidence of inducibility. However, the greatly increased rate of production achieved in the post-logarithmic phase, when most of the α-amylase is secreted, is virtually the same irrespective of the carbon source used and this clearly cannot be described as inducible.

In summary, the results show that there is a low but definite synthesis of α-amylase from the moment the cells start to grow until the end of the logarithmic phase. The maximum secretion of enzyme occurs after the end of the logarithmic phase when the cell mass is increasing at a reduced rate. The results further demonstrate that the major part of the α-amylase formation is not an inducible process—there being no proof that the remainder is inducible.

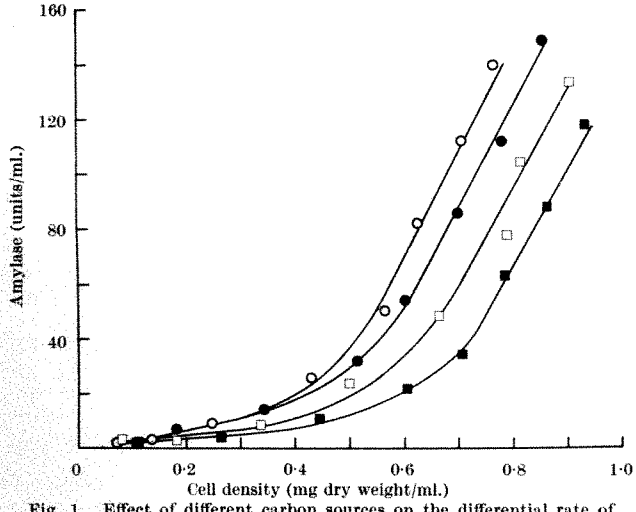


Fig. 1. Effect of different carbon sources on the differential rate of α-amylase formation. A series of T-tubes each containing 40 ml. of a defined medium was inoculated from a suspension of washed *B. subtilis* (Takamine) spores in distilled water and incubated as described by Coleman and Elliott (ref. 1). Tubes were taken in duplicate at 2-h intervals over a period of 14 h, commencing early in the logarithmic phase. On each occasion the cell mass and the α-amylase content of the supernatant fraction of the culture were determined as described by Coleman and Elliott (ref. 1). The composition of the defined medium was as follows: ammonium monohydrogen phosphate, 34 mM; potassium chloride, 5 mM; magnesium sulphate, 1 mM; calcium chloride, 0·125 mM; zinc sulphate, 0·0125 mM; sodium citrate, 2·125 mM; ferric chloride, 0·5 mM; 'trace metal ion' solution, as described by Coleman and Elliott (ref. 5), 0·25 ml./l.; vitamin solution, 50 ml./l., and a mixture of equal amounts (by weight) of nineteen naturally-occurring L-amino-acids (cyst(e)ine omitted) to give a total amino-acid concentration of 0·5 per cent. The medium was adjusted to *p*H 7·2 with 10 per cent (v/v) phosphoric acid and supplemented with either starch (●), maltose (○), glucose (■) or glycerol (□), each at a concentration of 1 per cent. The vitamin solution consisted of riboflavin, 0·1 mg, and 1·0 mg each of inositol, biotin, choline chloride, folic acid, nicotinamide, calcium pantothenate, pyridoxal-5-phosphate and thiamine (aneurin) in 1 l. of water

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Rate-determining Step in the Action of L-Amino-acid Oxidase

ALTHOUGH detailed information concerning the substrate specificity[1,2], co-factor requirement and physico-chemical properties[2] of L-amino-acid oxidase is available, our knowledge of the mechanisms of the reactions catalysed by this enzyme leaves much to be desired. Radda[3] has recently proposed a mechanism for the oxidation of phenylglycines by L-amino-acid oxidase which involves the elimination of the α-hydrogen as a hydride ion (or radical) followed by hydrolysis of the imine and dissociation of the enzyme-product complex.

Electron-withdrawing substituents in the phenyl ring of phenylglycine increased, within limits, the rate of the overall reaction and it was concluded that the initial loss of the α-hydrogen, as a hydride ion, was not rate-determining[3]. Furthermore, a Hammett plot gave a value for ρ of 1·8 which is similar to that observed for base-catalysed ester hydrolyses. Radda suggested that hydrolysis of the α-amino-acid is the rate-determining step. However, the Hammett plot showed a break to a line of negative slope with strongly electron-withdrawing substituents and it was suggested that this might represent a change in the rate-determining step of the reaction with the loss of the α-hydrogen becoming energetically significant.

Several lines of evidence led us to question the proposed mechanism. Previous work with flavoproteins (sarcosine oxidase[4], glucose oxidase[5], and possibly monoamine oxidase[6]) has demonstrated that removal of the α-hydrogen (probably as a proton) constitutes the rate-determining step. Furthermore, hydrolysis of α-imino-acids is better compared to the hydrolysis of Schiff's bases than to base-catalysed ester hydrolysis. In this connexion, Cordes and Jencks[7] have shown that the mechanism of hydrolysis of Schiff's bases from substituted benzaldehydes and aliphatic amines is *p*H-dependent. At neutral *p*H (6·5–7·5) the principal reaction is an attack by hydroxyl ions on the protonated Schiff's base. In this region (*p*H optimum of L-amino-acid oxidase, 7·2–7·5) (ref. 2) the effects of substituents are minimal as they exert opposing influences on the attack by hydroxyl ions and the protonation of the Schiff's base—ρ is approximately zero. Hence, the ρ value of 1·8 for the oxidation of phenylglycines appears inconsistent with a mechanism in which the hydrolysis of the α-imino-acid is rate-determining. The positive value of ρ simply indicates that negative charge is being developed in the transition state and is consistent with the removal of the α-hydrogen as a proton in the rate-controlling step.

The present investigation is based on the use of kinetic isotope effects, a technique which has been a powerful tool in the elucidation of reaction mechanisms[8–9]. α-Deuteriotyrosine and α-deuteriophenylglycine were prepared by standard synthetic procedures[10–12] under

Table 1. RATES OF OXIDATION OF AMINO-ACIDS BY L-AMINO-ACID OXIDASE (*Crotaleus adamanteus*)

	Rate in μl. Oxygen/min/mg*	k_H/k_D
D,L-Phenylglycine	2·1	1·41
D,L-α-Deuteriophenylglycine	1·5	
D,L-Tyrosine	11·8	1·41
D,L-α-Deuteriotyrosine	8·4	

* Average of three separate experiments which agreed within 5 per cent. Flask contained 30 μmoles substrate in 3 ml. of *tris* buffer (0·2 M, *p*H 7·2). With phenylglycine, 1 mg of L-amino-acid oxidase (σ, type IV) was used, and with tyrosine, 0·5 mg of a crude venom preparation obtained from Calbiochem.

conditions which give 95 per cent deuterium labelling in the desired positions. The purity of the products was determined by paper and thin-layer chromatography. The rates of oxidation were followed manometrically and comparison of the rates of oxidation of tyrosine with its α-deuterated analogue showed an isotope effect, $k_H/k_D = 1{\cdot}41$. Under conditions identical to those described by Radda, a similar isotope effect was found for the oxidation of phenylglycine (Table 1).

These isotope effects clearly indicate that the removal of an α-hydrogen is rate-determining in the oxidation of tyrosine and phenylglycine.

It is of great interest to note that the isotope effect observed ($k_H/k_D = 1{\cdot}41$) is substantially below the theoretical maximum value ($k_H/k_D = 7$) and is, in fact, approximately equal to the value calculated from simple considerations of the mass difference between hydrogen and deuterium ($k_H/k_D = \sqrt{2} = 1{\cdot}42$) (ref. 13). Wiberg[13] has pointed out that the magnitude of the isotope effect depends on the extent to which zero point energy is conserved in the transition state. The low values observed in this investigation indicate that stretching of the carbon–hydrogen (or carbon–deuterium) bond has proceeded only to a small extent in the transition state. Aebi[14] has arrived at similar conclusions for the peroxidatic formate oxidation by catalase.

These findings do not provide any explanation for the break in the Hammett plot observed by Radda[3], since it would be anticipated that increased electron-withdrawal would facilitate the removal of an α-hydrogen as a proton. As previously suggested[3], this anomaly could represent a change in the rate-determining step or steric factors hindering Michaelis-complex formation. We are at present extending these investigations to substituted phenylglycines in order to differentiate between these possibilities.

Note added in proof. Since acceptance of this communication, Coffey *et al.* (*J. Biol. Chem.*, **240**, 4058; 1965) have suggested, in the case of D-amino-acid oxidase, that the reaction proceeds through a mechanism involving proton abstraction; and Neims *et al.* (*Biochemistry*, **5**, 203; 1966) have demonstrated a biphasic Hammett plot in the case of D-amino-acid oxidase and have suggested the use of deuterium isotope effects to clarify this anomaly.

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Metabolic End-products of Anaerobic Spermatozoan Metabolism

DURING anaerobic fructolysis by mammalian spermatozoa, fructose is reportedly metabolized by way of glycolysis to lactic acid[1,2]. Results from many anaerobic metabolic investigations in our laboratory, however, in which bovine spermatozoa were incubated for 4 h at 37° C have consistently yielded low lactic acid values in relation to the fructose utilized. This failure to account for all the metabolized fructose as lactic acid led us to investigate the possibility that other metabolic acids were being formed and, if so, to determine if the level of carbon dioxide in the gaseous phase, previously reported to influence spermatozoan metabolism[3], affected the formation of these acids.

In order to eliminate the substrates present in the seminal plasma, exogenous substrate-free epididymal-like spermatozoa (ELC) were used. The detailed method for the collection and preparation of ELC has been reported[4]. They are collected directly into 100 ml. of an inhibitory diluent, washed once, and finally resuspended in saline. A total of 18×10^9 cells from pooled ejaculates were used for six gas treatments as follows: 100 per cent nitrogen or nitrogen containing 5, 25, 50, 75 or 100 per cent carbon dioxide. The 3×10^9 cells in each treatment were placed in four separate flasks each containing 750×10^6 cells for the 4-h incubation at 37° C in a citrate–bicarbonate medium[5] containing 0·5 per cent fructose. After incubation the contents of the four flasks in each gas phase were combined, deproteinized with barium hydroxide and zinc sulphate, and filtered. The filtrate was reduced to near dryness by evaporation under vacuum at 55° C and absorbed on a small quantity of silicic acid. The acids present were then separated by column chromatography[6]. Chloroform containing increasing amounts of *t*-butyl-alcohol was used to elute the acids present, which were then titrated against 0·005 normal potassium hydroxide. They were identified by the use of known acids both by paper and column chromatography techniques. The organic acids present in the spermatozoa and incubation diluent before and after incubation are reported as the micro-equivalents of each acid per 10^8 cells.

The carbon dioxide evolution was determined manometrically[7], and the fructose disappearance was measured colorimetrically[8]. The carbon dioxide evolved from the bicarbonate of the diluent in response to the acids produced is shown in line 1, Table 1, with the fructose utilized by the cells during the 4-h incubation period showing directly below the carbon dioxide data. As was expected, the highest utilization of fructose and evolution of carbon dioxide occurred under the 5 per cent carbon dioxide–95 per cent nitrogen[9].

The acids, accompanied by the standard error (*S.E.*) for the two replicates, are listed in the order of their elution from the silicic acid column. The presence of these acids in the incubation medium indicates that all these metabolic acids were produced at least in detectable levels under all anaerobic gaseous régimes. Low levels of each acid present in the initial portions taken before incubation were apparently in the sperm cells.

All the acids listed in Table 1, with the exception of fumaric and oxalic, have previously been reported to be present in semen immediately after collection[10–12]. The absence of seminal plasma in the incubation diluent, however, eliminates it as a source of any of these acids. After anaerobic metabolism in the presence of fructose, only acetic[13] and lactic[1,2] have previously been detected. The production by spermatozoa of other acids during the 4-h incubation period shows that metabolic pathways, in addition to the Embden–Meyerhof one, function in spermatozoa under anaerobic conditions and probably serve as as source of energy for both motility and biosynthetic purposes.

Table 1. UTILIZATION OF FRUCTOSE, EVOLUTION OF CARBON DIOXIDE, AND ORGANIC ACIDS PRODUCED BY BOVINE SPERMATOZOA (ELC) FROM FRUCTOSE DURING 4-H ANAEROBIC INCUBATION AT 37° C IN WARBURG RESPIROMETER (PER 10^8 CELLS) (n = 2)

Incubation gas	(Initial)	100% nitrogen	5% carbon dioxide 95% nitrogen	25% carbon dioxide 75% nitrogen	50% carbon dioxide 50% nitrogen	75% carbon dioxide 25% nitrogen	100% carbon dioxide
Carbon dioxide evolved (μM)		5·0 ±1·18	6·3 ±0·31	4·6 ±0·45	3·2 ±0·19	2·8 ±0·21	2·1 ±0·31
Fructose utilized (μM)		2·2 ±0·54	4·1 ±1·73	2·8 ±1·88	2·5 ±1·58	1·1 ±0·41	0·6 ±0·42
Acids produced (μequiv.)							
Acetic	0·16 ± 0·06	0·47 ± 0·05	0·56 ± 0·09	0·68 ± 0·34	0·51 ± 0·20	0·53 ± 0·24	0·50 ± 0·19
Formic	0·15 ± 0·08	1·00 ± 0·42	1·49 ± 0·88	0·50 ± 0·34	1·79 ± 1·60	0·37 ± 0·08	0·80 ± 0·65
Fumaric	0·07 ± 0·01	0·20 ± 0·06	0·19 ± 0·10	1·33 ± 0·90	0·51 ± 0·13	2·57 ± 1·57	1·74 ± 1·58
Lactic	0·46 ± 0·20	10·79 ± 0·15	10·46 ± 0·65	9·49 ± 4·39	5·43 ± 2·21	4·85 ± 0·27	3·37 ± 0·58
Succinic	1·12 ± 0·86	1·76 ± 1·40	0·94 ± 0·22	2·07 ± 0·98	3·08 ± 0·27	1·74 ± 1·06	1·00 ± 0·85
Malonic	0·00 ± 0·00	0·13 ± 0·18	0·17 ± 0·05	0·68 ± 0·24	0·18 ± 0·04	2·17 ± 0·07	0·71 ± 0·58
Glycollic	0·08 ± 0·02	4·31 ± 2·43	1·70 ± 0·10	1·62 ± 0·02	0·99 ± 0·12	1·43 ± 0·36	1·44 ± 0·61
Oxalic	0·08 ± 0·04	0·38 ± 0·24	1·42 ± 0·05	0·60 ± 0·25	0·83 ± 0·64	0·33 ± 0·01	0·90 ± 0·33
Malic	0·58 ± 0·15	0·33 ± 0·05	0·25 ± 0·12	0·77 ± 0·08	0·87 ± 0·25	0·24 ± 0·03	1·00 ± 0·70

Of the acids shown in Table 1, only lactic acid accumulation is highly correlated with the overall metabolic rate as measured by the evolution of carbon dioxide or utilization of fructose for each level of carbon dioxide in the gaseous phase. Fumaric acid accumulation shows the opposite trend, however, and increases as the level of carbon dioxide in the gas phase is increased. As evidenced by the *S.E.*, considerable variation between replicates was present in the levels of some of the acids produced.

The accumulation of fumaric, succinic, malonic and malic during anaerobic metabolism is evidence for the activity of the tricarboxylic acid (TCA) cycle even in the absence of oxygen. The increase in the concentration of fumaric, malonic and malic acids during incubation of the spermatozoa under the higher levels of carbon dioxide suggests that this may be the result of the uptake of carbon dioxide which occurs under anaerobic conditions[9].

The accumulation of these acids by spermatozoa during anaerobic metabolic studies would definitely explain the frequently reported failure to account for all the fructose utilized as resulting in lactic acid. The production of these acids as by-products of anaerobic spermatozoan metabolism would also explain some of the differences cited by Rothschild[14] between the values of anaerobic heat production and the disappearance of fructose in bovine sperm samples.

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Electron Spin Resonance Examination of Charge Transfer Complexes of Polycyclic Carcinogens and Nucleic Acid Bases

THE extensive discussions in the literature on the ability of polycyclic hydrocarbons to form charge-transfer complexes with various molecules[1], for example, iodine, and the attempts which have been made to correlate this property with the carcinogenicity of the hydrocarbon[1,2], prompted us to examine the electron spin resonance spectra of the charge-transfer complexes known to be formed[3] by polycyclic hydrocarbons and the DNA and RNA bases.

Szent-Györgi *et al.*[2] reported that certain carcinogenic hydrocarbon-iodine complexes involved complete transfer of an electron, detectable by electron spin resonance, and we wished to ascertain whether or not this could be observed for the corresponding purine and pyrimidine complexes. For the purposes of comparison, the electron spin resonance spectra of the iodine complexes were also recorded.

The iodine complexes were prepared in chloroform solution in a quartz tube of 8 mm outside diameter from 3–5 mg of the carcinogen and a 1–2 mole equivalent excess of iodine. The solutions were evaporated to dryness before examination by electron spin resonance. The DNA- and RNA-base hydrocarbon mixtures were obtained similarly by evaporation of a suspension of both compounds in benzene-95 per cent ethanol. In each case blanks containing each component alone were subjected to the evaporation procedure and the electron spin resonance spectra examined. In no case was a signal detected for the starting compounds alone.

The results obtained are summarized in Table 1.

Table 1

Compound	Iodine: Colour of complex	Iodine: Electron spin resonance signal* (spins/mole)	Adenine, guanine, cytosine, uracil
3,4-benzpyrene	Black	$1·8 \times 10^{21}$ ($2·5 \times 10^{20}$)	All mixtures colourless
20-methylcholanthrene	Red-black	$1·4 \times 10^{20}$ ($8·0 \times 10^{20}$)	No electron spin resonance signal detectable for any charge-transfer complex
1,2-benzanthracene	Light-brown	—	
1,2 : 5,6-dibenzanthracene	Brown	—	
2-aminofluorene	Black	$3·4 \times 10^{21}$ ($1·8 \times 10^{20}$)	
2-acetylaminofluorene	Red-brown	—	
5,7-dimethyl-1,2-benzacridine	Dark-brown	—	
Oestrone	Brown	—	
17β-oestradiol	Brown	—	
Stilboestrol	Dark-brown	—	

* Numbers in parentheses give values reported in ref. 2.

The carcinogenic oestrogens, oestrone, oestradiol, and stilboestrol were also examined in the hope that some correlation with the carcinogenic polycyclic hydrocarbons might become apparent, since steroids also form charge-transfer complexes with purines and pyrimidines[4].

The negative results obtained suggest that the complete transfer of an electron by carcinogens to give free radicals does not occur in carcinogen-DNA or carcinogen-RNA interactions, and the data provide additional circumstantial evidence in support of the conclusion drawn by Epstein *et al.* that charge-transfer tests are unreliable as criteria for evaluating the carcinogenicity of polycyclic compounds.

The electron spin resonance spectra were recorded on a 'Varian *V*-9500' X-band spectrometer. As shown in Table 1, the quantitative data obtained for the iodine complexes with a positive electron spin resonance signal are in good agreement with the values quoted by Szent-Györgi *et al.*[2].

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MICROBIOLOGY

Preparation of Pure Blue-Green Algae

IN the course of measuring the resistance of blue-green algae to γ-radiation from a cobalt-60 source, initially contaminated blue-green algae were found to be free from bacteria after irradiation. Since difficulties are encountered in the purification of Cyanophyta[1] (less than 30 per cent of the organisms in the Indiana University Collection are bacteria-free[2]), γ-radiation may afford an easy, reliable method for purifying algae from bacteria and be of advantage in physiological and biochemical investigations of these organisms. Practical conditions for the purification of a group of typical Cyanophyceae were, therefore, determined.

Filamentous algae were grown in 10 ml. of supernatant Chu[3] medium in 20-ml. loosely capped vials so that the sides and bottom of the vial were covered with a thin filamentous mat. Unicellular algae were inoculated into similar media to form lightly coloured suspensions. Irradiation was provided by a 'Gammacell 220' (Atomic Energy of Canada, Ltd.) furnishing $6{\cdot}48 \times 10^4$ rads/h at the irradiation site. At the end of the irradiation period (Table 1), a sample of algae from each vial was streaked out on trypticase soy agar to test for bacterial growth while another sample was sub-cultured into fresh medium to observe algal growth. The results given in Table 1 show that 4 h irradiation (about 260,000 rads) is capable of producing bacteria-free cultures with no observable algal damage. The only observable change appeared to be an increase in the size of pseudovacuoles which returned to normal with ensuing growth.

The purified algae were sub-cultured three times at 1-week intervals and observed regularly for 6 weeks, during which time they remained pure and in healthy condition. Cultural behaviour and microscopic structure remained characteristic; in the case of some filamentous forms, a slight stimulation of growth was apparent. Tests for anaerobes were negative. Fungi were not observed.

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Copulation of Ultra-violet-irradiated and Unirradiated *Saccharomyces* Cells of Different Ploidy

THE inactivation of *Saccharomyces* strains of different ploidy induced by radiation has been investigated in detail by various authors[1]. For X-rays, 'recessive'- and 'dominant'-acting damages have been separated[2]. 'Dominant' lethal damage renders a zygote formed by fusion of an irradiated and an unirradiated cell unable to develop into a visible colony. Stein and Laskowski[3] took this type of damage into consideration when building a mathematical model to interpret the inactivation of *Saccharomyces*. Part of their theory was that ultra-violet light of wave-length 254 nm also causes this type of dominant damage. This communication presents results which show that this damage can indeed be observed after ultra-violet irradiation.

Table 1

Organism	Algal (*A*) or bacterial (*B*) growth	Radiation: 2 h intermittent (30 min on, 10 off) 130,000 rads	2 h 130,000 rads	3 h 194,000 rads	3·5 h 227,000 rads	4 h 259,000 rads	4·75 h 288,000 rads	8 h 518,000 rads	43 h 2,786,000 rads
Lyngbya estuarii	*A*	+	+	+		+		+	−
	B	+	−	−		−		−	−
Oscillatoria brevis	*A*	+		+	+	− +			−
	B	+		−	+	− +			−
O. tenuis	*A*	+	+			+			−
	B	−	−			−			−
Phormidium autumnale	*A*	+	+			+			−
	B	+	+			−			−
P. luridum	*A*					+	+ +	+	−
	B					−	+ −	−	−
Plectonema boryanum	*A*		+		+		+		
	B		+		+		−		
P. calithricoides	*A*		+			+			−
	B		−			−			−
Microcoleus vaginatus	*A*	+				+			−
	B	+				−			−
Schizothrix calcicola	*A*	+	+			− +	+		
	B	+	+			− −	−		
Scytonema hoffmannii	*A*	+	+		+	+			−
	B	+	+		−	−			−
Nostoc linkia	*A*				+		+		
	B				+		−		
N. muscorum	*A*					+	+		
	B					−	−		
Coccochloris peniocystis	*A*				+ +	+	−		
	B				+ −	−	−		
C. stagnina Spreng	*A*					+ +	+ +		−
	B					+ −	+ −		−
Anacystis thermalis "B"	*A*	+			+ +	+ +			
	B	+			+ −	+ −			
A. thermalis "R"	*A*	+			+				
	B	+			−				
Anabaena variabilis	*A*						+		
	B						−		

Key: + Growth occurred. − Growth did not occur.
+ + / + − } Growth in some cultures, but at least one bacteria-free isolate could be obtained.

A diploid (266) and a tetraploid strain (407) of *Saccharomyces* were used for the irradiation experiments. These strains are more or less isogenic and homozygous, of mating type constitution $\alpha\alpha$ and $\alpha\alpha\alpha\alpha$ respectively, and serine prototrophic[4]. The unirradiated copulation partner was a haploid strain (211-1*bN*) of mating type *a*, which was serine auxotrophic. Ultra-violet irradiation was produced using a low-pressure mercury lamp (*HNS* 12, Osram) with a dose rate of $3{\cdot}1 \times 10^3$ erg/cm²/min with 10 per cent variation. The diploid and the tetraploid strain were incubated before irradiation for 40 h on a solid yeast extract medium (YP: 2 per cent agar, 0·5 per cent peptone, 1 per cent yeast extract (Difco), and 2 per cent glucose). The haploid strain was taken from a 20 h old shaker culture (liquid YP medium). Both were incubated at 30° C.

Before irradiation the strains were washed three times, and the suspended cells were centrifuged at low speed. This procedure gave about 98 per cent single cells in the supernatant and these were brought to a concentration of about 10^6 cells/ml. After irradiation the cells were mixed with the unirradiated haploid cells in a ratio of 1 : 5 and incubated on a solid YP medium for about 3 h at 30° C. From the resulting mixture of zygotes and cells, zygotes were extracted with a 'Fonbrune' micromanipulator. Zygote formation decreased with increasing dose of ultra-violet irradiation. In the control experiments without ultra-violet irradiation, the frequency of zygote formation was about 40 per cent. To avoid selection in the process of isolation, all the zygotes in one visual field of the microscope were taken.

After incubation at 30° C for 48 h the zygotes were inspected microscopically on the solid YP medium. Two types were observed: (1) zygotes that formed one or (very seldom) up to 200 descendants which were not able to grow up in liquid YP medium (lethal zygotes); (2) visible colonies with about 10^7 cells; these small colonies were transferred to fresh solid YP medium and checked for their mating type constitution by crossing with test strains by a technique described elsewhere[5]. Three different types were found. (*a*) Cultures giving no zygotes, either with the *a*- or with the α-test strain. (*b*) Cultures forming zygotes with the α-test strain. (*c*) Cultures forming zygotes with the *a*-test strain (these were very seldom noticed). Colonies of the first class were always serine prototrophic. This type included all cultures grown from real zygotes, while it is possible that types (*b*) and (*c*) resulted from incomplete formation of zygotes or, although this is very improbable, from the isolation of a haploid or diploid cell. Results of the copulation experiments are shown in Table 1.

Table 1. COMPARISON OF SURVIVAL FREQUENCY OF ULTRA-VIOLET-IRRADIATED DIPLOID AND TETRAPLOID *Saccharomyces* STRAINS AND THE COLONY-FORMING ABILITY OF ZYGOTES, FORMED BY IRRADIATED DIPLOID AND TETRAPLOID AND UNIRRADIATED HAPLOID CELLS

Irradiated strain	Ultra-violet dose (min)	Survival frequency	No. of isolated zygotes	Results of the examination of the cultures grown up from the isolated zygotes: Bogus zygotes, Irradiated strain	Bogus zygotes, Haplont	Real zygotes vital *v*	Lethal zygotes *l*	Frequency of lethal zygotes $\frac{l}{l+v}$
266	0·0	1·00	40	1	1	38	0	0·00
	6·5	0·23	47	0	5	25	17	0·41
	7·5	0·13	53	1	9	11	32	0·74
407	0·0	1·00	40	0	2	36	2	0·05
	5·0	0·51	50	2	1	27	20	0·42
	7·5	0·14	90	0	12	30	48	0·62
	8·0	0·07	50	0	6	11	33	0·75
	8·5	0·04	20	0	4	5	11	0·69

The frequency of lethal zygotes increases with the dose of ultra-violet radiation (last column in Table 1). The high rate of isolated zygotes, which later gave rise to parental haploid cells (column 6 in Table 1), showed that some processes may be involved after ultra-violet irradiation which 'save' most of the unirradiated part of the 'semizygotes'.

First observations, using the phase-contrast microscope with a special technique[6], made clear that the membrane between both copulating cells is dissolved in almost all the zygotes inspected after ultra-violet irradiation, although it was impossible to be sure about the fusion of the nuclei. It seems possible that 'dominant' ultra-violet damage will act only in real zygotes with fused nuclei, and that without fusion the unirradiated partner has a chance of survival. This means that 'dominant' lethality does not operate like a poison, but consists of damage within the nucleus or the mitotic apparatus as has been supposed for X-ray-induced 'dominant' damage in *Saccharomyces*. This suggestion is supported by the observation that most of the lethal zygotes begin to form one, and only one, daughter cell after ultra-violet irradiation.

When the results of ultra-violet irradiation are compared with those observed for X-rays[1,2], it seems that both types of irradiation act similarly in producing 'dominant' lethality. A comparison of the inactivation rate (l-s) with the frequency of lethal zygotes $l/l+v$ (see third and last column in Table 1) shows that in diploid and tetraploid strains the whole ultra-violet inactivation is larger than the 'dominant' damage. Although the main part of the inactivation can be explained in this way, the assumption of Stein and Laskowski[3], that 100 per cent of the ultra-violet inactivation of a tetraploid strain is caused by this 'dominant lethality', needs to be modified.

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Antibiotic Production in the Dacrymycetaceae

UP to the present time only four species of Dacrymycetaceae have been tested for antibiotic activity against bacteria[1,2]. Using sporophore extracts, Wilkens and Harris found no activity against *Bacterium* (*Escherichia*) *coli* or *Staphylococcus aureus* by *Femsjonia luteoalba* Fr., *Calocera cornea* (Batch.) Fr., *Calocera viscosa* (Pers.) Fr. or *Dacrymyces deliquescens* (Bull.) Duby. The present investigation was undertaken after a zone of inhibition produced by a culture of *Dacrymyces palmatus* (Schw.) Bres. was pointed out to me by Dr. R. H. Petersen.

Cultures were grown on a complete medium of malt extract, yeast extract and dextrose and a defined medium of glucose, asparagine, minerals and vitamins. One species, *Dacryopinax spathularia* Martin, was tested for antibiotic activity against various bacteria and fungi. Assay of antibiotic activity was made by the following techniques: (1) A filtrate of a culture of *D. spathularia* was placed in holes bored in agar medium seeded with selected test organisms. The zone of inhibition was measured after 24 h. (2) Photometric measurements were made at 400 mμ of turbidity increases of liquid cultures after 24-h exposure to medium in which *D. spathularia* had grown for 3 weeks. The *D. spathularia* was removed by membrane filtration. The second method proved to be the more consistent of the two. The addition of fresh nutrients did not change the results, which are shown in Table 1.

The antibiotic was active against all ten species of bacteria and four species of fungi tested by the photometric method. In several tests, however, *Saccharomyces cerevisiae* and *Candida albicans*, after initial inhibition,

Table 1. ACTIVITY OF *D. spathularia* AGAINST BACTERIA AND FUNGI

Test organism	Plate method	Photometric method
Staphylococcus aureus	+	+
Bacillus circulans	+	0
Bacillus mycoides	+	0
Bacillus subtilis	+	+
Sarcina lutea	+	+
Gaffkya tetragena	+	0
Staphylococcus epidermidis	+	0
Micrococcus spp.	+	0
Serratia marcescens	−	+
Proteus mirabilis	−	+
Escherichia coli	−	+
Aerobacter aerogenes	−	+
Pseudomonas aeruginosa	−	+
Proteus vulgaris	−	+
Pseudomonas fluorescens	−	+
Saccharomyces cereviseae	+	+
Candida albicans	+	+
Cryptococcus neoformans	0	+
Schizophyllum commune	−	+

+, Activity; −, no activity; 0, not tested.

Table 2. ACTIVITY OF VARIOUS SPECIES OF DACRYMYCETACEAE AGAINST *Bacillus subtilis*

Species tested	Result
Calocera cornea (Batch.) Fr.	−
Calocera viscosa Fr.	−
Dacrymyces deliquescens (Merat) Duby var. *deliquescens*	+
Dacrymyces deliquescens (Merat) Duby var. *ellisii* (Coker) Kennedy	+
Dacrymyces palmatus (Schw.) Bres.	+
Dacrymyces punctiformis Neuh.	+
Dacryopinax elegans (Berk. and Curt.) Martin	+
Dacryopinax spathularia (Schw.) Martin	+
Ditiola radicata Fr.	+
Femsjonia luteoalba Fr.	+

Activity was tested by placing plugs of test cultures on seeded agar plates.

showed growth after several hours exposure, presumably by the selection of resistant mutants, or the formation of adaptive enzyme.

To test the cause of the lack of response of Gram-negative bacteria to the plate method, *E. coli* and *Bacillus subtilis* were grown in media with varying concentrations of antibiotic and the activity tested by photometric assay of growth. No significant differences were found in the response of the two species. In a further experiment, these two species and *S. cereviseae* were exposed to the antibiotic for 24 h. The cells were centrifuged, washed with fresh media and plated. All three species grew. Because no resistant forms had ever been found previously among the bacteria, it would seem that the antibiotic is inhibitory and not lethal, at least at 24-h exposure.

A number of other species of Dacrymycetaceae were tested for activity by removing plugs from agar cultures and placing them on plates seeded with *B. subtilis*. The results shown in Table 2 and Fig. 1 indicate that antibiotic production is widespread in the Dacrymycetaceae. The lack of activity in the original screening[1,2] was probably owing to a dilution effect caused by the large quantity of "jelly" present, or lack of production of the antibiotic in fruit bodies. The absence of activity in the genus *Calocera* (Table 2) may be of taxonomic importance.

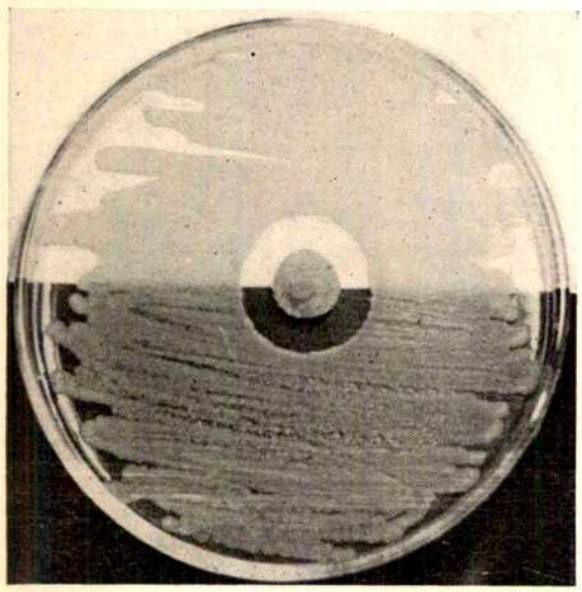


Fig. 1. Activity of a plug from a culture of *Dacrymyces palmatus* against *Bacillus subtilis*

The antibiotic, which can be produced on a simple chemically defined medium, is heat resistant (120° C for 1 h) and is stable to storage at room temperature (examined up to 6 months). The isolation of pure material has not been successful because of the difficulties imposed by the presence of a water-soluble polysaccharide which is produced by *D. spathularia*, and other members of the family that have been examined. Studies are at present under way to determine the mode of action of the antibiotic.

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In vivo Transfer of Infectious Drug Resistance

INFECTIOUS drug resistance that can be transferred *in vitro* from a donor bacterium to a sensitive recipient bacterium can also be transferred *in vivo*. Transfer has been shown to occur in mice which have had their normal intestinal flora reduced by the preliminary feeding of antibiotics. In such mice the donor and recipient strains become well established and multiply in the intestine[1,2].

A much simpler method of investigating the transfer of infectious drug resistance *in vivo* has now been developed. The success of this new method depends on the administration of donor and recipient strains of bacteria to newly hatched chickens which have no alimentary bacterial flora. The choice of donor and recipient strains for the following experiments was based on the results of previous *in vitro* transfers. Two strains of *Escherichia coli*, *C*9 and *C*15, were used as donor strains and both were resistant to tetracycline and streptomycin. They were wild-type strains which had been isolated from normal fowls. Two recipient strains of *E. coli* were used which were ampicillin resistant mutants[3] of two pathogenic serotypes 02 and 078; they were both fully sensitive to tetracycline and streptomycin. The original strains were isolated from cases of fowl coli septicaemia. A strain of *Salmonella typhimurium* (N.C.T.C. 5710) was also used as a recipient and this also was fully sensitive to tetracycline and streptomycin.

Chickens were collected from the incubator as soon as possible after hatching and often when they were still partly in their shells. They were infected by mouth with ten drops of an overnight broth culture of the donor bacterium and 6 h later received by mouth ten drops of an overnight broth culture of the recipient bacterium. Food and water were then made available and no special precautions were taken to provide sterile conditions. Tap water was used for drinking and the food was a proprietary chick mixture without antibiotics. The chickens were kept on wire mesh floors to prevent contact with faeces. They were observed for 10 days after being fed the bacterial strains, and faecal samples were cultured each day after the second day and examined for the presence of converted recipients.

Three experiments were carried out, and Table 1 shows the strains used in each experiment. It also shows that

Table 1

Experiment	Donor strain	Recipient strain	No. of chicks used	No. of chicks in which transfer occurred
(1)	*E. coli* C15	*E. coli* (078)	8	8/8
(2)	*E. coli* C15	*E. coli* (02)	16	16/16
(3)	*E. coli* C9	*S. typhimurium*	30	12/12*

* This figure represents a random sample at one particular time.

transfer of drug resistance occurred in all the chickens tested.

In these experiments there was only an occasional death among the groups of chickens after being fed both donor and recipient strains. In preliminary experiments, however, it was found that when the recipient strain *E. coli* 078 was fed without the donor strain many more deaths, about 25 per cent, occurred. These deaths were shown by bacteriological examination to result from coli septicaemia and were probably due to lack of bacterial competition in the alimentary tract.

In the first two experiments where both the donor and recipient were strains of *E. coli*, selection of the converted recipients was achieved by making use of their non-transferable ampicillin resistance. Daily samples of faeces from individual chickens were suspended in nutrient broth containing 20 μg ampicillin/ml. and incubated with gentle agitation at 37° C for 2 h. The samples were then plated on MacConkey agar containing 20 μg ampicillin/ml. and the lactose fermenting colonies which developed were identified by slide agglutination with specific antisera. Transfer of drug resistance could be detected as early as 24 h after feeding the two strains and the converted recipients could be isolated throughout the 10-day observation period. At first the converted recipients isolated from the faecal specimens formed a large proportion of the total faecal coliform count, but as the experiments progressed the proportion declined considerably.

In the third experiment, in which the recipient was *S. typhimurium* and the donor *E. coli C9*, the faecal specimens from individual birds were plated in an undiluted form and also after being diluted 10^{-2} in phosphate buffered saline. The selective medium used was brilliant green agar containing 25 μg oxytetracycline/ml. In addition, undiluted faecal samples, after being enriched with selenite broth, were plated on this medium. The converted recipients recovered were identified by slide agglutination with specific antiserum. Converted recipients were isolated as early as 24 h after feeding the two strains and their excretion continued for the 10 days of the experiment. During the first few days, the converted recipients were so numerous in the faeces that they could be isolated by direct plating on brilliant green agar containing oxytetracycline. Towards the end of the 10-day period their isolation could only be made from those samples enriched with selenite broth. However, by adding oxytetracycline to the drinking water at this stage the converted recipients could be isolated by direct plating of the faecal samples.

In both the transfer from coli to coli and from coli to salmonella, oxytetracycline resistance was transferred to the same level as that of the donor strain. In contrast, streptomycin resistance was transferred either at the same level or at a much lower level than the donor.

The results of these experiments show that transfer of drug resistance occurs as readily in the intestine of the chicken as it does when the two bacterial strains are mixed in a broth culture. Preliminary experiments have also shown that infectious drug resistance can be transferred in the chicken from resistant donor *E. coli* derived from pigs and calves to a sensitive recipient strain of *E. coli* (026) derived from man. It is, therefore, possible to obtain drug transfer *in vivo*, using chickens, even when the donor and recipient strains used are derived from different species.

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VIROLOGY

Absence of Serological Evidence of Myxoma Virus Infection in Humans exposed during an Outbreak of Myxomatosis

MYXOMATOSIS is a viral disease of rabbits regarded by some investigators as a neoplastic disease[1,2]. Epizootics have been observed in domestic rabbits (*Oryctolagus* spp.) in several areas of the Americas since 1898 when the disease was first reported by Sanarelli in Uruguay[3]. In California, myxomatosis outbreaks have been reported in domestic rabbit colonies along the coast from San Diego County to San Mateo County[4,5]. The disease in the *Oryctolagus* spp. is acute and highly fatal. The native wild rabbits (*Sylvilagus* spp.) are more resistant, and some develop benign fibromatous tumours[1]. In California, the brush rabbit (*Sylvilagus bachmani*) has been shown to be a reservoir host of the virus[5]. The virus has been shown to be transmitted naturally by a mosquito vector[6].

The host range of the virus disease appears to be very narrow. In his early work, Sanarelli[3] found that most animals were refractory to the disease. In attempting to infect several dogs he reported that one developed myxomatous neoplastic changes in the cutis. Two human subjects injected with infected rabbit serum developed a transient conjunctivitis. The animal work later was repeated by Bull and Dickinson[7]. They tested sixteen varieties of native Australian animals; none of the animals developed the disease. While there is considerable evidence of a narrow host range, the absence of observable signs of disease does not preclude the occurrence of inapparent infections nor of chronic effects after prolonged incubation.

With the increased interest in the possible viral aetiology of human cancer, evidence of transmission to man of viruses such as myxoma is of value. An opportunity to investigate for possible natural transmission of myxoma virus to man was presented in the summer of 1963 when myxomatosis occurred in a colony of domestic rabbits at a boys' camp in Santa Barbara County, California. An investigation was undertaken, and this report describes the outbreak and the results of a survey for serological evidence of myxoma infection in the boys and camp supervisor.

The outbreak began in June 1963 and resulted in daily losses of rabbits during the summer. Almost all the 600 rabbits in the colony died by the end of September when the epizootic ended. A similar epizootic had occurred in the same colony the preceding year.

The diseased rabbits demonstrated typical oedematous swellings of the head and vent. Histological examination of the lesions revealed myxomatous changes. Mosquitoes were observed feeding on the rabbits. Because of their role in the transmission of myxomatosis, adult mosquitoes from the rabbitry and larvae from nearby water sources were collected for identification. The adults proved to be *Anopheles freeborni* and *Culex tarsalis*, and the larvae *Anopheles franciscanus*, *Culex tarsalis*, *C. peus*, *C. bohardi* and *C. apicalis*. No viral isolations were attempted, but Marshall[5] demonstrated myxoma virus in pooled specimens of *A. freeborni* collected at the site of a myxomatosis outbreak in San Mateo County, California, in 1959.

That myxoma virus was the causative agent of the Santa Barbara outbreak was confirmed in the following manner. Portions of the inflamed eyelids and spleen were removed from an infected rabbit soon after death. Equal amounts of these tissues were pooled, ground, and saline was added to make a 10 per cent suspension. A rabbit, shown by serum neutralization (SN) testing to be devoid of myxoma antibody, was inoculated intradermally with 0·5 ml. of the suspension. A second rabbit, which had been immunized with myxoma vaccine 10 months previously, was inoculated in the same manner[6]. The susceptible rabbit died 9 days after inoculation with typical symptoms of myxomatosis. The temperature of the immune rabbit became elevated on the fourth day, but the animal remained clinically normal.

Blood specimens were taken from twelve boys, ranging in age from 12 to 15 years, and from one adult staff member. None of the persons tested reported any unusual or acute illnesses during this period. Mosquito bites were occasionally reported among the boys during the epizootic. In addition they all worked daily in or around the rabbit colony. All the boys lived within 300 yards of the colony during the 4 months of the outbreak, with the exception of two boys who left the camp after 3 months' exposure. The first sera were taken on August 8, 1963, approximately 2 months after the outbreak began, and second specimens were collected 7 weeks later. Six control specimens were taken from adults living in Berkeley, California; two of these were obtained from persons known to have antibodies against vaccinia virus. In addition, the serum of one of us (J. K. S.), who had worked with the myxoma virus in the laboratory for more than 14 years, was also tested.

The presence of myxoma antibody was determined by means of the SN test conducted in roller tube cultures of a rabbit kidney cell line as described by Saito and McKercher[8]. The antigen used was the *MSA* strain of myxoma virus which has a cytopathogenic effect on rabbit kidney cells. Myxoma reference serum was obtained from a vaccinated rabbit that was subsequently challenged with virulent virus. All sera used in the test were inactivated at 56° C for 30 min.

In the test procedure the virus was diluted in cell culture medium containing 3 per cent lamb serum in ten-fold steps, beginning with the 1 : 5 dilution. The sera were diluted 1 : 2·5 and mixed with an equal volume of the appropriate dilution of virus to give a final dilution of 1 : 5 of serum in the presence of decimal dilutions of virus. The serum–virus mixture was held at room temperature for 90 min and then added in a volume of 0·2 ml. per tube to cell cultures from which the medium had just been withdrawn. Five tubes were used for each serum–virus mixture. Ninety min at room temperature were allowed for adsorption to take place, and then 1·0 ml. of medium containing 3 per cent lamb serum was added to each tube of cell culture. Controls were included, and the cultures were incubated in stationary racks in an incubator set at 33·6° C. The final reading was made on the seventh day. The neutralizing capacity of the serum was expressed as the neutralizing index derived from the method of Reed and Muench[9].

None of the human sera had demonstrable antibodies against myxoma virus as tested by the SN technique, and there was no cross-neutralization observed in the two sera with vaccinia antibodies. Inactivation of human sera at 56° C for 30 min was necessary to eliminate non-specific neutralizing activity. Inactivation at this temperature had no effect on the antibody titre of the immune reference serum.

It cannot be concluded from this survey that inapparent human infection with myxoma virus never occurs. However, these persons had ample opportunity to become infected in an area where rabbits were dying daily with myxomatosis and where mosquitoes were present to perpetuate the epidemic. The lack of serological evidence of human infection suggests that under such conditions myxoma infection in humans is probably an unusual event, if it occurs at all. The negative SN test in the subject with years of varied laboratory exposure reinforces this view. Whether other circumstances, such as a vector with different feeding preferences or a more dense vector population, would result in human infections can only await opportunities for further investigation.

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Comparative Cytopathology of Rinderpest and Bovine Parainfluenza 3 Viruses in Cell Cultures

BECAUSE cytopathology may be useful in the detailed classification of viruses, we explored the comparative cytopathic changes induced by bovine parainfluenza 3 and rinderpest viruses in *MS* and dog kidney cell cultures.

Attenuated rinderpest virus was obtained from W. Plowright[1]. The virus had been serially passed a hundred times in bovine kidney cells and titred to $10^{4.5}$ $TCID_{50}$/ml. before it was grown in *MS* and dog kidney cells. Parainfluenza 3 virus was isolated[2] from sick cattle and was serially grown fifteen times in calf kidney tissue cultures and titred to 10^6 P.F.U./ml.

MS and dog kidney cells were grown in a medium consisting of 0·5 per cent lactalbumin hydrolysate in Hanks's balanced salt solution on 7 × 22 mm glass coverslips. The amount of inoculum per culture tube was 0·2 ml. of an undiluted seed virus. At intervals, infected coverslips together with uninfected controls were removed and stained with haematoxylin and eosin, using the modified technique of Reissig *et al.*[3].

Rinderpest virus failed to elicit any visible cytopathic effects but produced eosinophilic cytoplasmic inclusions and giant cells in *MS* cells (Fig. 1). About 90 per cent of the cells showed these inclusions 7 days after the inoculation of the culture. The inclusions were generally surrounded by a halo and some had a granular structure. A few cells showed intranuclear eosinophilic inclusions on the ninth day. Giant cells often contained the first inclusion. Acidophilic cytoplasmic inclusions and few giant cells were produced by rinderpest virus in dog kidney cells. Giant cells often contained the first, and at times the only, inclusions that appeared in a culture.

Bovine parainfluenza 3 virus showed distinct cytoplasmic inclusions and giant cells on the third day after inoculation in *MS* cells. About 83 per cent of the cells showed cytoplasmic as well as intranuclear inclusions. On the eighth day almost all the cells showed intracyto-

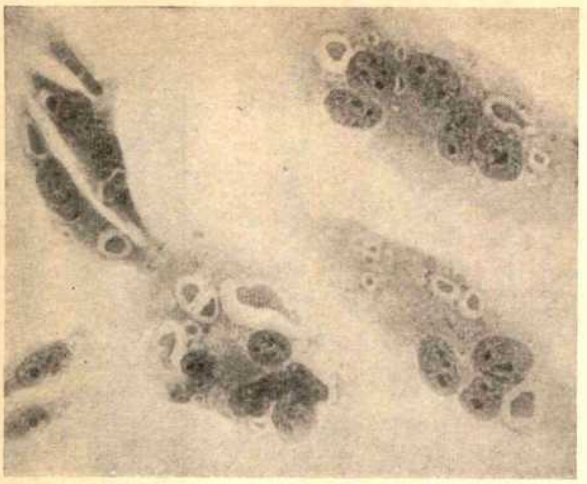


Fig. 1. *MS* cells 7 days after inoculation with rinderpest virus. Giant cells and eosinophilic inclusion bodies are present. Zenker's haematoxylin and eosin (×*c.* 500)

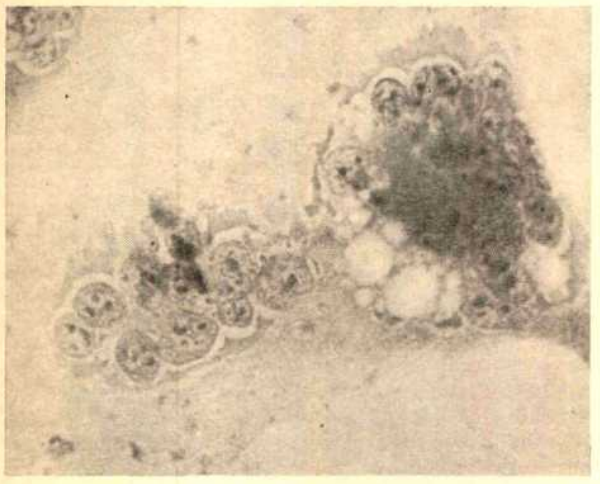


Fig. 2. *MS* cells 8 days after inoculation with bovine parainfluenza 3 virus. Giant cells and eosinophilic intranuclear and intracytoplasmic inclusions are present. Zenker's haematoxylin and eosin (×*c.* 500)

plasmic and intranuclear inclusions (Fig. 2). Few cytoplasmic inclusions and giant cells were seen in dog kidney cultures. No visible cytopathic effects were observed in the infected cultures.

These observations suggest that bovine parainfluenza 3 virus, a member of the myxovirus family[4], and rinderpest virus produce distinct and similar cytopathology in *MS* and dog kidney cells. Such findings support the decision taken in Montreal in August 1962 by the Viral Subcommittee of the International Nomenclature Committee[5] to accept that rinderpest, measles, dog distemper and respiratory syncytial virus are all members of the myxovirus group.

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Serological Properties of Coxsackie *A7* Virus

DURING epidemiological work on enteroviruses, four strains of Coxsackie *A7* virus were isolated from faecal specimens of healthy children cultured in cynomolgus monkey kidney tissue. Experiments carried out with these isolates and other representative strains of Coxsackie *A7* virus revealed that differences in antigenicity exist among strains of Coxsackie *A7* virus and emphasized the importance of the selection of working sera to identify isolates.

Virus strains: The prototype strain of Coxsackie *A7* virus, strain Parker, was obtained from two laboratories. One was sent in 1961 from Dr. H. Gelfand, Communicable Disease Center, Atlanta, as a suckling mouse torso suspension designated as 'Parker *M*34': this virus is here referred to as 'Parker (CDC)'. The other prototype strain was received in 1961 from Dr. M. K. Voroshilova, Institute of Poliomyelitis and Viral Encephalitides, Moscow, as a 10 per cent suspension of muscles of suckling mice. This virus had originally been sent by Dr. S. Gard, Stockholm, to Dr. Voroshilova in 1957, and had since undergone four passages through suckling mice. This strain is referred to as 'Parker (Moscow)'.

AB IV strain was first isolated by Dr. Voroshilova in 1952[1], and was then adapted to rhesus monkey kidney tissue cultures and identified as Coxsackie *A7* virus in 1957 by Dr. K. Habel, National Institutes of Health, Bethesda[2]. We received the strain from Dr. Voroshilova in 1961, as *AB* IV-*K.H.* No. 2435 culture fluid. The strain 119 (368) was isolated by Dr. Voroshilova in 1961 from a case of paralytic poliomyelitis in Moscow and sent to us in 1964 as culture fluid. The strain *NYS*-58182 was a tissue-culture adapted virus derived from a suckling mouse isolate from a poliomyelitis case in 1958 and sent to us as *TC*2-3 pool in 1964 from Dr. H. A. Wenner, University of Kansas. The strain Kyoto-435 was isolated in 1963 by Dr. Ashihara[3] by the inoculation of faecal extract of a clinical poliomyelitis case into a suckling mouse and was received in 1964 as the second passage.

Antisera: Monkeys and rabbits were immunized against representative strains. Undiluted tissue culture lysate after one freeze-thawing and a light centrifugation or a 10 per cent homogenate of infected suckling mouse torsos after a light centrifugation served as antigen for immunization. In most cases the antigen was subjected to a fluorocarbon treatment and was stored at −25° C. Rabbits and monkeys were, as a rule, immunized by subcutaneous and intravenous routes. Serum was inactivated at 56° C for 30 min before use. Anti-*AB* IV horse serum H–419 was supplied by Dr. Voroshilova in 1964.

Neutralization test: For tissue culture neutralization tests primary cynomolgus monkey kidney tube cultures were used. On the day before the test, the growth medium was replaced with serum-free maintenance medium (Earl's balanced salt solution containing 0·5 per cent lactalbumin hydrolysate and 0·11 per cent sodium bicarbonate, 100 U. of penicillin and 100 μg of streptomycin per ml.) and this was replaced with serum-free maintenance medium before inoculation. An equal amount of virus containing about 100 $T.C.I.D._{50}$/0·1 ml. and serial four-fold dilutions of serum was mixed and kept standing at 36° C for 3 h followed by standing at 5° C overnight. 0·2 ml. of the mixture was inoculated into each tube, containing 1 ml. of maintenance medium. Observation was carried out for 7 days and 50 per cent neutralizing end-point was calculated. Four tubes were used per dilution of the test serum. For suckling mouse neutralization tests, new-born mice from several litters were randomized and re-distributed to minimize variance in susceptibility. An equal amount of virus containing about 200 $L.D._{50}$/0·03 ml. and serial four-fold dilutions of serum were mixed and incubated at 36° C for 2 h. 0·03 ml. of the mixture was inoculated subcutaneously into each mouse, using one litter, in some cases only half the litter, per

dilution of the test serum. Daily observations were made for 14 days and 50 per cent neutralizing end-points were also calculated.

Experimental results:

(1) Isolation and identification of four strains of tissue culture isolates. Table 1 shows the data on the isolation of these strains. Three of them were isolated in our laboratory and the fourth strain, Natori-32, was isolated by Dr. H. Ujiie, Miyagi Institute of Public Health, and sent to us for identification. Attempts to identify these isolates by our routine procedures, using antisera against poliovirus 1, 2, 3, Coxsackie *A*9, *B*1-6, Echovirus 1–7, 9, 11–27 and Reovirus 1, failed to indicate to which class these agents belonged. Further investigations revealed that all these isolates are neutralized by the antiserum against *AB* IV strain of Coxsackie *A*7 virus. Three of these strains are also pathogenic to new-born mice by subcutaneous inoculation, manifesting generalized myositis and degeneration of the striated muscle.

Table 1. ISOLATION OF COXSACKIE *A*7 VIRUS IN MONKEY KIDNEY TISSUE CULTURES

Strain name	Collection of specimen			First appearance of CPE	Suckling mouse pathogenicity
	Year	Area	Source		
Z–C–61–7	1961	Nagaoka	Faeces (healthy child)	1st passage (9th day)	+(3)*
Z–C–52–7	1961	Nagaoka	Faeces (healthy child)	1st passage (11th day)	−(4)
63–*G*–35–4	1963	Takatsu	Faeces (healthy child)	2nd† passage (4th day)	+(2)
Natori-32	1963	Miyagi	Faeces (healthy child)	1st passage (9th day)	+(5)

* Numbers in parentheses indicate the tissue culture passage level of each strain, at which suckling mouse pathogenicity was examined.
† The first passage showed no cytopathogenic effect (CPE) up to the sixth day after inoculation.

(2) Cross-neutralization tests. The cross-neutralization test was carried out using antisera against various strains of Coxsackie *A*7 virus and some representative results are summarized in Table 2. These indicate that all the tissue culture adapted strains were equally neutralized by anti-*AB* IV, -63–*G*–35–4 or -Natori-32 immune sera. These sera also neutralized the Parker (Moscow) strain according to suckling mouse neutralization tests, but scarcely so in the case of Parker (CDC) and Kyoto-435 strains. Antiserum against Parker (Moscow) strain also neutralized tissue culture adapted strains in the same manner as the homologous strain, but less strongly so against Parker (CDC) and Kyoto-435 strains. On the contrary, immune sera against the prototype Parker (CDC) of Kyoto-435 strain neutralized tissue culture adapted strains to a lesser extent than the homologous strains. As a rule, immune sera against the strain Parker (CDC) neutralized Kyoto-435 strain as well as the homologous strain, and vice versa. Two immune sera, that is, *M*–5807–2 (monkey immune serum against Parker (CDC) strain) and *SR*–9–2 (rabbit immune serum against Kyoto-435 strain), were rather exceptional, heterologous neutralization titres between these two strains being quite low compared with the homologous titres.

The reason why the two lines of the prototype Parker strain of Coxsackie *A*7 virus show a shift in antigenicity has yet to be explained. It is interesting, however, that all the tissue culture adapted strains of Coxsackie *A*7 virus examined so far seem to be closely related to the prototype Parker (Moscow) strain, whereas one suckling mouse isolate, Kyoto-435, is closely related to the other line of the prototype strain, Parker (CDC). The findings demonstrate that monkeys or rabbits will respond in some cases, though not so frequently, to an agent more or less specifically, producing rather strain-specific antibody, as was observed with the sera *M*–5807–2 and *SR*–9–2. Extensive investigations using numerous strains of Coxsackie *A*7 virus, both tissue culture and suckling mouse adapted strains, are now under way. Complement-fixation and haemagglutination inhibition tests will also be included in further investigations. The data reported here would thus seem to indicate the importance of the selection of an adequate number of working sera for the identification of Coxsackie *A*7 isolates.

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Herpes Simplex Virus Particles in the Nerves of Rabbit Corneas after Epithelial Inoculation

THIS article demonstrates, for the first time, infection of deep ocular structures by herpes simplex virus as revealed by the presence of herpes particles in the cells of the stromal nerves.

Inoculation of the corneal epithelium of rabbits with herpes simplex virus produces an experimental disease that closely resembles naturally occurring human herpes simplex corneal infection (dendritic keratitis) in several ways: in both man and rabbit the epithelial lesions have a branching form, there is anaesthesia of the cornea, and the superficial ulcers respond to treatment with anti-herpetic drugs[1]. A certain number of human herpes simplex corneal infections are followed by inflammation of the deep layers (disciform keratitis)[2]; in such deep lesions, herpes virus is demonstrated much less frequently than in dendritic keratitis[1,3]. Under proper conditions, similar deep keratitis can be produced in the rabbit by certain strains of herpes simplex virus, despite the fact that herpes virus and antigens appear to be limited to the corneal epithelium or endothelium[1,4,5].

We have investigated rabbit eye tissues by light and electron microscopy after infecting the corneal epithelium with a strain of herpes simplex virus which regularly produces lesions in the stroma[6]. At intervals after in-

Table 2. CROSS-NEUTRALIZATION OF COXSACKIE *A*7 VIRUS

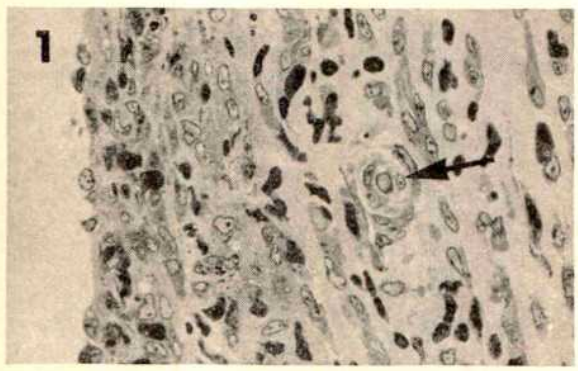
Antisera against strain	Serum code	Testing virus: Tissue culture adapted					Suckling mouse adapted		
		AB IV	119 (368)	*NYS*–58182	63–*G*–35–4	Natori-32	Parker (CDC)	Parker (Moscow)	Kyoto-435
AB IV	*SR*–4–3	5·3	5·3	> 4·0	3·6*	4·0	< 1·0	4·5	1·0
AB IV	*M*–5824–2	4·6	4·7	5·0	4·8*	5·0	< 1·0	< 4·2	< 1·0
AB IV	*H*–419	3·3	3·3	> 3·0	2·5*	2·7	< 1·0	2·4	< 1·0
63–*G*–35–4	*SR*–14–2	6·5	7·5	6·7	6·5	8·0	< 1·0	7·0	1·6
Natori-32	*SR*–24–1	4·0	5·0	6·5	6·0	5·0	< 1·0	5·5	1·0
Parker (CDC)	*SR*–12–2	2·0	1·5	2·5	1·5	1·5	7·3	3·5	7·5
Parker (CDC)	*M*–5807–2	2·3	3·5	4·0	2·7	4·3	7·7	4·7	1·5
Parker (Moscow)	*CR*–4	3·7	5·0	5·7	4·5	5·5	1·5	5·4	< 1·0
Kyoto-435	*SR*–8–2	⩽ 1·5	2·5	2·5	2·0	2·5	5·4	2·3	6·5
Kyoto-435	*SR*–9–2	⩽ 1·5	< 1·0	< 1·0	< 1·0	< 1·0	⩽ 1·5	< 1·0	⩾ 6·5

Antibody titres are expressed as reciprocals of $\log_4$ serum dilution, showing 50 per cent neutralization end-point against 34–300 $T.C.I.D._{50}$ or $L.D._{50}$ of the challenge virus.
* 1,000 $T.C.I.D._{50}$ was used as challenge virus.

fection, specimens were taken from the central cornea and corneoscleral junction (limbus). Small blocks of tissue were fixed in 3 per cent glutaraldehyde buffered with 0·067 M cacodylate, and then washed in a 0·1 M cacodylate solution containing 0·25 M sucrose. The tissues were post-fixed with 1 per cent osmium tetroxide in phosphate buffer, dehydrated in graded alcohols, and embedded in 'Epon' resin. Thin sections of this material were contrast-stained with uranyl acetate and lead acetate, and examined in an RCA 3*E* electron microscope; after the thin sectioning, thick sections (1–2 μ) for light microscopy were cut from each block and stained with toluidine blue.

Particles with the characteristic morphology of herpes simplex virus[7,8] were found in the corneal epithelial cells and in the nerves at the limbus. A herpes-infected corneal nerve in the limbal plexus, as described by Whitear[9], is shown in Fig. 1. Within the nucleus of one Schwann cell in this nerve there are characteristic herpes particles bounded by a single membrane ("naked virus particles") about 75–100 mμ in diameter and containing a dense central nucleoid (Fig. 2). Other virus particles are present at the cell membrane (Fig. 2); these are similar in structure to the nuclear virus particles, but possess a second limiting membrane or envelope. The cell was identified as a Schwann cell by the nerve axon in the cytoplasm and by the typical amorphous basement membrane just outside the cell membrane. The extracellular virus particles are lying between the plasmalemma and this basement membrane (Fig. 2).

Since herpes simplex virus acquires its outer envelope from the cell membrane by a process of budding[8], the position of these extracellular virus particles makes it probable that they have budded out from the cell membrane and have not penetrated the thick basement membrane surrounding the cell. The presence of single-membrane particles in the nucleus, and of double-membrane particles between the plasmalemma and basement membrane, indicates that this Schwann cell has been the site of formation of both naked and enveloped virus particles.



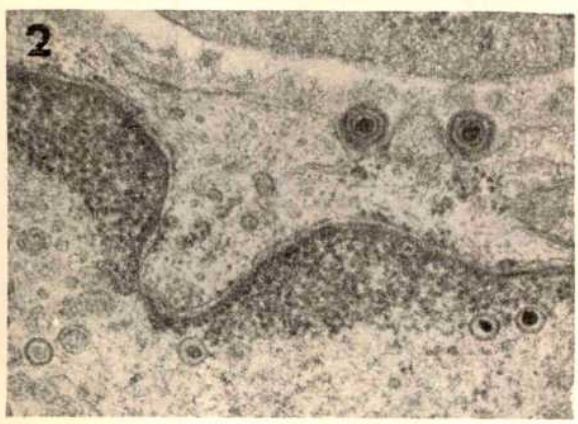


Fig. 1. Light photomicrograph of a section from the limba region of a rabbit eye infected with herpes simplex virus 48 h previously. Much of the superficial epithelium is missing; the infected peripheral nerve (arrow) lies in the anterior layers of the stroma. Toluidine blue stain. (× 480)

Fig. 2. An electron photomicrograph of the same Schwann cell in subsequent serial section. In the nucleus (bottom) there are three dense centred virus particles bounded by a single-membrane. At the cell surface there are two virus particles with two limiting membranes (upper right). The position of these virus particles between the cell membrane and the basement membrane would suggest that they had acquired an outer envelope from the cell membrane, but had not yet passed through the basement membrane. (× 43,000)

Infection of the limbal nerves has been detected 48 h and 72 h after inoculation; further investigations are now in progress to determine the duration and extent of this deep ocular infection. Typical particles have been found regularly in the corneal epithelial cells, but none of the other stromal cells (keratocytes) of the cornea or limbus show similar evidence of infection despite the marked inflammatory response of these tissues.

The presence of virus particles in these Schwann cells is the first direct morphological evidence that herpes simplex virus proliferates in the deep stroma of the cornea and sclera. It has been known for many years that herpes virus infection of the cornea, or other peripheral sites in the rabbit, often produces encephalitis and that the virus can reach the central nervous system through the peripheral nerves[10]. A recent re-evaluation of the pathogenesis of herpes encephalitis in mice by means of an immunofluorescent technique has shown that the virus reaches the central nervous system in blood-borne macrophages (in young animals) or by an ascending infection of the peripheral nerves[11]. Although the strain of herpes simplex used in these experiments rarely caused encephalitis in rabbits, the agent did infect the Schwann cells of nerves beneath the corneal epithelium. If such a local dissemination of virus occurs in dendritic keratitis in man, this involvement of stromal nerves would offer an explanation for the corneal anaesthesia and involvement of deeper structures of the anterior segment of the eye found so regularly in human cases of herpes simplex keratitis.

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ENTOMOLOGY

Micro-determination of Insect Blood Volume

Methods of estimating insect blood volume depend either on haemocyte counts before and after an injection of a measured volume of saline or on the dilution of amaranth dye by the haemolymph[1]. The latter method

has been recommended for repeatable and consistent results with locusts[2]. Neither method, however, is likely to be practical for experiments with small insects, for example, the housefly, because the volume of liquid used for dilution (0·1–0·2 μl.) is too small to be administered with accuracy.

The following method, which is being used in our investigations of altering the ionic ratios in insect blood, has proved satisfactory in determining small blood volumes, and is based on the exchange of a cation which exists in abundance in the blood with ion-exchange resin. Sodium in both housefly and blowfly exists largely in the haemolymph.

Cation exchange resin '*IR*-120' sieved to a uniform mesh of 36 is thoroughly activated to the (H^+) form and tested for complete absence of sodium by ashing a sample and testing with a flame photometer. This resin is strongly acidic and has an exchange capacity of about 16 μg Na^+/10 beads at 20° C.

Weighed houseflies are held by suction on their dorsal or ventral side. An incision is made with a sharp hypodermic needle in the last abdominal segment, or inter-muscularly in the thorax, or even in the head capsule. Ten beads for flies weighing up to 20 mg are inoculated with watchmakers' forceps. After 10 min when the entire haemolymph has circulated the resin and the exchange reached equilibrium, the beads are carefully removed and with a little experience should be secured clean. They are washed with de-ionized water, ashed, and their sodium content determined with a flame photometer. This represents the total blood sodium in equilibrium with the resin.

A random sample of 1 or 2 μl. of blood drawn into a micro-pipette is mixed on a watch glass with the same number of beads for a few minutes. The resin is washed with de-ionized water and the amount of Na^+ exchanged/μl. is determined. The volume of blood =

$$\frac{\text{total blood } (Na)^+ \text{ exchanged}}{(Na)^+ \text{ exchanged}/\mu l.}$$

The ion exchange equilibrium constant $(Na)^+$ solution/$(Na)^+$ resin, for up to 5 μl. of housefly blood using ten beads, is 0·3 at room temperature and it remains constant within the range 15°–25° C. It is therefore necessary that both experiments are carried out within that range.

Four- to six-day-old houseflies fed on milk and kept at 25° C and in 50 per cent relative humidity showed a blood volume of 0·17 ± 0·02 μl./mg wet body-weight. A similar result was obtained for the blowfly (*Phormia terraenovae*), where again ten beads/20 mg body-weight were used. The blood volume of both species varied according to age and diet.

The advantages offered by this method include: (1) it takes into account any blood which exudes from puncturing the insect, because the resin will exchange ions with it through the process of inoculation; (2) it can be applied to insects much smaller than the housefly using beads of suitable mesh; (3) it yields results very rapidly.

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HAEMATOLOGY

Gm(g)—A "New" Gamma-globulin Factor

THE four antigenetically different heavy polypeptide chain sub-types[1–3] of γG-globulin* are probably determined by genes at different genetic loci[4–7]. Two of these, the 'We' and 'Vi' loci, are closely linked and related to the Gm system[4–7]. Gm^1 and Gm^4 are alleles belonging to the 'We' locus. Myeloma proteins or isolated γG-antibodies of the We sub-group are Gm(1) or Gm(4) (refs. 4 and 8). Gm^5 belongs to the 'Vi' locus where various partly identical genes are present[9]. Isolated γG-antibodies of the Vi sub-group are either Gm(5) or lack any known Gm factor[4–6]. It has been suggested that Gm(–5) antibodies of the Vi sub-group are determined by a hypothetical unidentified gene Gm^-, which is the true allele of Gm^5 (refs. 4 and 6).

This communication reports a new γG-globulin factor, called Gm(g), determined by a gene, Gm^g, behaving as an allele of Gm^5 at the 'Vi' locus. Gm(g) can be identified by a haemagglutination-inhibition test[10] using anti-Gm(g) 3978 and red cells coated with incomplete Gm(g) anti-D 4752.

Incomplete anti-D 4752 from a Gm(1,–2,4,5,g), Inv(–1, –2) donor was characterized as a γG-antibody by preparative ultracentrifugation and various tests with specific rabbit antibodies to γG-, γA- and γM-globulin respectively[11]. Red cells (O,R_1R_2) coated by anti-D 4752 in excess were strongly agglutinated by antiglobulin sera but not by several anti-Gm((1),(2),(4), (5),(6),(8),(9),(10), (11),(12)) or anti-Inv ((1),(2)) reference reagents. Red cells coated with pepsin-digested (Fab) anti-D 4752 were tested with anti-γ-globulin factors detected by Fab γG-globulin. For convenience, such anti-γ-globulin factors are called 'pepsin-agglutinators' and they show specificity for heavy chain sub-group determinants[11,12]. Similarly, reference anti-D antibodies were also tested with at least two anti-Gm factors and four 'pepsin-agglutinators' of each specificity (Table 1). All the Gm(1) and Gm(4) anti-D antibodies belonged to the We sub-group and Gm(5) anti-D to the Vi sub-group. Anti-D 4752 was a Gm(–5) antibody of Vi sub-group.

Table 1. HEAVY CHAIN SUB-GROUP AND Gm SPECIFICITY OF VARIOUS ANTI-D ANTIBODIES

Anti-D	Gm-type of donor	Specificity of anti-D antibody: Heavy chain sub-group	Gm-type
3091	1,2,–4,–5,g	We	1,2,–4,–5,–g
3311	1,–2,4,5,g	We	1,–2,–4,–5,–g
3083	–1,–2,4,5,–g	We	–1,–2,4,–5,–g
3358	–1,–2,4,5,–g	We	–1,–2,4,–5,–g
3109	–1,–2,4,5,–g	Vi	–1,–2,–4,5,–g
3500	–1,–2,4,5,–g	Vi	–1,–2,–4,5,–g
4752	1,–2,4,5,g	Vi	–1,–2,–4,–5,g

By using red cells coated with whole anti-D 4752 a corresponding haemagglutinating substance in serum 3978 was found (titre 128). Activity was also detected by anti-CD Ripley. No reaction was obtained with red cells coated by each of thirty-six other strong incomplete anti-D antibodies specific for at least one of the factors, Gm(1),(2),(4),(5),(6),(7),(8),(9),(10),(11),(12) or Inv (1),(2). Another serum (3765) showed a weak reaction with anti-D 4752 and a strong reaction with reference Gm(1) anti-D. Activity detected by anti-D 4752 was only absorbed by red cells coated by anti-D 4752 but not by using Gm(1) anti-D. On the other hand, the anti-Gm(1) activity of serum 3765 was only removed by Gm(1) anti-D and not by using anti-D 4752. Sera 3978 and 3765, both Gm(–1,–2, 4,5,–g) were from patients with rheumatoid arthritis. There was no prozone in the agglutination of red cells coated by anti-D 4752 or anti-CD Ripley.

The haemagglutinating substances of sera 3978 and 3765 were inhibited by native pooled γG-globulin and by γG-globulin of serum 4752, as characteristic for anti-Gm

* The nomenclature used in this paper is that drafted in *Bull. Wld. Hlth. Org.*, **33**, 721 (1965).

factors. No inhibition was obtained with Fab γG-globulin, which indicated that the reactive site was part of the Fc fragment. Results of density gradient ultracentrifugation and tests with specific rabbit antisera[11], and of mercaptoethanol-treatment[11], indicated that the anti-Gm(g) factors were γM-globulins.

Fifteen Gm(1,–4,–5) Caucasian sera which were Gm(2) positive or negative and fifteen Gm(–1,–2,4,5) sera were tested in dilutions 1/4, 1/6 and 1/8 for inhibition of 2,4 and 8 agglutinating units[10] of anti-Gm(g) 3978. All the Gm(1) sera inhibited the agglutination while the Gm(–1) sera did not. Similar results were obtained when isolated γG-globulin[12] (2 mg/ml.) of the same sera was used. Gm typing[10] of sera from a hundred unrelated Caucasian blood donors (Table 2) showed the following frequencies : Gm(1), 52 per cent; Gm(2), 25 per cent; Gm(4), 91 per cent; Gm(5), 91 per cent; Gm(g), 49 per cent. All the Gm(2) sera were also Gm(1). Except for two sera which gave intermediate reaction in Gm(g) typing, all but one serum showed concordance between Gm(1) and Gm(g) types. Four agammaglobulinaemia sera were Gm(–g). Each of five isolated Gm(1) myeloma proteins kindly provided by Dr. M. Harboe were all Gm(1,–g). In a two-dimensional haemagglutination-inhibition test[10] the Gm(g) inhibiting capacity was weaker than that of Gm(1) in a given serum positive for both factors.

Of thirteen American and twenty West Indian Negro sera, kindly provided by Dr. K. Kano and Dr. E. R. Gold, respectively, twenty-five were Gm(1,–4,5,–g), five Gm(1,4,5,–g), one Gm(1,–4,5,g), one Gm(1,–4,–5,g) and one Gm(–1,4,5,–g). One West African Negro serum was Gm(1,–4,5,–g).

Results of the serological tests indicated that Gm(g) anti-D 4752 and anti-Gm(g) 3978 determined a γG-globulin factor distinct from previously known factors[6,9,13,14]. The Gm(g) anti-D 4752 did not contain other Gm factors. According to the 'one gene–one polypeptide chain' concept (see ref. 6), Gm(g) is determined by a separate gene.

Various findings indicated that *Gmg* is the true allele of *Gm*[5]:

(i) Anti-D 4752 was a Gm(–5) γG-antibody of the Vi sub-group.

(ii) Anti-Gm(g) was found in Caucasian Gm(–1,–2,4.5,–g) sera, in one case coexisting with anti-Gm(1).

(iii) All sera tested were either Gm(5,–g), Gm(–5,g) or Gm(5,g). In Caucasians, Gm(g) and Gm(1) were usually inherited together, just as Gm(4) and Gm(5). Only one Caucasian serum was Gm(1,4,5,–g). Although the genes at the 'We' and 'Vi' loci are closely linked, 'crossing-over' occurs[6], which would explain this phenotype. The intermediate reactions are probably due to the very small amounts (0·25–1·1 mg/ml.) of Vi γG-globulin in normal sera[1]. In Negroes Gm(1) and Gm(5) are inherited together, and Gm(4) is very rare in a Negro population without Caucasian genes[14]. Accordingly, phenotype Gm(1,–4,5,–g) was most frequent in American and West Indian Negroes and was also found in the single West African Negro. The other phenotypes were explained by Caucasian ancestry in American and West Indian Negro populations.

(iv) Gm(g) antibodies were uncommon. Two-dimensional haemagglutination inhibition experiments further indicated that only a small proportion of the γG-molecules in a Gm(g) serum carries the Gm(g) specificity. *Gmg* thus takes part in the synthesis of a relatively small amount of γG-molecules, just as *Gm*[5].

Since *Gmg* is probably equivalent to the unidentified gene *Gm*– at the 'Vi' locus[4,6] (Fig. 1), the common gene complexes in Caucasians are *Gm*[1]*Gmg*, *Gm*[1,2]*Gmg* and *Gm*[4]*Gm*[5]. Further investigations of Gm(g) are at present being carried out.

Table 2. Gm-TYPES OF 100 NON-RELATED CAUCASIAN SERA

	Gm(g)	Gm(–g)	Intermediate
Gm(1,–4,–5)	9	0	0
Gm(–1,4,5)	0	48	0
Gm(1,4,5)	40	1	2

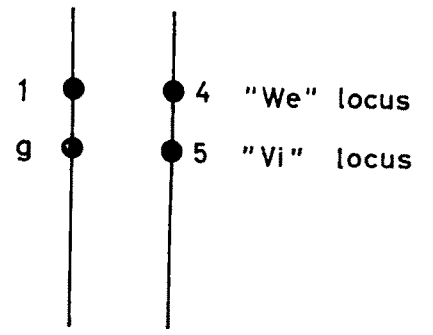


Fig. 1. *Gm* genes at the 'We' and 'Vi' loci in Caucasian Gm(1,4,5,g) individuals. Probable localization of *Gmg*

I thank Drs. H. Fudenberg, E. R. Gold, K. Halvorsen, M. Harboe, K. Kano, E. van Loghem, L. Mårtensson, C. Ropartz, A. G. Steinberg and M. Waller for generous provision of reagents which made this work possible. This study was aided in part by a grant from L. Meltzers Hoyskolefond, Bergen, Norway.

Note added in proof. Twenty-two African Negro sera kindly provided by Drs. T. E. Cleghorn and F. Lothe were all Gm(1,–4,5,–g). Gm(g) has been given the designation Gm(21) by the WHO International Reference Centre for Genetic Factors of Human Immunoglobulins.

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PATHOLOGY

Polyarteritis Nodosa-like Disease in Outbred Mice

Helyer and Howie[1] described a disease of *NZB/BL* × *NZY/BL* and *NZB/BL* × *NZW/BL* F_1 hybrid mice closely resembling human systemic lupus erythematosus (SLE) in renal histology. A breeding nucleus of these mice was provided by Drs. F. and M. Bielschowsky and Dr. Howie, to allow confirmation of their histological findings. Similar lesions were found in *NZB/BL* mice, although the age of onset is later than in the *B* × *W* hybrids. Outbred albino Palmerston North (*PN*) mice, initially intended as controls, showed renal and arterial lesions more closely resembling human polyarteritis nodosa than SLE. A comparison of the two types of lesion is summarized in Table 1. Spontaneous arteritic lesions have been described in rats by Skold[2] and in mink by Leader[3]. Deringer[4] has described arteritis of the ovarian and small bowel arteries in *BL/De* mice, but generalized spontaneous arteritis in mice does not appear to have been described previously.

The *PN* mice were bred in a small colony at the Palmerston North Hospital for 10 years, then used to

Table 1. COMPARISON OF RENAL CHANGES IN MICE OF *PN* AND *NZB/BL* STRAINS

		Arterial fibrinoid	Perivascular cells	Tubular dilatation and/or casts	Glomerular change
All renal lesions	*PN*	17	21	16	18
	NZB/BL	1	16	11	22
Minimal lesions excluded	*PN*	11	15	14	13
	NZB/BL	1	4	7	20

Twenty-two *PN* and twenty-two *NZB/BL* mice of comparable age and sex were used. All show either glomerular change or arterial fibrinoid.

establish a specific pathogen-free colony by Glaxo Laboratories, Ltd. We are indebted to this firm for providing mice retired from intensive breeding at approximately 6 months of age. Of 169 mice under investigation, 90 mice have been examined *post-mortem*, and 28 of these show lesions. Three of the latter were found dead and three were killed prematurely. The remainder were killed when they became ill. The group is not homogeneous in that there were only 45 males and some were selected for albuminuria from a larger group at 6 months of age.

Seventeen animals showed renal changes of moderate or marked degree, and five showed minimal changes. The renal tubules were dilated and in most cases contain casts. The glomerular affection was less severe than in the *NZB/BL* mice, wire loop lesions being clearly shown in only three *PN* mice but in the majority of *NZB/BL* mice. Marked perivascular cuffing with lymphocytes and plasma cells occurred in both groups of mice. Arterial fibrinoid necrosis, often localized to a segment of the artery, was seen in most of the affected *PN* kidneys. Eleven of these, and three additional mice, showed arteritis of other vessels often of a more acute type with polymorphonuclear and eosinophil infiltration. Coronary arteritis with myocardial scarring occurred in three mice. The splenic arterioles were involved in five cases, and the carotid, femoral, salivary, lymph node, and small bowel arteries were affected in two cases each. Fibrinoid change with loss of elastic tissue and localized aneurysm formation was seen in the ascending aorta in three animals and another died of a ruptured aneurysm of the abdominal aorta. This lesion was seen in only one *NZB/BL* mouse, which was not one of the comparison sample. Arteritis of medium-sized vessels was rarely encountered in *NZB/BL* and *B* × *W* hybrids. The fibrinoid in arterial lesions and glomeruli resembled that of human periarteritis and Aleutian disease of mink[3] in that it stained red with picro-Mallory, pink with periodic acid–Schiff and green with fluorescent antiglobulin. Pyronine staining was weak except in the glomerular loop lesions. Staining with the Feulgen reagent was negative. The arterial lesion stained faintly with alcian blue. Thioflavine *T* did not stain, except in two animals with amyloidosis as well as arteritis. Five of forty-eight animals without arteritic lesions showed amyloidosis. No lesions were seen in mice killed at 6–12 months after neonatal injection with a cell-free saline extract of kidney from *NZB/BL* mice affected by SLE-like disease. One *PN* mouse developed lesions at 9 months, but the majority developed lesions later, nine of forty-one at 9–12 months, and six of twenty-nine at 12–15 months. Adequate sections of the thymus were available in forty mice. Germinal centres[5] were clearly discernible in four out of ten with arteritic lesions, and in one of thirty without. None of the eleven mice with lymphoma showed arteritis. This does not appear to be a result of earlier death from this lesion. The numbers are too small to comment on these findings or on the relationship to inter-current disease. Homogeneous antinuclear staining previously described in outbred mice by Norins and Holmes[6] and in an inbred strain by Friou and Teague[7] did not show a clear correlation with arteritis. Positive staining occurred in six of sixteen affected mice and in four of forty-four others. Two other mice with renal lesions showed transient nucleolar fluorescent staining. *LE* cells were not found in any of forty-four *PN* mice. One animal with leukaemia had the only positive Coombs test of sixty-one terminal sera examined.

The degree of the differences between the lesions of the *PN* mice and the *NZB/BL* mice makes it probable that they are separate but closely related conditions, one resembling polyarteritis nodosa and the other human systemic lupus erythematosus. This provides a further spontaneous animal model for the investigation of this group of diseases.

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PHYSIOLOGY

Serum Cholesterol Levels in Alloxan Diabetic Rats after Loading with Various Alimentary Fats

IT has been shown[1] that large amounts of lard taken orally for several days raised the serum cholesterol levels of alloxan diabetic rats above 300 mg per cent, whereas those of normal rats after the same loading remained at about 100 mg per cent.

It is well known that dietary fats, especially those of vegetable origin, affect the serum cholesterol content. Russell *et al.*[2] demonstrated that 28 days of a diet containing 30 per cent lard produced a marked elevation of the serum cholesterol in alloxan diabetic rats. A 30 per cent corn oil diet, however, had no effect.

The purpose of the investigation reported here was to investigate the effect of a brief excessive load of vegetable fat on the serum cholesterol content of alloxan diabetic rats. We used coconut oil, which is rich in saturated fatty acids, and sunflower oil, known for its relatively high content of unsaturated fatty acids.

The experiment was carried out with male Wistar rats 8–12 weeks after a subcutaneous injection of alloxan. Only the rats with blood ketone body levels above 10 mg per cent 3 days after injection, and excreting more than 5 g glucose within 24 h 6 weeks later, were used as diabetics. The techniques and methods were as described previously[1]. The fat loading of the alloxan diabetic and the control normal rats was carried out as follows: on the first day the rats received five doses of coconut oil or sunflower oil (0·5 ml./100 g body-weight) through stomach tubes. On the second day they received the same oils in five doses each of 1 ml./100 g body-weight. 15 h after the last feeding they were bled and the serum cholesterol content was determined using the micro-

Table 1. SERUM CHOLESTEROL LEVELS IN NORMAL AND DIABETIC RATS (mg/100 ml.)

	Normal	Diabetic	*P* value*
No fat loading	99 ± 3·7 (12)†	97 ± 3·4 (16)	—
Coconut oil	150 ± 4·4 (5)	285 ± 20·0 (7)	< 0·01
Sunflower oil	95 ± 7·0 (7)	334 ± 37·0 (7)	< 0·01

* Results of Student's "*t*" test.
† Numbers in parentheses represent the number of animals in each group.

method of Rappaport and Eichhorn[3]. During the course of the investigation it was found that the normal rats did not tolerate loading with coconut oil; one third of them died. The average values and their standard deviations of the serum cholesterol levels for all groups of rats are given in Table 1.

Table 1 shows that only coconut oil elevated the serum cholesterol levels of normal rats. In the alloxan diabetic rats, however, both vegetable oils caused the same striking rise of serum cholesterol as had the lard in the previous investigation. These findings seem to be in accordance with the theory based on the work of Brady and Gurin[4] that the diabetic liver cannot convert acetate to long-chain fatty acids, but can synthesize cholesterol at the usual rate. On the other hand, impairment of cholesterin synthesis has recently been reported in alloxan diabetic animals[5,6].

The fact that we could produce a threefold increase of the serum cholesterol content of alloxan diabetic rats within 60 h with various alimentary fats shows that in the diabetic animals it is possible to metabolize the two-carbon fragments broken down from the alimentary fats through an elevated synthesis of cholesterol.

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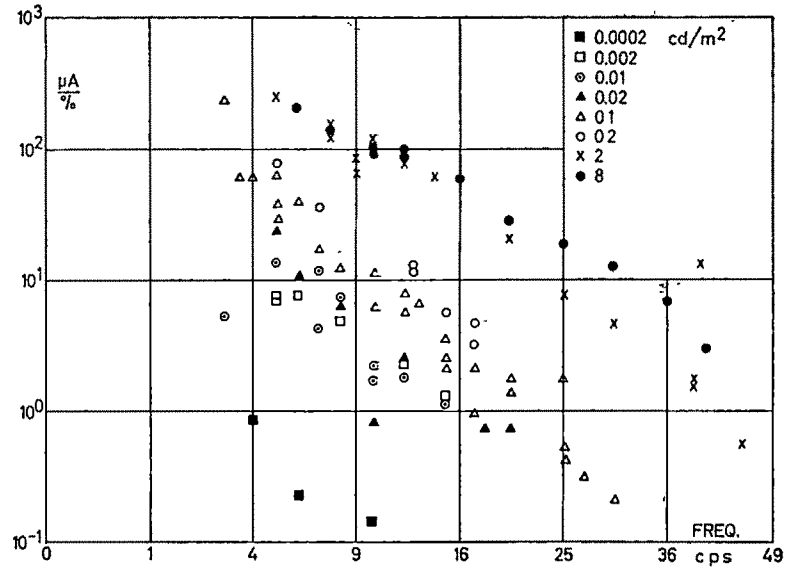


Fig. 2. Attenuation of foremost human visual mechanisms. Same conditions as Fig. 1. Ordinate shows current amplitude divided by corresponding modulation depth of light on a logarithmic scale. Frequency scale is parabolic

Electro-optical Interaction in the Retina

WE have advocated[1,2] the use of combined electrical and optical stimulation of the human eye as a research tool, and have discussed[3] some experimental results obtained by Brindley[4] with this method. This article amplifies some of our previous[2] experimental results.

In a series of experiments, we measured the angles $\varphi + \pi$ between modulated lights $L(t) = L_0(1 + M \sin \omega t)$ and currents $i(t) = i_0 \sin(\omega t - \varphi - \pi)$ applied to the eye simultaneously, which yield minimal total flicker perception. These angles were measured as a function of the frequency $\omega/2\pi$ over a wider range of brightnesses L_0 than before[2].

We checked once more[2] that the value φ obtained in this way does represent the phase angle by which L must lead i for maximal total flicker perception and thus probably is the phase shift applied to the light-signal by the foremost part, or parts[4], of the human visual system. It turns out that φ is independent of i_0 and M at fixed L_0. Experimental photopic and scotopic results are presented in Fig. 1.

It should be noted that, although in all runs the light stimulus was presented on a 40° test field, actual photopic measurements bear on a few degrees in the centre of the field. Scotopic phase shifts bear on the periphery of the test field, where the electrical phosphene is much more conspicuous at these levels of adaptation.

These data, which were obtained using only one subject and with pinkish light from a 1*B*59 tube, should illustrate, nevertheless, the general behaviour. An artificial pupil was not used.

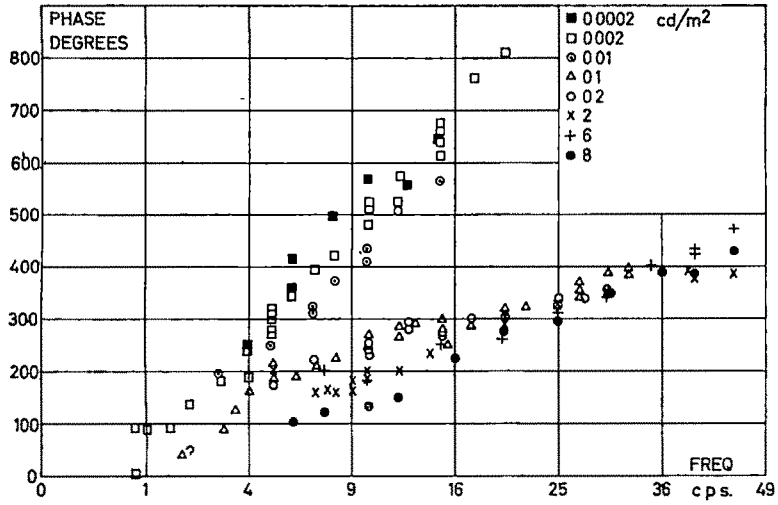


Fig. 1. Phase shift of foremost human visual mechanisms. Subject F.V., left (leading) eye; optical test field 40°, pinkish light from 1*B*59 glow modulator tube. No artificial pupil. Frequencies are plotted on a parabolic scale

In the same series of experiments, the current amplitudes were set for optimal cancelling of light-induced and current-induced flicker by means of a procedure which we described earlier[2]. Session-to-session variability of these settings prevented a definition of the curves shown in Fig. 2 better than about 0·3 log unit.

A considerable low pass behaviour of the amplitude-versus-frequency curves occurs at all levels of brightness, as would be expected from the phase measurements (but we prefer to postpone a detailed discussion of this property for the time being).

It is certain, however, that the amplitudes obtained are not nearly proportional to the prevailing brightness L_0. On the other hand, strict proportionality between i_0 and M at fixed L_0 exists; the proportionality constant, i_0/M, depends only slightly on the brightness; it fluctuates from one half-hour session to the next. In the experimental series described here, at least 10 min of adaptation elapsed before any measurements

were made. This was necessary because of the slow adaptational changes exhibited by the threshold for the electrical phosphene[5,6].

The 1*B*59 light source was used in a feedback circuit described earlier. As no mydriasis or artificial pupil was used, our data at low frequencies and medium brightnesses may be slightly influenced by pupillary movements.

A current source of at least 120,000Ω internal impedance injected current up to 0·5 m.amp root mean square by way of a 10 cm² ring-electrode placed over the upper and lower left eyelids of the subject. A large electrode on the right arm was connected to earth. The right eye was tightly closed. Zero phase angle in the foregoing means that maximum light flux and positiveness of the eye-electrode occur at the same time.

The family of photopic curves probably represents some average activity of the three kinds of cone receptors recently[7] demonstrated. Whether or not this curve can be split up by using differently coloured or monochromatic light, to show the relative phase shifts demonstrated by de Lange[8] and Walraven[9] in flickerphotometry, will have to be examined later.

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Interference of Injected Tetra-*n*-Propylammonium Bromide with Outward Sodium Ion Current in Squid Giant Axons

IT was demonstrated recently[1] that tetraethylammonium (TEA^+) chloride affects the potassium permeability of a squid giant axon in a very interesting way. After TEA^+ is injected into the axon to an axoplasmic concentration of about 40 mM, membrane resistance to outward K^+ current is unusually high, while resistance to inward current remains about normal; that is, after injection the K^+ permeable membrane has the property of "anomalous rectification". This was interpreted to mean that TEA^+ ions enter the membrane from the axoplasm and stop up the K^+ channels unless the channels are swept clear of TEA^+ by inrushing K^+ ions. While testing other quaternary ammonium salts for this property, it was found that tetra-*n*-propylammonium ($Tn\text{-}PA^+$) bromide affected in this way not only the K^+ current but also the Na^+ current.

The experiments reported here were performed on giant axons of the Chilean squid *Dosidicus gigas*, and the techniques and artificial sea-water used were similar to those previously described[1].

In Fig. 1 are plotted current-voltage curves of the membrane, taken during the period of high Na^+ permeability by the method indicated in the inset: the membrane was depolarized to 10 mV to increase the Na^+ permeability, and a second voltage step was applied at the peak of the Na^+ current transient so obtained. Current was measured immediately after the second step. $Tn\text{-}PA^+$ at an axoplasmic concentration of 8 mM causes marked rectification of the current, while TEA^+ at the same concentration does not affect it (Fig. 1). Control curves similar to that shown (preceding which nothing was injected) were recorded after injection of both potassium chloride and potassium bromide in sufficient quantity to raise the axoplasmic K^+ concentration by 10 mM or more. Fig. 2*a* shows the currents produced by longer depolarizing steps, at an axoplasmic $Tn\text{-}PA^+$ concentration of 10 mM. The first peak in the curves is the Na^+ current, while later current is K^+. In the depolarization to 90 mV, which is beyond the Na^+ equilibrium potential, changes can be seen in both the Na^+ and the K^+ current. When the trace first reappears, the current (Na^+) is decreasing in magnitude rather than increasing as it does in the control curve (Fig. 2*b*). This implies that the rectification of Na^+ current after a voltage step is not established instantaneously, and that we observe here its time course. The K^+ current displays a hump resembling that produced by 2·4 mM TEA^+ (Fig. 2*b*): the K^+ current increases, decreases, then increases slowly.

These results raise two questions: what properties of these quaternary ammonium ions cause them to compete with Na^+ and K^+ for the current channels, and why do they stop up the channels instead of simply passing through? The most obvious answer to the first question is that these two ions, in solution, may be similar in size

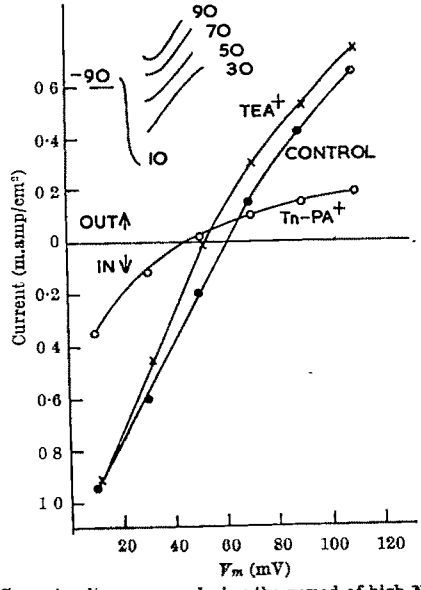


Fig. 1 Current-voltage curves during the period of high Na^+ permeability, taken by the method shown in the inset. Numbers in the inset give membrane potential (V_m). $Tn\text{-}PA^+$ had this effect only when injected into the axon

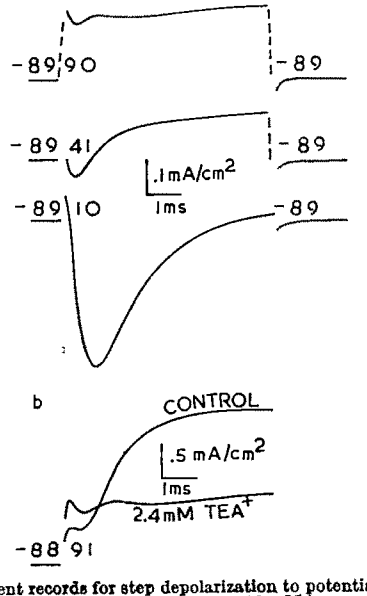


Fig. 2. Current records for step depolarization to potential given (mV). Axoplasmic $Tn\text{-}PA^+$ was 10 mM in *a*

to hydrated Na^+ or K^+ as the case may be. In crystals, at least, the sizes of the ions can be compared easily. In $KAl(SO_4)_2.12H_2O$ the K^+ ion is surrounded by six water molecules at a distance from K^+ to oxygen of 2·94 Å (ref. 2) (all distances will be centre-to-centre distances). The distance to the centre of the entire water molecule is perhaps 3 Å. Adding to this 1·45 Å, which is half the distance between water molecules in pure water, the maximum radius of the K^+ ion with one hydration shell is about 4·45 Å. This is similar to the maximum radius of TEA^+ in crystalline tetraethylammonium iodide[3], which is 4·6 Å if the radius of the terminal $—CH_3$ group is taken as the van der Waals radius of 2 Å (ref. 4). TEA^+ (and also Tn-PA^+) probably does not have a hydration shell in solution[5], so nothing more need be added to the 4·6 Å. The fact that the larger Tn-PA^+ ion (5·9 Å by the same calculation, with arms fully extended[6]) interferes with the Na^+ current suggests that in solution Na^+ may be more hydrated than K^+. In $NaAl(SO_4)_2.12H_2O$ the Na^+ ion is surrounded by six water molecules at a distance of about 2·5 Å (ref. 2). In solution, the first hydration shell may be more complete, since it is sterically possible to fit in more than six water molecules. A second layer added to a closely packed first layer (that is, with water molecules 2·9 Å apart) would be at a distance of about 4·2 Å from the Na^+, making the maximum radius about 5·65 Å. These radii must not be taken too seriously, but they do suggest that in solution the hydrated K^+ ion is similar in size to TEA^+, and the hydrated Na^+ similar to Tn-PA^+. Tn-PA^+ perhaps competes with K^+ when its hydrocarbon arms are not fully extended. The reason why TEA^+ and Tn-PA^+ stop up the channels is less apparent. These ions are less radially symmetrical than Na^+ and K^+, and this may allow them to approach more closely and thus bind more tightly to a (hypothetical) negatively charged group in the membrane. The fact that both TEA^+ and Tn-PA^+ are effective only when inside the axon indicates a difference between the inside and the outside of the membrane.

Fortified with this evidence that the K^+ ion in solution has one hydration shell while the Na^+ ion has two (a view that is widespread but not conclusive), it is easy to proceed to a simple theory of the cation selectivity of the membrane. In Fig. 3 the large circle represents a site selective for Na^+. The site has a negative charge, but its precise character is unimportant so long as it is a good fit for a hydrated Na^+ ion and its circumference is rigidly fixed. Entry of a hydrated K^+ ion (medium size circle) would only partially fill the site, leaving an appreciable space. Water molecules, the smallest available molecules, could not fit, and the space would remain empty, even though its total volume was equal to that of a number of water molecules. Creation of such a space would require a great deal of energy, for to make a hole the size of a single water molecule in an aqueous solution requires as much energy as the transport of a water molecule from the aqueous to the vapour phase. Still more energy would be required to lower the dielectric constant of the space[7]. The large energy barrier resulting from these two factors would prevent the entry of the K^+ ion. The hydrated Na^+, on the other hand, would be too large to enter a K^+ selective site, which would be similar to the Na^+ site but smaller. This selectivity hypothesis does not depend on the exact value of the radii used here, which are not likely to be absolutely correct.

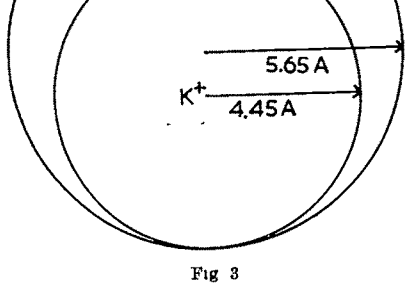


Fig 3

I thank my wife for dissecting the axons used, all members of the Biophysics Laboratory for help and discussion, and the Estación de Biologia Marina for facilities.

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PSYCHOLOGY

A Two-hour Reading Course

IT seems likely that the improvements brought about by reading efficiency courses are largely due to such factors as increased concentration rather than the training of eye movements or 'perceptual skills'[1,2]. It has been suggested[3,4] that increasing one's concentration while reading, or 'reading more actively' (a term frequently used by instructors), results in an increasing use of the redundancy of the passage being read, making the immediate stimulus more easy to recognize and process further.

To test the practical implications of such a hypothesis a single session reading course was designed to include the following features:

(1) An initial 450-word passage followed by a comprehension test consisting of ten simple open-ended questions.

(2) Short lectures on bad reading habits: (*a*) inadequate concentration; (*b*) vocalization or sub-vocalization; (*c*) unnecessary regressions.

(3) Reading a short selection of statistical approximations to English (first, third and sixth orders) to illustrate the existence of constraint in the language without reference to meaning.

(4) An illustration of the effects of context on word recognition. This was accomplished by flashing several words on a screen, initially in isolation then with a context sentence which predicted it to a small degree, finally with a context sentence which predicted the word to a high degree. The exposure time and contrast were chosen so that about a third of the students saw the word on the first exposure, a further third on the second exposure and nearly all when the word was briefly exposed with a strong context.

(5) Illustrations of the notion of backwards redundancy on the level of both words and ideas, indicating to what extent regressions may be unnecessary.

(6) Five 3-min sessions of silent reading of a 'Pelican' or similar non-fiction volume, during which students were expected to keep in mind what they had just been told.

(7) A final passage for reading with a comprehension test.

The whole procedure lasted just less than 2 h, and there were interspersed frequent statements of the type "You *can* all improve your reading efficiency" and similar exhortations and encouragements.

Table 1

	Sub-group 1 ($n=36$)		Sub-group 2 ($n=26$)	
	Initial test—*A*	Final test—*B*	Initial test—*B*	Final test—*A*
Mean efficiency	211	243	161	242
Mean efficiency	211	252	158	252
Mean improvement	33 per cent		80 per cent	
Median improvement	19 per cent		45 per cent	
Wilcoxon T	140		11	
	($P<0.002$)		($P<0.0001$)	

Note: The word 'efficiency' is defined as the product of speed of reading in words per minute and the fractional comprehension.

The students were personnel on a U.S. Air Force base; three lectures have been given, with a total of sixty-two students. Two passages, *A* and *B*, were used, some of the students reading *A* initially and *B* finally and the rest vice versa. The time for reading was taken by the students to the nearest 5 sec and the reading efficiency was calculated as the product of the speed of reading in words per min and the fractional comprehension score. The results are shown in Table 1.

It is clear that the group as a whole improved its reading efficiency in the 2 h. The best estimate of the average improvement is about 30 per cent, one student improving by 500 per cent and fifteen students performing less well on the final test than on the initial test. The mean group improvement found on commercial courses, which take from 15 to 27 h, is about 50 per cent.

Three points must be made about the present course. First, the course was held in the evening following a full day's work for the students, and the course itself was reportedly exhausting. Accordingly, the final scores might be artificially low. Secondly, as opposed to this, the only real test of a course of this kind is the long-term effect. It is likely that many of the students in the absence of further encouragement will not maintain their improved performance. Thirdly, the course makes no direct attempt to extend the students' capacity, merely to show them what kinds of relevant knowledge they already have. For some of the people, for example, an extensive vocabulary training would be necessary. This was pointed out to the classes, as were a few fairly obvious "reading techniques" such as the advantages of reading the summary of a book before tackling the main body, and the different strategies which one might use when reading material for specific purposes; but for some people more detail of this kind would be necessary. It was clear, however, that many of the students had never realized that language was redundant, nor that they need not read every word of a text. In some cases a short course of this kind could be as effective as a longer one.

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When Recognition is No Better than Recall

RECENT findings have thrown doubt on the validity of the generalization that it is easier to recognize than to recall. Davis *et al.*[1], using a list of fifteen two-digit numbers as stimuli, found that if all ninety possible numbers are presented in a recognition test, performance is no better than recall when both are tested immediately after the presentation.

I have made a similar comparison of recognition and recall using a list of English counties as stimuli. (This research was carried out before the recent boundary changes.) Counties preserve the advantage numbers have of being a clearly defined, limited, and enumerable category, but whereas all subjects can generate all ninety two-digit numbers at will, they are not able to list all the counties.

Table 1. MEAN RECALL AND RECOGNITION SCORES FOR COUNTIES

Stimuli	Recall	Recognition
Commonly named	5·45	5·27
Rarely named	3·86	5·27
Average	4·65	5 27

The experiment was carried out in two stages. In the first, response availability was examined. Eighty-six young newly-enlisted men, tested in groups of 10–20, were asked to write down the names of all the English counties. Ten minutes were allowed, by which time all had stopped writing. A mean number of 19·99 out of a possible 40 were listed (*S.D.*, 5·45). The frequency with which any particular name was listed ranged from 93 per cent of subjects (Hampshire) to 9 per cent (Huntingdonshire).

In the second stage, two lists of counties were selected as stimuli for memory experiments in which free-recall and recognition measures were compared. One list consisted of the six most frequently named counties. The other consisted of the six least frequently named ones. To lengthen the lists of stimuli sufficiently to exceed the memory span and thus induce some errors, six additional counties from the middle of the response-availability range were added, three at the beginning and three at the end. The same six buffer items were used with both lists and they were not counted when assessing performance.

The stimuli were printed on large cards and were presented one at a time with a 5-sec exposure and a 1-sec interval between items. Memory was tested immediately after presentation. Alternate members of each group were required to recognize the stimuli from a list of all forty counties typed in random order, while the remainder had a blank sheet of paper on which they had to write out the twelve they had seen in any order. Three minutes were allowed for this test. Three groups of fresh subjects were tested with each list, the order of presentation of the critical stimuli being varied each time. Altogether, twenty-two subjects were tested under each condition.

The mean performance on the critical stimuli is shown in Table 1. It can be seen that although recognition is markedly better than recall for the rarely named counties and is better overall, with the commonly named counties recall is slightly superior.

Similar tests have been carried out with a 40-min delay between presentation and test, during which time the subjects were kept busy with other, unrelated, experimental tasks. With the commonly named counties the mean performance on recognition was 5·07 (fifteen subjects), while that on recall was 4·31 (sixteen subjects). The superiority of recognition under this condition was statistically significant ($P < 0.02$). With two-digit numbers, however, introduction of the delay did not lead to significant advantage for recognition. The relevant scores were: recognition, 8·07 (forty subjects); recall, 7·85 (forty subjects) $P > 0.25$.

The picture is thus rather complex, but it would appear that on immediate test recognition is no better than recall for readily available responses. A fuller account of these experiments and their implications will appear elsewhere.

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Vertical Sinusoidal Vibration as a Psychological Stress

MOST modern vehicles move, by design or accident, to some extent in all six theoretical axes (movement along and rotation around the three orthogonal axes, fore-aft, side-side, and up-down). Whole-body vibration is likely to be applied to human occupants most frequently and with greatest power in the vertical (seat to head) direction,

however[1]. Investigations of major physiological and psychological effects of vertical vibration have, in addition, usually used sine-wave forcing functions as a necessary research convenience[2].

Reviews of vertical sinusoidal vibration effects[1-4] related to psychomotor performance generally report two areas of impairment; fine motor control and vision. Both effects derive from body resonance phenomena, which are primarily dependent on frequency. For example, vertical oscillation at about 5 c/s produces a marked resonance of shoulder-girdle and abdominal contents, which in turn means that manual dexterity is degraded, and unpredictable relative motions obtain between the eye and the visual target. These are examples of the specific effects of vibration stress.

Non-specific stressor action, resulting in experimentally demonstrable impairment of mental function, has only rarely been reported for vibration. Theoretically[5,6] man has a finite capacity for handling information, and if some of this is devoted to persistent anxiety about his organic integrity it should be possible to show that a limited capacity remains for carrying out tasks. Two experiments are reported which set out to test this hypothesis[7].

The psychological task chosen combines mental arithmetic and immediate memory. Subjects have a list of digits read out to them, and are required to give the sum of the last two digits presented. As an example, the series 5 . . 7 . . 3 . . 11 . . 8 has solutions 12 . . 10 . . 14 . . 19. The task thus involves storing one item from an operation while performing that operation. Interpolated items of this type are known to accelerate the normal decay of immediate memory[8].

In the first experiment, five adult male subjects were exposed to vertical sinusoidal vibration at 4·8 and 6·7 c/s, at an acceleration-amplitude of 0·5 *g* applied to the seat, for two 15-min exposures at each frequency. Two sets of digit lists were prepared, using the numbers 1–5 (four problems) and 4–20 (sixteen problems) in random order. Possible learning or adaptation effects were reduced by arranging the vibration conditions and digit lists in a different random combination for each subject. The presentation of and response to the tasks were tape-recorded, and two measures of performance derived. The first, the time taken to complete a list, included the total time the subject used in computation; the time which the experimenter spent in reading out digits was subtracted. The second measure was the total number of addition errors made. A final index of performance, indicating the time spent achieving each correct addition, was derived from these two measures. An analysis of variance of these results demonstrated that performance on the sixteen-problem lists was adversely affected by vibration at either frequency, compared with performance when stationary. There was no difference between the conditions at 4·8 and 6·7 c/s, even though the former is by general agreement the more uncomfortable in terms of body transmissibility. The four-problem list is considered to have been too easy to be sensitive to stress effects.

In the second experiment, eight adult males who were then military jet pilots were experimented on. They were exposed to the same vibration conditions, but for two 10-min exposures only at each frequency. Two new sets of digit lists were constructed, using the numbers 1–10 (nine problems) and 5–30 (twenty-five problems). Eight subjects allowed possible order effects to be balanced out over the total experimental design, a procedure which has some advantages over randomization. Identical measures of performance were used to derive the metric time utilized per correct response. Analysis of variance showed that vibration had impaired performance of the nine-problem list, but not of the twenty-five-problem list, which was judged to have been too difficult relative to the learning opportunity available. Again, the conditions at 4·8 and 6·7 c/s did not give rise to significantly different impairments.

Table 1

Time spent on each correct solution (secs)		Hypothetically increasing stress level → Static	0·5*g* at 6·7 c/s	0·5*g* at 4·8 c/s
Hypothetically increasing task difficulty ↑	Digits 5–30	2·7	2·7	2·7
	Digits 4–20	2·1	2·3	2·4
	Digits 1–10	1·3	1·4	1·5
	Digits 1–5	1·2	1·4	1·6

Table 1 presents means for these data. Although these cannot be tested statistically (because of different subject populations and experimental treatments) this table does lend support to the theory, partially confirmed elsewhere[5,6], that stress level and task difficulty operate synergistically to diminish human information processing ability at higher levels.

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AGRICULTURE

Bract Pigmentation in Bananas

SIMMONDS[1] investigated the pigmentation of the bracts of wild banana species and edible cultivars. Of the two wild species from which the Eumusa series of edible bananas are derived[2] *Musa acuminata* was found to contain a mixture of delphinidin, petunidin, malvidin, cyanidin, and peonidin, that is, both cyanidin, delphinidin and their methylated derivatives, whereas *Musa balbisiana* contained only a mixture of delphinidin and cyanidin. Methylated anthocyanidins were absent. The bracts of edible bananas were found to have "broadly, the pigments expected on taxonomic and genetic grounds but show an (unexplained) tendency towards a generally lower level of methylation of the anthocyanidins"[1]. That is to say, some cultivars clearly derived from *M. acuminata* nevertheless only contained non-methylated anthocyanidins. At the time of the 1954 investigation the various geographical subspecies of *Musca acuminata*[3] were not yet clearly defined and little material from Melanesia was available. We now have available in Honduras a much larger *Musa* collection[4] of which 526 accessions of *Musa acuminata*, *Musa balbisiana*, and their cultivars having bracts pigmented with anthocyanin have been examined.

The methods of extraction, hydrolysis, and paper chromatographic separation were as described by Simmonds[1].

The results with the wild species are summarized in Table 1.

Clones in which the anthocyanidins are not methylated are found not only in *M. balbisiana*, but also in the *banksii* (New Guinea) and *errans* (Philippine) geographical subspecies of *M. acuminata*. The lack of methylation is not, therefore, confined to *M. balbisiana* as Simmonds believed.

Table 1. ANTHOCYANIDINS IN *Musa acuminata* AND *Musa balbisiana*

Species or sub-species	No. of accessions tested	No. containing only cyanidin-delphinidin	No. also containing methylated anthocyanidins
M. acuminata burmanica	3	0	3
M. acuminata siamea	6	0	6
M. acuminata truncata	6	0	6
M. acuminata zebrina	6	0	6
M. acuminata microcarpa	7	0	7
M. acuminata malaccensis	19	0	19
M. acuminata errans	4	1	3
M. acuminata banksii	15	9	6
M. balbisiana	14	14	0
Totals	80	24	56

Table 2. ANTHOCYANIDINS IN THE BRACTS OF *M. acuminata* EDIBLE CULTIVARS (353 ACCESSIONS)

Presumed derivation of cultivars*	Diploid		Triploid		Total	
	U†	*M*†	*U*	*M*	*U*	*M*
siamea	0	1	0	11	0	12
zebrina	0	16	6	29	6	45
microcarpa	31	37	0	5	31	42
malaccensis	1	15	51	53	52	68
errans	1	8	11	0	12	8
banksii	22	22	6	27	28	49
Totals	55	99	74	125	129	224

* D. L. Richardson (unpublished work)
† *U*, Number of accessions with only cyanidin and delphinidin. *M*, Number of accessions also containing methylated anthocyanidins.

This makes it less surprising to find cultivars derived from *M. acuminata* cultivars lacking in methylated anthocyanidins which, as Table 2 shows, are relatively common.

Half of the diploid cultivars derived from *banksii* have bracts which contain only non-methylated anthocyanidins. This is not unexpected, as a similar distribution is found in the wild sub-species. It is, however, unexpected to find so many cultivars of microcarpa derivation which do not contain methylated anthocyanidins. These include such common edible bananas as 'Eda-an' and 'Pisang jari buaya'.

Triploid acuminata cultivars lacking methylated anthocyanidins are also common. These are not confined only to the Cavendish group as believed by Simmonds[1], but also include 'Pisang susu' in the *malaccensis* group and the Philippine Lacatans among others.

In agreement with Simmonds[1] it was found that among these accessions derived from both *M. acuminata* (*AA*) and *M. balbisiana* (*BB*) the *AB* diploids (three tested) contained methylated anthocyanidins, the *AAB* triploids were mixed (thirty-one *U*, sixteen *M*), and the *ABB* triploids (thirty-seven tested) contained only unmethylated pigments.

Of the naturally occurring tetraploids[4], Atan (*AAAB*, two accessions) and Kalamagol (*AABB*, two accessions) contain methylated anthocyanidins, whereas Klue Teparod (*ABBB*, two accessions) contains only unmethylated pigments. A high proportion of genic contribution from *Musa balbisiana* obviously results in bracts lacking in methylated pigments as with *Musa balbisiana* itself.

Of greater importance to a banana breeding programme is hybridization within the acuminatas and bract pigmentation may be a convenient genetic marker. Accordingly, the inheritance of pigment type within *Musa acuminata* is being investigated.

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Mode of Action of L-Threo-β-phenylserine as a Chemotherapeutant of Cucumber Scab

CERTAIN amino-acids, such as L-threo-β-phenylserine, have been shown to reverse the action of indoleacetic acid on root growth of cucumbers, bud development of beans and epinasty of tomatoes. It was thought that a relation might exist between the activity of such compounds as auxin antagonists and their chemotherapeutic activity against certain fungal diseases including cucumber scab[1].

Competitive inhibition of auxin action by these compounds seems less probable than an indirect effect. I have shown that after application of 0·02–0·005 M L-threo-β-phenylserine to the roots of cucumber seedlings, crude extracts of the hypocotyls and cotyledons possess higher IAA oxidase activity than extracts of untreated plants. This might explain the antiauxin activity of L-threo-β-phenylserine.

If there is a relation between the increase in IAA oxidase activity and the decrease in the IAA content of the plants on the one hand and the increase in resistance against *Cladosporium cucumerinum*[1,2] on the other hand, other substances influencing IAA oxidase activity may be expected to influence the susceptibility as well. Thus *B* 995 (*N*-dimethylamino succinamic acid) and CCC ((2-chloroethyl)trimethylammonium chloride), which produce an increase in IAA oxidase and peroxidase activity in cucumbers[3], should increase the resistance. Gibberellic acid should have an opposite effect[3,4].

The influence of these substances on the susceptibility of cucumbers for *C. cucumerinum* was investigated by applying the compounds to the roots of 7-day-old cucumber seedlings var. 'Lange Gele Tros', or by spraying twice on the green parts. The seedlings were inoculated with a suspension of spores of *C. cucumerinum* after treatment. The severity of the disease was assessed according to the rate of infection of the hypocotyls after incubation for 3–6 days at 20° C in a moist atmosphere. The disease indices given in Tables 1 and 2 have been calculated using a scale from 0 (healthy) to 4 (severely diseased).

Table 1 shows that both *B* 995 and CCC (obtained by courtesy of G. Ligtermoet and Zn.) have a protective effect. Higher concentrations caused injury, particularly to the roots. The protective effect of *B* 995 and CCC and, to a lesser extent, that of L-threo-β-phenylserine (S.A.F. Hoffmann La Roche and Co.) was reversed by simultaneously administering gibberellic acid (potassium salt, Nutritional Biochemicals Corporation). The effect of gibberellic acid alone was investigated by applying it to the roots in various concentrations and determining the disease index 3, 4 and 5 days after inoculation. Untreated plants showed the maximum index, 4, at the fifth day after inoculation, but plants treated with gibberellic acid 450, 90 and 18 p.p.m. reached this stage already after 3, respectively 4 days. Even treatment with 3·6 p.p.m. seemed to result in a somewhat faster development of disease symptoms. The increase in susceptibility caused by gibberellic acid could also be demonstrated by inoculation with a spore suspension of a concentration that would not cause maximum infection in untreated plants, but would do so in plants treated with gibberellic acid. Moreover, under our experimental conditions only seedlings inoculated 7–9 days after sowing show the typical symptoms of a heavily diseased hypocotyl, whereas in older plants the first leaf is attacked instead. After treatment with gibberellic acid, however, the hypocotyl appeared to remain susceptible for a longer time. The fact that disease symptoms appear particularly in the younger growing parts of the plant may be important in itself in this connexion, since these usually contain more IAA.

Table 2 shows that spraying with IAA also increased the susceptibility of the seedlings. This result points to a direct relation between the IAA content of the plants

Table 1. PROTECTION OF CUCUMBER SEEDLINGS AGAINST *C. cucumerinum* BY TREATMENT WITH VARIOUS SUBSTANCES AND REVERSAL OF THE EFFECT BY GIBBERELLIC ACID

	Concentration (p.p.m)	Disease index—gibberellic acid 0	Disease index—gibberellic acid 50 p.p.m
L-threo-β-phenylserine	905	0·9	1·7
"	452·5	2·4	3·8
B 995	250	0·8	3·9
"	125	2·9	4·0
CCC	250	1·8	3·8
"	125	2·7	4·0
Water		3·7	4·0

Disease index determined 5 days after inoculation.

Table 2. INCREASE IN SUSCEPTIBILITY OF CUCUMBER SEEDLINGS FOR *C. cucumerinum* CAUSED BY SPRAYING WITH INDOLEACETIC ACID AS DEMONSTRATED BY COMPARING DISEASE INDICES AT DIFFERENT TIMES AFTER INOCULATION

Concentration of indoleacetic acid (M)	Days after inoculation 4	5	6
0·001	2·9	3·6	4·0
0·0002	2·7	3·3	4·0
0·00004	2·3	3·4	3·7
0·000008	2·1	3·4	3·6
Water	1·9	2·8	3·4

and their susceptibility to *C. cucumerinum*. Thus it may be considered to support the hypothesis that the chemotherapeutic action of phenylserine is mediated through its influence on IAA oxidase activity. The activity of the enzyme *in vitro* was not influenced by adding phenylserine. This means that the increase in activity in treated plants must be effected in an indirect way. Elema[4] found that gibberellic acid in peas inhibits formation of *p*-coumaric acid, a co-factor for enzymatic IAA oxidation: addition of the compound to extracts of plants treated with gibberellic acid restored IAA oxidase activity.

It is tempting to explain the effect of phenylserine by assuming increased production of *p*-coumaric acid or other compounds acting as co-factors for IAA oxidase in treated plants.

Only preliminary experiments have so far been carried out on the mechanism of action of other chemotherapeutically active amino-acids. It seems probable that other mechanisms than interference with IAA oxidase activity may also be involved.

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SOIL SCIENCE

Seleniferous Soils in Parts of England and Wales

GEOCHEMICAL reconnaissance by stream sediment sampling of 3,000 square miles in the Midlands, North Wales and South-west England disclosed extensive areas characterized by abnormally high molybdenum contents of 5–60 p.p.m. compared with normal background of < 2 p.p.m. The technique used was similar to that applied in Eire[1], and in view of the association of selenium and molybdenum in that country, a selection of the British samples was also analysed for selenium. The results showed selenium to be present in stream sediments in each of the anomalous molybdenum areas, with maximum values ranging from 3·8 to 9·0 p.p.m. selenium compared with the normal background of < 0·2 p.p.m. (Table 1).

Following up the stream sediment indications, preliminary soil samples have been collected from selected anomalous catchment areas. Sample sites were chosen on low-lying poorly drained organic soils, which Irish experience[1,4] has shown to be favourable for the accumulation of selenium and molybdenum. The analyses show that the stream sediment values are related to soils that contain moderately anomalous concentrations of these two elements rising to 7·0 p.p.m. selenium and 45 p.p.m. molybdenum (Table 2).

Table 1. RANGE AND MEAN CONTENT OF SELENIUM AND MOLYBDENUM IN STREAM SEDIMENT

Area	No. of samples	Se (p.p.m.)	Mo (p.p.m.)
North Staffordshire and Derbyshire	22	0·2–9·0	5–40
		2·8	19
Caernarvon	16	< 0·2–4·0	1–25
		1·3	13
Devon	23	0·2–3·8	5–10
		1·9	6
Normal background		< 0·2	< 2

Data refer to minus 80-mesh fraction; selenium was determined colorimetrically (ref. 2) and molybdenum spectrographically (ref. 3).

Table 2. RANGE AND MEAN CONTENT OF SELENIUM AND MOLYBDENUM IN POORLY DRAINED SOILS IN ANOMALOUS CATCHMENT AREAS

Area	No. of samples	Sample depth (in.)	Se (p.p.m.)	Mo (p.p.m.)
North Staffordshire and Derbyshire	8	12	1·5–7·0	8–45
			4·0	20
Caernarvon	4	0–6	1·7–5·0	5–20
			3·0	10
Devon	4	12	0·2–4·0	10–20
			1·5	13
Normal background			< 0·2	< 5

Data refer to minus 80-mesh fraction.

Table 3. SELENIUM AND MOLYBDENUM CONTENT OF SELECTED ROCKS FROM THE ANOMALOUS AREAS

Area	Rock-type	Se (p.p.m.)	Mo (p.p.m.)
North Staffordshire and Derbyshire	Lower Carboniferous		
	Marine shales	2–24	10–30
	Sandstone	2	15
	Limestone	0·2–1·0	< 2
Caernarvon	Ordovician		
	Dark grey slates	0·7	4
	Black pyritic slates—upper bed	4·5–6·5	9–60
	Tuff	1·3	3
	Black pyritic slates—lower bed	0·2–1·8	13–17
Devon	Culm Measures		
	Grey-black marine shales	< 0·2–3·5	< 2–15
	Pyritic shales	2·5–6·0	15–30

The principal bedrock source of the selenium and molybdenum seems to be certain marine shale facies of the Lower Carboniferous in both Staffordshire and Devon, and of the Ordovician in North Wales (Table 2).

Herbage studies have not yet been completed, as much of the work was carried out in the late autumn. Analysis of several samples of mixed grasses from the anomalous regions indicates both selenium and molybdenum contents to be well above those considered normal, and more detailed investigations will be undertaken during the present growing season.

Hypocuprosis is recognized in cattle grazing pasture in part of the molybdenum anomalous region in North Staffordshire, but has not been reported from the Devon and Caernarvon areas. Two suspected cases of chronic selenosis have been reported from Derbyshire, but not from the specific area described in this communication[5]. No animal selenium studies have been made in Devon or North Wales. Nevertheless, the geochemical data indicate the possibility of animal disorders, probably at the sub-clinical level, associated with both selenium and molybdenum in all three areas examined.

More detailed studies are being undertaken, but the preliminary results reported here are of immediate interest in that they provide the first evidence of the existence of seleniferous soils in Britain and lend support to the view that geochemical drainage surveys have potential value in detecting and delineating suspect areas where trace element imbalances may constitute an agricultural problem.

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VETERINARY SCIENCE

Transition of Developmental Stages of *Theileria parva in vitro* at High Temperature

Theileria parva is responsible for East Coast fever of cattle—a disease which is usually fatal and which is characterized by an acute thermal reaction, a lymphoid hyperplasia followed by exhaustion of the lymphoid tissues and leucopenia.

T. parva has been cultivated in its 'macroschizontal'[1] stage over several months in tissue cultures of bovine lymphocytes associated with baby hamster kidney cells[1,2]. The mode of multiplication has been investigated in these cultures and it has been shown that the parasite does not destroy its host cell, but propagates within the multiplying lymphoid cells. In established cultures the nuclear particles of *Theileria* divide at about the same rate as the host cells—the mean number of parasite particles and the percentage of infected cells remain constant. During division of the host cell, the theilerial body closely associates with the mitotic apparatus and is pulled apart and distributed to both daughter cells in late mitosis.

A similar mechanism of multiplication has been assumed for the intralymphoid stage of the disease in the animal. The lymphoid hyperplasia largely arises from mitotic division of lymphoid cells, many of which are infected with *T. parva*. Proof that this is the only way by which the number of the cells infected with *Theileria* increases in the bovine animal is still lacking. There is, however, no evidence for a new infection of lymphoid cells by disintegrated single theilerial particles. It is possible that in cattle an ideal host–parasite relationship is not established, but that the parasite finally destroys the host cell.

In the animal, in the later stages of the disease, another form of the parasite appears in some of the lymphoid cells, which consists of a large number (100 or more) of single particles—the so-called 'gamont' or 'microschizont'[1]. These particles have been assumed to infect erythrocytes. The tick host, *Rhipicephalus appendiculatus*, mainly feeds on blood and the intra-erythrocytic forms are presumed to give rise to the life-cycle in the tick.

The first form (often called the agamont or macroschizont) was cultivated for several months *in vitro* at 37° C without showing any of the microschizontal forms. When incubated at 40° C, however, occasional microschizonts appeared in most cultures.

Two strains of *T. parva* were cultivated according to Hulliger's method[2] for 122 and 233 days, respectively; these were then transferred to a waterbath adjusted to 41°–42° C. Maximum temperatures were read once a day with a clinical thermometer; these were found to range from 41·3° to 42·4° C in the first and from 40·2° to 42·2° C in the second experiment. The cells were initially incubated for 1–2 h in 100 per cent bovine serum, then transferred to normal medium which consisted of 80 per cent Eagle's medium[3], 10 per cent tryptose broth and 10 per cent serum. Normal calf serum and serum from animals in the late stages of East Coast fever were used. Supernatant fluid was taken off and smears made at intervals of 2–3 days. New medium was added on these occasions.

Cell growth. There was very little cell multiplication. A few mitoses could occasionally be seen, but multiplication was so limited that no expansion was necessary.

Appearance of microschizonts. The first microschizonts appeared on the sixth day in the first experiment and on the ninth day in the second. In both experiments, irrespective of the type of serum used, large numbers (28–36 per cent) of the parasitized cells contained microschizonts and their precursors. The cell numbers in the cultures decreased in the next few days and the numbers of macroschizonts diminished. A few microschizonts and macroschizonts could be seen over 2–4 days more, but the cells then gradually degenerated if left at the high temperature.

Morphological changes. The following sequence of events was reconstructed from the chronological appearance of the stages in these two experiments and also on rare occasions in other cultures at 40° C: (*a*) Macroschizonts with very large particle numbers (thirty or more) appeared in some of the parasitized cells (Fig. 1) while the average particle number in untreated cultures is fifteen. (*b*) After one or two days, the large macroschizonts had increased in number and, in many, the chromatin particles had become bulky (2–3μ in diameter) and densely staining. The host cells were often disrupted in smears. (*c*) Bodies morphologically similar to microschizont particles and about 1μ in diameter appeared; these were associated with the large chromatin particles of the macroschizonts. These were seen at the edge of the large particles, often in clusters. (*d*) Large agglomerations of these microschizont-like particles were evident, filling the cytoplasm of the host cells. In many, it was not possible to discern the host cell membrane. Portions of some of the bulky chromatin particles remained within most of the microschizont masses (Fig. 2).

It proved possible to produce the microschizontal stage of *T. parva* in cultures of macroschizonts. The process seemed to be associated with slower or arrested mitosis of the host cells while the theilerial particles continued to multiply without inhibition. This resulted in large parasites containing many nuclear particles which then

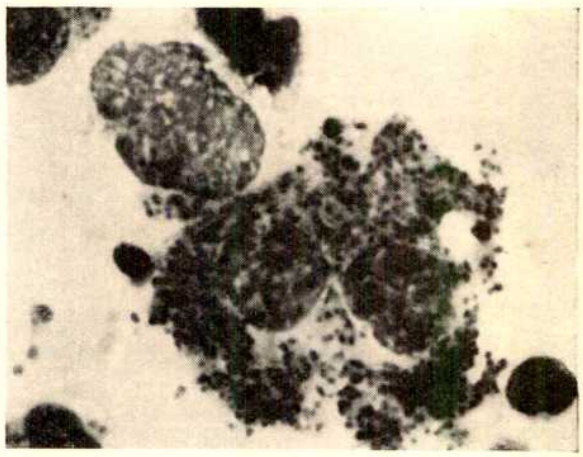


Fig. 1. Macroschizont of *T. parva* with a large number of particles. (Giemsa staining. × *c.* 1,200)

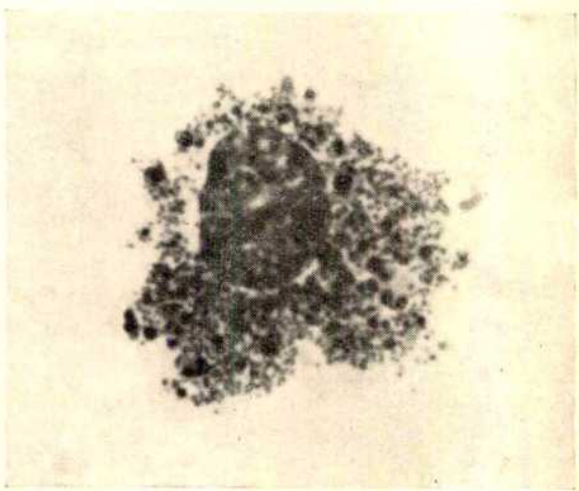


Fig. 2. Microschizont with a few large chromatin particles left. (Giemsa staining. × *c.* 1,500)

increased in size. The single microschizontal forms were produced from these large particles. Some of the large nuclear bodies often remained.

A similar process occurs in cattle, but, more often than not, nuclear division of the microschizont precursor is more complete than in cultures—less of the residual chromatin being seen.

Macroschizonts multiply by a complex process: the single nuclear particles divide by binary fission within one cytoplasmic body. The resultant multinucleated body divides into two during the mitosis of its host cell, one part going to each daughter cell. The production of microschizonts would seem to be by a process of multiple division, or by rapid successive binary divisions of nuclei and cytoplasm leading to parasites each consisting of one nuclear particle in a separate cytoplasmic envelope. The host cells then disrupt and the parasites are freed.

The part played by the high temperature is not clear. It definitely slows down multiplication of the host cell, but not of the parasite. As yet, microschizont formation has not been achieved in cultures by means other than high-temperature incubation. Partially deficient medium, varying oxygen tension and low temperature, while they slow down the rate of cellular multiplication, have failed to bring about microschizont production. The high temperature seems to be essential for the development of this stage. The disease caused by *T. parva* is characterized by high temperatures up to 42·4° C during the period of microschizont development. A reciprocal adaptation of the disease in the host and development of the parasite might therefore be considered. Temperature changes have also been suggested as a factor in the development of other protozoa[4].

The infectivity of these tissue culture microschizonts for the tick vector is at present under investigation.

We thank Miss K. Cowl, D. Mugo and K. Mugo for their technical assistance.

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[1] Hulliger, L., Wilde, J. K. H., Brown, C. G. D., and Turner, L., *Nature*, **203**, 728 (1964).
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GENERAL

Hypothesis-testing in Classification

A RECENT paper by Williams and Lance[1] considers the place of probability in classification, and regards the concept as inapplicable where the population classified is finite. In limiting themselves to probabilities associated with sampling, however, they seem to ignore an important part which probabilistic considerations can and should play in classification.

Taxonomy is always concerned with decisions between 'splitting' and lumping'. Should two groups of individuals be placed in different classes or the same class? This is not the problem of discrimination, where classes have already been distinguished and defined; the question is whether the classes should be separated in the first instance.

The usual principles of hypothesis-testing call for a null hypothesis leading to a well-defined distribution of observational statistics. In classification problems, the most useful type of null hypothesis is generally that the individuals studied form a single class. If it is shown that this hypothesis must be rejected, progressively more complex hypotheses (of two, three or more distinct classes) may take its place until the simplest hypothesis which can be accepted at the selected significance level is found.

If the attributes on which the classification is to be based are metric, then their values in the individuals observed may be regarded as forming a sample from a continuous multivariate distribution which will usually be unimodal if the null hypothesis of a single class is true. It may often be considered appropriate to include in the null hypothesis an assumption that (possibly after transformation in some cases) the joint distribution of attribute values is multivariate normal. The parameters of this distribution can then be estimated from the observations and the hypothesis tested. Rejection will most often indicate multimodality. The distinct modes must then be identified and boundaries between them delineated in the zones of minimum frequency, in order to enable each individual to be allotted to one of the classes (modes) thus distinguished.

If the attributes are qualitative, a significance test of this sort cannot be based on a single attribute only. A suitable null hypothesis is then independence of the various attributes, which may readily be tested by contingency tables or otherwise. If the different attributes occurring in each individual are independent of one another, the whole set of individuals may be regarded as a single class in which the different attribute values are assorted at random. If, on the other hand, certain combinations of attribute values occur in preference to others, the individuals with these combinations may be regarded as constituting distinct classes.

If both quantitative and qualitative attributes occur, the foregoing tests may be applied separately to each of the two types of attribute; if the null hypothesis of a single class is still tenable, the evidence in respect of the two types can in principle be combined by comparing the multivariate distribution of values for the quantitative attributes in each cell of the contingency table representing the qualitative attributes.

The approach outlined in the preceding three paragraphs is perhaps a counsel of perfection. But approximate tests may readily be envisaged—involving, perhaps, step-by-step procedures and order statistics—and should be developed for routine application.

It may be noted that these tests of the null hypothesis are as applicable when the sets of individuals and attributes studied are completely known as when either or both are samples from an infinite set. The problem is comparable with that of testing contingency tables. The figures in a table may be complete, and not a sample of a larger population, yet nevertheless the question of independence still has meaning and a significance test is appropriate. Likewise it has meaning to ask whether attributes in a set of individuals occur independently of one another, irrespective of whether the set is or is not a sample.

Williams and Dale[2] argue that significance tests in classification are pointless because they will normally be disregarded. If the set under consideration is large, the classifier will want to divide it, whatever the results of a significance test. Whether this is a correct description of the behaviour of classifiers in most fields is questionable. In any event, the fact that a significance test may be ignored is scarcely a reason for not having one.

Significance tests of the type proposed here can serve as statistical guidance for the classifier confronted with the usual dilemma of 'splitting' versus 'lumping'. For most purposes, he will be content to accept the simplest hypothesis in accord with the facts, and the simplest hypothesis is usually one involving fewest classes. He splits only where splitting seems unavoidable. Such decisions are not served by most techniques for numerical taxonomy. They show 'how' observations may be classified, not 'whether' they should be classified. It is for this latter purpose that the similarity and deviant indices already proposed[3–6] have been designed, and for

which a complete technique of cluster analysis is in preparation.

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[1] Williams, W. T., and Lance, G. N., *Nature*, **207**, 159 (1965).
[2] Williams, W. T., and Dale, M. B., *Adv. Bot. Res.*, **2**, 35 (1965).
[3] Goodall, D. W., *Nature*, **203**, 1098 (1964).
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Rapid Method for determining Numerical Indexes for Time-course Curves

IN reporting experiments with biological processes such as abscission, germination, and dormancy, where results are observed and recorded periodically, most investigators have found it convenient to present the data as time-course curves. While time-course curves such as those shown for abscission in Fig. 1 are useful in indicating the initiation and the total amount of abscission, as well as the slope of the response curves (rate), they cannot readily be compared numerically. For comparison of the results of large numbers of treatments in individual experiments, or of groups of experiments, and for statistical analyses, numerical designations are necessary. The time required for 50 or 100 per cent abscission and the percentage abscission at an arbitrary time after treatment are designations commonly used. Neither of these methods allows a satisfactory comparison of data frequently encountered in research. For example, comparison of the data represented by curves 1, 2, and 3 in Fig. 1 on the basis of the time to 50 or 100 per cent abscission is impossible as not all the curves reach either of these levels by the end of the experiment. This problem is intensified when treatments retard or inhibit abscission. Furthermore, the relationship of the curves changes with time, and thus comparison of the percentage abscission at an arbitrary time represents only a small part of the information in the curve.

The 'abscission index' described here incorporates all the attributes of a time-course curve into a single number. The data are first plotted as time-course curves on graph paper, ruled 12 by 20 units to the inch (K. and E. No. 358–21). Each side of the graph is 5 in. and the area is

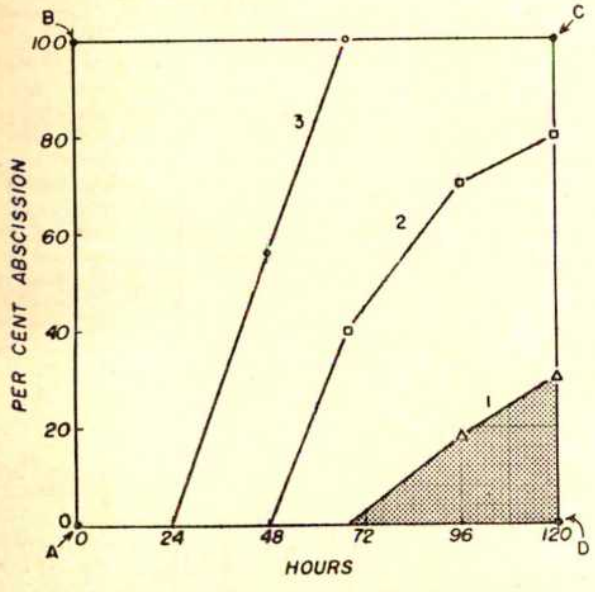


Fig. 1. Time-course curves representing abscission data

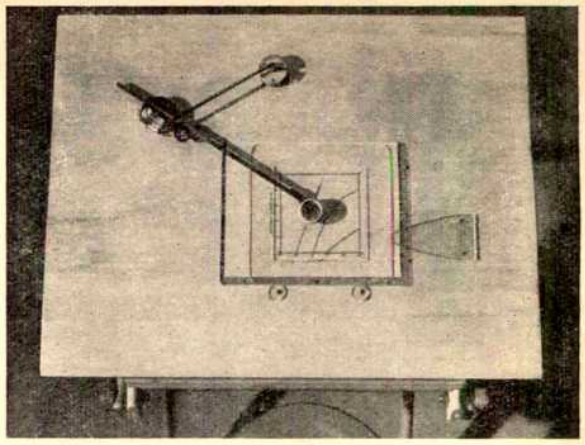


Fig. 2. Apparatus used for the precision measurement of time-course curves

25 in.^{2} (in Fig. 1, the area of the rectangle connecting points A, B, C, and D). To obtain the index, the area under each curve (for example the shaded area under line 1) is measured with an optical planimeter (Filotecnica Salmoiraghi model 236A). The tracer arm has been modified and adjusted to a position allowing the vernier scale reading to be exactly 100 when the area enclosed by lines connecting $ABCD$ is traced. Consequently, when any curve is traced, the vernier reading is the area under that curve expressed as per cent of the total area of $ABCD$. As the instrument is calibrated so that 25 in.^{2} reads 100, the areas determined can readily be converted to any other desired units. Adjustment of the length of the planimeter tracer arm enables indexes to be obtained from various sizes and shapes of graphs; such indexes can be directly compared.

Fig. 2 is a photograph of the planimeter set up on a drawing board that has been modified to make the index determinations rapid and accurate. A section $8 \times 11{\cdot}25$ in. has been removed from the centre of the board and replaced by clear 'Plexiglas' positioned over a light box. Grooves in the 'Plexiglas' allow the graph to be held in position by a vacuum produced by a small blower mounted under the board. The graph paper is positioned so that the axes are in conjunction with lines engraved on the top surface of the 'Plexiglas' inlay. An accurately machined plastic template resting against three stops serves as a guide for the lens on the end of the tracer arm. When the circumferential surface of the lens is held firmly against the inner edge of the template, the hardstone centre of the lens exactly follows the boundaries of the graph. Use of this template contributes greatly to both the speed and accuracy of the determinations.

The abscission index described is the area under a time-course curve expressed as a percentage of the area of a rectangle of which one dimension represents 100 per cent abscission and the other dimension represents the time-duration of the experiment. This index is similar in concept to that recently described by Finlay[1], but the method described here has the advantages of versatility and speed, as well as simplicity of operation. This method and the resulting indexes are adaptable to many types of biological investigations and should prove to be a valuable research tool.

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Department of Agronomy,

R. J. COFFELT

Department of Agricultural Engineering,
University of California,
Davis.

[1] Finlay, K. W., *J. Inst. Brew.*, **66**, 51 (1960).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, July 18—Friday, July 22

ROYAL MICROSCOPICAL SOCIETY (at Imperial College, London, S.W.7), at 10 a.m. daily—Conference on "The Role of the Microscope in Scientific Investigation".

Thursday, July 21

UNIVERSITY OF LONDON (at the Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Prof. J. H. Wilkinson (Pennsylvania): "Iso-Enzymes—Their Biological and Clinical Significance".*

Friday, July 22

UNIVERSITY OF LONDON (at the Royal Veterinary College, Royal College Street, London, N.W.1), at 5 p.m.—Dr. Howard A. Schneider (Chicago): "Some Nutritional Parameters in the Ecology of Infectious Disease".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

ASSISTANT (honours graduate) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (July 20).

ASSISTANT LECTURER IN ZOOLOGY IN THE BIOLOGY DEPARTMENT—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 20).

LECTURER OR ASSISTANT LECTURER IN THE PHYSIOLOGY DEPARTMENT—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8 (July 20).

TUTORIAL RESEARCH FELLOW OR STUDENT IN GEOGRAPHY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (July 24).

ASSISTANT LECTURER IN SOCIAL ANTHROPOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (July 25).

HOSPITAL PHYSICIST (Senior Grade) IN THE DEPARTMENT OF PATHOLOGY, Queen Elizabeth Hospital for Children, Hackney Road, London, E.2—Group Secretary, Hackney and Queen Elizabeth Group H.M.C., Hackney Hospital, London, E.9 (July 27).

LECTURER/ASSISTANT LECTURER (with a special interest in social/urban geography) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, The University, Aberdeen (July 30).

LECTURER IN BIOCHEMISTRY—The Secretary, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1 (July 30).

LECTURER/ASSISTANT LECTURER (with a good honours degree or equivalent and special qualifications in any of the following fields: (i) biogeography; (ii) meteorology; (iii) surveying and photogrammetry; (iv) cartography) IN THE DEPARTMENT OF GEOGRAPHY, University of Malaya—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Kuala Lumpur and London, July 31).

LECTURER OR ASSISTANT LECTURER (with a keen research interest in control engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, to join a team, directed by Prof. J. M. Nightingale, working on the application of modern control theory to practical problems in a variety of fields including electrical machine control, process control, bio-medical engineering, and digital systems—The Deputy Secretary, The University, Southampton (July 31).

LECTURERS OR ASSISTANT LECTURERS (2) IN EXPERIMENTAL PSYCHOLOGY—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (July 31).

SENIOR LECTURERS/LECTURERS IN THE DEPARTMENT OF BOTANY; and SENIOR LECTURERS/LECTURERS, SENIOR TEACHING FELLOWS/TEACHING FELLOWS IN THE DEPARTMENT OF PHYSICS, Monash University, Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar of the University (July 31).

POSTDOCTORAL FELLOW (biochemist with some experience in plant biochemistry and enzymology) IN THE CHEMICAL LABORATORY, to study the biosynthesis of alkaloids in higher plants—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex (August 1).

SENIOR LECTURER (Psychology) (with an honours degree in psychology and preferably specialized in psychometrics and measurement in education) IN THE SCHOOL OF EDUCATION, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 1).

SENIOR LECTURER or LECTURER (with a postgraduate degree or requisite research experience in any of the following: (*a*) animal nutrition with interest in either mono-gastric or ruminant animals; (*b*) meat technology; (*c*) dairy technology; (*d*) animal genetics and breeding with an interest in poultry) IN THE DEPARTMENT OF AGRICULTURE, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 6).

LECTURER or ASSISTANT LECTURER IN INORGANIC OR PHYSICAL CHEMISTRY (preferably inorganic) IN THE DEPARTMENT OF CHEMISTRY, University of the West Indies, Jamaica—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 8).

PROJECT SCIENTIST (with a higher degree in electrical engineering or physics and extensive experience in electronic instrumentation or data processing systems) IN THE DEPARTMENT OF CONTROL ENGINEERING, School of Electrical Engineering, University of New South Wales, Australia, to work initially on a project aimed at achieving the optimal control of a steam generating plant by means of a digital control computer—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 8).

ARTS or SCIENCE GRADUATE (interested in engineering, and prepared to be trained by the Science Librarian) with a view to taking charge of the Engineering Sciences Library in twelve months' time—The Librarian, University College, Gower Street, London, W.C.1 (August 10).

CHAIR OF ELECTRICAL ENGINEERING at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, August 12).

CHAIR OF MECHANICAL ENGINEERING at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, August 12).

READER OR SENIOR LECTURER IN SOCIOLOGY in the Faculty of Arts and Social Sciences, Ahmadu Bello University, Northern Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 14).

ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF GEOGRAPHY—The Registrar, King's College (University of London), Strand, London, W.C.2 (August 15).

LECTURER (specially qualified in animal ecology or behaviour), ON THE STAFF OF THE DEPARTMENT OF NATURAL HISTORY, but attached to Culterty Field Station, Newburgh—The Secretary, The University, Aberdeen (August 15).

SENIOR LECTURER (preferably with an interest in the application of sociological theory to the study of industrial societies) IN SOCIOLOGY—The Secretary and Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. 66/74 (August 15).

SECOND CHAIR OF ELECTRICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 19).

READER OR SENIOR LECTURER IN ANATOMY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 22).

ASSISTANT LIBRARIAN (qualified librarian, with a good honours degree, preferably in some branch of the physical sciences)—The Registrar, Bradford Institute of Technology, Bradford 7 (August 27).

JUNIOR LECTURER, LECTURER OR SENIOR LECTURER IN DAIRY OR SHEEP HUSBANDRY at Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or the Registrar of the University (New Zealand and London, August 31).

LECTURER (physicist with theoretical or experimental research interests) IN THE DEPARTMENT OF NATURAL PHILOSOPHY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 44/66 (August 31).

SENIOR LECTURER/LECTURER IN PHARMACY at Rhodes University Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

SENIOR LECTURER (with experience in the fields of industrial and applied sociology) IN SOCIOLOGY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

CHAIR OF ARCHAEOLOGY OF THE ROMAN PROVINCES AT THE INSTITUTE OF ARCHAEOLOGY—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 2).

AGRICULTURAL STATISTICIAN, SCIENTIFIC OFFICER GRADE, in London, for duties which will be concerned with the collation of technical data from scientific experiments—The Establishment Officer, Potato Marketing Board, 50 Hans Crescent, Knightsbridge, London, S.W.1.

ASSISTANT BIOCHEMIST (Basic Grade) (with a first- or second-class honours degree in chemistry or biochemistry) IN THE DEPARTMENT OF PATHOLOGY—The Hospital Administrator, Crumpsall Hospital, Manchester, 8, quoting Ref. 4884.

COMPUTER MANAGER (with experience of operating modern time-sharing computers) to have overall responsibility for the day-to-day operation of the computer and ancillary equipment—The Assistant Registrar (Establishment), The University of Sussex, Stanmer House, Stanmer, Brighton, Sussex.

ENGLISH ELECTRIC RESEARCH FELLOW IN THE SCHOOL OF ENGINEERING SCIENCE for work in either of the following fields: automatic control, metal fatigue—The Registrar, The University of Warwick, Coventry, Warwickshire.

INSECT PATHOLOGIST (microbiologist, preferably with experience in bacteriology, mycology, virology or protozoology) to study insect diseases with their possible utilization in pest control—The Division of Entomology, University of California, Berkeley, California 74720, U.S.A.

MYCOLOGIST (with good taxonomic knowledge and physiological interests) for work on the production of ergot alkaloids and other biologically active substances of fungal origin by fermentation methods—The Secretary, Biochemistry Department, Imperial College, London, S.W.7.

ORGANIC CHEMIST OR BIOCHEMIST (with an interest in analytical techniques) to teach chemistry within the Department of Biological Sciences—The Registrar (RS), Portsmouth College of Technology, Hampshire Terrace, Portsmouth.

PHYSICS TECHNICIAN (with at least O.N.C. or City and Guilds Intermediate Certificate, but higher qualifications preferred) IN THE ISOTOPE LABORATORY—The Establishment Officer, Charing Cross Hospital, London, W.C.2.

PHYSIOLOGIST/BIOPHYSICIST to study physiological response to controlled changes in environmental conditions—D. C. Page, Head of Personnel Services, the Electricity Council, Research Centre, Capenhurst, Chester, quoting Ref. N/28.

RESEARCH ASSISTANT (preferably with experience of crystallography or electron microscopy/diffraction) IN THE DEPARTMENT OF PHYSICS to work on studies of epitaxial growth using low energy electron diffraction in a well-equipped established laboratory—The Registrar, University of York, Heslington, York.

RESEARCH ASSISTANTS (2) (honours graduates in pharmacy, physical chemistry, physics or mathematics) IN PHYSICAL PHARMACY—The Registrar (RS), Portsmouth College of Technology, Hampshire Terrace, Portsmouth.

RESEARCH FELLOW IN THE DEPARTMENT OF APPLIED MATHEMATICS to work on atomic and molecular processes—Prof. D R. Bates, F R.S , Department of Applied Mathematics, The Queen's University, Belfast, Northern Ireland.

RESEARCH FELLOW (preferably with research experience in plasma physics, optics, or spectroscopy) for a Science Research Council supported investigation into the coherence and ionizing properties of laser light—Prof. D. J. Bradley, Department of Physics, Queen's University of Belfast, Belfast 7, Northern Ireland.

SCIENTIFIC OFFICER (with an honours degree or equivalent and preferably some research experience) to undertake research on physiological problems arising from the adaptation of large animals to different conditions of environment and nutrition—The Secretary, Institute of Animal Physiology, A.R.C., Babraham, Cambridge.

TECHNICAL OFFICER (with a recognized degree or national diploma in agriculture) IN THE CEREALS SECTION OF THE TRIALS BRANCH—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

TRAINEE OFFICERS (2) (preferably between 20 to 25 years of age, with a degree in agriculture, agricultural botany or botany or the National Diploma in Agriculture with suitable experience)—The Secretary, National Institute of Agricultural Botany, Huntingdon Road, Cambridge.

VIROLOGIST, BASIC GRADE (preferably graduate in microbiology or bacteriology) to assist in the development of research into paediatric virological problems—The Group Secretary, Booth Hall Hospital, Manchester, 9, quoting Ref. 4901.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

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UNPUBLISHED LITERATURE

NEXT to downright villainy, misguided zeal is one of the most dangerous forces in society. At this stage, it is only proper that those who have designed the scheme known in the United States as PIE (for *Physics Information Exchange*) should be given the benefit of the doubt. There is nothing to show that they are villains, but their zeal is alarmingly apparent. What they propose is an experimental scheme for the rapid circulation of documents in high energy physics. The promise of rapidity, however unreal, cannot fail to be attractive. The risks are great, and deserve to be weighed thoroughly by the scientific community as a whole. *Physics Today*, the official journal of the American Institute of Physics, has done a public service by ventilating the issue and inviting comment. With luck, there is now a chance that the conduct of the experiment will be moderated by the weight of interested opinion. There is even sense in hoping that the experiment will never be mounted in the form originally proposed.

Physics Information Exchange is really a scheme for rationalizing the present practice of circulating what are called 'preprints'. In many fields of research it has become habitual for intending authors to circulate copies of their manuscripts to colleagues likely to be particularly interested. In one sense, the practice is in line with the way in which scholars have traditionally sought out the constructive criticism of their colleagues. It is a measure of the competitiveness of modern science that preprint circulation lists are often very large, and that some scientists seem to use the same lists of names for preprints and for Christmas cards. One immediate consequence of PIE would be to lighten the burden of circulating preprints. Those with something to show would send a single copy to an office at the U.S. Atomic Energy Commission in Washington, whence copies would be manufactured and sent to each of a number of chosen preprint libraries, which would in turn serve as secondary distribution centres. The supporters of PIE appear to have convinced themselves—though not their critics—that three hundred preprint libraries would cover the whole world, and that a modest bundle of documents each week would suck dry the literary talents of those who write on high-energy physics. Nobody would have to pay.

As with all closed communities, the most obvious question is how the membership of PIE would be decided. In principle, according to the sponsors of the scheme, everybody with a legitimate interest in theoretical high-energy physics should have a right to membership. But what kind of interest is legitimate? And who decides? With the best will in the world, these are not questions to be dismissed with the beguiling formula that "researchers in theoretical high energy physics throughout the world could qualify for membership". Dr. Michael Moravcsik of the Lawrence Radiation Laboratory, Berkeley, who has at least had the courage to let his name go forward as the chairman of the PIE planning group, cannot really expect this form of words to be sufficient. Either he and his collaborators must face up to the invidious task of defining what they mean by qualification, and thus perpetuate in their own system some of the clubbiness of which they complain in the present preprint networks, or they must throw their doors open to all who ask for membership and run the risk that administration will get the better of them.

Worse still, there is a danger that under the arrangements so far described, the confidence of the preprint authors will be betrayed. One illustration of this difficulty is that under the present informal arrangements for circulating preprints, plagiarists can be quickly struck off the lists. Under the much more impersonal arrangements for PIE, when authors will have no means of knowing where their preprints finish up, plagiarism will be hard to recognize, impossible to prove, and difficult to control. Will it, for example, be necessary for an act of plagiarism to be followed by the excommunication of the whole of a preprint library, or will central PIE insist that the library should excommunicate the offender? And who will act as arbitrator? It is hard to imagine that scientific authors, not renowned for their tolerance of imitators, will willingly trust their manuscripts to PIE unless it is a much more tightly-knit group than at present planned or, on the other hand, much more carefully controlled.

This is the essential contradiction in the proposals for PIE. The intention is to combine the speed and informality of personal communications with the wide dissemination of formal publication. It is disingenuous of Dr. Moravcsik to pretend that his plans for PIE are distinct from an act of publication simply because the documents to be circulated will be fragile, bulky, unscrutinized, un-edited and without indexes. It would be as sensible to exclude from the set of formal publications the great volume of the pulp literature to be found on some bookstalls simply because the grammar in it is defective, the printing inadequate and the words indelicate. The truth is that the essence of the act of publication is that of making literature publicly available. In the liberal interpretation of PIE, preprints would be made available to all and sundry, and that would constitute publication. One of the reasons why the planners of PIE have retained so much of their original optimism is that they have chosen to dodge the implications of this conclusion. For practical purposes, they are proposing to produce a new kind of scientific journal—one whose pages will be open to all who claim space in them, and which will lack the benefit of criticism by referees and constructive interchange between authors and editors. To meet the tedious need that scientists should be able to retrieve

what colleagues publish, or to refer to it, the organizers of PIE are hoping that established journals will undertake the chore of republishing material which has already done the rounds of the preprint libraries.

This, of course, is why the proposals for PIE are so offensive. The organizers are planning a scientific journal the outstanding quality of which will be the subordination of discrimination to speed. There is nothing much wrong with the present preprint system, and a little thought could enable participating scientists to gain more benefit from it. Equally, there is nothing wrong with proposals for new scientific journals, and of course there is everything to be said for using the techniques of modern communication to reduce the time-lag before publication to a few weeks or so. But there is no evidence at all to support the proposition implicit in the plans for PIE that what theoretical high energy physics needs most urgently is a bad scientific journal—one in which the safeguards on which contributors and readers have come to rely are gaily thrown overboard. It is, of course, entirely understandable that the editors of *The Physical Review* and of *Physical Review Letters* should feel most directly threatened by the proposals for PIE, but there is more than mere self-interest in the declaration of Dr. Simon Pasternack, the editor of *The Physical Review*, that the proposal for PIE is "a serious threat to physics communication and to the physics research community". The irony is that these two American journals have won their present place in modern physics by the sheer excellence of the discrimination to whose virtue and necessity the organizers of PIE are apparently indifferent. Thus the complaints that the proposals are probably unworkable, and that they are riddled with contradictions, although true, are not the most serious to be made against those who have invented PIE. It is much more serious that they are apparently insensitive to those qualities which distinguish good literature from bad. That is the most telling of many reasons for hoping that PIE will be stillborn.

NEW MATHEMATICS

THE educational system in Britain seems to be responding with accustomed confusion to the arrival of what is sometimes called the new mathematics. In secondary schools of various kinds, a number of experiments have been under way for several years. Though the School Mathematics Project under Professor Bryan Thwaites is the best known of them, it is by no means alone in the field. Other experiments have sought to turn the ingredients of the new mathematics to more practical account. Elsewhere, things have gone the other way, and experimental courses in mathematics are to be found which owe something to the axiomatic experiments in the United States, and which promise to cater most adequately for those schoolboys and schoolgirls who know in their bones that they are born mathematicians. In itself, this diversity has been a good thing. Many innovations have been tried, and teachers of different inclinations have been able to engage their enthusiasm where it seemed that there would be the greatest benefit. But diversity is now becoming an impediment to reform. For one thing, those teachers of mathematics wishing to renovate their syllabuses are at a loss to know which innovations to adopt. Worse still, those to whom change is abhorrent are more able to practise old ways without interference. And then the institutions which depend on the secondary schools for an education of some kind in mathematics—employers of all kinds, but particularly the universities—are puzzled to know what is going to happen. With all the confusion, there is a danger that the hard work of the last decade or so may nullify itself.

This prospect is particularly frustrating because a coherent development of mathematics in the schools is necessary if there is ever to be an improvement of the relationship between British schools and British universities on mathematics. At present, the means by which the universities recruit undergraduates in mathematics exerts an unreasonable—and a hostile—influence on mathematics teaching in the schools. Entry to most university departments of mathematics still requires that a pupil should spend roughly two-thirds of his time between sixteen and eighteen studying mathematics. Not merely is his education unbalanced, but he (or she) has to gamble on a career in mathematics at an age when very few pupils can be certain of their talents (and also correct in their estimation of them). It would be surprising if this strange practice were not a part of the reason why several university departments of mathematics have found recruitment difficult in recent years. The same system probably provides a great many young people who finish up as scientists with an education that is less rounded than it should be. The obvious solution is to design a system in which the departments of mathematics are restrained from asking intending entrants to specialize heavily in mathematics in the last two years at school. The ideal would be a system in which intending mathematicians and scientists alike were taught the same mathematics, which should be largely determined by the need—in the broadest sense—to provide a mathematical foundation for science teaching. The mathematicians should be enabled to select their bright young men and women not by seeing which of them can cover the most ground, but by more qualitative and more meaningful tests.

Though the heads of some departments of mathematics in the universities are sensitive to the need of a more liberal regimen in the schools, it is unlikely that the universities will abandon their entrenched position of their own accord. There is a kind of Gresham's Law which ensures that bad practice is more stable than the good. But the schools are unlikely to be able to force through a change until they know what they would like to see. Who, then, is to take this cause in hand? Of the British institutions likely to be influential, only the Schools Council is suitably independent yet free to advocate an opinion.

By all accounts, the council is hoping for great things from a conference in the autumn at which it hopes to win approval for a plan to make a synthesis of experiments in the schools. With luck, the universities may be persuaded to give the project something like a blessing. So far the strongest opposition seems to have come from pundits in the Department of Education and Science who argue that the diversity of current practice in the schools is a proof that the schools do not particularly wish to change their ways. In reality, of course, in teaching as elsewhere, diversity may be a sign of rapid evolution.

SUBMARINE RAILWAY

IT is not surprising that the Prime Ministers of France and Britain should have been grateful for a few crumbs of agreement to fill out the communiqué after their recent meeting in London. By all accounts, British membership of the European Economic Community was not a fruitful topic of conversation. It is therefore paradoxical that the two Prime Ministers should have considered this a suitable time for a decision in principle that the tunnel beneath the English Channel should in due course be built. It would have been easy to understand if their predecessors had come to such an agreement at any time in the last century. But now the case for a tunnel between France and Britain is wearing thin. Alternative ways of crossing what is, after all, a narrow strip of water have become more attractive, particularly in the last decade. At the same time, doubts have grown about the capacity of the proposed rail tunnel to cater adequately for the traffic between Britain and the mainland of Europe. It looks very much as if the two governments may be making the right decision at the wrong time.

The case for the tunnel is based on surveys of the traffic between Britain and the mainland of Europe which were carried out in the late fifties. Even when first published, it appeared that the demand for facilities to cross the English Channel had been underestimated. Experience has borne out that awkward suspicion, and one obvious result has been the rapid growth of cross-Channel ferry services in the sixties. Compared with the proposed tunnel, these surface and air routes have the advantage that they do not require that great volumes of traffic should be funnelled through two railheads. They are also increasingly cheap and quick. Meanwhile the chances are that the construction of a bridge across the Channel would not be relatively simpler (and cheaper) than seemed likely five years ago. In the circumstances it is to be hoped that the two governments will have another close look at the wisdom of the course to which they have committed themselves before the digging of a tunnel actually begins. If then they decide to go ahead, it is essential that the financing of the tunnel should not somehow confer an unfair commercial advantage on what promises to be an old-fashioned way of travelling from Britain to France.

NEWS AND VIEWS

Charter for Botanists

A PLAN to spend $1,500 millions during the next decade on the development of the plant sciences has been drawn up by a committee of the U.S. National Academy of Sciences (*The Plant Sciences, Now and in the Coming Decade*, Government Printing Office, Washington, $5.00). The starting point for the argument is frankly practical, and is based on the fact that applied botany is also the oldest of all industries and still the only way of converting solar energy into food. As part of its vision of the future, the committee makes much of the potential agricultural applications of plant growth regulators, propagation from fragments of plants and even single cells, the relationship between trace elements and fertility, ferrodoxin as a spur to the study of nitrogen fixation, and phytotrons as means of optimizing methods of cultivation.

Chambers for controlling the environment of plant growth would eat up half the capital expenditure the committee recommends. It would like to see 850 small chambers installed each year for the next decade at universities and other research establishments, and would also have the U.S. Government spend $9 millions on a new phytotron unit as well as half that sum on extensions to existing installations. Roughly $70 millions would be spent on electron microscopes in the same period, while botanical gardens would receive a total of $35.3 millions in the coming decade. The budget as a whole works out as follows, with amounts in millions of dollars:

	Research	Capital
Environment and nutrition	180	208·0
Biochemistry and biophysic	354	7·9
Growth and development	94	0 6
Plant structure	67	69 8
Genetics	77	—
Taxonomy	49	35·3
Plant pathology	60	12·5
Economic botany	—	57·5
Manpower	135	8·5
Computers	—	10·1
	1,016	410 2

The assumptions made by the committee about the cost of supporting individual researchers will be of particular interest outside the United States. At present the cost of a year's research in taxonomy is a mere $10,000, though the committee expects that this will rise to $16,000 by 1974. In biochemistry, by contrast, present costs amount to $35,000 a year, chiefly because of the costs of materials and laboratory assistance. By 1974, the committee supposes, it will cost $45,000 to keep a qualified plant biochemist working for a year. In other words, the committee paints a cheerful picture of the future of the plant sciences and their applications. It remains to be seen how many of its proposals will eventually be backed by hard cash, for this prospectus is only one of several now in circulation in Washington.

Universities in Clover

BRITISH universities are better off than they will admit, to judge from the statement of Mr. Anthony Crosland, Secretary of State for Education and Science, at the University of Birmingham last week. Commenting on the very rapid increase in the number of both

undergraduates and postgraduate students, nearly double that of nine years ago, Mr. Crosland said that "most of the credit for this quite remarkable achievement lies with the universities themselves". But the government was well aware of the high priority necessary for higher education and research and had backed this decision with increasing financial support. Mr. Crosland rejected the notion that the universities have been meanly treated by successive governments. Within the past five years the University Grants Committee has increased university expenditure from £80 million a year, about eight per cent of all educational spending, to £207 million, or 12½ per cent. In spite of the great expansion of students, the ratio of students to staff had remained constant at about seven-and-a-half. At the same time, resources devoted to university building over the last ten years had increased tenfold. In response to the Flowers Report, the Government had also allocated about £20 million to universities over the next six years for new computers and related costs. "This is a very substantial additional expenditure which perhaps has been too little noticed in the universities," Mr. Crosland added.

Both undergraduate and graduate studies are far ahead of the Robbins Report projections. The undergraduate enrolment for 1966–67 should be 189,000, or 2,000 more than originally estimated, while nearly a quarter of last year's graduates—13,500—stayed on for postgraduate work, thus more than doubling the estimated growth rate. This development, said Mr. Crosland, "has resulted from the cumulative decisions of all the universities in the country, and only incidentally from national policy in matters such as postgraduate awards." He concluded that the Government's role is to keep national issues in the forefront of academic discussion, and to create a climate of opinion in which close involvement with the social and economic needs of the nation is a respectable aspiration for a university.

Uranium in Europe

THE Euratom organization persists in taking what the stock exchanges would call a bullish view of the uranium market. In 1963, when uranium was something of a drug on the market and when the British Government was having to explain to the Canadians why the United Kingdom Atomic Energy Authority wished to stretch out its contracts to buy uranium from Canadian mines, Euratom published a report which drew attention to the likelihood of scarcity (EUR 414e—*The Problem of Uranium Resources and the Long-Term Supply Position*). Briefly, the estimates compiled in 1963 suggested that Europe as a whole would need something like 100,000 metric tons of uranium metal in the decade 1970–80, and that only some 15,000 tons of this would be supplied from within Europe at a reasonably competitive price of around $8 for a pound of U_3O_8. One immediate result, within the six countries of Euratom, was to stimulate uranium prospecting in Europe. Some of the results of this work are now described in the document *Les Ressources en Uranium dans la Communauté Européenne* published at Brussels.

The most remarkable change in the past three years has been a cheerful upward revision of the estimates of the potential uranium resources of Europe. It is true that the Benelux countries (Belgium, Luxembourg and the Netherlands) are written off as contributors, but it appears that France, West Germany and Italy are better off than has hitherto been thought. More detailed examination of uranium ore deposits known for some years has led to increases of estimates of their potential. Hunting for new kinds of uranium deposits has also turned out to be successful. The result is that the Working Party responsible for the survey considers that the proved reserves of uranium ore, known to be equivalent to 31,000 tonnes in 1964, could be increased by up to 40,000 tonnes by means of a sufficiently energetic programme of exploration. Some of this addition to present reserves would come from the discovery of new deposits, and some from the extension of deposits at present being worked. France is thought to contain half the uranium as yet undiscovered, while Italy and West Germany share the rest. The Working Party has a number of suggestions to make about the future exploration for uranium in Europe, not the least constructive of which is the suggestion that there should be better liaison between the geologists. But, for all its optimism about the future of uranium supplies, there remains an unhappy discordance between potential supply and likely demand. Even if prospecting turns out to be as successful "as it seems reasonable to hope, the reserves will, however, remain insufficient for the needs of the community as a whole in the decades ahead . . ."

Drilling Deep

IT should soon be known whether the National Science Foundation will have to buy its way out of the contracts signed for the equipment needed for drilling the *Mohole*, or whether the project will be allowed to limp along on a somewhat straitened budget. Earlier this year the appropriate committee of the United States House of Representatives declined to vote money for next year's operations, and thus put in jeopardy the construction of the *Mohole* drilling barge, estimated to cost $27 millions and due to start experimental drilling in the Pacific during 1967. Now it is up to the Independent Offices Appropriations Subcommittee of the United States Senate to decide whether there shall be some loosening of the public purse-strings. At this stage it is unlikely that the sponsors of the project will get the whole of the $127 millions now thought necessary to complete the *Mohole*, though it would be entirely sensible if the Senate decided to complete the barge on which work has begun, and to provide funds for a modest programme of experimental drilling in 1967 and 1968. After all, nobody questions the value of deep boreholes, but merely the wisdom and feasibility of going in one leap to the Mohorovicic discontinuity on a fixed budget, even if that budget is eight times the cost originally estimated.

Some of the artlessness of the *Mohole* project can be found in the record of the symposium on *Drilling for Scientific Purposes* held in Ottawa last year under the auspices of the International Upper Mantle Committee (Queen's Printer, Ottawa, 75 cents). At the symposium Professor H. H. Hess of Princeton described a meeting in Washington in March, 1957, at which eight people were gathered "to analyse projects submitted from earth scientists of the country. We had something like sixty projects to review in two days . . . we were rather tired, and Walter Munk mentioned that none of these proposals was really fundamental to an under-

standing of the Earth, although many of them were very good. If someone wanted to study the clay minerals of such and such a formation to find out about its diagenesis, well, this was a good thing to do. However, it would not solve a major problem about the Earth. If someone wanted to study the stability of anthophyllite, well yes, I would like to know about its stability too. But still, it is a relatively small point. We had gone through sixty of these proposals, most of which we rated as being very good projects. They should be supported." But then emerged the proposal that "we should have projects in earth science—geology, geochemistry, geophysics—which would arouse the imagination of the public and which would attract more young men into our science". That was the beginning of the *Mohole*.

The overall effect of the report of the symposium is, however, more a vindication than a condemnation of deep drilling. There is an impressive account of how a Canadian team has studied a basic intrusion in the Canadian Shield with three modest holes. The JOIDES programme, in which the three principal oceanographic institutions in the United States participate, has learned a great deal about ocean sediments since the Pliocene by drilling into the continental shelf off Florida. The Russians are looking hard, from the Baltic Shield to where Asia runs out into the Pacific, for profitable sites at which to drill holes of up to 7 or even 10 km. (One hole described at the symposium had then passed 3,800 metres, a third of it in granite.) The Swiss would drill into the Alps, the Japanese into the roots of their island arc and the Icelanders into the succession of lava flows which supports them and the Faroe Islands. Then there is great interest in the oceanic basalts above the Mohorovicic discontinuity whose magnetization may be responsible for magnetic anomalies recently measured. Obviously there will be plenty of work to keep a deep drilling barge busy even if *Mohole* itself is still a long way off.

specification of the site should help to thin out the field. It is laid down that the spot selected should be roughly 20 square kilometres in area, at least 3·5 kilometres across at its narrowest point, and flat to within 20 metres over the whole area. The ground must be stable to within 0·15 mm in 100 metres, and there must be a power supply capable of providing 100 MW by the time the accelerator is in operation, and with the possibility of 300 MW if storage rings are ever added. Something like 2 cubic metres of cooling water a second may be necessary, while access to the site by air and on the surface must be easy. Finally, the specification states, there must be towns nearby capable of absorbing the 15,000 people needed to run the accelerator. There is even a passage which refers to the way in which it will be easier to recruit staff in areas which are attractive to live in. It will be fascinating to see how the Council votes if it should ever be necessary to choose, on grounds of amenity, between a site in southern Europe—between Nice and Marseilles, for example—and one in the north.

Fossil Mammals

VERTEBRATE palaeontologists would do well to take note of four recent works on fossil mammals: Savage, Russell and Louis have made a painstaking reassessment of the European Eocene equids with special emphasis on some French material (*Univ. Calif. Publ. Geol. Sci.*, **56**: *European Eocene Equidae* (*Perissodactyla*); 1966). The equid faunas at any one time in the Eocene are seen to comprise several species, which differ mainly in size, and this may reflect diverse habitats. Recent living

(*Mus. Comp. Zool., Harvard Univ.*, **133**, No. 6: *The Fossil Elephant Shrews*; 1966). He supplies a diagnosis and achieves a neat rehousing of some African Tertiary mammals which had previously been misplaced among marsupials, mixtodectid insectivores and hyracoids. The order has only one family, the Macroscelididae; it is entirely African, and ranges from Lower Oligocene to Recent. The extinct sub-families were essentially herbivorous while the living members are more markedly omnivorous.

Reed and Turnbull have described the insectivore genera *Arctoryctes* and *Cryptoryctes* from the Oligocene and Miocene of North America (*Fieldiana: Geol.*, **15**, No. 2; 1966). They provide a detailed account of the functional anatomy of the bones of the forelimb and shoulder girdle of the fossil forms, and compare them with living talpid moles. The trends in the two groups are broadly parallel but differ in detail; the arctryctines are more specialized while the talpids have the lead in overall efficiency of the limb.

Domestic into General Science

QUEEN Elizabeth College of the University of London is taking another important step in the transition from its original role as King's College of Household and Social Science to a general science college. After 20 years as principal, Miss M. J. Sargeaunt is retiring in September and Dr. Kenneth G. Denbigh, professor of chemical engineering at Imperial College, London, since 1961, will be the new principal. Although he has [illegible] formal plans, Prof. Denbigh hopes the college [illegible] scientific centre. Because space [illegible] large and [illegible] than

[illegible] for Locusts

Lister Institute—1966

THE London Lister Institute of Preventive Medicine has had another good year, as judged from the annual report for 1966. Although it gets no money from the University Grants Committee, the Institute is one of the recognized schools of the University of London for postgraduate research, and during 1965–66 there were sixteen postgraduate research workers at the Institute registered for higher degrees. Also embodied within the Lister are three units of the Medical Research Council—the Trachoma Research Unit, the Blood Group Research Unit and the Blood Group Reference Laboratory.

The research being undertaken involves the genetics, physiology and morphology of bacteria, trypanosomiasis, various aspects of virus diseases and bacterial infections, immunology, human blood group substances, biochemistry of lipids, macro-molecular components of blood, and human plasma proteins. In recent years, increasing emphasis has been placed on various aspects of virology and immunology. In virology, there have been considerable developments in studies of trachoma agents, and of special importance has been the success in passaging trachoma and inclusion conjunctivitis agents in the lungs of mice. The possibilities are being investigated of producing a potent vaccine free from those substances in nervous tissue which are responsible for the demyelinating accidents which sometimes occur during the treatment of rabies. New fields of research in immunology include investigations to test the possibility that generalized eczema may result from the incorporation of bacterial or fungal antigens into cells of the skin. There is also an investition of the possibility that nodular vasculitis may be caused by complexes of antigen and antibody.

Parliament in Britain

OPENING a debate in Committee on the Civil Estimates in the House of Commons on July 5, Sir Edward Boyle and other speakers strongly challenged the adequacy of the Government's allowance of £25 million for the university building programmes for 1965–69. In his defence the Secretary of State for Education and Science, Mr. A. Crosland, was content to emphasize the increase of numbers of students to 189,000 by October 1966, with a further 47,000 full-time students in technical colleges. In the course of his speech he announced that Professor B. H. Flowers had agreed to become chairman of the Computer Board; the Secretary of State also agreed that 44 university institutions might really be too many as compared with the same number of students in fewer but larger institutions. He, and other speakers, emphasized the importance of a balanced distribution of reserves between the universities and other educational institutions. The White Paper, *A Plan for Polytechnics and Other Colleges*, issued in May received favourable comment in the debate, but on both sides of the House it was urged that rigidity in higher education should be avoided. The geographical distribution of the proposed polytechnics was, however, criticized, particularly the absence of such institutions from eastern England.

There was some evidence of fresh thinking about public control of universities, and in replying on the debate, the Minister of State, Department of Education and Science, Mr. G. Roberts, said that there was no intention of creating a rigid pattern, irrespective of traditions and individual circumstances. The changes proposed in the White Paper were essentially flexible and evolutionary, and the Minister would not designate a college or group of colleges for status as a polytechnic until there had been full consultation with the Regional Advisory Councils and with the local education authorities concerned.

REPLYING for the Government in an adjournment debate in the House of Commons on colour television on July 6, the Postmaster General, Mr. E. Short, said that the decision to introduce colour television on the 625-line definition standard was determined by four main considerations which had led the Television Advisory Committees last December to reaffirm the earlier decision of 1962. First, given the decision to change the line standard from 405 to 625 lines, it would be a retrograde step to start colour on 405 lines. Secondly, dual standard colour sets would be more expensive to install and maintain, and might be so complex as to jeopardize the success of a colour service. Thirdly, sets designed to receive colour on both line standards would cost 7–10 per cent more than sets designed for 625-line colour only. Fourthly, it was questionable whether a 405-line colour transmission would permit an acceptable quality of colour reception for those with colour receivers, or of black-and-white reception for those without. The latter for years to come would constitute the great majority of viewers. By the end of 1967 it was estimated that 65 per cent, and by the end of 1968 about 75 per cent, of the population would be able to receive BBC 2. Mr. Short emphasized that, whether transmitted on u.h.f. or v.h.f., the 625-line standard provided a better picture than the 405-line standard. Uniformity, he said, was desirable.

University News:

Birmingham

DR. J. M. BISHOP has been appointed to the newly established second chair of medicine.

Bristol

DR. J. M. MARSTRAND has been appointed to a chair of pure mathematics; Dr. M. H. Rogers has been appointed to a chair in the Computer Unit.

London

PROF. J. N. MORRIS, director of the Social Medicine Research Unit of the Medical Research Council and professor of social medicine at the London Hospital Medical College, has been appointed to the chair of public health tenable at the London School of Hygiene and Tropical Medicine.

Loughborough

DR. G. W. KIRBY, at present lecturer in chemistry in the Imperial College of Science and Technology, has been appointed to a chair in organic chemistry at Loughborough University of Technology.

Reading

THE following professors have been appointed: Dr. J. C. Bowman (animal production); G. Williams (zoology).

Appointments

THE chairman of the Schools Council from October 1, 1966, will be Mr. Alan Bullock, Master of St. Catherine's College, Oxford. He will succeed Sir John Maud, who has been chairman of the Schools Council since its formation two years ago, and who is now to be chairman of the Royal Commission on Local Government.

It has also been announced that Mr. Geoffrey Caston, an assistant secretary at the Department of Education and Science, is to succeed Mr. Derek Morrell as one of the three joint secretaries of the Schools Council.

THE American Institute of Physics has appointed Dr. Ralph A. Sawyer as acting director in succession to Dr. Van Zandt Williams, who died suddenly on May 13, 1966. Dr. Sawyer acted as director of the Institute for a short period from October 1, 1964, to April 1, 1965, before Dr. Williams took up his appointment. He is a spectroscopist and has been closely associated with the University of Michigan.

MR. I. G. K. WILLIAMS, at present head of the Administrative Division in the Health and Safety Branch of the United Kingdom Atomic Energy Authority, has been appointed deputy director general of the European Nuclear Energy Agency.

Announcements

THE following have been elected full members of the Soviet Academy of Sciences: A. N. TIKHONOV (mathematics); N. G. BASOV, L. F. VERESHCHAGIN, S. V. VONSOVSKY, V. L. GINSBURG and A. M. PROKHOROV (general and applied physics); M. A. MARKOV and A. B. MIGDAL (nuclear physics); V. I. POPKOV (physical and technical problems of energetics); V. V. MENNER, M. A. SADOVSKY and A. V. SIDORENKO (sciences of the earth); V. P. BARMIN, P. D. GRUSHIN, V. P. MISHIN, V. V. NOVOZHILOV, N. A. PILYUGIN and M. K. YANGEL (mechanics and control processes); I. V. PETRYANOV-SOKOLOV, G. A. RAZUVAYEV and N. M. EMANUEL (general and technical chemistry); I. P. ALIMARIN, S. T. KISHKIN and A. M. SAMARIN (physico-chemistry and technology of inorganic materials); N. D. IERUSALIMSKY, J. W. PEIVE and G. M. FRANK (biochemistry, biophysics and the chemistry of physiologically active compounds); P. K. ANOKHIN, E. M. KREPS, V. V. PARIN and B. V. PETROVSKY (physiology); B. L. ASTAUROV and N. P. DUBININ (general

biology); A. A. GUBER (history); B. M. KEDROV (philosophy and law); A. M. RUMYANTSEV and T. S. KHACHATUROV (economics); V. M. ZHIRMUNSKY and M. B. KHRAPCHENKO (literature and language); G. K. BORESKOV, N. N. VOROZHTSOV, A. B. ZHUKOV, YU. A. KUZNETSOV, L. A. MELENTYEV, A. V. NIKOLAYEV and V. V. STRUMINSKY (Siberian branch). The following have been elected corresponding members: N. P. BUSLENKO, V. YA. KOZLOV, S. P. NOVIKOV, YU. V. PROKHOROV and A. A. SAMARSKY (mathematics); L. D. BAKHRAKH, A. F. BOGOMOLOV, V. S. BOROVIK-ROMANOV, L. A. VAINSHTEIN, L. P. GORKOV, E. M. LIFSHITS, R. V. KHOKHLOV and I. S. SHKLOVSKY (general and applied physics); V. P. DJELEPOV, L. B. OKUN, E. L. FEINBERG, L. P. FEOKTISTOV and A. E. CHUDAKOV (nuclear physics); N. S. LIDORENKO (physical and technical problems of energetics); YU. D. BULANZHE, P. N. KROPOTKIN, V. V. RZHEVSKY, A. B. RONOV, E. F. SAVARENSKY, E. M. SERGEYEV, A. I. TUGARINOV and V. E. KHAIN (sciences of the earth); G. S. BYUSHGENS, S. V. VALLANDER, S. S. LAVROV, N. N. MOISEYEV, I. F. OBRAZTSOV, G. S. POSPELOV, V. S. PUGACHOV, V. B. RAUSHENBAKH and G. P. SVISHCHEV (mechanics and control processes); N. S. YENIKOLOPOV, YA. M. KOLOTYRKIN, A. A. PETROV and A. S. SADYKOV (general and technical chemistry); R. S. AMBARTSUMYAN, V. V. KAFAROV, B. N. LASKORIN and E. M. SAVITSKY (physico-chemistry and technology of inorganic materials); M. V. VOLKENSHTEIN, M. N. KOLOSOV, M. A. PROKOFIEV, A. S. SPIRIN and A. A. SHLYK (biochemistry, biophysics and chemistry of physiologically active compounds); O. G. GAZENKO, P. G. KOSTYUK and A. M. UGOLEV (physiology); A. P. ANDRIYASHEV, M. S. GILYAROV, A. L. TAKHTADZHYAN and S. S. SHVARTS (general biology); YU. V. BROMLEI, YA. S. GROSUL, YU. A. POLYAKOV and V. L. YANIN (history); G. A. AKSENYONOK, D. A. KERIMOV and T. I. OIZERMAN (philosophy and law); V. A. VINOGRADOV, A. G. MILEIKOVSKY, T. V. RYABUSHKIN, V. G. SOLODOVNIKOV and T. T. TIMOFEYEV (economics); M. N. BOGOLYUBOV and D. F. MARKOV (literature and language); A. A. BOROVKOV, G. S. GORSHKOV, M. I. KARGAPOLOV, N. N. PUZYREV, M. G. SLINKO, V. P. SOLONENKO, L. V. TAUSON and N. N. YANENKO (Siberian branch).

THE U.S. National Academy of Sciences and the Czechoslovak Academy of Sciences have agreed on a programme of scientific exchanges lasting for the next 3 years. Visits of from one month to one year by individual scientists are included under the agreement, and it is expected that a total of 40 man-months of visiting a year in each direction will take place.

THE U.S. Naval Research Laboratory has agreed with the University of Maryland and with Cornell University to conduct joint programmes of research on plasma physics. The objective of the two bilateral agreements is that the laboratories concerned should plan their work in consultation with each other and that there should be frequent interchanges of students and scientists between the institutions. The Naval Research Laboratory's programme is being directed by Dr. Alan C. Kolb, now superintendent of the Plasma Physics Division in which he has worked for the past decade.

THE Council of the Royal Society of Edinburgh has announced that Dr. S. Evans, a member of the Scott Polar Research Institute, has been awarded the Dr. W. S. Bruce Memorial Prize for 1966, in recognition of his many applications of physics to scientific exploration in the Antarctic and particularly for his development of radio echo sounding of ice. The Council has also announced that the David Anderson-Berry Prize for 1966 has been awarded as follows: Dr. G. F. Whitmore, for his investigations into the effects of radiation on the underlying cellular and chemical events which occur at various stages during the growth and division of mammalian cells; *proxime* to Dr. L. A. Stocken and Dr. M. Ord, for their work on the effects of X-irradiation on the synthesis of deoxyribonucleic acid.

MR. D. WALLIS has been awarded the Miles Prize by the National Institute of Industrial Psychology, in recognition of his work on new training techniques at the Navy Department of the Ministry of Defence. He is the first recipient of this new Prize. Dr. D. McKie Kerslake has been awarded the Vernon Prize by the Institute.

THE one hundred and third meeting of the British Pharmaceutical Conference will be held in Manchester during September 5–9. Further information can be obtained from the Pharmaceutical Society of Great Britain, 17 Bloomsbury Square, London, W.C.1.

THE third international Congress of Human Genetics will be held in the University of Chicago during September 5–10. Further information can be obtained from Dr. B. S. Strauss, University of Chicago, 1307 East 60th Street, Chicago, Illinois.

AN international symposium on "Selenium in Biomedicine" will be held at Oregon State University during September 6–8. Further information can be obtained from the chairman of the symposium, Dr. O. H. Muth, Nutrition Research Institute, Oregon State University, Corvallis, Oregon.

THE NIGHT SKY IN AUGUST

All times are in Universal Time

MOON

New Moon	16d 12h
Full Moon	1d 09h
	31d 00h

CONJUNCTIONS WITH THE MOON

Venus	14d 20h, 4° S.
Mars	14d 10h, 4° S.
Jupiter	14d 08h, 4° S.
Saturn	5d 13h, 2° N.

PLANETS

Times of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_φ (10^6 miles)	Zodiacal position
Mercury	*R*	Unfavourable	3h 05m	Unfavourable	+0·4	81	Cancer
Venus	*R*	2h 00m	2h 35m	3h 15m	−3·3	144	Cancer
Mars	*R*	2h 05m	1h 55m	1h 50m	+1·9	223	Gemini
Jupiter	*R*	2h 40m	1h 55m	1h 15m	−1·4	563	Gemini
Saturn	*R*	21h 40m	20h 40m	19h 40m	+1·0	813	Pisces

D_φ is the distance of planet from the Earth on the 15th of the month

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	*R*/*D*	Time	Mag.
56 Tau	*R*	11d 01h 46·0m	+5·3

(*D*, disappearance; *R*, reappearance)

METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Perseids	July 27–Aug. 17	12d	46° R.A., +58° Dec.	Favourable

OTHER PHENOMENA

4d 02h, Venus 1° S. of Mars.
7d 17h, Venus 0·1° S. of Jupiter.

TECUITLATL; A GLIMPSE OF AZTEC FOOD TECHNOLOGY

By DR. W. V. FARRAR

University of Manchester Institute of Science and Technology

THE unicellular yellow-green alga *Chlorella* (Chlorophyta), and species related to it, has often been discussed in recent years as a possible source of cheap food. Its production, which needs only sunlight, water and inorganic salts, would be economical of labour and land; experimental work has been going on in many countries[1]. A great impediment to this project is the unpalatability of *Chlorella*, however prepared and disguised, to human beings[2], and no solution to this problem seems to be in sight. This article presents evidence that the pre-Conquest inhabitants of Tenochtitlán (the present Mexico City) ate considerable amounts of a *blue-green* alga (Cyanophyta) of unknown species, which both the natives and the Spaniards found palatable. While it was being written, a Belgian Saharan expedition reported[3] that Africans in the vicinity of Lake Chad were also in the habit of eating a blue-green alga, *Spirulina platensis*.

Tenochtitlán was built on an island in a lake of brackish undrinkable water (Lake Tezcoco), connected to the mainland by three causeways. The small part of the lake enclosed by the causeways contained fresh, but still undrinkable, water; it was always at a slightly higher level than the rest of the lake. Drinking-water came from several miles away, by means of an aqueduct. The size of the city astonished Cortés and his companions on their entry in 1521. Their estimates of its population have often been derided as exaggerations; but the report made by Cortés of 60,000 families agrees reasonably well with Humboldt's much later estimate that the old city must have been about three times as big as the one he visited, the population of which was about 137,000 (ref. 4). At a conservative estimate (say 250,000) it was much larger than the largest towns in Europe.

How was such a large urban population fed, in a country of primitive farming, where all land transport was on the human back? There were fish in the lake, but there were no large, edible domestic animals. The staple foodstuff was maize, but the varieties then cultivated were not high-yielding. From the descriptions by the *conquistadores* of the wares offered for sale in the great market of Tenochtitlán, it would seem that the people, though by no means starving, were pressing hard on their resources. Nothing edible was neglected, not even snakes, 'lice', or the usually despised dog. It was no doubt the pressure of necessity which led to human sacrifice on a vast scale, followed by cannibalism; and to the invention of "*chinampas*" or "floating gardens", though the latter either fell out of use[5], or never existed[6] except as an ingenious system of irrigation.

With this background of knowledge, it was interesting to find in Prescott's *Conquest of Mexico* two brief references[7] to a "slime" gathered from the lake and eaten by the people of Tenochtitlán*. Prescott's first authority was the eighteenth-century historian Clavigero[8]:

> "Not contented with feeding on living things, they ate also a certain muddy substance that floats on the water of the lake, which they dried in the sun and preserved, to make use of it as cheese, which it resembled in flavour and taste. They gave to this substance the name of Tecuitlatl, or excrement of stones."

Clavigero can only be considered as a secondary source; but the other reference is to Bernal Diaz, who was among the first Spaniards to enter Tenochtitlán. His memoirs, written in his old age in simple and vigorous language, are a classic of Spanish literature. In his wide-eyed account of the great market[9], he finds room for half a sentence about the "slime":

> ". . . then there were fishmongers, and others who used to sell little loaves which they make out of a sort of slime which they gather from the great lake, which they thicken and make loaves of it, having a flavour like cheese." (' . . . *pues pescaderos y otros que vendían unos panecillos que hacen de una como lama que cogen de aquella gran laguna, que se cuaja y hacen panes de ello que tienen un sabor a manera de queso.*")

A search among the other sixteenth-century chroniclers of the conquest of Mexico revealed a few more references. Cortés in his letters to the Spanish Court does not mention the "slime", unless his reference to the collection of "salt" from the lakeside[10] refers to tecuitlatl. But Cortés, returned from conquest, and in retirement in Spain, provided the material for a book written by his secretary, Gómara, who was himself never in Mexico. Gómara wrote[11]:

> "[they eat] a sort of earth, for with nets of very fine mesh they collect, at a certain time of year, a soft substance which breeds on the water of the lakes of Mexico, and they thicken it, though it is neither plant nor earth, but a sort of mud. It is plentiful, and they collect a lot of it; they empty it out on floors, as if they were making salt, and there it thickens and dries. They make it into cakes like bricks, which they sell, not only in the market [of Tenochtitlán] but carry it to others outside the city, and far off. They eat this as we eat cheese, and it has rather a salty taste, which is delicious with *chilmolli*†. They say that so many birds come to the lake for this food, that often in winter some parts are covered with them." (". . . *y aun tierra; porque con redes de malla muy menuda abarren en cierto tiempo del ano una cosa molida que se cria sobre la agua de las lagunas de Méjico, y se cuaja, que ni es yerba ni tierra, sino como cieno. Hay dello mucho y cogen mucho; y en eras, como quien hace sal, lo vacian, y allí se cuaja y seca. Hácenlo tortas como ladrillos, y no solo las venden en el mercado, mas llévanlas tambien á otros fuera de la ciudad y léjos. Comen esto como nosotros el queso, y así tiene un saborcillo de sal, que con chilmolli es sabroso. Y dicen que á este cebo vienen tantas aves á la laguna, que muchas veces por invierno la cubren por algunas partes.*")

The Franciscan friar Toribio (known as Motolinia, "the poor man") lived among the Mexicans for many years, speaking their language and sharing their food and way of life. His *Memoriales* (written about 1541) appear to be notes for, or a first draft of, his better-known *Historia*

* Ref. 7 has misleading footnotes (pp. 284 and 536) which equate the 'slime' with *ahuahutle*, the egg-masses of some water-insect, which are eaten as a sort of caviare. The two materials are quite different.

† A pungent sauce.

General, in which the following passage does not appear, though the relation between the two works is not very clear. His account[12] of the preparation of tecuitlatl is more detailed than that of Gómara:

"There breeds upon the water of the lake of Mexico a sort of very fine slime, and at a certain time of year when it is thickest, the Indians gather it with a very fine-meshed net until their *acales* (as they call their boats) are laden with it; on shore they make on the earth or on sand a very smooth plot, two or three *brazas*‡ wide and a little less in length, and they throw it down to dry, enough to make a cake two *dedos*‡ thick. In a few days it dries to the thickness of a worn ducat; and they cut this cake like broad bricks. The Indians eat a lot of it, and it keeps so well that they go with this merchandise to all the markets of the land, while those among us who share the tastes of the Indians find it very pleasant; it has a salty flavour." (*"Crianse sobre el agua de la laguna de México unos como limos muy molidos, y á cierto tempo del ano que están más cuajados, cógenlos los indios con unos redejoncillos de malla muy menuda, hasta que hinchen los acales ó barcos dellos, y á la ribera hacen sobre la tierra ó sobre arena unas eras muy llanas con su borde de dos ó tres brazas en largo y poco menos de ancho, y échanlos allí á secar; echan hasta que se hace una torta de gordor de dos dedos y en pocos dias se secar hasta quedar en gordor de un ducado escaso; y cortada aquella torta como ladrillos anchos, cómenlo mucho los indios y tiene se buenos anda esta mercaduria por todos los mercaderes de la tierra, como entre nosotros los que son de la salsa de los indios es bien sabroso, tiene un saborcillo de sal."*)

Another Franciscan friar, Bernardino de Sahagún, knew the natives so well that his *Universal History* (dealing mainly with religions and social customs) was first written in Náhuatl about 1550; he made a Spanish version thirty years later, although it was not published until 1830. His account[13] of tecuitlatl is brief but of particular interest:

"There is an *urrosas*§ which breeds upon the water, which is called Tecuitlatl; it is of a clear blue colour; after it is well thickened and coarse it is gathered, and spread on ashes on the ground, and afterwards they make cakes of it, and eat them cooked." (*"Hay unas Urrosas§ que se crian sobre el agua que se llaman Tecuitlate; son de color de azul claro; despues hacen unas tortas de ello, y tostadas las comen."*)

The most valuable authority would be the naturalist Francisco Hernandez, who was sent by the Council of the Indies to report on the flora, fauna, and minerals of New Spain and their possible economic exploitation. This report was compiled about 1550–60, but the manuscripts were scattered and lost, and there was no reasonably complete publication until 1790. This[14] does not mention tecuitlatl, but there is a summary of his unpublished notes[15] in which it is given as a chapter-heading under "minerals". (The notes presumably exist in manuscript in the Spanish archives.) The Spaniards were evidently confused about the proper classification of tecuitlatl; they could not (lacking the microscope) identify it as a plant, although it 'bred'; but the 'breeding' of minerals was still a common belief in the sixteenth century.

At some time unknown, but probably not long after 1550, the practice of making tecuitlatl must have ceased. The native population was decimated by repeated outbreaks of smallpox, and in the first confused years of Spanish occupation many traditions were lost. Cervantes de Salazar[16], writing about 1560, refers to it, but the passage is lifted entire from Gómara. Acosta[17], who was in Mexico during the years 1586–87, does not mention it.

‡ *braza* equals 1·7 m; *dedo* equals 1·8 cm.

§ 'Urrosas' (or 'urronas') is a puzzle. It is not Spanish, and is either a word from some Mexican language or a mistranscription.

The curious character, Thomas Gage, an English Dominican friar who turned Puritan preacher, wrote[18] as though it were still used during his sojourn in Mexico (1625–26), but a close examination of the passage shows it again to be plagiarized from Gómara, this time (with some modernization of spelling) from the English translation by Nicholas[19]. Humboldt[4], who missed nothing, has no word of it; but by his time (1803) the draining of the lake had gone so far that it occupied only a fraction of its area at the Conquest.

The key phrase among these quotations is that of Sahagún, who refers to the 'clear blue' colour of tecuitlatl. This identifies it almost certainly as a member of the Cyanophyta, or blue-green algae. These primitive plants owe their colour to the presence of phycocyanin, a blue tetrapyrrole-protein pigment, which is sometimes accompanied by the related reddish phycoerythrin. On some sheets of water in the United States and Canada they grow in abundance at some seasons, and are known as "water bloom". They thrive in both fresh and brackish water, and some species are able to make use of atmospheric nitrogen[20], though this may not have been necessary in Lake Tezcoco, which presumably received most of the sewage from the city. The chroniclers do not make it clear whether tecuitlatl grew on the fresh or salt-water portions of the lake; but the latter was by far the larger, and the accounts stress the salty taste and good keeping qualities of the product. Humboldt[21] gave a figure for the density of the water which corresponds to about 2·5–3·0 per cent of dissolved salts, which were mainly sodium chloride and carbonate; sulphate was absent.

No published work has been traced on the use of any species of Cyanophyta as food. Indeed, cases of cattle poisoning have been ascribed on good evidence to "water bloom", and toxic substances have been isolated from the genera *Microcystis* and *Anabaena*[22]. Tecuitlatl, however, seems to have been eaten without ill effect, and even enjoyed. There is probably now little hope of identifying the alga on the spot; the great drainage schemes of 1607–1789 and even more of 1885–1900, followed by huge urban expansion, have radically altered the terrain of the Valley of Mexico. Indeed, the disuse of tecuitlatl may have been the consequence of a slight change of environment, causing the replacement of a palatable dominant species by an unpalatable or even toxic one.

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THE EXISTENCE OF THE CROSSROADS POLICEMAN

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In the physical world, according to the Second Law of Thermodynamics, there is a general tendency towards a decrease of order. Maxwell once lamented the lack of demons. He said that the fight against increasing entropy could be won if there were demons who could sit by a door in the side of a vessel containing a gas at some temperature above absolute zero, and who would open the door and let through particles satisfying some definition of "fast". The same demons would otherwise do nothing. (This is a slight reformulation of the principle; a single attribute dimension of linear velocity has been used to name the particle, and a certain value on it, namely, "faster than n ft./sec", has been taken as the criterion for the decision of the demon. The demon has the relatively simple task* of recognizing these names.)

It would seem that in the biological world the equivalents of Maxwell demons exist as there is a possibility that there is increasing organization in the same sense as that which the Second Law of Thermodynamics infers. If we were able to construct a machine that would make use of the patterns of inputs and outputs from a process and extract them from the "rules of ordering" that the process exhibited in its performances, then it would be possible for the machine to construct in part of itself a sub-machine that would mimic, in every way known† to the machine, the process under observation[2].

This article briefly sketches the nature of a mechanical procedure whereby the presence of an elementary class* of Maxwell demons can be detected and machines constructed automatically to mimic their behaviour. No weighting or averaging processes are used as were applied, for example, by Selfridge[3]. The idea here is that if demons, in making decisions about events, make use only of distinctive features of events, then these must ultimately be accessible to an external observer. If a demon's decision rule is thought of as a relational structure embodying these distinctive features linked to each other by the appropriate ordering relation, then it is possible to discover the nature of this structure simply from the performance of the demon. This is done by noting that each individual ordering produced by the demon gives rise to a set of ordered distinctive features; every observation is used to reveal fragments of different possible relational structures. As the decision rule is determinate, it is found that, as experience accumulates, only one set of fragments when collected together produces a coherent structure. The process makes use of both a passive initial process, which is sensitive to and extracts the set of distinctive features (which may be thought of as something like Hubel and Wiesel[4] units) and the ordering relations between them, and then later an active process which makes use of this information to form the relational structure which represents the decision rule used by the demon; an analogy with present-day approaches to the central nervous system is intended. The procedure is more of the nature of a discovery process than a learning process for the reason that there is no component in the procedure equivalent to reinforcement. (Elsewhere, I have sketched out[5] the procedure more fully and have outlined a mechanical scheme that extracts a recursive rule system using only example productions of that system. Moreover, in a forthcoming paper, I shall extend the scheme so that a machine learns a systematic way of providing descriptions of objects sensed by it in its environment. The description provided comes from within a closed system of descriptions in the sense of that proposed by Grimsdale *et al.*[6].)

The nature of the problem and its solution can be stated in very simple terms, using an everyday example. A number of important assumptions will be involved; these will be indicated by $A1$, $A2$, $A3$. The problem is:

> P: "By observing only the patterns of time intervals between cars entering and leaving a crossroads, where the cars involved have distinctive features, can we decide whether or not the state of affairs is the same as if a policeman were at the crossroads directing the traffic in an orderly fashion ?"

To make the problem quite explicit, it must be considerably idealized. There are two main one-way roads. No left or right turns are allowed; neither are crashes, overtakings and spontaneous creations, and furthermore the crossing point must be clear of cars before observation is started (that is, the initial state of affairs is known). In other words, we assume ($A1$) that the specific ordering of the cars that prevailed when they were observed, on the two roads, just before they entered the cross-over point is preserved when they are observed leaving it. Only the pattern of inter-car time intervals is disturbed; this is because cars on the two roads will occasionally coincide and, because they can only cross one at a time, they must be ordered, and in the process one car will suffer a relative delay. The crucial assumption ($A2$) is: If the policeman imposes certain distinctions Dp on the cars so that he can direct them, then the number of these is finite and, moreover, the observers of the process must be able to make a finite number of distinctions Do which include at least Dp, that is, $Do \supseteq Dp$. The "shape" of the name by which these distinctions are known to both parties involved is not important so long as they are distinct for the parties concerned; arbitrary names can be assigned. The policeman may be following the rule (PR): "Whenever there is a coincidence of cars at the crossroads, always allow the make 'X' through before the make 'Y' and 'Y' before 'Z'. And when two cars of the same make coincide, allow that on road one ($r1$), the road on my right, through first. Otherwise, do nothing." The observers may, however, know the makes that the policeman calls "X", "Y" and "Z" by the names "A", "B" and "C", and the roads that he calls "$r1$" and "$r2$" they may call "$R1$" and "$R2$"; it does not matter. Clearly the policeman's rule must be comprehensive; if there are n makes of car (n finite), it must include an ordering of all the n makes; he must not be confronted with a make that he does not recognize. In our example we shall assume that there exist just four makes of cars in all the world, called by the observers (A, B, C, D).

* If the demon's task were to let through particles named with grammatical, but not necessarily sensible sentences, and not let through any named with non-grammatical sentences—for example, to let through a particle named "Colourless green ideas sleep furiously" and not to let through "Furiously sleep ideas green colourless"—then the automatic procedure discussed here would be inadequate. A more competent demon would be required; we must posit a range of classes of demons with varying competences. (The example given is derived from Chomsky[1], whose publication also provided most of the inspiration for the work reported here.)

† By "every way known" I mean that the mimic will exhibit behaviour which will correspond to a rule system (an input/output transformation matrix) derived from the experience that it has gained, so far, of the process controlled by the Maxwell demon; for every example of behaviour, exemplifying a particular rule, emitted by the process controlled by the Maxwell demon, the mimic will be able to provide an example illustrating the same rule. If the Maxwell demon has never had occasion to exemplify one of its rules, then clearly the mimic has been denied the appropriate information, but soon after receipt of such an example the mimic will be able to supply an example performance which will also exemplify that. This is an important point.

To show how the process observers could be mechanized the nature of their task must be shown quite clearly. The arrangement of process observation is shown in Fig. 1.

It will be seen that there are four clerks (iC_1, iC_2, oC_1, oC_2), who quite passively react to events on the television screens. They have each been provided with a coding sheet and a print-out clock. When a car passes, the clerks record both its time of passing and its make. A fragment from a typical record written by clerk iC_j, $j=1,2$, might be:

$$\ldots, ({}_0t_{10}, R_j, A), ({}_0t_{11}, R_j, B), ({}_0t_{12} R_j, D), ({}_0t_{13}, R_j, A), \ldots \quad (1)$$

where ${}_0t_{10}$ denotes the magnitude of the tenth time interval, the recording of which was occasioned by the passing of a car and it was a car of make "A". (The time at which the observation period was begun is arbitrary, but all the clocks were started at the same time and synchronized.) The clerk oC_j uses a similar recording procedure but slightly different marks, so that the fragment of his record that relates to the same cars (we know they are the same due to $A1$) will appear as:

$$\ldots, ({}_0t'_{10}, R_j, A), ({}_0t'_{11}, R_j, B), ({}_0t'_{12}, R_j, D), ({}_0t'_{13}, R_j, A), \ldots \quad (2)$$

I think it is clear from the duties of the clerks as stated that their task could be mechanized quite easily. The four makes of car could each carry a plate engraved with a two-place binary code, A-00, B-01, C-10, D-11, and photocells could then activate the print-out clock and assign one of the binary coded names. Thus records such as (1) and (2) could be produced mechanically.

At the end of the observation period, which is of an arbitrary length (but see †), the clerks bring their records to the co-ordinator (CO). He co-ordinates the two input records and the two output records by ordering the individual observations in order of time of observation. As the clock supplies a time assignment of finite length, if there have been any crossroad coincidences then the CO is bound to find cars within the input records with identical time assignments. (The problem is slightly glossed over here, but it is clearly possible to state the proper conditions for this specific problem.) A typical record that the CO forms, by amalgamating the records of iC_1 and iC_2, might be:

$$I/P: \; //({}_0t_1{}^{(1)}, R1, A), \left\{\begin{matrix}({}_0t_2{}^{(2)}, R1, A)\\ ({}_0t_1{}^{(2)}, R2, B)\end{matrix}\right\}, ({}_0t_3{}^{(3)}, R1, C), ({}_0t_2{}^{(4)}, R2, A), \left\{\begin{matrix}({}_0t_4{}^{(5)}, R1, B)\\ ({}_0t_3{}^{(5)}, R2, C)\end{matrix}\right\},$$
$$({}_0t_4{}^{(6)}, R2, C), \left\{\begin{matrix}({}_0t_5{}^{(7)}, R1, B)\\ ({}_0t_5{}^{(7)}, R2, A)\end{matrix}\right\}, \left\{\begin{matrix}({}_0t_6{}^{(8)}, R1, B)\\ ({}_0t_6{}^{(8)}, R2, C)\end{matrix}\right\}, ({}_0t_7{}^{(9)}, R2, C), \left\{\begin{matrix}({}_0t_7{}^{(10)}, R1, A)\\ ({}_0t_8{}^{(10)}, R2, A)\end{matrix}\right\} // \quad (3)$$

where "//" is a beginning and an end marker, and k in ${}_0t_k{}^{(j)}$ is the time ordering before the amalgamation of the records, while (j) is the time ordering after. The ordering depends only on a magnitude judgment and can easily be mechanized (it is one-dimensional). Clearly in the O/P record there will be no coincidences; those that appear in the I/P record will be ordered. The output record formed by the CO from the records of oC_1 and oC_2 which corresponds to the foregoing can now be presented:

$$O/P: \; \overset{1.}{//({}_0t_1{}'^{(1)}, R1, A)}, \overset{}{({}_0t_2{}'^{(2)}, R1, A)}, \overset{}{({}_0t_1{}'^{(3)}, R2, B)}, \overset{}{({}_0t_3{}'^{(4)}, R1, C)}, \overset{}{({}_0t_2{}'^{(5)}, R2, A)},$$
$$\overset{2.}{({}_0t_4{}'^{(6)}, R1, B)}, \overset{}{({}_0t_3{}'^{(7)}, R2, C)}, \overset{3.}{({}_0t_4{}'^{(8)}, R2, C)}, \overset{}{({}_0t_5{}'^{(9)}, R2, A)}, \overset{}{({}_0t_5{}'^{(10)}, R1, B)}, \quad (4)$$
$$\overset{4.}{({}_0t_6{}'^{(11)}, R1, B)}, \overset{}{({}_0t_6{}'^{(12)}, R2, C)}, \overset{5.}{({}_0t_7{}'^{(13)}, R2, C)}, \overset{}{({}_0t_7{}'^{(14)}, R1, A)}, \overset{}{({}_0t_8{}'^{(15)}, R2, A)}//$$

Given that the records as shown in (3) and (4) are marked as complete (the beginning and end are marked with "//"), can we now decide whether the ordering is systematic or not?

We shall now consider the coincidences marked "1" to "5" in (4). If there is any ordering, according to $A2$, it must arise from distinctive features. We can now consider a record reduced just to the relations:

$$//(R1/R2, A/B), (R1/R2, B/C), (R2/R1, A/B), (R1/R2, B/C), (R1/R2, A/A)// \quad (5)$$

where "/" means "precedes" and is transitive.

Bearing in mind $A2$ and introducing a further assumption ($A3$)—namely, that policemen are determinate beings, that is to say that once they have decided to follow a certain course of action they do not change, we can allow the CO to search for identical relations exhibited in (5). The CO does not have to take all the distinctions Do that he knows and form preliminary "hypotheses"; in the first term of expression (5) there are already two suggestions. The CO now takes these and systematically searches among the others (that is not difficult to arrange) for others identical to them. Only an "identical or different" judgment is involved here; the process of taking an item, comparing it with a set of other items and making such a judgment is an everyday task for a computer if the item can be represented in a binary code—here it can. Thus, this process can be mechanized. As a result of his search and comparison procedure, the CO finds that the only set of relations that does not (on the information available to him, see †) transgress $A3$ is:

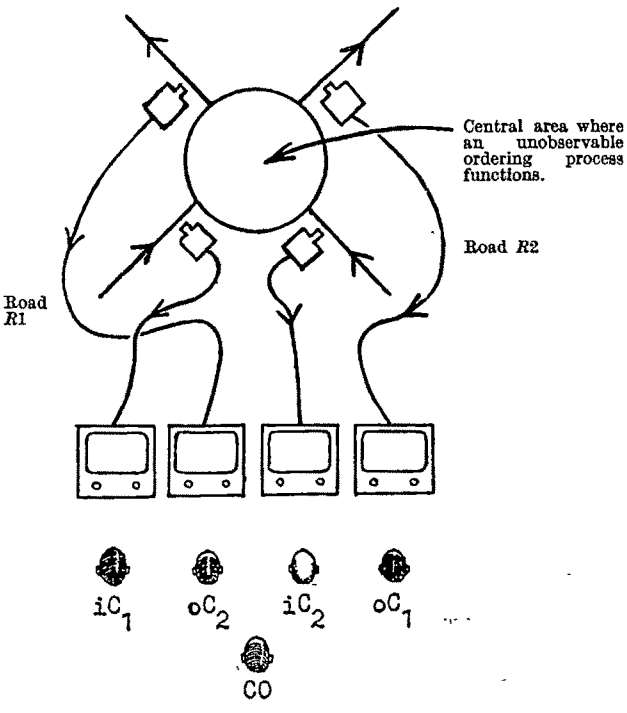


Fig. 1

$$//(A/B), (B/C), (A/B), (B/C), (A/A)// \quad (6)$$

These may be summarized by:

$$\begin{bmatrix} A/B/C \\ A/A \end{bmatrix} \quad (7)$$

In expression (6) the last term (A/A) suggests the possibility of a further distinction. If it were found that

associated with every relation, such as A/A, there was some other specific relation, for example (fragment):

$$\ldots (R1/R2,A/A), \ldots, (R1/R2,C/C), \ldots, (R1/R2,B/B), \ldots \quad (8)$$

then, with the information which we have so far (see †), we could summarize the policeman's hierarchical decision rule as:

$$\begin{bmatrix} A/B/C \\ R1/R2 \end{bmatrix} \quad (9)$$

This means that he is behaving as if he were following a rule like the PR one already mentioned. If the relations between the distinctions mentioned on the top line of (9), that is, "$A/B/C$", are insufficient to make a decision, then he ignores them and turns to the relation between the distinctions mentioned on the next line of (9), that is, $R1/R2$.

The sketch of the discovery procedure is now in essence complete. The CO as a result of his search among the two sets of relations used here, the $(R1,R2)$ set and the (A,B,C,D) set, may conclude that so far as it is known (see †) the (A,B,C) set is systematic and that the state of affairs at the crossroads is the same as if there were a policeman there directing the traffic according to the hierarchical decision rule (9). However, the way in which the CO and the clerks do this and the way in which it may be mechanized has been sketched and thus they may all be replaced by a general-purpose computer provided with photocell inputs and a suitable programme. In this way the computer is able to structure part of itself, by following the procedure already outlined, so that as a result of observing the pattern of inter-car time intervals at the inputs to the crossroads, it could activate something which would order the cars leaving the crossroads in an identical fashion to that which has just been demonstrated with a policeman. (There is an inherent and very important time lag.) Thus the computer has mimicked the behaviour of a living thing; actually the behaviour of the policeman with respect to the cars was so systematic that he could have been replaced by a machine in the first place, but that is a peculiarly human intuitive judgment and not one that a computer could make.

We must now briefly discuss what would happen if the policeman were not there. If, and only if, the pattern of relations (10) had been obtained:

$$//(R1/R2,A/B),(R2/R1,B/A),(R1/R2,B/C),(R1/R2,C/B)// \quad (10)$$

then no systematic set of relations can be found and we must assume that it is just as if a policeman were not there and that individual motorists were deciding for themselves using their own individual 'precedence criteria'.

We note in this example that the policeman did not ever encounter the make of car that the observers knew as "D"; thus although the policeman's rule system contained a rule appropriate to "D" and was, in the terms of the names assigned by the observers:

$$\begin{bmatrix} A/B/C/D \\ R1/R2 \end{bmatrix} \quad (11)$$

the observers have only sufficient information to construct (9). The discussion in relation to the specific problem is now complete.

I would now like to discuss the generality of the procedure and to state a problem. Perhaps it will be best to begin by discussing the nature of decision rules. $A2$ is equivalent to an assumption that if the policeman is using some distinction related to the cars, then it must be possible to code that distinction (in, say, binary code). If that is the case, then it is not too difficult to see how the procedure could be extended to cope with a finite number of attribute dimensions each containing a finite number of distinctions. What now happens if the policeman uses a table of random binary digits such that when there is a coincidence and his table shows an "0", he lets the car on his right go first, and when it shows "1" he lets the car on his left through? In this event, the CO decides that the state of affairs is the same as if there were no policeman there at all. If the policeman is consulting a table of numbers generated by a specific infinite non-recurring decimal, then the CO can only discover this by pursuing the specific hypothesis that this is so and cannot work in the manner described, that is, without a hypothesis. The assumptions $A1$ and $A3$ must now be discussed. Assumption $A3$ is essential in this sense; the procedure here takes time to extract the policeman's rule system, but would eventually achieve the characterization (11) of the policeman's system. The policeman might now reverse all his rules for a while and then change back to the system (11); in this event the procedure would extract:

$$|\underline{\rightarrow RS1 \rightarrow RS2}|$$

(where $RS1$ is $\begin{bmatrix} A/B/C/D \\ R1/R2 \end{bmatrix}$, $RS2$ is $\begin{bmatrix} D/C/B/A \\ R2/R1 \end{bmatrix}$, and "$\rightarrow$" means "... followed by ...")

so long as the rate of change of the rule systems by the policeman was sufficiently slow for the procedure to work, otherwise the CO would decide that the policeman was behaving in a random manner.

I would now like to state a problem—it is in two parts: "Can the individual decision rules of the policeman be extracted in the situation depicted in Fig. 2, under the following conditions: (i) when assumption $A1$ still holds? (ii) when the restriction on left or right turns is relaxed?

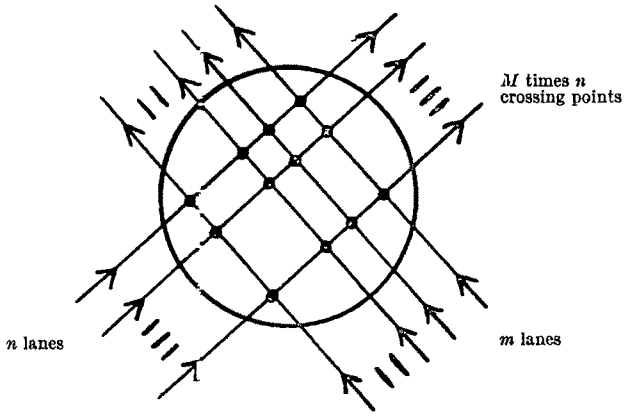


Fig. 2

A solution of this problem will have: (*a*) if (i) is solved, a relevance for automatic discovery procedures for phrase structure grammars; (*b*) if (ii) is solved, a relevance for transformational grammars. Only transformational grammars are adequate for natural language[1]. The possibility that the solution to (ii) will provide a specification for automatic machines capable of describing patterns will be considered elsewhere.

I thank Dr. Alan Gauld, who pointed out the similarity between the policeman problem and the Maxwell demon situation, and Dr. John Newson for a discussion which forced me to think even more about these problems.

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BOOK REVIEWS

THE GEORGIAN PLANET

The Planet Uranus

A History of Observation, Theory and Discovery. By A. F. O'D. Alexander. Pp. 316. (London: Faber and Faber, 1965.) 70*s*.

URANUS, the "Georgian Planet" as William Herschel liked to call it after his discovery on March 13, 1781, is the third largest planet in the solar system, and with its five satellites, Uranus has provided amateur observers, astronomers and mathematicians with some fascinating problems over the past 180 years.

The planet is remote from the Earth, at twice the distance of Saturn. It is therefore too far away to excite the attention of even the most advanced planners of space travel. However, it is almost visible to the naked eye, and it can certainly be easily examined, when suitably placed in the sky, by anyone using binoculars and a small telescope. Dr. Alexander wrote an extremely well-documented account of the planet Saturn in 1962, and he has now followed this up by an equally scholarly book on Uranus. He has compiled from primary sources an encyclopaedic history, arranged chronologically, of the initial discovery, and subsequent observations up to 1963, of Uranus and its satellites. The wealth of detail provided and the historical references cannot be found elsewhere within one volume. There are quotations (translated), diagrams, data and photographs from many of the original sources and from reports of past astronomers, both American and European. Those who are interested in names will be delighted with the discussions on how the "Georgian" planet changed its name to Uranus and how the names chosen for the satellites have a relationship with Shakespeare. Those who like the thrill of detection can read how the satellites were lost and how in looking for them three others were found, and also how difficulties encountered in the calculation of the orbit of Uranus led to the mathematical discovery of the planet Neptune. For the spectroscopist and physicist, the spectral investigation will be of considerable interest, since they finally proved that, as was first recorded in *Nature* (134, 418; 1934), methane is a major constituent of the atmosphere of Uranus. It was on February 16, 1948, that the fifth satellite, Miranda, was photographed by P. P. Kuiper for the first time. The satellite is 123,000 km from Uranus and of about the seventeenth magnitude. It had not previously been observed visually. The exceptional thing about Uranus and its system is that Uranus rotates and its satellites revolve in the retrograde sense, when referred to the ecliptic. Dr. Alexander tells us that during 1966 the Sun passed through the plane of the satellite orbits during February, and the Earth in March with another passage to come in July.

The Planet Uranus is a standard work and reference source for use by both specialists and others. The masterly description, though fully detailed, is pleasant to read. It is almost too thrilling to leave before completion. Doubtless during the next decade there will be many new exciting discoveries to add, as astronomy (both from the Earth and from space) is now in a stage of rapid advance, and it is to be hoped that Dr. Alexander will not only continue his series of volumes on the planets, but will also follow up his present book with a sequel, including perhaps any radioastronomical observations of interest which have been omitted from *The Planet Uranus*.

S. WEINTROUB

GRAVITATIONAL COLLAPSE

Gravitation Theory and Gravitational Collapse

By B. Kent Harrison, Kip S. Thorne, Masami Wakano and John Archibald Wheeler. Pp. xvii+177. (Chicago and London: University of Chicago Press, 1965.) 6.50 dollars; 48*s*.

GRAVITATIONAL collapse occurs when a collection of matter is of such a size that the inward gravitational attraction is greater than the elastic forces generated by compression. It is obvious that collections of matter of about planetary mass are stable, but it was found by Landau in 1932 that when an amount of cold matter of a critical mass of the order of the mass of the Sun is assembled together the system becomes unstable. During the late 'thirties a beginning was made in studying such collapse, notably by Oppenheimer and Volkoff. Although such collapse could be studied in Newtonian gravitation it is evident that eventually the matter will reach such a state of compression that general relativity becomes essential. As a result of the theoretical analysis of this problem which has been pursued for the past few years, two questions present themselves urgently for consideration: (1) Does the theory introduce any restrictions which would prevent collapse from proceeding to completion? (2) When matter collapses can the baryons in it escape destruction?

Wheeler's opinion is that at least in the case of cold matter, for which he has carried out the analysis in detail, there is no escape from eventual collapse and no escape from the disappearance of baryons. By the same token there is no escape from the serious contradiction between general relativity and any quantum field theory which holds to the conservation of baryon number. In the present book the authors expound their theory starting from the basic principles. The starting point could be described by saying that a first-rate genius ignorant of general relativity would be able to read the book without trouble from the beginning. Lesser mortals may well find training in general relativity and quantum mechanics an advantage.

C. W. KILMISTER

GROUP THEORY IN CLASSICAL AND QUANTUM PHYSICS

Theory of Groups in Classical and Quantum Physics

By Théo Kahan, in collaboration with P. Cavaillès, T. D. Newton, G. Lochak, R. Gouarné, G. Rideau and R. Nataf. English translation edited by A. R. Edmonds. Vol. 1: Mathematical Structures and the Foundations of Quantum Theory. Translated by H. Ingram. Pp. xxiii+566. (Edinburgh and London: Oliver and Boyd, Ltd., 1965.) 210*s*.

SINCE the beginning of the nineteenth century, the influence of the physical sciences on the development of mathematics has gradually diminished until, at the present time, the theoretical physicist depends to a large extent on the pure mathematician for the mathematical techniques that he may require in his attempts at creating order out of the data provided by the experimental physicist. Regrettably, many theoretical physicists still remain unacquainted with the methods of modern abstract mathematics even though they are well aware that without the pre-existence of abstract algebra and in

particular group theory, the remarkable recent developments in the study of the fundamental symmetries of elementary particles would have been seriously retarded.

The volume under review is the first of a treatise written by Théo Kahan and several eminent collaborators which is devoted mainly to the group structure. It was originally written in French and published in 1960 by Dunod, of Paris, under the title *Théorie des Groupes en Physique Classique et Quantique*. The translation into English by H. Ingram is good. The main objectives of the treatise are, first, to provide an account of axiomatized mathematics and the theory of abstract groups directed specifically towards the theoretical physicist, and secondly, to investigate with considerable thoroughness the different applications of group theory to the various branches of physics.

The book is divided into seven parts. The first of these is entitled "Theory of Groups and Axiomatized Mathematics for the Use of Physicists" and was written by P. Cavaillès and T. Kahan following the axiomatic method of the team of French mathematicians calling themselves Nicolas Bourbaki. The first part concludes with an appendix by G. Lochak on integration over the topological groups. The next part of the book is concerned with the "Inhomogeneous Lorentz Group" and was written by T. D. Newton. This is followed by a part on the "Theory of Abstract Groups", written by R. Gouarné. Inevitably, a certain degree of overlap with the contents of the first part of the book occurs here. Some repetition is, of course, quite helpful to the reader although it would have been advantageous if the authors had used the same notation. Parts IV and V, written by G. Rideau, deal with the "Theory of Representations" and the "Permutation Group", respectively. Then comes a part written by T. Kahan concerned with the "Theory of Groups and Axiomatics of Quantum Mechanics". It is quite short and develops the elements of quantum theory from five axioms, the Dirac approach being followed fairly closely and the connexion with group theory discussed. Lastly, there is a part on the "Group of Rotations", written by R. Nataf.

The general aim which motivated the preparation of this treatise is certainly a good one and, on the whole, the first volume has turned out very well, although it could have been written in a more unified manner by a single author. However, the translation of the first volume is very welcome particularly when taken together with the second volume, so far unpublished, which deals with the applications of group theory to the various branches of physics. B. L. MOISEIWITSCH

SURFACE IMPACT

Atomic and Ionic Impact Phenomena on Metal Surfaces

By Manfred Kaminsky. (Struktur und Eigenschaften der Materie, Band 25.) Pp. xii+402. (Berlin and New York: Springer-Verlag, 1965.) 58 D.M.

A MONOGRAPH on a large subject imposes rigid limits on an author. Dr. Kaminsky decided to restrict *Atomic and Ionic Impact Phenomena on Metal Surfaces* strictly to metal surfaces and even within this framework he had to be selective. He states plainly in the preface that his selection of topics reflects principally his own interests in the various activities in the field and is not intended to reflect their relative importance.

In five introductory chapters Dr. Kaminsky gives brief descriptions of the structure of metal surfaces, forces which can act on an atom or molecule impinging on the surface and the energetics of surface reactions. Each of these topics could easily become a subject of a separate monograph, and readers familiar with these fields will feel that perhaps less than justice has been done to their subjects. I was certainly disappointed to find no reference to the work of Farnsworth, Landner and Morrison and others on the atomic structures of metal surfaces, but this was compensated by very full treatment given to some of the other topics.

The chapter on sputtering by ion bombardment is long and detailed and much information and data are also to be found in the chapters dealing with accommodation coefficients, surface ionization, ion scattering, ion neutralization and the kinetic emission of electrons by bombardment with charged and uncharged particles. There are briefer chapters which deal with the elastic collisions, formation and emission of negative ions and the de-excitation of metastable atoms and ions on metal surfaces.

There is a considerable degree of uniformity in presentation throughout the book. Each chapter starts with a clear physical picture of the phenomenon which is to be discussed and this is followed by the theoretical treatment, experimental methods and results. Whenever possible experimental data published by a large number of workers are collected into tables most convenient for easy reference and comparison. Chapters are divided under numerous sub-headings which are very useful when the reader is hunting for a specific information or reference; in consequence, however, the list of contents is difficult to read and looks monotonous. Printing main chapter titles in heavier type would help to relieve the monotony and make it easier for the reader to find his bearings.

The rate of growth of surface science is considerable and appears to be increasing. There are new graduates entering the field and older research workers established in other fields of science are developing interest in surfaces. Dr. Kaminsky's book satisfies, therefore, a real need in providing a very readable account of an important topic. The book should be of value to senior students and research workers. There are more than 800 references to papers, books and review articles published before 1963, including many classics published at the beginning of this century or earlier. J. ADAM

INTERPRETING ORGANIC SPECTRA

Interpretation of Organic Spectra

Edited by D. W. Mathieson. Pp. ix+179. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965. Published in association with the Royal Institute of Chemistry.) 42*s*.

NUMEROUS text-books have been written on the interpretation of infra-red, nuclear magnetic resonance and mass spectra, in which group frequencies, chemical shifts and fragmentation patterns have been discussed and tabulated. With these to hand, the main hurdle to be faced by the novice spectroscopist is the interpretation of actual spectra.

In *Interpretation of Organic Spectra*, based on seminar sessions at one of the Summer Schools in Organic Chemistry held under the aegis of the Royal Institute of Chemistry, about ten spectra of each type are analysed in detail. For pedagogic reasons the amount of ancillary information regarding each sample is kept to a minimum, in order that both the strength and the limitations of each technique may be apparent. Additional infra-red and nuclear magnetic resonance spectra of unknown compounds are included as problems.

In each section the samples have been well chosen to show the capabilities of the particular technique, and the substances are obviously representative of those examined in a typical analytical laboratory. As such, a number of them contain impurities, which are usually indicated in the worked examples and may themselves provide useful experience. However, at least two of the unknown infra-red spectra include extraneous bands, which can only cause confusion. The novice is also liable to be confused by the three different formats used for the infra-

red spectra and by the absence of a τ scale from several of the nuclear magnetic resonance spectra which are nevertheless interpreted in terms of τ values.

The treatment of the three sections varies in the amount of background information given. The nuclear magnetic section, contributed by J. A. Elvidge, includes sufficient data on chemical shifts, spin-coupling constants, etc., for it to be used without reference to other works. In the interpretation it is assumed that the empirical formula of each sample is known, a circumstance not always achieved in actual practice. The infra-red section, by J. K. Brown, K. J. Morgan, C. J. Timmons and D. Whiffen, is based on assignments published in specialist works, such as that of Bellamy, and some such reference book is required for the interpretation of the unknown spectra. In the section on mass spectrometry, by A. Quayle and R. I. Reed, the examples are chosen to illustrate the fragmentation behaviour of most classes of compound, and appropriate literature references are given. Each spectrum is interpreted either on the basis of the "z-number" or of the relative abundance of the parent and $(P+1)^+$ ions. Complete identification is frequently only possible by reference to published spectra.

For anyone concerned with only one branch of spectroscopy the price may seem a little high, but the book has an added value in providing a unique comparison of the scope of these techniques and of the different approaches required for interpretation of the three types of spectra.

R. J. MESLEY

PRACTICAL INFRA-RED SPECTROSCOPY

Laboratory Methods in Infrared Spectroscopy

Edited by R. G. J. Miller. Pp. 164. (London: Heyden and Son, Ltd., 1965.) 45*s*.; 6.50 dollars.

ONLY a few years ago infra-red spectroscopy was the almost exclusive province of the specialists, who knew every optical and mechanical principle involved in the construction of their spectrometers and who by trial and error had developed effective means of extracting spectral information from even the most intractable samples. To-day, with the easy availability of relatively inexpensive spectrometers, it is inevitable that many of them are in the hands of operators with little spectroscopic experience. The aim of this book is to pass on to such people some of the accumulated "know-how" of the experienced spectroscopists and the specialists in particular fields.

There are chapters on the operation of a spectrometer, the recording of a spectrum and on infra-red optical materials, but the bulk of the book is concerned with sample preparation. In addition to a general chapter on the handling of solid, liquid and gaseous samples, there are sections on high- and low-temperature spectroscopy, chromatography as a means of sample preparation, and special types of sample such as aqueous solutions and dispersions, natural and synthetic fibres, and insoluble polymers and rubbers. A final chapter on the orientation of crystals differs from the remainder in introducing a number of theoretical calculations, but for the most part this is essentially a practical manual.

The contributors, who are too numerous to mention individually, are acknowledged experts in their respective fields, and between the covers of this slim volume may be found a wealth of practical suggestions and recommendations based on years of experience. The book is intended primarily for those with little spectroscopic experience, with the object of improving the quality of the spectra they produce, but there can be few spectroscopists who will not find in it some useful hints or stimulus for further work.

R. J. MESLEY

TISSUES AND ORGANS

Organogenesis

Edited by Robert L. DeHaan and Heinrich Ursprung. Pp. xi+804. (New York and London: Holt, Rinehart and Winston, 1965.) n.p.

FOR those who might expect *Organogenesis* to be a compilation of embryo morphology there are some surprises. We are introduced to work which should be better known and to younger and older workers whom we might not otherwise encounter. In the first section, Heinrich Ursprung reviews biochemical genetics, but does not mention the essential chemical contributions of Gobind Khorana. The author's principal purpose is to imply that specific non-histone molecules stimulate normal development by attachment to and detachment from particular loci, at the right times and within the right cells. This has not been shown. E. H. Mercer describes the histogenesis of the skin of the tadpole and J. P. Trinkaus discusses morphogenetic movements.

Bengt Kallen contends that early proliferations and migrations within the brain determine its morphogenesis. Ray Watterson discusses, principally, the migration of pre-mitotic and post-mitotic nuclei, to and from the ependyma. R. W. Sperry concludes that the growing tip of a neurone must possess a unique specificity which determines its growth, connexions and functions. This paper should be read by every young biologist. Rita Levi-Montalcini and Pietro Angeletti describe recent work with the nerve growth factor discovered in consequence of Elmer Bueker's discovery, in 1948, of the neurogenic effect of mouse sarcoma 180. Vincent Wigglesworth reminds us that arthropod nervous systems display differentiations as interesting as those of vertebrates. Alfred Coulombre assesses the development of the vertebrate eye with the aid of 274 references. This is a formidable conclusion to a sterling section.

Rudolpho Amprino reviews the dispute about the inductive role of the apical, ectodermal ridge of the chicken limb bud. The dispute is placed in context by J. Milaire, who discusses the genetics of abnormal limb development in mammals. Benjamin Moffett concludes that normal joints undergo morphogenetic change throughout the life of the individual. Elizabeth Hay confirms the occurrence of dedifferentiations following the amputation of the urodele limb. Hay argues that dedifferentiation strips muscle and cartilage cells of their differences and both kinds of dedifferentiated cells form cartilage and muscle during regeneration. Irwin Konigsberg describes the fusion of mononuclear cells to form skeletal muscle. There is a reference to the difficulty of preparing pure skeletal myosin from embryos, but it is not pointed out that myosin preparations from adults are variable and inhomogeneous also. (Kay, C. M., in *Muscle*, edit. by Paul, W. M., *et al.*, 109. Pergamon, 1965.)

Eugene Bell reports that feather follicles accumulate polysomes which do not begin to incorporate amino-acids into protein until late on the thirteenth day of incubation of the chicken egg. At this time the RNA strands of the polysomes become susceptible to degradation by exogenous ribonuclease and to unknown, endogenous agents. The endogenous degradation is slow. Bell does not report experiments which distinguish between low levels of endogenous ribonuclease and resistance to the endogenous ribonucleases which are ubiquitous in living tissues according to Wilfred Razzell.

The formation of the amniote heart is linked to the formation of the liver. Contact with presumptive heart mesoderm endues some of the endoderm with the capacity to form liver, and the presumptive heart cells then migrate caudad over the surface of the endoderm which, itself, shifts anteriad and mediad as both tissues swing into the midline during the formation of the foregut; the cardiac mesoderm in front, the hepatic endoderm behind, and

additional mesoderm between them. This additional mesoderm induces the hepatic endoderm to form the liver. The formation of the foregut is essential to the full fusion of the cardiac primordia (Szepsenwohl, J., *Arch. d'Anat. d'Hist. et d'Embryol.*, 17, 307; 1933). Excepting the omission of references to Szepsenwohl, to the apparent inability of the medial endoderm to facilitate fusion except by forming the foregut, and to C. Dareste who first produced double heart embryos and inferred that the heart arises from a pair of bilateral primordia, the readable review by Robert Dehaan of cardiac morphogenesis is comprehensive. Y. Croisille and N. M. LeDouarin discuss the morphogenetic and biochemical differentiations of the liver. The discussion of lactate dehydrogenase might have made fuller use of the quantitative data of Joel Solomon. On p. 450, the authors strip the subject of growth regulation of its semantic gloss and reveal its chemical inadequacies.

Sergei Sorokin, Thomas Shepard and Robert Auerbach discuss the lung, the thyroid, and the thymus, respectively. Sorokin's paper is a model of authoritative elegance and Shepard's is a clear exposition. The functions of the thymus have been elucidated because of Auerbach's restatement of the origin of the thymus. There must be very few instances of embryological work which prompted new insight into adult physiology.

Adesanya Grillo and Theodore Torrey discuss the pancreas and the kidney, respectively. Grillo's discussion includes the effects of insulin on the embryo. Torrey argues for the concept of a 'holonephros' for which some of the evidence has been provided by the electron microscopy of T. S. Leeson.

The final section is devoted to the reproductive system. L. G. Gallien reviews the genetic control of sex differentiation. On p. 595, in referring to fertile ovaries of *XO* mice, the wording should read "an insufficient quantity of masculinizing genes". Alfred Jost suggests that the mammalian testis secretes an inhibitor of the ovary and the Müllerian duct which is not identical with the steroid androgens. Dorothy Price and Evelina Ortiz suggest that the inhibitor is a steroid androgen. Giovanni Chieffi places this difference of opinion in perspective by comparing mammals with other vertebrates. He infers that the gonads secrete sex-determining substances which are not identical with the classical oestrogens and androgens. Lemen Wells briefly reports some of the evidence for endocrine function in the foetus. Terrell Hamilton and Ching-sung Teng report increased ribonuclease and decreased RNA in regressing Müllerian ducts. Helene Charniaux-Cotton summarizes the hormonal control of sex among the invertebrates.

The text is unusually free of bromides: "it is tempting to" (conclude or postulate) occurs twice. The authors and editors deserve congratulations for a coherent, sometimes eloquent, summation and projection of animal development. Their task took effort and co-operation which show. We find here a re-assertion of the importance of tissue structure in determining development and a recurrent implication that the essential mechanisms are located in the periphery of each cell. The assertion and the implication are timely reminders that metazoan cells depend on each other and their environment in ways that microbes do not know. R. F. RUTH

INVESTIGATING ACCIDENTS

Accident Research

Methods and Approaches. By William Haddon, jun., Edward A. Suchman and David Klein. Pp. xvi+752. (New York and London: Harper and Row, Publishers, 1964.) 15 dollars.

THERE is evidence in this book of the extent to which accidents cut across established medical disciplines and how many approaches are required before a composite picture begins to emerge. There is clear evidence of the difficulty of interpreting retrospective investigations where the level of reliability of the information on which these are based is variable; also of planning realistic prospective studies. This leads to "the use of generalizations based upon data known by informed research workers to be faulty". Such generalizations are then contradicted, leading to confusion and lack of confidence in the result of such enquiries. Many examples of conclusions based on inadequate information and issued with official authority are cited, particularly in relation to road traffic accidents. It is argued that "in many situations it is not practicable to conduct research that is completely adequate in every respect; but some would hold that so long as the research is done honestly and with complete awareness of the deficiencies of both material and methods employed such efforts may produce worth while contributions". This is a considerable pitfall in accident research. For example, because investigators are convinced that one or two factors play an important part in accidents of all or any kind, they investigate only these factors and even ignore the need for controls. An example given here is the statement that 20 per cent of those involved in accidents exceeded the speed limit, therefore 80 per cent of those involved were not exceeding the speed limit, therefore if 50 per cent of drivers exceed the speed limit they are safer than the ones who do not! The same kind of argument can be applied to accidents in the home, in industry, age groups, etc. As already mentioned, this is pointed out in the book, but should perhaps appear a bit more often and in bolder type.

The risk taken in crossing a road is reported from "studies representing highly original experimental work on the kind of decisions that are made by individuals in everyday life". The authors afterwards became aware that similar work had already been done and reported that the agreed conclusion was that "pedestrians are concerned with a time gap and not a distance gap in the traffic".

The risk of driving under the influence of drink, or drugs, is another case in which confusion has been produced by conflicting statistics, and support can be found in this volume for almost any point of view. Figures bearing on the influence of this factor in accidents vary from 0·5 to 50 per cent. However it is investigated, there is no doubt that an increasing quantity of alcohol influences first judgment then performance, and the two together in direct proportion to the quantity.

There is no doubt that some people are "accident-prone", and investigations are reported into various aspects of this problem. It is not surprising in view of factors already mentioned that no very firm conclusions are reached in, for example, an investigation "designed to test the hypotheses that accident prone children have greater aggressive drive as a result of nurturance frustration in an authoritarian home environment and greater aggression anxiety as a result of punishment for expression of aggression when compared with accident free children".

There is much valuable information painstakingly collected and appraised, making a start at trying to apply scientific parameters to preventive medicine in the accident field. There are many difficulties and pitfalls, but the effort and the volume are very worth while. J. C. SCOTT

MYXOMATOSIS

Myxomatosis

By Frank Fenner and F. N. Ratcliffe. Pp. xiv+379+15 plates. (London: Cambridge University Press, 1965.) 100*s*. net; 19.50 dollars.

IN 1859 a Thomas Austin imported twenty-four rabbits into Victoria, Australia. Rabbits had previously been imported into the country from the time of the first

settlement, but they had been mainly domestic. The 1859 consignment included some wild rabbits, and on liberation they multiplied rapidly. By 1886 the rabbits had reached all States in Australia and were proving a pest, mainly by competitive grazing on pastures with sheep and damage to young shoots and trees. This rate of spread was, however, paralleled and surpassed by that of the myxomatosis virus introduced in 1951. Originating in South America, where it was first noted as a disease affecting European rabbits kept in the laboratory, myxomatosis was the main cause of the reduction of the Australian rabbit population in certain areas to 20 per cent of the original numbers by 1955 and made it possible to control the menace. Similar introductions of the disease into France, England and Chile also caused a reduction in rabbit numbers. The choice of the correct strain, whether of rabbits or virus, led to successful spread of the virus, and it is appropriate that two scientists from Australia, where the rabbit problem was urgent and important and its investigation and control given scientific and financial support, should collect published and unpublished results and produce an authoritative work on myxomatosis.

Myxomatosis, which is caused by the myxoma virus, is characterized in the European rabbit, *Oryctolagus cuniculus*, by the mucinous tumours it produces on the skin of infective animals, leading eventually to death. In the American rabbit, *Sylvilagus brasiliensis*, the virus has formed a symbiotic relationship with its host, in which it only causes tumours at the sites of infection. In the book Prof. Fenner and Mr. Ratcliffe cover all aspects of the interaction of virus with the hosts it may affect. The chapters on the behaviour and ecology of rabbits contain many new findings based mainly on investigations carried out on animals kept in enclosures and show that the European rabbit is essentially gregarious with a striving for each individual to be part of a social group. Possibly because the main emphasis was on applied investigations, little has been reported on the fundamental aspects of myxovirus, despite the attractive dust cover, but the behaviour of the virus in tissue culture, its pathogenesis in animal hosts and the development of immunity are extensively covered. Studies in the laboratory and in the field on the transmission of myxovirus show the importance of winged insects, especially mosquitoes, and much basic information is given on the mechanical spread of virus from infected rabbit skin tumours to clean rabbits by way of the mouth-parts of the insect.

Of special interest are the investigations of the virulence of the virus after the initial outbreaks. The virus virulence was reduced from 99 per cent for laboratory rabbits to about 90 per cent. At the same time, there is evidence of genetic resistance developing in European rabbits.

The final chapters deal with the history and evolution of myxomatosis in North and South America, Australia and Europe. It is regrettable that in South America the relationship between myxoma virus and its natural host, *S. brasiliensis*, has not been extensively studied, despite the pioneer work of the late Dr. H. Aragão in Brazil, since it is possible that eventually a similar symbiotic relationship may exist in Europe and Australia between genetically resistant European rabbits and an attenuated myxoma virus. The spread of the virus in Australia and in France was equally rapid due to transmission by mosquitoes. In England it was slower, since transmission was by the rabbit flea. In California, where a different strain of virus is found, European rabbits have been equally at risk. In the Argentine an interesting epidemiological position exists, with European rabbits affected with myxomatosis in the south and American rabbits affected in the north.

The book throughout is interesting and pleasant to read, and contains many informative figures, tables, photographs and maps. Besides providing much information to workers in many disciplines on epidemiological methods and the hazards of biological control, it also emphasizes how much both of fundamental and applied importance can be discovered as a result of concentration on a practical problem.

R. F. SELLERS

WAYS OF WATERFOWL

Handbook of Waterfowl Behaviour

By Paul A. Johnsgard. Pp. xiv + 378 + 20 plates. (London: Constable and Co., Ltd., 1966.) 75*s*.

THE coming of the theory of evolution by natural selection resulted sooner or later in a rebirth, or at least a reorientation, of almost every branch of biology. But although Darwin himself wrote a pioneer book on behaviour, namely, *The Expression of the Emotions in Man and Animals*, the evolutionary way of looking at animal behaviour was slow to develop. Although Darwin's book had a great effect, it was a delayed effect—and it was not, in fact, until the coming of the modern ethologists that the species-characteristic fixed-action patterns of animals began to be sufficiently well described to use as taxonomic characters. This development represented a new tool that could be used to develop the comparative study of behavioural characters so as to throw new light on the evolution and relationships of species and also of higher categories. Among the pioneers in this field perhaps as many as four-fifths chose to work with birds. This was understandable. The Aves are characterized by the possession of highly elaborate stereotyped species-specific display activities ideal for comparative investigation. Among the groups most intensively studied were the *Anatidae*, the ducks, geese and swans, and the names of Heinroth and Lorenz will always be associated with the ethology of this group. Lorenz, in his classic comparative study of the movements of *Anatidae* (1941), was perhaps the first to show the great promise of this method. But no one man can get far with the inventory, the itemization, of the behaviour patterns found in a big group such as this without having access to a very extensive collection of material maintained under the best possible conditions. Even Lorenz in 1941, great wildfowl fancier though he is, lacked the facilities to carry the investigation beyond a certain point. Modern students of wildfowl behaviour have a unique advantage over all their predecessors. Mr. Peter Scott, by establishing the Wildfowl Trust at Slimbridge in Gloucestershire, and endowing it with the finest collection of waterfowl in the world, has provided research facilities which are unsurpassed. Many ethologists have worked at Slimbridge, but none has done more thorough and intensive work there than Mr. Paul A. Johnsgard of the University of Nebraska. This book gives the conclusions resulting from many years of investigation both at Slimbridge and in the field in many parts of the world. It is a fine piece of work containing detailed descriptions and more than a hundred pages of illustration. It will be indispensable to students of ethology, especially those concerned with bird behaviour.

W. H. THORPE

High Speed Pulse Technology

By Frank B. A. Früngel. Vol. 1: Capacitor Discharges—Magnetohydrodynamics; X-rays—Ultrasonics. Pp. xiii + 620. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 192*s*.

High Speed Pulse Technology is a translation, brought up to date, of the second edition of Dr. Frank Früngel's book in German. The translation is the work of three of his German collaborators; and though the English often falls short of the standards of Fowler, it is adequate.

Capacitor discharges have an enormously wide field of application in physics and in technology. They can be used first of all to produce very high-voltage pulses, as in the Marx generator, and shaped discharges of all sorts, from purposes ranging from the operation of magnetrons and klystrons to electric fishing. They can be also used for producing megamp current pulses for the operation of theta-pinch devices and the like. The energy can be converted into light-flashes for spectroscopy and for high-speed photography or into X-ray flashes for high-speed radiography. It can be converted into heat as in spot welding or into strong magnetic fields, which can find such varied applications as plasma generation, magneto-optical shuttering, and even metal forming by intense electrodynamic forces. In many of these applications the maximum of power is reached in a few nanoseconds and the current rise can be of the order of 10^{11} amp/sec. The sudden discharge of energy in gases and in liquids produces shock waves which can be used in echo-sounding and even for the explosive shaping of metals.

All these applications are thoroughly covered in the 600 pages of the book. Any physicist or engineer who is interested in impulsive processes in which the energy is stored capacitatively in the first place can find full descriptions of the state of the art in any of the branches, amply illustrated, and with a complete list of references. It is a standard work of reference which will be a valuable time and labour saver for anybody working in these fields.

D. GABOR

The Riemann Hypothesis and Hilbert's Tenth Problem

By S. Chowla. (Mathematics and Its Applications, Vol. 4.) Pp. xv+119. (London and Glasgow : Blackie and Son, Ltd., 1965.) 40*s*.

PROF. CHOWLA discusses a number of more or less related matters in number theory linked with various forms of the Riemann hypothesis or with Hilbert's problem of discovering a finite algorithm for determining all integer solutions of the equation $f(x, y) = 0$, where f is a polynomial with integer coefficients. The book treats character sums, Gaussian sums, the law of quadratic reciprocity, Burgess's estimate of the least quadratic non-residue of a prime, the results of Jacobsthal, Eisenstein *et al.*, on the expression of a prime as the sum of two squares, the Mordell–Weil work on the generation of rational points on a cubic, and a discussion of various diophantine equations. There is also a useful list of conjectures and unsolved problems.

The book thus contains a variety of interesting and important material, some of very recent date, and it is unfortunate that the author appears to have put it together in some haste and has not exercised any considerable gifts of exposition. Certain easy matters are expounded rather fully while difficult steps are often left to the skill of the reader. Since the material is on the whole by no means simple, the claim that "not much knowledge of mathematics" is assumed seems to me to be without substantial foundation. In addition, the proof reading is quite exceptionally defective: wrong or inconsistent symbols and fount are frequent, while punctuation is erratic and full stops almost (but not quite) always omitted in arguments conducted in mathematical notation. A sample check showed no page to be free of several such defects, though I had some consolation from the sentence with three full stops and an exclamation mark in the middle, and from the attribution of Hilbert's problem to the International Congress of 1800. The style varies greatly, sometimes flowery and sometimes in the form of headline notes. There is a good deal of unintentional repetition and a couple of pages are, for no very clear reason, left in their original French.

All this is a pity, for the book contains substantial and rewarding work which may well repay a reader of patience, perspicacity and fortitude.

S. R. TIMS

MEETINGS

ATHEROSCLEROSIS

ATHENS—1966

AN international symposium on "Recent Advances in Atherosclerosis Research" was held in Athens during May 30–June 2, under the presidency of Prof. B. Malamos, with Dr. A. N. Howard as general secretary. The main purpose of the symposium was to encourage multi-disciplinary discussion of epidemiology, lipid metabolism and thrombosis in relation to arterial degenerations.

C. Aravanis (Athens) reported his group's epidemiological investigations in Crete and Corfu, where ischaemic heart disease is responsible for only 1–5 per cent of the death-rate. He found a low incidence of hypertension and obesity among these islanders; their serum cholesterol is low by European and American standards, while they tend to eat little animal fat and be engaged in much hard physical work. J. Stamler (Chicago) re-emphasized the role of hypercholesterolaemia, hypertension and cigarette smoking in the aetiology of atherosclerosis. However, J. J. Groen (Jerusalem) pointed out that hypertension and cerebrovascular disease are just as common among Jewish immigrants from Afro-Asian countries as they are in those from Europe and the United States, yet only the latter group is peculiarly prone to coronary heart disease. Thus, hypertension clearly plays a significant contributory part in atherogenesis, but some other factor appears to be the major determinant of the disease. Most speakers tacitly, or more vocally, implied that this other aetiological factor is the effect of the diet on the serum lipids. H. C. McGill (New Orleans) summarized his geographical investigations and showed that atherosclerosis is more severe in Negroes domiciled in a North American community than in those living in less affluent countries. These collective findings —as well as the relationship between obesity and serum lipid levels (B. Rifkind, Glasgow)—suggest that the prevalence of hyperlipaemia and obesity in Western societies is the most important hurdle to be overcome in the effective prophylaxis of atherosclerosis. However, although epidemiological studies often prompt ideas for further investigation, they seldom themselves provide that degree of proof which can be used as a sheet-anchor in scientific argument.

The atherogenic effect of saturated dietary lipids was emphasized by D. Kritchevsky (Philadelphia), who showed that triglycerides containing palmitic and myristic acids cause more atheroma in the cholesterol-fed rabbit than does "polyunsaturated" corn-oil. R. Paoletti (Milan) mentioned that polyunsaturated fatty acids act as precursors for prostaglandins. These latter substances inhibit catecholamine-induced lipolysis in adipose tissue and thus reduce the serum lipid level. The theme of polyunsaturated fatty acids was pursued by L. W. Kinsell and his colleagues from Oakland, California; they showed that dietary trilinolein increases human biliary excretion of cholesterol and bile acids. T. Miettinen (Helsinki) confirmed previous conclusions that excess dietary cholesterol suppresses sterol synthesis in the rat liver but, like many other investigators, he obtained inconclusive results about the occurrence of such "feed-back" control in man.

E. H. Ahrens (New York) made the important point that cholesterol is chemically degraded in the human intestine; this process could well help to protect man against the effects of excess dietary cholesterol. S. Gerö (Budapest) tentatively suggested that cholesterol absorp-

tion may be influenced by gastro-intestinal secretion of mucopolysaccharides; he reported that the amount of gastric mucin is inversely proportional to the cholesterol level of the blood. Clearly, degradation and absorption of sterol in the human intestine are worth-while problems for more detailed investigation.

The lipoprotein vehicles for plasma lipid transport were discussed by many speakers. D. S. Frederickson (Bethesda) outlined techniques for isolating lipoproteins by electrophoresis or ultracentrifugation, and considered the diagnostic value and limitations of these methods in disease states. A. Scanu and his colleagues from Chicago described the molecular structure of the apolipoprotein moiety of these vehicles; lipids play no part in maintaining the secondary structure of the protein, but they do link the protein sub-units together. Thus, it can be inferred that peroxidation or other chemical changes in the lipid moiety would so alter the dispersion forces within the macromolecule that the lipoprotein would become physically unstable. Such instability of β-lipoprotein could directly result in lipid deposition within the arterial wall, as was first postulated by I. H. Page in 1954. Results presented by K. W. Walton (Birmingham) indicate that the metabolic turnover of low-density lipoprotein in the plasma may also be an important factor in atherogenesis. Because the metabolic turnover of low-density lipoprotein is twice as fast in premenopausal women as in men, it is possible that relative metabolic inertia is responsible for the accumulation of such lipoprotein in the male's plasma.

The interesting curiosity of "autoimmune hyperlipaemia" was reported by J. L. Beaumont (Paris); circulating anti-β-lipoprotein antibody was identified in two patients who clinically presented with severe atherosclerosis and excessively high serum cholesterol and triglyceride levels.

The entry of lipid into the arterial wall was discussed by S. Christensen (Aarhus, Denmark); he measured the transfer rates of radioactive cholesterol, phospholipid and protein across the intimal surface of the cockerel's aorta. The entry rates of phospholipid and protein into the aortic wall were closely correlated with each other in both normal and hyperlipaemic birds. This biochemical evidence suggests that lipid enters the arterial wall in lipoprotein form. Using fluoroscein- or ferritin-labelled specific antibodies, M. D. Haust (Ontario) and K. W. Walton (Birmingham) confirmed microscopically that low-density lipoprotein, albumin and fibrin are present in human atherosclerotic lesions. Haust noted—as had B. Watts and R. W. Wissler—that lipoprotein is more abundant in early than in old sclerotic plaques. She considered that such protein partly enters the arterial wall by "insudation"; this process is essentially the same as Virchow's "plasma imbibition" and Lendrum's "plasmatic vasculosis". G. H. Rothblat, D. Kritchevsky *et al.* (Philadelphia) presented evidence that the uptake of cholesterol by the cell is a non-enzymatic physical process; they hinted that it may depend on the binding of cholesterol by phospholipid in the cell wall.

Lipid metabolism in the arterial wall is clearly an important element of atherosclerosis; it could play an important part in either initiating or resolving the lesion. D. E. Bowyer, A. N. Howard and their colleagues at Cambridge reported that normal rat, rabbit and baboon arteries synthesize triglycerides and phospholipids—but not cholesterol esters—from carbon-14-labelled fatty acids. Likewise, H. B. Lofland (Winston-Salem, North Carolina) showed that normal arteries from the 'White Carneau' pigeon synthesize triglycerides and phospholipids, but atherosclerotic arteries from these birds divert lipid synthesis towards cholesterol esters. Because recent studies show that polyunsaturated cholesterol esters are rapidly mobilized from tissues and promote less tissue reaction than free cholesterol, Lofland's demonstration of cholesterol esterification in atherosclerotic arteries delineates a possibly important defence mechanism of the diseased tissue against cholesterol deposition.

J. Patelski (Poznan, Poland)—in co-operation with the Cambridge group—characterized phospholipase *A*, lipase and cholesterol esterase in animal arteries; the first two enzymatic activities are increased in rats fed an atherogenic diet, but cholesterol ester hydrolysis is suppressed by this dietary regime. In double-labelling experiments on human and canine arteries, Y. and O. Stein (Jerusalem) demonstrated that these tissues synthesize lecithin by acylation of lysolecithin. However, J. D. Billimoria (London) reported that the isolated rat aorta incorporates considerably more phosphorus-32 into cephalin phospholipids than it does into either lecithin or sphingomyelin. Following Zilversmit's pioneer investigations, it is now widely accepted that the arterial wall synthesizes phospholipid. However, previous biochemical and histochemical analyses have shown that sphingomyelin and, to a lesser extent, lecithin are the characteristic phospholipids of normal and atherosclerotic arteries. The importance of Billimoria's finding is that phospholipids which are synthesized most rapidly (that is, cephalins) may have an even faster metabolic turnover, so that they do not accumulate in the arterial wall.

The metabolic implications of ischaemia of the ageing arterial wall were discussed by several speakers. Histochemical and cytochemical studies, reported by C. W. M. Adams (London), showed that oxidative and esterolytic activities decline in the middle of the muscular coat of the ageing human aorta. This effect was attributed to ischaemia of the medial muscle-fibres at the watershed between the blood-supply from the adventitial vasa vasorum and outward diffusion from the lumen. Lipase activity declines throughout the whole thickness of the vessel, while lactic dehydrogenase activity increases in the ischaemic mid-zone. T. Zemplényi (Prague) attributed the increased activity of the latter enzyme to its anaerobic "*M*" isoenzyme. Zemplényi also described decreased activity of Krebs cycle enzymes in the ageing abdominal aorta in man and in various animals; he attributed this metabolic impairment to age-dependent changes in the aortic smooth-muscle fibres. A. Lazzarini Robertson (Cleveland, Ohio) postulated that the tunica intima also suffers from incipient hypoxia. From tissue-culture studies of human "intimacytes" he found that lipid deposition in these cells increases their oxygen requirements and thus initiates a vicious circle of hypoxia. These various observations indicate that the local catabolism and outward transport of lipids are impaired by ischaemia of the ageing artery.

Discussion of the pathology of atherosclerosis was mainly centred on the disease in primates. T. Clarkson (Winston-Salem, North Carolina), A. N. Howard (Cambridge) and R. W. Wissler (Chicago) described the atherosclerotic process in squirrel monkeys, spider monkeys, baboons and rhesus monkeys; the main emphasis of these reports was on the similarity of atherosclerosis in man to that in other primates.

G. A. Gresham (Cambridge) stressed the genetic factors that operate in atherosclerosis. For example, the 'White Carneau' breed of pigeon develops severe spontaneous atherosclerosis (Clarkson and Lofland), while mice with genetic diabetes mellitus suffer from generalized lipid deposition (A. E. Renold, Geneva).

In the pharmacological field, R. G. Gould (Palo Alto, California) described the hypotriglyceridaemic effect of *p*-chlorophenoxyisobutyrate ('Atromid *S*'); he tentatively suggested that inhibition of hepatic cholesterol synthesis may depress lipoprotein secretion and, as a result of this, the plasma-triglyceride level falls. J. M. Thorp and M. F. Oliver (Macclesfield and Edinburgh) considered that 'Atromid *S*' reduces the binding of thyroxine by plasma albumin, so that the hormone accumulates in the liver and acts locally against lipid synthesis. In this connexion, R. Pick (Chicago) reported

that hypothyroidism reduces the protective effect of oestrogens on the coronary arteries of cholesterol-fed chicks.

The final session of the symposium was concerned with the mechanism of thrombosis. Ultrastructural features of platelet aggregation were clearly illustrated by J. E. French (Oxford). G. V. R. Born (London) expressed the view that adenosine diphosphate (ADP) released from the vessel-wall may initiate platelet aggregation; a chain-reaction is set up by the production of ADP from the adenosinetriphosphate within such aggregated platelets. Born reported that adenosine and its 2-chloro derivative are strong *in vitro* inhibitors of ADP-induced platelet aggregation. Moreover, these inhibitors suppress thrombogenesis in the experimental animal. Both Born and J. F. Mustard (Toronto) mentioned the possible thrombogenic effect of collagen exposure in the injured vessel. In this connexion, P. Constantinides (Vancouver) presented histological evidence that fissures in atherosclerotic plaques frequently underlie arterial thrombi.

Mustard considered that the platelet aggregates formed during the initial stage of thrombosis are later stabilized by the deposition of fibrin fibrils. C. Hawkey (London) produced experimental evidence to support this concept; she showed that experimental thrombi progressively disintegrate if the plasma fibrinolysin system is activated with streptokinase. This observation indicates that fibrin "cements" the platelet aggregates in the thrombus and explains the efficacy of anticoagulant therapy in some forms of human thrombosis. C. W. M. ADAMS

MUSEUMS ASSOCIATION 1966

THE seventy-second annual conference of the Museums Association was held in Sheffield during July 4–9 under the presidency of Sir Frank Francis, director of the British Museum, London. In his presidential address, Sir Frank deplored the distinction now being made between "living" and "dead" art, and said it was ludicrous that while the Government White Paper recognized that museums might play the part of local art centres, they were not included in the grant proposals. He emphasized the value of the relatively recently formed Area Museum Councils and made a plea for scholarly research by museum staffs. Museums throughout Britain were becoming more and more attractive to the communities they served and were endeavouring to kill the traditional image that they had unfortunately acquired.

The relationship of the national and provincial museums formed the general theme of the conference, and this was discussed in the fields of archaeology, art and natural history. Speakers included Mr. R. L. S. Bruce-Mitford (British Museum), Dr. G. B. Corbet (British Museum (Natural History)) and Mr. M. Levey (National Gallery) for the national institutions, and Mr. D. T.-D. Clarke (Colchester and Essex Museum), Mr. I. M. Evans (Leicester Museum and Art Gallery) and Mr. H. Scrutton (Walker Art Gallery, Liverpool) for the provincial institutions. At many times during the papers and in subsequent discussions the present friendly co-operation between national and provincial staffs was emphasized, but it was strongly felt that this help should be co-ordinated. The exchange of staffs was advocated, though it was emphasized that this would entail standardizing the status and salaries of national and provincial personnel. It was also considered that the problem varied in the different disciplines dealt with by museums and art galleries and was a simpler matter in archaeology and natural history than in art. In the former subjects the acquisition and display of local and regional objects were desirable in all but the larger institutions, but in art the solution of the problem was more complicated.

The concentration of natural history and geological type specimens in national institutions was considered to be a matter for further discussion, for although the larger provincial museums could deal with various groups in an adequate manner it was certainly not desirable for the smaller institutions to hold type material.

At the annual general meeting, Mr. G. L. Conran, director of the City Art Galleries, Manchester, was elected president for 1967. The next conference will be held in Glasgow during June 26–30, 1967. F. S. WALLIS

OBITUARIES

Prof. K. J. Franklin

PROF. K. J. FRANKLIN, emeritus professor of physiology in the University of London, died on May 8 at the age of 68. "K. J.", as he was known to a wide circle of friends and colleagues, held the chair of physiology at St. Bartholomew's Hospital Medical College from 1947 until 1958. He came from Oxford with a reputation as an authority on the renal circulation and with a wide background of circulatory physiology.

Franklin was born in London in November 1897. He was educated at the Bluecoat School, and in 1915 won a scholarship in classics to Hertford College, Oxford. Instead of going to Oxford, however, he joined the Royal Artillery and served with a field battery in France until the Armistice. He went up to Oxford in 1919, and decided to study medicine rather than classics. He went to St. Bartholomew's Hospital as a clinical student in 1922, qualified in 1924 and returned to Oxford as a fellow of Oriel College. From 1924 until 1938 he was demonstrator of pharmacology at Oxford and combined that duty with that of tutor in physiology and librarian at Oriel College. When the Nuffield Institute for Medical Research was established in 1935, Franklin became assistant director and continued in this post until he was appointed professor of physiology at Bart's in 1947.

His ability to do several tasks at once had been proved at an early age when he had combined clinical studies with the post of part-time demonstrator in physiology under Prof. (now Sir Charles) Lovatt Evans at Bart's and, in his final year, that of part-time lecturer in physiology at Brasenose College, Oxford. As a good classical scholar, Franklin turned to a study of medical history. He also combined the techniques of cinematography and radiography in a study of the renal circulation. Between 1947 and 1958, Franklin was occupied with his work on the renal circulation, his classical interests in making new translations of some of the works of William Harvey, and in supervising the construction of the new buildings which house the Departments of Physiology and Pharmacology. He was elected a Fellow of the Royal Society in 1955.

His last series of experiments was aimed at determining whether the phenomenon of cortical necrosis of the kidney, which is a serious complication in concealed accidental haemorrhage, could be explained on the basis of a utero-renal reflex. Ill-health unfortunately forced him to retire before any firm conclusions could be drawn.

Those of us who worked with him will always remember him as a tall and impressive figure with a shy manner and very softly spoken. Further acquaintance soon revealed that he had a fine sense of humour and a most kindly personality.

Prof. Franklin is survived by his wife and one daughter.

F. J. AUMONIER

GALACTIC COSMIC-RAY SOURCE AND LOSS PROBLEM

By PAUL BAKER, JUN.*, PROF. R. L. CHASSON and V. J. KISSELBACH

University of Denver, Denver, Colorado

SUBSTANTIAL direct evidence of a sidereal anisotropy is indicated for the charged galactic cosmic radiation:

$$(> 20\,\frac{gV}{c}\ \text{primary rigidity})$$

The results have been obtained with a technique of celestial scanning, using a battery of paired narrow-angle muon telescopes, two of which are vertical, and the others with axes tilted at about 38° with respect to zenith and in the north, south, east and west geographic planes. (The technique is referred to as "crossed-telescopes".) The apparatus is located in Denver at 1,600 m altitude and with a Quenby-Wenk threshold of about $3\frac{gV}{c}$. Details of the instrumentation have been given elsewhere[1], and in this article we shall discuss the first year of data obtained with the S and E pairs of tilted telescopes. These results are additionally interesting as they are directly concerned with the recently-concluded observational programme of the International Years of the Quiet Sun and, furthermore, afford some verification of basic assumptions underlying certain techniques of the new cosmic-ray astronomy. (More detailed remarks regarding these direct scanning techniques can be found in a recent review by Reber[2].)

The telescopes operate in directional pairs, and the total counting rate from each direction is about $4{\cdot}5 \times 10^4$ per hour. The directional response is such that the telescope opening angle at half-maximum of intensity is about 17° (about 1 h in right ascension). The integrated counts from each directional channel are recorded every solar minute, and the results are eventually computed in 6-min sidereal time intervals by a cumulative time-shifting formula. The sidereal 6-min intervals lag the solar intervals by about 1 sec in solar time; thus in 15 days our time conversion is in error only by about 1 min, and we process data each 15 days as a unit.

The findings to be discussed here are derived from data taken from two isolated periods of time: (1) 60 days bracketing the summer solstice of 1965; and (2) 84 days bracketing the winter solstice of 1965. The two solstice periods were considered and intervening data ignored in order to avoid spurious interference-type variations having basic solar periodicity that may be introduced into the sidereal scaling as a result of the unique one-day difference between the sidereal and the solar year[3]. Final reduction of observations consisted of summing the 6-min data into half-hour spans, computing south-minus-east half-hourly differences, and then superposing the results to give half-hourly means of sidereal diurnal variation. The taking of such differences accomplishes two important purposes: (*a*) we eliminate the major portion of the complex of meteorological corrections ordinarily required to reduce muon intensity to a standard basis as the telescopes view through essentially the same air mass; and (*b*) the telescope tracking in right ascension (east leading south) gives a check on variations observed.

The latter point is brought out more clearly in Fig. 1, which is a plot of the asymptotic directions of arrival[4] for primary particles giving rise to muons detectable by the tilted telescopes. Since the cut-off at Denver is about $3\frac{gV}{c}$, we estimate the lower limit of primary rigidity to be $20\frac{gV}{c}$ for production of a muon just capable of reaching our apparatus. The $P^{-2.5}$ differential rigidity spectrum for such charged primaries yields the value of $60\frac{gV}{c}$ for the mean primary rigidity to be considered in our observations, corresponding to a range typical of galactic cosmic rays. It should be noted from Fig. 1 that the east telescope leads the south by about 3 h in right ascension, with south sensing almost exclusively along the celestial equator and east sensing to a celestial latitude of 25° north and almost entirely in its meridian plane.

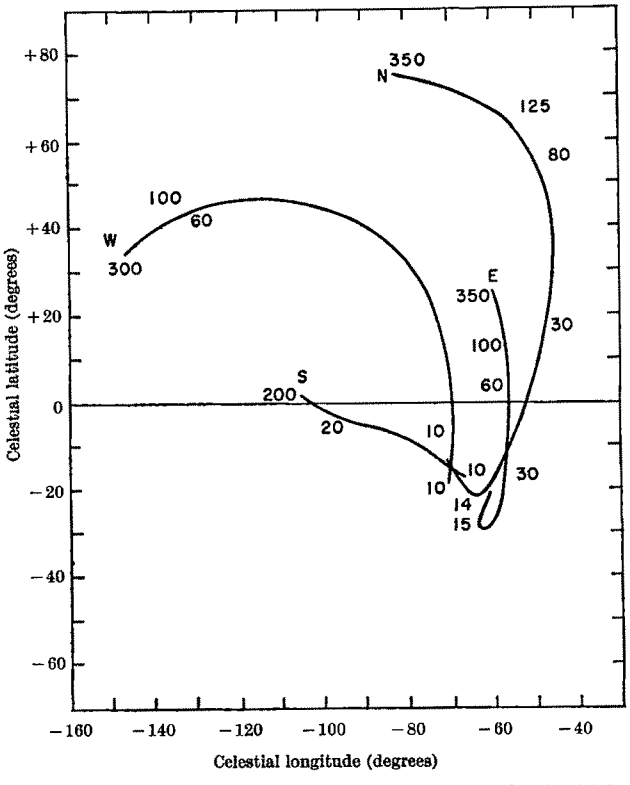


Fig. 1. Asymptotic directions of approach of primaries (ref. 4) for tilted telescopes. Numbers on curves indicate magnetic rigidities in $\frac{gV}{c}$

One then examines the sidereal south-minus-east results for characteristic oscillatory signatures in the trend of daily variation; since east leads south, the signature will first tend to be negative and then reverse to positive if the scanning pair encounters a region of excess intensity,

* On a research appointment from the U.S. Air Force Academy.

where the zero baseline is that of the daily average difference. Conversely, the sign performance will be positive to negative if a region of reduced intensity is encountered.

Fig. 2 contains the results of the two isolated solstice periods and, finally, their superposition, giving a sidereal diurnal plot where solar effects are largely cancelled due to the 180° phase shift within sidereal time. Error bars are based on the standard error of the total counting statistics going into the differences (about 3×10^6 total counts per 30 min for the accumulated 144 days). Solar-time plots (not shown) for the equivalent period, taking south and east telescopes independently, gave typical strong maxima at about 1500 h local solar time with an amplitude of about 0·5 per cent. This solar diurnal variation is consistent with the results of many other investigators[5]; its directional and time dependence is also of considerable interest, and will be discussed in future publications.

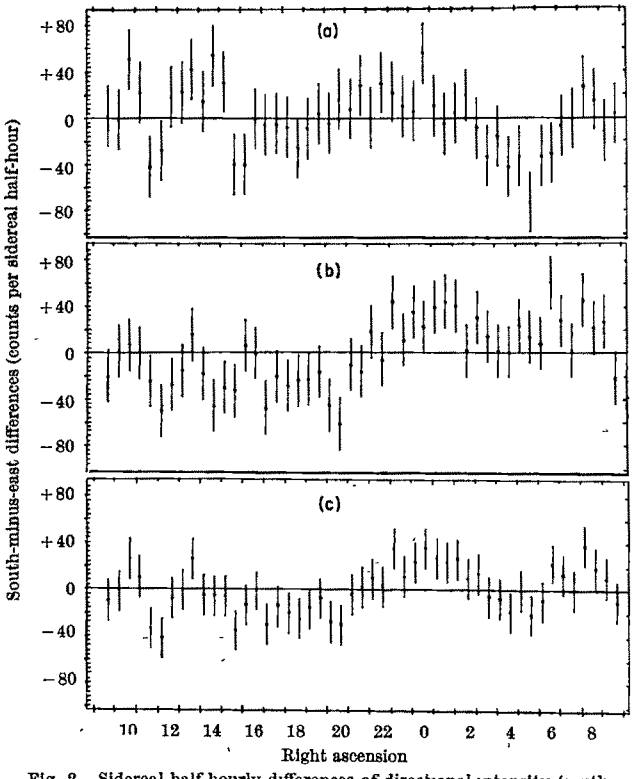


Fig 2. Sidereal half-hourly differences of directional intensity (south-minus-east) averaged over: (*a*) 60 days bracketing summer solstice 1965 (May 18–July 20); (*b*) 84 days bracketing winter solstice 1965 (November 20–February 23); (*c*) combination of above results. (Referred to zero counts per half-hour average difference)

The sidereal anisotropy indicated in Fig. 2*c* is of the order of 0·06 per cent, quite in agreement with the magnitudes found previously[5] using techniques of harmonic analysis. Without attempting to invoke specific causes for each detail of this curve, we wish to direct attention to the striking peak at about 0 h right ascension (R.A.), apparently corresponding to a diminution of intensity as it is followed so well by a related dip in the signature starting at 3 h. Because the south telescope lies in the local meridian plane, its axial orientation is regarded as the fiducial one in reckoning the direction in space for assignment of R.A. of structure found in the results; thus we assign the location of this feature to lie between 3 and 5 h R.A. There is also an apparent non-random sequence of points indicating a lowering of the galactic intensity centred at 11 h, and a similar, but less strong, indication at 9 h R.A. We did not observe anisotropy at all approaching the magnitude reported by Standil and Bukata[6].

A runs analysis of the values shown in Fig. 2*c* yielded eight runs (consecutive oscillations of sign about the mean south-minus-east difference). Now, to force a conclusion that our sign change sequence is random (a 48-point sample) one should expect between fourteen and twenty-eight such runs to exceed the 5 per cent confidence limits. Our markedly low number of runs leads us to say, then, that the 144-day sample departs very significantly from randomness and, further, to assert that the fluctuating sequences are possibly related to some astrophysical processes or galactic structure features. The apparent minima at 4, 9 and 11 h are associated with viewing directions roughly perpendicular to the winter plane of our galaxy. The long, consistent sequence of points between 13 h and 20 h is considered to be "standard background" and spans the summer plane of the Milky Way. Argument that the feature between 13 h and 20 h is not a source irregularity is as follows: the south–east difference is roughly constant; thus either the eastern intensity keeps increasing linearly as the south follows it by 3 h, causing a constant south–east difference, or south and east are looking at equal intensity values for the whole period. We assume the latter, as it is very difficult to believe the existence of such a vast and constantly increasing intensity structure, even though it does span the summer plane of the galaxy, and, finally, a similar feature does not appear in the traversal of the winter plane.

The following mechanisms may be responsible for a decreased intensity from a localized direction: (1) a large expanding cosmic ray trap operating in a neighbouring part of the galaxy, which could have some extremely interesting time-dependent properties; or (2) extensive magnetic modulating mechanisms. (The known fields in the near spiral arm at about 6 h R.A. and in the Crab Nebula at 5·5 h are noted.) Or we can consider the possibility of (3) reduced density of cosmic-ray sources in certain directions as compared with the general background, or (4) excessive absorption along the paths from certain source directions. (In the last-mentioned case, unreasonably high interstellar densities might be required if nominal trajectories, say $\lesssim$ 1,000 parsec, were assumed for the primary particles the progeny of which are detected by our instruments.)

Further calculations are planned involving the recent equinoctial period and similar selected data to be collected by the continuing operation of the telescopes. Other directional differences will also be taken to isolate strips of the celestial sphere with limited extent in declination, as provided by the dissimilar asymptotic cones viewed by the various telescopes. We will then continue our mapping as we look, for example, at the latitude of Cygnus and Cassiopeia and also hope to be able to delineate the position of the Crab Nebula if it is a source of anisotropy within our range of rigidities.

This work was supported by the National Science Foundation. We thank the staffs of the computing centres of the University of Denver and the U.S. Air Force Academy for their help, Prof. H. Bondi and Dr. A. G. Wilson for useful discussions, and Prof. K. G. McCracken for making the asymptotic direction computer programme available. We also thank Prof. Mario Iona for his advice.

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ROTATION OF NEUTRON STARS

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THIS discussion is prompted by some calculations of Zee and Wheeler concerning energy loss by gravitational radiation from the quadrupole oscillations of a neutron star. Zee and Wheeler[1] estimate a half-life for radiation of the quadrupole vibrational energy of the order of seconds. Meltzer and Thorne[1] have further suggested that if the neutron star is rotating, the rotational flattening will provide a coupling between the purely radial oscillations of the star and the quadrupole oscillations, in which the radial vibrational energy will be drained away through the quadrupole mode with a half-life of about a day even with very moderate rotational flattening. As energy storage in radial vibrations has been suggested as a reservoir giving rise to X-rays from the circumstellar regions of neutron stars by synchrotron and bremsstrahlung mechanisms[2], it is of interest to examine the degree to which rotational flattening may be expected to persist after formation of a neutron star in a supernova implosion.

Recent hydrodynamical calculations on the supernova collapse problem by W. D. Arnett (ref. 3 and personal communication) show that the collapse of a star of several solar masses will leave a remnant in the range 0·5–2 solar masses, the uncertainty partially representing differences in starting conditions and partially representing the approximations which have so far been made in handling the equation of state during the collapse. We have recently shown that the maximum stable mass of a neutron star probably lies in the range 1–2 solar masses[4,5]. It may readily be shown that if a star $\sim$ 10 solar masses is rotating on the verge of rotational instability at the equator while on the main sequence (as is usually observed to be the case), and if it continues to rotate rigidly (owing to magnetic and turbulent viscosity) after evolving to the red giant phase, then the central one solar mass will rotate at much less than the verge of rotational instability after being suddenly compressed to neutron star dimensions with local conservation of angular momentum. This consideration is strengthened if one includes the effects of angular momentum loss accompanying mass loss in the red giant phase, but it is weakened to the extent that turbulence and magnetic fields may not be able to maintain rigid rotation of the core with the envelope in the red giant phase. For complete generality we discuss the case of a fast-rotating neutron star remnant.

Hoyle, Narlikar and Wheeler[6] have suggested that the remnant of a supernova explosion will be a rapidly-rotating flattened disk. If the disk is very thin, the implication is that the hydrodynamic time scale of the collapse will be lengthened and the gravitational potential energy release will be less localized. For this reason energy transfer by neutrino diffusion will be very inefficient and Arnett's calculations would then suggest that a strong shock wave is not formed to eject the outer envelope. Thus the disk will be very massive, and if there is outward transport of angular momentum and inward transport of mass as a result of turbulence, the central part of the disk is likely to collapse through its Schwarzschild radius, thus precluding the possibility of long-term storage of energy.

It must also be enquired whether a very flat disk may not be unstable to deformation into a Jacobi ellipsoid with three unequal axes, thus forming a mass quadrupole. A related question has been investigated by Roberts[7], who found that a rapidly spinning polytrope will deform into a Jacobi ellipsoid if the polytropic index is less than about unity. We have assigned rough effective polytropic indices to our models of neutron stars[5] by comparing the ratio of central to mean densities for our models with those for polytropes. The results are shown in Fig. 1.

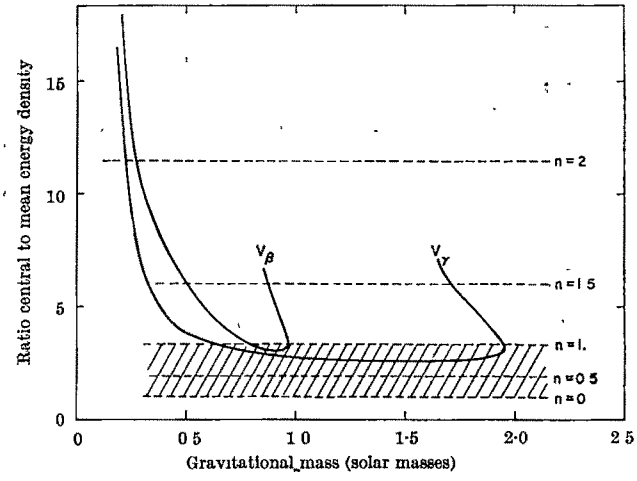


Fig 1. Plot of the ratio of the central to the mean proper energy density for the Tsuruta–Cameron neutron star model sequences characterized by the Levinger–Simmons potentials V_β and V_γ. Also given are the ratios of central to mean densities for various polytropic indices n. The shaded region below $n = 1$ will be unstable to deformation into Jacobi ellipsoids

The two model sequences shown in Fig. 1 represent two different expressions for the nuclear interaction potential which enters the equation of state used in the construction of the models. The correct model sequence may well lie between these two. It may be seen that the effective polytropic index of the models in the vicinity of one solar mass is indeed near or below unity. Thus there is a good chance that neutron stars can deform into Jacobi ellipsoids if they are spinning rapidly enough. The resulting mass quadrupole can emit gravitational radiation, and this will slow the spin rate until the ellipsoid relaxes to a spheroid. Chin has shown that the characteristic time for this is of the order of a second[8].

We now consider the slowing of the spin of a neutron star due to loss of angular momentum when an external magnetic field exerts a torque on mass which is flowing away in the form of a stellar wind. The stellar wind is likely to arise from the heating of a corona around the neutron star by shock waves and hydrodynamic waves generated by the radial vibrations[2,9]; its expansion will be

assisted by radiation pressure owing to the high surface temperature at the photosphere. The outflowing plasma will tend to pull the magnetic field radially away from the star, and the rotation will tend to wrap the field lines into a spiral. The following calculation of angular momentum loss is similar in principle to one previously carried out by Schatzman[10] in connexion with the Sun.

We may expect that close to the neutron star the magnetic field causes the corona to co-rotate with the star, but at large distances the corona rotates slower and the field is wrapped into a tight spiral. The condition of co-rotation may be expected to break down at roughly that distance r such that the radius of curvature of the field lines is also r. The Coriolis pressure gradient[7] on the gas $2\rho v\omega$ will be approximately equal to the magnetic tension:

$$2\rho v\omega = B^2/4\pi r \qquad (1)$$

where ρ is the gas density, v is the expansion velocity, ω is the angular velocity of the neutron star and B is the magnetic field. We take the mass flow f through any spherical surface concentric with the star to be constant:

$$f = 4\pi\rho r^2 v = \text{constant}$$

If we designate physical quantities at the neutron star surface by subscripts zero, then:

$$\frac{\rho v}{\rho_0 v_0} = \left(\frac{r_0}{r}\right)^2$$

For the radial magnetic field:

$$\frac{B}{B_0} = \left(\frac{r_0}{r}\right)^2$$

Thus from equation (1) we obtain:

$$r^3 = \frac{B_0^2}{8\pi}\,\frac{r_0^2}{\rho_0 v_0 \omega}$$

Let the angular momentum of the neutron star be:

$$L = KMr_0^2\omega$$

where M is the stellar mass.

The rate of loss of angular momentum is:

$$\frac{dL}{dt} = -fr^2\omega = KMr_0^2\,\frac{d\omega}{dt}$$

The solution to this equation is:

$$\omega_i^{2/3} - \omega^{2/3} = bt$$

where t is the time required for the angular velocity to be reduced from an initial value ω_i to a smaller value ω, and

$$b = \frac{2}{3}\,\frac{f^{1/3}}{KM}\left[\frac{B_0^2 r_0}{2}\right]^{2/3}$$

Thus the time required to remove the initial angular momentum is:

$$t = \frac{3KM\omega_i^{2/3}}{2^{1/3}f^{1/3}B_0^{4/3}r_0^{2/3}} \qquad (2)$$

Let us estimate some reasonable values of the quantities in connexion with equation (2). We take as rough values for a neutron star $M = 2 \times 10^{33}$ g, $r_0 = 10^6$ cm, and $K = 0{\cdot}2$. The Kepler orbital velocity at the surface is $(GM/r_0^3)^{1/2} = 1.15 \times 10^4$, so we take $\omega_i = 10^4$ radians/sec. The initial rotational energy is:

$$\tfrac{1}{2}KMr_0^2\omega^2 = 2 \times 10^{52} \text{ ergs}$$

This must be dissipated during the slowing of the spin; let us suppose this energy goes into mass loss in order to get a crude estimate of f. The resulting mass ejection is roughly $Kr_0^3\omega^2/2G \approx 10^{32}$ grams. Thus $f \approx 10^{32}/t$, and equation (2) becomes:

$$t \approx 3 \times 10^{31}/B_0^2 \qquad (3)$$

Now Woltjer[11] has estimated that a neutron star is likely to be formed with a magnetic field B_0 of the order of 10^{14}–10^{16} gauss. If this is correct, then from equation (3) we find that the spin of a neutron star is likely to be eliminated on a time scale of seconds.

Thus we conclude that there is no assurance that rotational flattening of a neutron star will be responsible for eliminating radial vibrational energy by providing a coupling to the quadrupole oscillation mode. Furthermore, it appears that rotational energy storage in a neutron star is most unlikely to provide a reservoir which can lead to energetic radiation processes for very long after the star is formed. More careful detailed considerations of all these processes are needed.

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INTERCONTINENTAL CONODONT CORRELATION OF THE PALAEOZOIC SEDIMENTS OF THE BONAPARTE GULF BASIN, NORTH-WESTERN AUSTRALIA

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A PRELIMINARY investigation of the geology of the Bonaparte Gulf Basin was published by the Australian Bureau of Mineral Resources in 1955 (ref. 1). In 1963, the Bureau began a detailed study of the geology of the basin; the initial observations were recorded by Veevers *et al.*[2] (see Fig. 1 for the localities mentioned herein). Previous published accounts of the geology include McWhae *et al.*[3] and Thomas[4], who summarized the knowledge of Carboniferous stratigraphy up to 1958. Geological investigations before 1952 have been concisely reviewed by Noakes *et al.*[5].

Conodonts were first discovered in the Bonaparte Gulf Basin by Öpik[1,6], who recorded the presence of a Lower Ordovician (Tremadocian) fauna in a glauconitic sandstone which he named the Pander Greensand. A preliminary investigation of our material supports Öpik's conclusion. Glenister[7] noted the occurrence of Carboniferous conodonts in the upper parts of both the Burt Range Limestone (herein referred to as the Burt Range Formation) and the Septimus Limestone. The conodont locality (*c*) of Glenister (ref. 7, p. 214), which he tentatively referred to the Spirit Hill Limestone, is on brachiopod evidence[4,8] the same age as the Septimus Limestone.

Conodont zonations of European and North American Devonian and Carboniferous standard sections have been

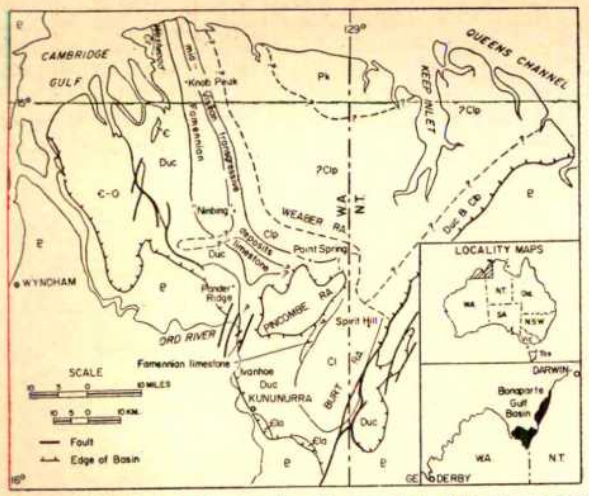


Fig. 1. Geological sketch-map of southern portion of Bonaparte Gulf Basin. e, Proterozoic; Cla, Lower Cambrian Volcanics; C, Cambrian; C-O, Cambrian and Ordovician undifferentiated; Duc, Cockatoo Sandstone; Cl, Lower Carboniferous undifferentiated—includes Burt Range Formation, Enga Sandstone and Septimus Limestone; Clb, Burt Range Formation; Clp, Point Spring Sandstone; PK, Lower Permian Keep Inlet Beds

published by Bischoff[9], Bischoff and Ziegler[10], Collinson, Scott and Rexroad[11], and Ziegler[12]. British Carboniferous conodont faunas have been studied by Austin, Druce and Rhodes[13]. Information contained in these works has enabled us to correlate the Upper Palaeozoic succession of the Bonaparte Gulf Basin with the classical sections of the northern hemisphere (Fig. 2). Such information will provide a stratigraphical framework for the detailed investigations being conducted by the Bureau.

Detailed palaeontological investigations of samples collected by a survey party led by J. J. Veevers in 1963 have disclosed abundant conodont faunas in limestone formations previously known to contain conodonts. In addition, conodonts were discovered in the unnamed Viséan calcarenite of Veevers *et al.*[2], in intercalated calcareous beds at the base of the Enga Sandstone and in a belt of limestone in the Ninbing region which Traves[1] referred to the Burt Range Limestone (Fig. 3).

Conodonts have only been recovered from the calcareous middle part of the Cockatoo Sandstone in the north-west part of the basin, at Westwood Creek. This fauna contains the species *Ancyrognathus* sp., *Polygnathus* cf. *brevilamina* Branson and Mehl[14], and *Polygnathus normalis* Miller and Youngquist[15], which indicate a Frasnian (toI) age. However, the upper boundary of the Cockatoo Sandstone may possibly be as young as the toII stage of the Famennian, because about 2,000 ft. of sandstone, which has so far yielded no conodonts, overlies the fossiliferous limestones, and is in turn succeeded by the limestones of the Knob Peak area, of upper toIIβ–lower toIIIα age (see following, and Fig. 1). Furthermore, the possibility that the non-fossiliferous basal beds may be of uppermost Middle Devonian age cannot be excluded.

Upper Devonian conodonts have also been discovered in limestones[16] exposed as a long narrow belt from Knob Peak southwards through Ninbing, to the east band of the Ord River at Buttons Crossing (5·5 miles north of Ivanhoe Homestead). The presence of the conodont species *Palmatolepis glabra elongata* Holmes[17] and *P. glabra pectinata* Ziegler[18] indicates that these beds belong to the upper toIIβ–lower toIIIα ammonoid zones of the German standard section. Traves[1] and Veevers *et al.*[2] have referred these limestones to the Burt Range Formation.

The conodont faunas of the Burt Range Formation, however, include the species *Polygnathus inornata* E. R. Branson[19], *Pseudopolygnathus* sp. of the *prima* group and *Siphonodella cooperi* Hass[20], which are comparable with those from the CuI–CuIIα ammonoid zones of the German standard section, and the Hannibal–Chouteau interval of the Upper Mississippi Valley. The presence of *Siphonodella duplicata* (Branson and Mehl)[21] within the basal strata clearly shows that the Burt Range Formation is restricted to the Lower Carboniferous.

Therefore, our evidence shows that the limestones at Ninbing are older than the Burt Range Formation, and are separated from it by a considerable hiatus. These limestones constitute a hitherto unnamed formation, which will be described and named in a forthcoming publication of the Bureau.

The conodont faunas discovered in the basal beds of the Enga Sandstone include species found in the upper part of the Burt Range Formation, such as *Polygnathus inornata* E. R. Branson[19], and species of the genus *Spathognathodus*. These indicate a CuIIα age for the basal portion of the Enga Sandstone, which confirms the

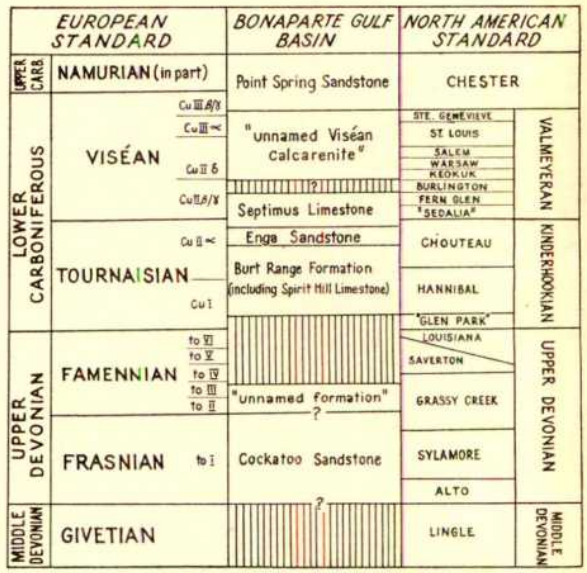


Fig. 2. Intercontinental correlation of the formations of the Bonaparte Gulf Basin. Vertical hatching denotes apparent absence of time-rock units; thicknesses of formations are not to scale

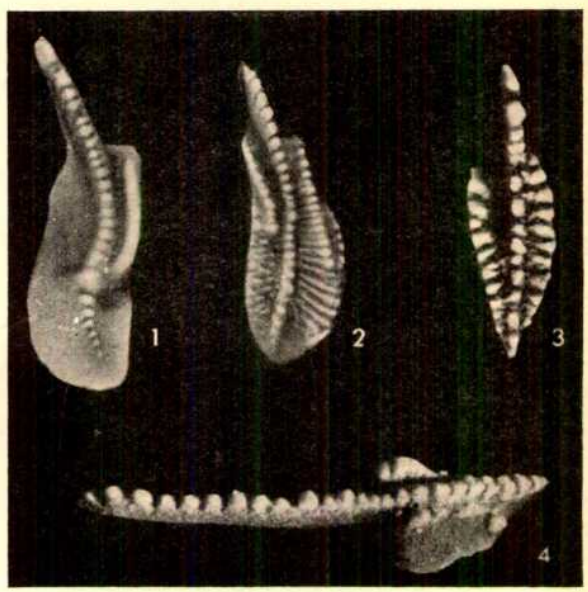


Fig. 3. Conodonts from the Bonaparte Gulf Basin. Oral views × *c.* 35. (1) *Palmatolepis glabra pectinata* Ziegler, 1960, from unnamed Famennian limestone. (2) *Siphonodella cooperi* Hass, 1959, from Burt Range Formation. (3) *Polygnathus nodomarginata* Branson, 1934, from Septimus Limestone. (4) *Gnathodus texanus* Roundy, 1926, from unnamed Viséan calcarenite

Tournaisian age previously assigned by Thomas[4], and the Kinderhookian age suggested by Öpik[1].

The late Tournaisian to early Viséan age of the Septimus Limestone, based on the brachiopod studies of Thomas[4,8] and Roberts (personal communication), is supported by our investigation of the conodonts. The fauna includes the species *Polygnathus nodomarginata* E. R. Branson[19] and new and re-described species from the *Z* coral–brachiopod zone of the type Avonian at Bristol[13].

The unnamed Viséan calcarenite of Veevers *et al.*[2] has yielded an abundant conodont fauna, including the species *Geniculatus claviger* Hass[22], *Gnathodus girtyi* Hass[22], *G. texanus* Roundy[23], *Mestognathus beckmanni* Bischoff[9], *M. bipluti* Higgins[24], and *Spathognathodus scitulus* (Hinde)[25]. This fauna indicates a CuIII age, although the presence of *Gnathodus* cf. *G. cuneiformis* Mehl and Thomas[26] in the basal portion suggests that the formation may be as old as the CuIIδ zone. The apparent absence of species characteristic of the CuIIIγ zone suggests that it is doubtful whether the top of the formation is any younger than the early part of the CuIII zone. In terms of the Upper Mississippi Valley standard succession[11], the formation can be correlated with part of the Valmeyeran stage; it is no older than the lower portion of the Burlington Formation, and no younger than the Ste. Genevieve Formation. Thus, the conodont evidence suggests a slightly younger age than the early Viséan age assigned to it by Thomas[27].

No conodonts have yet been found in calcareous samples of the Point Spring Sandstone; Thomas[8,27] and Roberts indicate, on brachiopod evidence, a late Viséan to early Namurian age for this formation.

The systematics and biostratigraphy of the conodont faunas from the Upper Palaeozoic sequence of the Bonaparte Gulf Basin are being investigated by one of us (E. C. D.) and similar studies on the Lower Palaeozoic succession are in progress (P. J. J.).

We thank Drs. J. J. Veevers and J. Roberts for valuable discussions in the field on the stratigraphy and structure of the basin, and for systematically collecting conodont samples in measured sections. We also thank Dr. Günther Bischoff, of Gewerkshaft Elwerath Erdölwerke, Hannover, West Germany, for examining and discussing these faunas, and Mr. A. T. Wilson, who prepared and picked the residues.

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TRANSPORT PROCESSES AND THERMODYNAMIC EQUILIBRIUM

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IN a recent series of articles[1–3], Prof. Melehy has presented a thermodynamic theory applicable to transport phenomena for liquids, gases and electrons in matter. Important quantities in his theory are the particle diffusion force and the corresponding diffusion potential. In cases where the system of particles is subject to long-range forces such as gravitational or electrostatic, he contends that the conventional condition of equilibrium expressed as:

$$f_e + f_d = 0 \qquad (1)$$

leads logically to results which contradict experimental observations like the Peltier effect. In (1) f_e is the force on the particle as a result of long-range forces, f_d is the particle diffusion force. Since (1) expresses that the net force on the particle is zero, it is argued[2] that the net work in transferring the particle between two points, whatever the gravitational or electrostatic potential difference may be, is zero, that is, the transfer would be at constant energy. It is further argued that direct consequences would be: (*a*) a denial of the existence of such effects as the Peltier effect; (*b*) the necessity in thermal equilibrium for the conduction bands to line up throughout a crystal containing a non-degenerate *p–n* junction.

The theory is used to prove[2] that the Fermi level of the crystal in case (*b*) cannot have the same value throughout the crystal.

In the most recent paper[3] a treatment generalizing this theory on a thermodynamic basis is given; here it is claimed that the usual proof of the constancy of the chemical potential throughout a system in thermodynamic equilibrium is invalid.

In the present article, it is shown that these results of Melehy's theory are themselves invalid. It will be convenient to consider first his criticism of the conventional treatment of the chemical potential, then to consider the properties of the particle diffusion force and to show that the work done in transferring a particle from point *A* to point *B* in a system is not solely due to that done against the forces f_e and f_d and hence zero. It will be shown that the contribution to the work done in changing the volume occupied by the particle from that at *A* to that at *B* must also be taken into account. This contribution is not necessarily zero, and taking it into account, for instance, leads to the result for a perfect gas that the work done in transferring a particle from *A* to *B* is equal to the potential energy difference per particle between *A* and *B*. Thus Melehy's argument that (1) cannot be true, since it denies the existence of well-known experimental results, breaks down. Equation (1) must be true since it is an expression of the constancy of the chemical potential throughout the system, and the empirical assumption made by Melehy that the left-hand side of (1) is not itself zero but has a zero vector curl is

wrong. In any case, the extra work term, introduced as here, makes it not only wrong but unnecessary to depart from the usual treatment of equilibrium.

Chemical Potential and Thermodynamic Equilibrium

Ref. 3 considers the increment dF of the Helmholtz free energy:

$$dF = -SdT - PdV + \sum_k \mu_k dN_k \qquad (2)$$

The argument goes on as follows: "The last term in (2) is a summation of more than one product, since it is needed here supposedly to test whether or not the particles would have the same chemical potential at different points.

"The conventional argument supposedly proving that for electrons in solids $d\mu = 0$ in thermal equilibrium proceeds as follows. First it is assumed that dF is zero. This particular assumption will be shown in the next section to be unjustifiable. For the time being, however, let us accept the conventional assumption that $dF = 0$ in thermal equilibrium. It is then reasoned conventionally that if dN particles were transferred from point A to point B at constant volume, then:

$$dF = (\mu_a - \mu_b)dN = 0 \qquad (3)$$

[(2) and (3) are equations (24) and (25) respectively of ref. 3]. From equation (25) [(3) here] it would follow that $\mu_a = \mu_b$.

"The weakness in the preceding argument is that if dN particles were transferred from point A of high particle concentration n_a to point B of low particle concentration n_b, this could not possibly be done at constant volume. To explain this point let us calculate specifically the volumes occupied by dN particles at point A and at point B. At the former point, the volume $dV_a = (dN)/n_a$, and at point B, the volume $dV_b = (dN)/n_b$. Thus $dV_b = (n_a/n_b)dV_a$.

"If points A and B are on either side of, for example, an interface between a metal and a p-type semi-conductor, respectively, the ratio of (n_a/n_b) could be 10^{16} or higher. Thus the assumption that dN particles could be transported at constant volume in any system is obviously invalid."

In fact, the conventional argument as outlined is not rigorous—it implies that dV is the change in the volume occupied by the dN particles. Further, equation (2) as quoted applies to a macroscopic system of volume V and entropy S, and should be used only when it has been proved that the μ_k are constant throughout the system. It is, in fact, possible to transport in the system dN particles from A to B without altering the volume of the system. The conventional treatment takes account of the work required to change the volume of the region outside the neighbourhoods of the points A and B, so that the total volume remains constant. Since the foregoing account of the conventional theory is not clearly rigorous, it would appear desirable to give a rigorous account which does not beg the question of the constancy of μ throughout the system. The proof to be given is in the spirit of that of Gibbs[4] but is not identical with it. It has the advantage of not assuming the presence of the last term in:

$$dU = TdS - PdV + \mu dN \qquad (4)$$

an assumption which is not obvious, for example, in the case of systems under non-hydrostatic stresses, where the work done in injecting dN particles into the system is not obviously independent of where the injection is carried out. For simplicity, a system of one chemical component is considered—the generalization to a multi-component system is obvious. Following Gibbs[5], the conditions of equilibrium of a system may be obtained on the supposition that the system is enclosed in a perfectly rigid and perfectly non-conducting impermeable envelope. Under these conditions the equilibrium condition is taken to be that $\delta U = 0$, for a virtual reversible infinitesimal displacement in which $\delta S = \delta V = 0$.

Let the system be imagined to be divided into many fixed small regions $i = 1,2, \ldots$. For example, two of these regions could be infinitesimal regions containing the points A and B of the argument quoted here. Let t_i, p_i, V_i, U_i, S_i, v_i, u_i, s_i and φ_i be respectively the temperature, pressure, volume, energy, entropy, molar volume, molar energy, molar entropy, and the molar potential energy of the material in region i. The regions are assumed sufficiently small so that the foregoing quantities may be taken to be uniform through each region. Consider a virtual reversible displacement of the whole system, involving an injection of δM_i moles of material into region i. Then:

$$\delta U = \Sigma[\delta U_i + u_i \delta M_i + \varphi_i \delta M_i] = 0 \qquad (5)$$

where:

$$\delta U_i = t_i \delta S_i - p_i \delta V_i \qquad (6)$$

we have the restrictions:

$$\delta V = \sum_i (\delta V_i + v_i \delta M_i) = 0 \qquad (7)$$

$$\delta S = \sum_i (\delta S_i + s_i \delta M_i) = 0 \qquad (8)$$

$$\delta M = \sum_i \delta M_i = 0 \qquad (9)$$

It should be noted that u_i is the molar energy at region i, that is, it is an intrinsic quantity independent of φ_i. On substituting (6) into (5), and introducing undetermined multipliers P, T, μ for (7), (8), (9) respectively, one obtains the set of three equations:

$$\begin{aligned} t_i &= T \\ p_i &= P \\ \mu &= u_i - Ts_i + Pv_i + \varphi_i \end{aligned} \qquad (10)$$

Thus it is seen how the chemical potential μ may be introduced as a constant throughout the system, which may have several phases. The validity of this proof cannot be in doubt, as it is based on a most general condition of thermodynamic equilibrium. Recently[6], I have generalized this treatment to a multi-phase system in chemical equilibrium under non-hydrostatic stresses, for which the expression for the work in (6) is, of course, different.

Equation (10) thus shows that the intrinsic potential $u - Ts + Pv$ plus the molar potential energy φ is a constant throughout the system. Thus if an increment dN of material is added to the system at any region i, (10) yields:

$$dU_a = TdS_a - PdV_a + \mu dN \qquad (11)$$

where $dU_a = (u_i + \varphi_i)dN$, $dS_a = s_i dN$, $dV_a = v_i dN$ are the increments to U, S and V, for the whole system, arising from the injection of mass dN. If, further, a deformation of the whole system occurs, with no change of total mass, the changes in U, S and V are given by:

$$dU_b = TdS_b - PdV_b \qquad (12)$$

Combining (11) and (12) one obtains for a general virtual displacement of the whole system:

$$dU = TdS - PdV + \mu dN \qquad (13)$$

where (13) has been shown to be independent of where in the system dN has been injected. The method of this proof shows that Melehy's objection is not valid and that the correct treatment takes into account the deformation of the surrounding medium, necessary for the total volume to remain constant under the transfer of dN particles from one point to another in the system.

Particle Diffusion Force and Potential

It is true that from the condition:

$$\mu^i + \varphi = \mu = \text{constant} \quad (14)$$

where μ^i is the intrinsic potential (see ref. 4, p. 146, for the introduction of this term), and φ the molar potential energy, one may obtain a force equation by differentiating, thus:

$$\nabla\mu^i + \nabla\varphi = 0 \quad (15)$$

and if one defines, after Melehy[3]:

$f_d = -\nabla\mu^i$, the diffusion force, and $f_e = -\nabla\varphi$, the long-range force (15) can be written:

$$f_e + f_d = 0 \quad (16)$$

Since f_e represents the long-range forces, f_d must be the force per mole exerted on a particle by the surrounding medium. It should be mentioned that the term "particle" as used by Melehy can be interpreted as a small part of the thermodynamic system constant in mass, for example, one electron. That is, the volume occupied by the particle may vary and would be mv where m is the mass in moles of the particle and v is the molar volume in the region occupied by the particle.

It is claimed[2] that it is a consequence of (16) that a transfer of a particle from point A to point B in the system would be at constant energy. The fallacy of this assertion lies simply in the fact that in the transfer the volume occupied by the particle changes and work is required to deform the surroundings of the particle sufficiently to accommodate it.

Consider a particle of small mass m (in moles) which occupies a volume mv at point A, v being the molar volume at that point. In a small displacement $\delta\mathbf{r}$ of the particle, the work which must be done on it is easily seen to consist of three terms, first, that required to translate the particle against the pressure forces of its surroundings $(\delta\mathbf{r}.\nabla P)mv$; secondly, the work against these forces required to change the volume of the particle $(\delta\mathbf{r}.\nabla v)mP$; thirdly, the work to be done against the long-range forces $(\delta\mathbf{r}.\nabla\varphi)m$. That is, the total work:

$$\delta W = \delta\mathbf{r}.(v\nabla P + P\nabla v + \nabla\varphi)m \quad (17)$$

$$= \delta\mathbf{r}.[\nabla(Pv) + \nabla\varphi]m \quad (18)$$

Thus for a perfect gas with a uniform temperature:

$$\delta W = (\delta\mathbf{r}.\nabla\varphi)m \quad (19)$$

Equation (19) is in agreement with the classical explanation of the Peltier effect, and may, in fact, be used to relate the electrostatic potential difference to the energy which must be provided for the transport of electrons in metals.

For a system with a uniform temperature (for example, see ref. 3):

$$\nabla\mu^i = v\nabla P \quad (20)$$

and it is thus seen that (17) contains not only the work required against f_e and f_d, but the additional third term involving the change of volume occupied by the particle. (18) shows that the work to be done in a displacement of a "constant mass particle" involves the gradient of Pv and not of μ^i.

(15) becomes:

$$-\nabla\varphi = v\nabla P \quad (21)$$

and expresses the fact that for thermodynamic equilibrium the net force on the particle must be zero, whereas in a virtual displacement the work done does not solely arise from these two forces.

This argument shows that the work done against the surroundings of the particle, in transferring it from one point to another in the medium, cannot be described as a diffusion potential the gradient of which yields the particle diffusion force, f_d of refs. 1 and 2, which is equal and opposite to the long-range force f_e in equilibrium. Also, since it has been shown here that the chemical potential is a constant of the whole system, the basis of Melehy's theory is invalid, and consequently, for example, the application to the discussion of the properties of a p–n junction.

[1] Melehy, M. A., *Nature*, **202**, 864 (1964).
[2] Melehy, M. A., *Nature*, **205**, 456 (1965).
[3] Melehy, M. A., *Nature* **209**, 670 (1966).
[4] Gibbs, J. W., *Scientific Papers*, 1, 147 (Longmans, Green and Co., London, 1906).
[5] Ibid., page 62.
[6] McLellan, A. G., *J. Geophys. Res.* (in the press).

MASS SPECTROMETRIC DETERMINATION OF THE AMINO-ACID SEQUENCE OF PEPTIDES*

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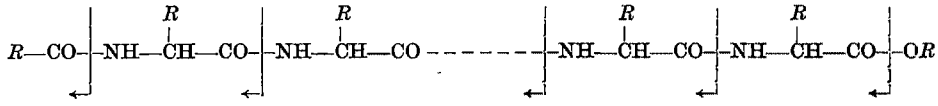
One of the most difficult and cumbersome tasks in the elucidation of the primary structure of proteins is the determination of the amino-acid sequence of peptides —a veritable barrier to the rapid solution of many chemical and biochemical problems. The use of mass spectrometry for this purpose with its great saving in time and in the requirement of material used (micro-amounts) could greatly facilitate this task.

* For preliminary communications, see refs 1–3.

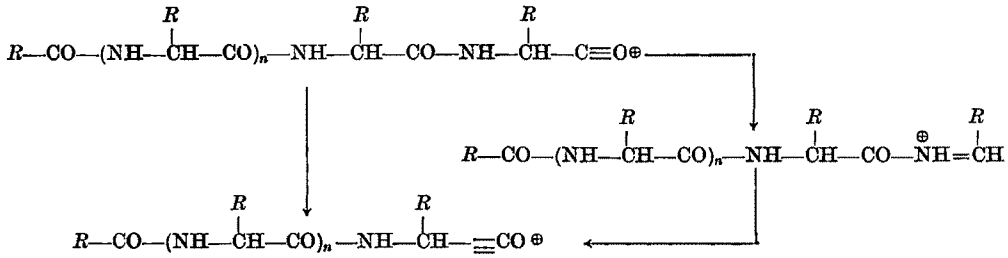
Because of their low volatility and tendency to undergo irregular fragmentation, the free peptides are not very suitable for direct mass spectrometric analysis[4]. More convenient are the methyl (*tert*-butyl) esters of the acyl-peptides which can readily be prepared from the peptides by a method described in one of our preliminary communications[2] (see also refs. 5–8).

The unit sequence in the peptides follows quite readily from an analysis of the amino-acid type of fragmentation[1],

which involves rupture of the amide bonds of the acyl-peptide ester, beginning with the C-terminus:



Although fragmentation of the peptide chain may start at any of the various amide bonds, this does not distort the over-all picture of the spectrum. The stepwise elimination of the amino-acid residues in the resulting fragments may proceed in one or in two stages, according to the following scheme:



Frequently such transformations can be confirmed by the corresponding metastable ions.

The data presented in Table 1 clearly illustrate the universality of the amino-acid type of fragmentation—peptides with the most varied amino-acid residues tending to undergo this kind of fragmentation. Mass spectrometry can therefore be considered as a novel technique for the determination of amino-acid sequences—a technique based on fundamentally different principles from those previously used. It should be added that each amino-acid makes its own contribution to the mass spectrum, which usually facilitates an analysis of the latter. Furthermore, the fragmentation picture is influenced by the position and chemical environment of the amino-acid in the peptide chain.

Specific types of fragmentation, dependent on the nature of the amino-acids, will be illustrated below for a number of examples.

Table 1*

Amino-acid	Peptides containing the amino-acid in the previous column	Sequence of elimination of amino-acid residues (*m/e* of the fragments)
Gly	Dec-Gly-Leu-Val-OMe (1)	M^+(455)→OMe(424)→Val(325)→Leu(212)→Gly(155)
	Dec-Gly-Gly-Leu-Gly-OMe (2)	M^+(470)→OMe(439)→Gly(382)→Leu(269)→Gly(212)→Gly(155)
Ala	Ac-Ala-Ala-Ala-Ala-Ala-Ala-OMe (3)	M^+(500)→OMe(469)→Ala(398)→Ala(327)→Ala(256)→Ala(185)→Ala(114)
	Dec-Ala-Ala-Ala-Asp(OMe)-OMe (4)	M^+(528)→OMe(497)→Asp(OMe)(368)→Ala(297)→Ala(226)→Ala(155)
	Dec-Gly-Try-Ala-Ala-OMe (5)	M^+(571)→OMe(540)→Ala(469)→Ala(398)→Try(212)→Gly(155)
	Dec-Try-Leu-Val-Pro-Ala-Pro-Val-OMe (6)	M^+(948)→OMe(917)→Val(818)→Pro(721)→Ala(650)→Pro(553)→Val(454)→Leu(341)→→Try(155)
Val	Dec-Val-Val-Gly-Try-OMe (7)	M^+(627)→OMe(596)→Try(410)→Gly(353)→Val(254)→Val(155)
	Dec-Pro-Ala-Ala-Val-OMe (8)	M^+(524)→OMe(493)→Val(394)→Ala(323)→Ala(252)→Pro(155)
	Ste-Ala-Leu-Gly-Leu-Val-Pro-Ala-Pro-Val-OMe (9)	M^+(1115)→OMe(1084)→Val(985)→Pro(888)→Ala(817)→Pro(720)→Val(621)→Leu(508)→→Gly(451)→Leu(338)→Ala(267)
Leu	Dec-Leu-Ala-Ala-Ala-OMe (10)	M^+(512)→OMe(481)→Ala(410)→Ala(339)→Ala(268)→Leu(155)
	Dec-His-Leu-Ala-Leu-OMe (11)	M^+(620)→OMe(589)→Leu(476)→Ala(405)→Leu(292)→His(155)
	Dec-Glu(OMe)-Leu-Val-Val-OMe (12)	M^+(640)→OMe(609)→Val(510)→Val(411)→Leu(298)→Glu(OMe)(155)
Ile	Dec-Glu(NH_2)-Ile-Ala-Ala-OMe (13)	M^+(569)→OMe(538)→Ala(467)→Ala(396)→Ile(283)→Glu(NH_2)(155)
	Dec-Cit-Ile-Ala-Ala-OMe (14)	M^+(598)→OMe(567)→Ala(496)→Ala(425)→Ile.Cit(155)
Pro	Dec-Gly-Gly-Pro-OMe (15)	M^+(397)→OMe(366)→Pro(269)→Gly(212)→Gly(155)
	Ste-Ala-Leu-Gly-Leu-Val-Pro-Ala-Pro-Val-OMe (9)	See above
	Dec-His-Ala-Pro-Val-OBut (16)	M^+(632)→OBu^t(559)→Val(460)→Pro(363)→Ala(292)→His(155)
	Dec-Pro-Ala-Ala-Val-OMe (8)	See above
Ser	Dec-Ser-Phe-Phe-Ser-OMe (17)	M^+(654)→OMe(623)→Ser(536)→Phe(389)→Phe(242)→Ser(155)
	Dec-Lys(Dec)-Phe-Phe-Ser-OMe (18)	M^+(849)→OMe(818)→Ser(731)→Phe(584)→Phe(437)→Lys(Dec)(155)
Thr	Dec-Lys(Dec)-Thr-Pro-Ala-OMe (19)	M^+(737)→OMe(706)→Ala(635)→Pro(538)→Thr(437)→Lys(Dec)(155)
Cys	Dec-Cys(CH_2CO_2Me)-Phe-Phe-Ser-OMe (20)	M^+(742)→OMe(711)→Ser(624)→Phe(477)→Phe(330)→Cys(CH_2CO_2Me)(155)
Asp	Dec-Ala-Ala-Ala-Asp(OMe)-OMe (4)	See above
	Dec-Ala-Asp(OMe)-Gly-OMe (21)	M^+(443)→OMe(412)→Gly(355)→Asp(OMe)(226)→Ala(155)
	Dec-Asp(OMe)-Val-Ala-Leu-OBu^t(22)	M^+(640)→OBu^t(567)→Leu(454)→Ala(383)→Val(284)→Asp(OMe)(155)
	Dec-Asp(OMe)-Leu-Val-Val-OMe (23)	M^+(626)→OMe(595)→Val(496)→Val(397)→Leu(284)→Asp(OMe)(155)
Asp(NH_2)	Dec-Asp(NH_2)-Ile-Ala-Ala-OMe (24)	M^+(555)→OMe(524)→Ala(453)→Ala(382)→Ile(269)→Asp(NH_2)(155)
	Dec-Gly-Asp(NH_2)-Ala-Ala-Phe-OMe (25)	M^+(646)→OMe(615)→Phe(468)→Ala(397)→Ala(326)→Asp(NH_2)(212)→Gly(155)
	Dec-Asp(NH_2)-Phe-Ala-Gly-Met-OMe (26)	M^+(706)→OMe(675)→Met(544)→Gly(487)→Ala(416)→Phe(269)→Asp(NH_2)(155)
Glu	Dec-Gly-Glu(OMe)-Tyr-OMe (27)	M^+(549)→OMe(518)→Tyr(355)→Glu(OMe)(212)→Gly(155)
	Dec-Ala-Pro-Glu(OMe)-OMe (28)	M^+(497)→OMe(466)→Glu(OMe)(323)→Pro(226)→Ala(155)
	Dec-Glu(OMe)-Leu-Val-Val-OMe (12)	See above
Glu(NH_2)	Dec-Glu(NH_2)-Ile-Ala-Ala-OMe (13)	See above
	Dec-Glu(NH_2)-Phe-Ala-Gly-Met-OMe (29)	M^+(720)→OMe(689)→Met(558)→Gly(501)→Ala(430)→Phe(283)→Glu(NH_2)(155)
Met	Dec-Gly-Met-Ala-Ala-OMe (30)	M^+(516)→OMe(485)→Ala(414)→Ala(343)→Met(212)→Gly(155)
	Dec-Asp(NH_2)-Phe-Ala-Gly-Met-OMe (26)	See above
	Dec-Glu(NH_2)-Phe-Ala-Gly-Met-OMe (29)	See above
Orn	Dec-Orn(Dec)-Ala-Ala-Phe-OMe (31)	M^+(743)→OMe(712)→Phe(565)→Ala(494)→Ala(423)→Orn(Dec)(155)
Cit	Dec-Cit-Ile-Ala-Ala-OMe (14)	See above
Lys	Dec-Lys(Dec)-Phe-Phe-Ser-OMe (18)	See above
	Dec-Lys(Dec)-Thr-Pro-Ala-OMe (19)	See above
Phe	Dec-Phe-Val-Gly-Val-OMe (32)	M^+(588)→OMe(557)→Val(458)→Gly(401)→Val(302)→Phe(155)
	Dec-Cys(CH_2CO_2Me)-Phe-Phe-Ser-OMe (20)	See above
	Dec-Gly-Asp(NH_2)-Ala-Ala-Phe-OMe (25)	See above
Tyr	Dec-Gly-Glu(OMe)-Tyr-OMe (27)	See above
	Dec-Val-Tyr-Val-Ala-OMe (33)	M^+(618)→OMe(587)→Ala(516)→Val(417)→Tyr(254)→Val(155)
	Dec-Tyr-Ala-Val-Ala-OMe (34)	M^+(590)→AlaOMe(488)→Val(389)→Ala(318)→Tyr(155)
His	Dec-Ala-His-Leu-OMe (35)	M^+(507)→OMe(476)→Leu(363)→His(226)→Ala(155)
	Dec-His-Leu-Ala-Leu-OMe (11)	See above
	Dec-Val-Leu-Ala-His-OMe (36)	M^+(606)→OMe(575)→His(438)→Ala(367)→Leu(254)→Val(155)
	Dec-His-Ala-Pro-Val-OBu^t (16)	See above
	Dec-His-Ala-His-Leu-OMe (37)	M^+(644)→OMe(613)→Leu(500)→His(363)→Ala(292)→His(155)
Try	Dec-Try-Val-Gly-Val-OMe (38)	M^+(627)→OMe(596)→Val(497)→Gly(440)→Val(341)→Try(155)
	Dec-Try-Leu-Ala-His-OMe (39)	M^+(693)→OMe(662)→His(525)→Ala(454)→Leu(341)→Try(155)
	Dec-Gly-Try-Ala-Ala-OMe (5)	See above
	Dec-Val-Val-Gly-Try-OMe (7)	See above
	Dec-Try-Leu-Val-Pro-Ala-Pro-Val-OMe (6)	See above

* The mass spectra were taken on commercial *MX*-1303 and *RMU*-6*D* (Hitachi) instruments at 150°–350° C with the sample introduced directly into the ion source.

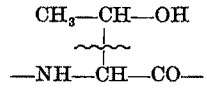
Aliphatic monoamino monocarboxylic acids (glycine, alanine, valine, leucine, isoleucine and proline) essentially do not complicate the general picture of the spectrum. It is true, however, that branched chain amino-acids may sometimes partly lose their chain; especially is that so in the case of leucine and isoleucine (see, for example, Fig. 1). The elimination may involve part of the chain (for example, methyl in valine or isoleucine) or the chain as a whole, depending on the neighbouring amino-acids. This usually gives low-peak intensities as compared with the peaks of the amino-acid type of fragmentation. The mass spectra of peptides containing proline residues often exhibit quite strong peaks, due to the ions resulting from elimination of the carbonyl groups and ring contraction:



As might be expected, the fragmentation of peptides containing amino-acid residues with functional substituents in the β-position is accompanied by elimination (usually only partly) of the function from the molecular ion or the resultant fragments:



Examples of such peptides are those containing serine, threonine and/or cysteine residues (see, for example, Fig. 2 and compare with ref. 5). A further type of side-chain elimination is also possible for threonine derivatives (compare ref. 6):



Elimination of the β-substituent is always observed in the fragmentation of peptides containing the residues of asparagine or the β-ester of aspartic acid. This process is characterized by its stepwise developments—first the elements of ammonia/alcohol and then CO are eliminated. Such mechanisms are often supported by the presence of the corresponding metastable ions.

The same two-stage elimination picture is shown by the γ-esters of glutamic acid and by glutamine (see, for example, Fig. 3), although here the relative intensity of the corresponding peaks is somewhat lower than for the aspartic acid derivatives. For the γ-substituted amino-acids (derivatives of glutamic acid and methionine), elimination of the entire side-chain also partially occurs, as can be seen in the example of the peptide (30) (Fig. 4).

Peptides containing citrulline may suffer loss of the $CONH_2$ group from the δ-substituent; weaker peaks can also be seen in the spectrum, and these are due to the ions resulting from the elimination of the 'fractions' of NH_3 or $NHCONH_2$.

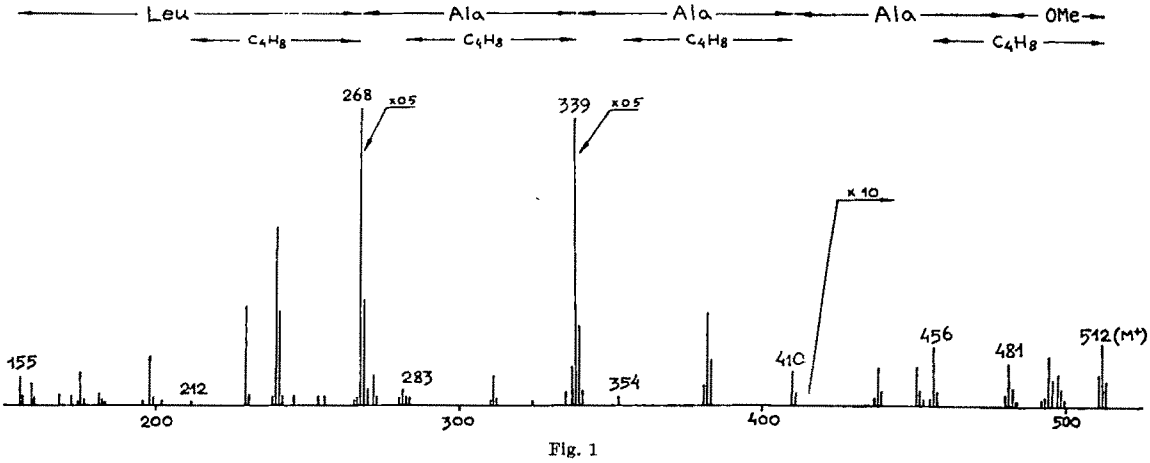


Fig. 1

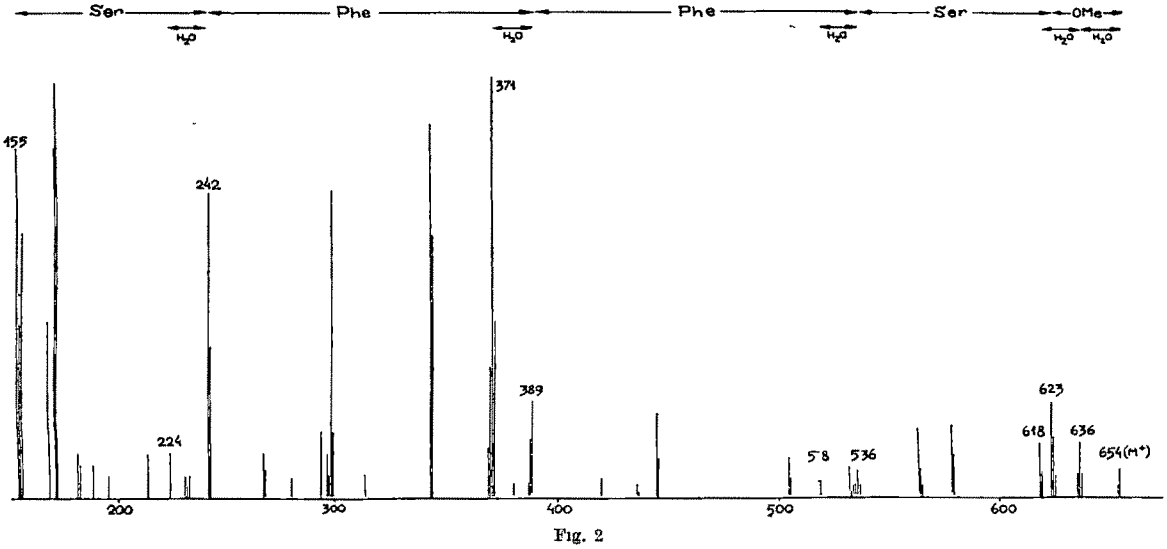


Fig. 2

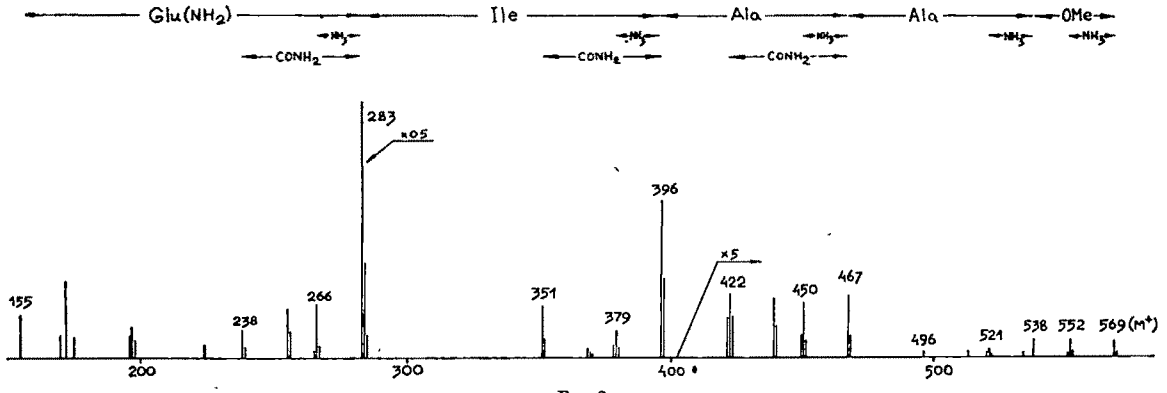


Fig 3

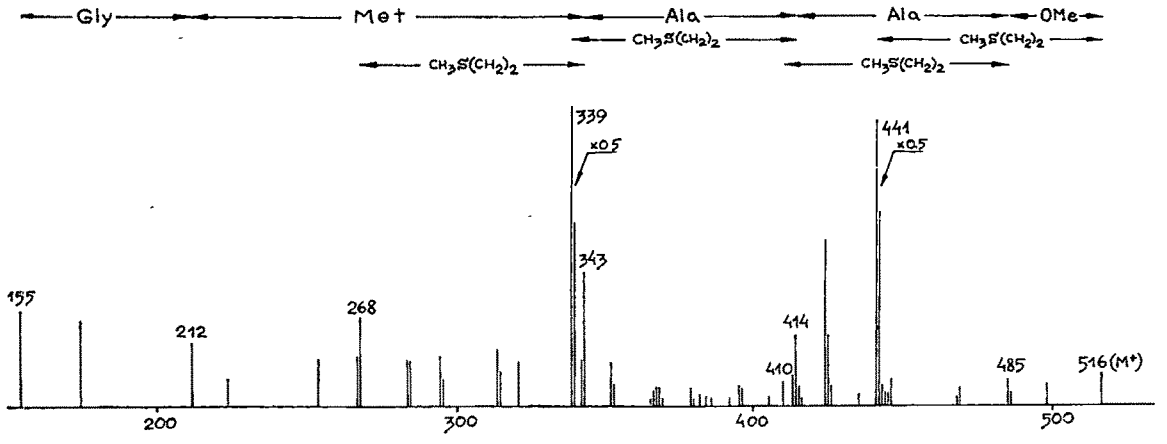
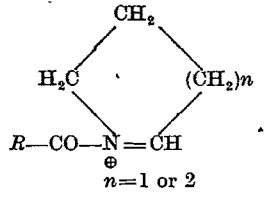


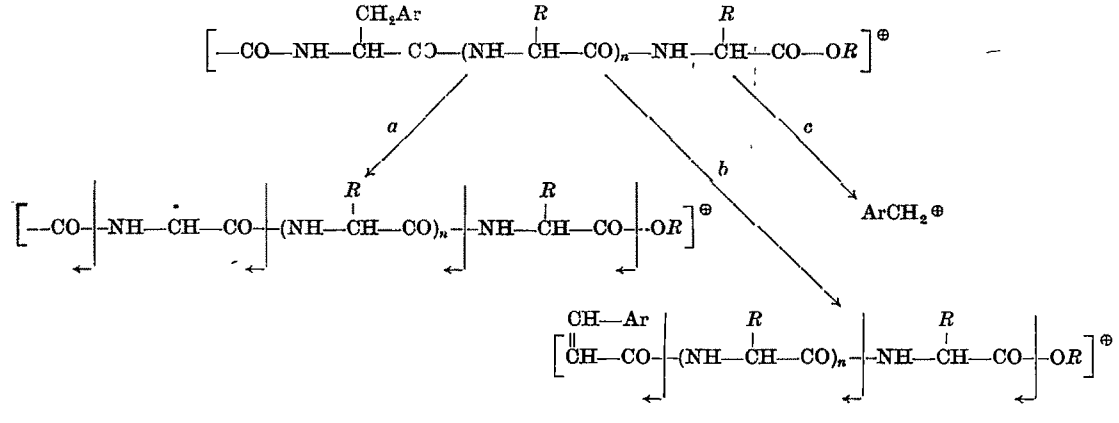
Fig. 4

Before mass spectrometry is carried out of peptides containing diamino monocarboxylic acids (lysine and ornithine), the ω-amino group must be protected by acylation simultaneously with the α-amino group of the N-terminal amino-acid. For peptides with N-terminal lysine and ornithine, one of the strongest peaks is due to ions of the type (see, for example, the *m/e* 238 peak in Fig. 5 and the *m/e* 224 peak in Fig. 6):



It would seem feasible that arginine residues in peptides containing this amino-acid are converted into citrulline or ornithine residues, after which such peptides readily undergo the amino-acid type of fragmentation (as already outlined).

Peptides containing aromatic and heterocyclic amino-acids (phenylalanine, tyrosine, tryptophan, histidine) are characterized by: (*a*) partial elimination of the side-chain as $ArCH_2^{\cdot}$ during the amino-acid type of fragmentation; (*b*) initial rupture of the N—C_α bond of the aromatic/heterocyclic amino-acid residue followed by the amino-acid type of fragmentation; (*c*) elimination of the side-chain in the form of $ArCH_2^{\oplus}$ (for example, tropylium in the case of phenylalanine) (see Fig. 7). All three processes are most clearly manifested in tryptophan-containing peptides:



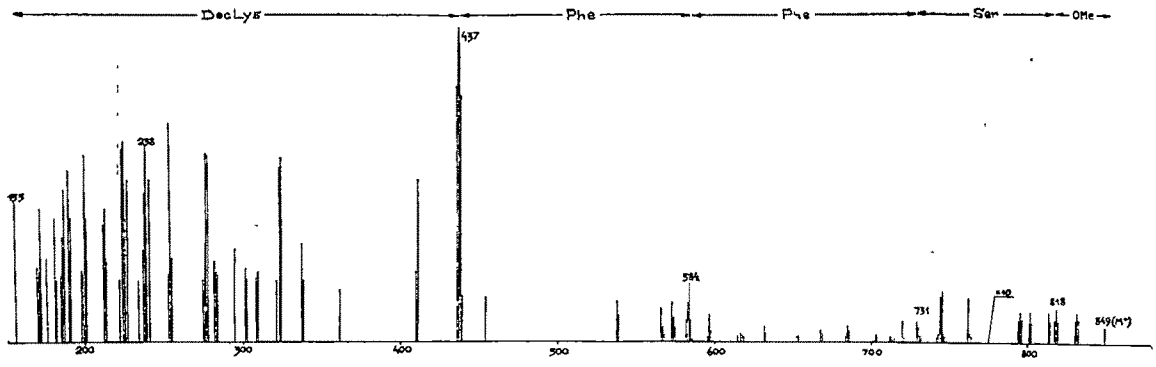


Fig 5

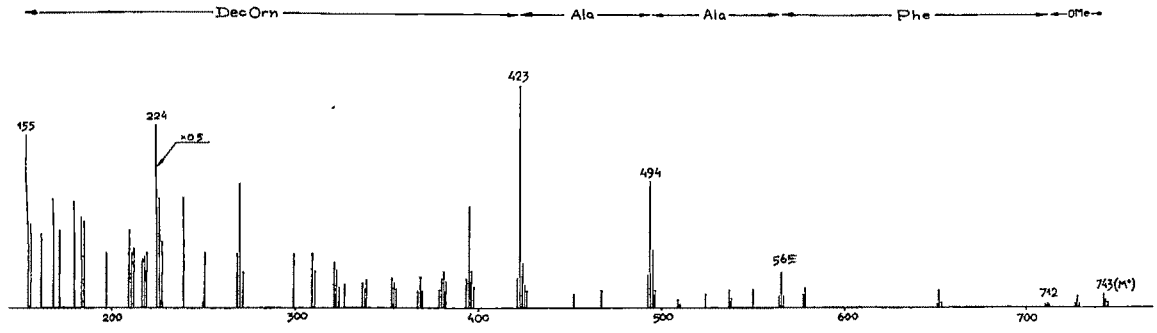
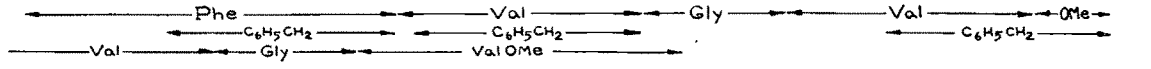


Fig. 6



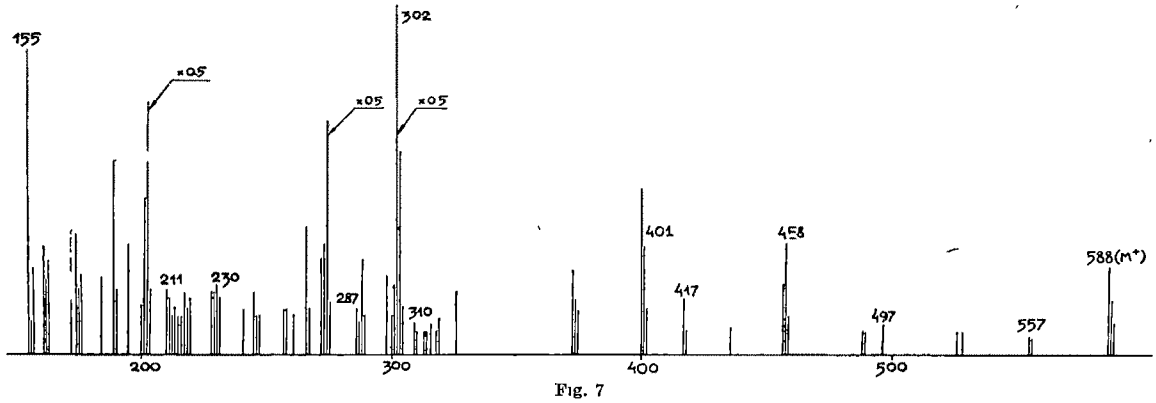


Fig. 7

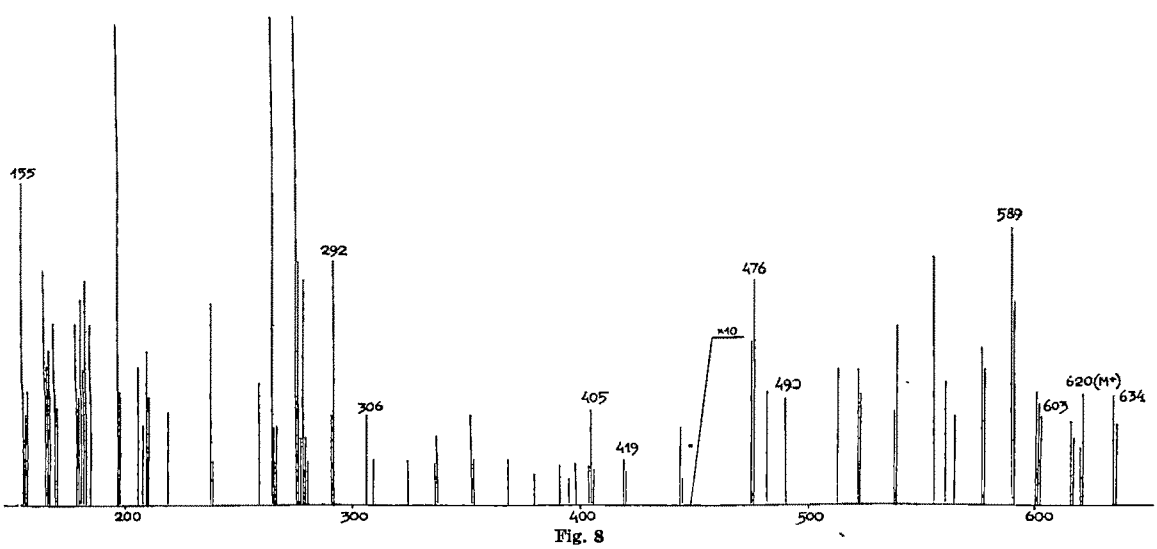


Fig. 8

Because of its strong basicity, the imidazole imino group suffers intermolecular methylation. Thus in peptides containing one histidine residue the peaks corresponding to the amino-acid type of fragmentation are accompanied by peaks shifted by 14 m.u. (see Fig. 8). Correspondingly, the mass spectra of peptides containing two histidine residues (for example, compound 37) exhibit two types of satellite peaks with 14 and 28 excess m.u.

The data presented here show that mass spectrometry can be used to determine the amino-acid sequence in peptides containing all the usual amino-acids. Analysis of the spectra is greatly facilitated if the amino-acid composition of the peptide is known beforehand. The method is limited only by the volatility of the acylpeptide ester, which depends on both the nature of the amino-acids and protective groupings. In the present work a very wide range of peptides, up to a nonapeptide (9), was investigated, but the latter is not the limiting case. The acylpeptide ester can be made more volatile by changing the protecting groups and thus broadening the possibilities of the method. Work is being carried out at present to determine the limits of the method, but even now the mass spectrometric analysis of peptides should make it possible to accelerate sharply and to simplify determinations of the primary structure of proteins.

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ESTABLISHMENT OF A LINE OF MOSQUITO (*Aedes aegypti* L.) CELLS GROWN *IN VITRO*

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THE causal agents of many diseases, both viral and non-viral, that affect man and animals are transmitted by mosquitoes. Some of these agents, after ingestion, multiply in the tissues of the mosquito. Examples of these are western equine encephalomyelitis, yellow fever, and malaria. An interesting feature of the viruses in this group is that they do not appear to cause a disease in the tissues of the mosquito vector. The successful culture of mosquito cells *in vitro* would permit more intensive investigations of these viruses than could be achieved by using infectivity assays or techniques on whole insects.

In vitro culture of mosquito cells has been attempted many times[1–6]. In the majority of experiments no growth has been obtained although the tissues have survived for many weeks as evidenced by muscular contractions. However, Kitamura[7] has recently obtained growth and survival of cells from ovarian tissues of *Culex pipiens molestus* Forsk. for up to 4 weeks. This article reports the establishment, *in vitro*, of a line of cells from *Aedes aegypti* L. larval tissues.

The mosquito tissues used in the experiments reported here were obtained from axenically grown larvae about to pupate[8]. The larvae were placed in insect culture medium[9] and the heads, tails and alimentary canals were removed. After discarding the peritrophic membranes, the alimentary canals and bodies were washed twice in fresh medium. The tissues were torn apart before cultivation.

Each culture consisted of the bodies and alimentary canals from three larvae in 1·0 ml. of medium in a $3 \times \frac{1}{4}$-in. round-bottomed tube slanted about 20° from the horizontal. The medium was the same as is used for the growth of the cell lines of *Antheraea eucalypti* Scott. established by Grace[9]. The cultures were incubated at 30° C.

In June 1963, four cultures were set up. Although the alimentary canals contracted vigorously when dissected the contractions stopped when they were first placed in the culture fluid, but resumed after about 15 h.

Two days after the cultures were made, cells had migrated from several masses of tissue. The number of cells continued to increase over the next 14 days and mitotic divisions were numerous. Most of the cells floated in the medium, but many remained in clumps attached to the surface of the culture tube.

Four weeks after the cultures were set up, the first subculture was made. Free-floating cells were transferred to a Porter flask containing 0·5 ml. of the medium in which the cells had been growing and 0·5 ml. of fresh medium. Since this time subcultures have been made at intervals of about 7 days. In the early subcultures, 3×10^5 cells in 3·0 ml. of medium were placed in 2-oz. half-flat medicine bottles. The later subcultures have consisted of 2×10^5 cells/ml. in 4-oz. and 8-oz. bottles (Fig. 1).

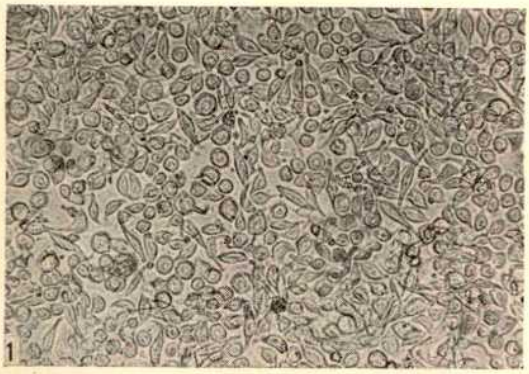
The cells have also been grown in suspension. Inocula for these cultures consisted of $2–4 \times 10^5$ cells/ml. of medium. In suspension the cells have a generation time of about 51 h at 30° C.

The cells have been deep-frozen at −80° C in medium containing 10 per cent glycerol and have been successfully regenerated after 8 months.

Initially there were a number of morphologically distinct types of cells in the cultures. However, after the cultures had been growing for about 3 months, many of the types had disappeared. At the present time the most common type is spindle-shaped, 40–50μ long and 8–10μ wide. These cells grow in clumps and usually remain attached to the culture vessel (Fig. 2). The second, slightly less common type of cell is round in outline, about 20μ in diameter and has a granular cytoplasm (Fig. 3). Some of these cells grow in clumps loosely attached to the surface of the culture vessel, but the majority float in the medium. Two other types of cells are present, but in very small numbers. One type consists of very large round cells, 55–60μ in diameter. The other type characteristically puts out long extensions which are very firmly attached to the glass.

It is not possible to determine from which tissues these cells have grown. In last-stage larvae about to pupate the imaginal disks that will develop into the adult gut, wings, gonads and other appendages have started to

grow and it is possible that the cells could have come from some of these tissues. From 1953 until the cultures just described were set up in June 1963, many cultures of mosquito tissues had been set up. In none of these did growth take place except to a limited extent in two cultures initiated in October 1962. In these cultures a few cells migrated from the tissues 7 months after they were set up. Over the next 4 weeks the number of migrating cells increased and several cells in each culture were seen to divide. Ten months after setting up the cultures there were still a few healthy cells but no mitotic divisions were seen and the cultures were discarded about 3 months later.





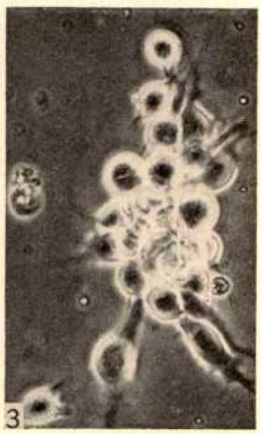


Fig. 1. Cells from the mosquito *Aedes aegypti*. From a culture 23 months old. (×*c*. 45)

Fig. 2. Spindle-shaped cells of the mosquito *Aedes aegypti* growing *in vitro*. Phase contrast. (×*c*. 90)

Fig. 3. Round cells of the mosquito *Aedes aegypti* growing *in vitro*. Phase contrast. (×*c*. 140)

In the past 18 months more than a hundred cultures of mosquito tissues have been made and in only two of these has growth comparable to that described for the cell line occurred. However, the growth continued for only 2·5 and 4 months respectively and then the cultures died. In these cultures the *A. eucalypti* haemolymph was replaced by 3 per cent *Antheraea pernyi* Guer. haemolymph.

Six months after the line had been established an investigation of the chromosome complement of the cells was made. In *Aedes aegypti* the diploid number of chromosomes is six, but many cells in the larvae are polyploid. Cells from the cultured line were stained and mounted as squash preparations in lactic-orcein[10]. The results were surprising in that no cells having the diploid number of chromosomes were observed. Most of the cells had chromosome complements of 96 (32*n*), while the next most frequent number was 48 (16*n*). A few cells contained many more than 96 chromosomes.

Originally the medium in which the cells were grown contained 5 per cent heat-treated haemolymph from *A. eucalypti*. As a constant and large supply of this haemolymph could not be assured, attempts were made to adapt the cells to grow in media containing either bovine plasma albumin (BPA), calf serum or the haemolymph from a related species of Saturniid, *Antheraea pernyi*, which can be obtained in large numbers. Media containing various concentrations of these supplements were tested; the most suitable were those containing either 3 per cent *A. pernyi* haemolymph or 1 per cent BPA or 1 per cent calf serum.

By December 1964, about 13 months after the adaptation experiments were begun, the growth of the cells in 3 per cent *A. pernyi* haemolymph and those in 1 per cent BPA were more satisfactory than the growth in medium containing 1 per cent calf serum. In none of the media, however, have the cells grown so well as those grown in media containing 5 per cent *A. eucalypti* haemolymph.

As the cells in the mosquito cultures are morphologically not unlike the cells from the ovarian cultures of *A. eucalypti* growing *in vitro*, it was necessary to confirm that the cells were of mosquito origin. To determine this, a serological method using the gel diffusion precipitation method (Ouchterlony technique) was used. Antiserum to the *A. eucalypti* cells was used. The antigens consisted of sonicated suspensions of (1) cells from the mosquito cultures, (2) mosquito larvae, (3) cells from the *A. eucalypti* cell lines and (4) ovarian tissues from *A. eucalypti* diapausing pupae.

The only precipitin lines which formed were between the *A. eucalypti* antiserum and the suspensions from the *A. eucalypti* cell lines and ovarian tissue.

Further evidence to support the view that the cells are of mosquito origin is furnished by the chromosome complements of the cells. In the line of cells described in this article the great majority of cells contained either 96 or 48 chromosomes and only a few contained more than 96 chromosomes. In the *A. eucalypti* cell lines the most frequent chromosome numbers were 100, 200 and 400, while cells of higher ploidy, although less numerous, were frequently seen[11]. The second piece of supporting evidence is that a virus, causing a cytoplasmic polyhedrosis in the *A. eucalypti* cell lines, would not grow in the cells described in this paper.

It is most unlikely that a group of cells from the *A. eucalypti* lines would change their serological characteristics, their chromosome number and their ability to maintain virus growth when other cells from the lines have retained these properties.

Cultures of the mosquito line have been sent to Dr. Earl C. Suitor at the United States Naval Medical Research Unit at Taipai in Taiwan. Suitor has successfully maintained the line and has adapted them to grow in medium containing 2 per cent and 0·5 per cent cynthia (*Samia walkeri* Dru.) haemolymph together with foetal bovine serum or bovine plasma albumin. Samples of these cells have been sent to me by Dr. Suitor and are being maintained in this laboratory.

I thank Mrs. N. Blunden for help in caring for and maintaining the cultures. This work was supported in part by a research grant from the U.S. Public Health Service.

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INDUCED MUTATION AFFECTING THE CONTROL OF MEIOTIC CHROMOSOME PAIRING IN *Triticum aestivum*

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HOMOEOLOGOUS chromosomes of wheat (*Triticum aestivum* $2n = 6x = 42$), which are genetically corresponding members of the three different genomes of which the chromosome complement is composed, are prevented from pairing at meiosis by a genetic activity of chromosome 5*B* (ref. 1). Although pairing is normally confined to fully homologous partners, homoeologues will pair in the absence of chromosome 5*B*. This effect is produced by the deficiency of no other member of the complement.

Chromosome 5*B* is heterobrachial and the activity by which homoeologous pairing is prevented is confined to the long arm, which is about three times the length of the short[2]. It seems reasonable to suppose that the effect derives from the activity of a single locus, but this cannot be proved as allelic variation is unknown.

An investigation (of which the experiment described in this article forms part) was initiated with the aim of inducing such allelic variation by mutation. The availability of mutants would both allow the completion of the analysis of the formal genetics of the system and more readily permit the study of its functional basis. In addition, the use of genetically induced homoeologous recombination in wheat improvement would be greatly eased by the availability of allelic variation in the 5*B* system.

The most difficult problem posed by any attempt to induce mutations of genes affecting meiotic characters is that of recognition. This is especially true of work with the 5*B* system of *T. aestivum* as the occurrence of multivalents, resulting from the synapsis of homoeologous as well as homologous chromosomes, could easily be overlooked because they could well be relatively infrequent. Consequently, in the present work potential mutants were scored at meiosis in hybrids between *T. aestivum* and rye (*Secale cereale* $2n = 14$).

This method was used because of the ready distinction between the meiotic behaviour of *T. aestivum* × *S. cereale* hybrids with and without chromosome 5*B*. When chromosome 5*B* is present in such hybrids there is very little chromosome pairing at meiosis, whereas in its absence bivalents and trivalents are frequent. This was initially shown by Riley, Chapman and Kimber[3], and was again demonstrated in some of the products of the present work (Tables 1 and 2). The experiment was thus designed to enable the recognition of mutants that changed the normal expression of meiotic pairing towards that occurring in the absence of chromosome 5*B*.

Ethyl methanesulphonate (EMS), which has been shown to be efficiently mutagenic in *T. aestivum*[4], was used to treat seeds of plants of *T. aestivum* variety 'Chinese Spring', in which chromosome 5*B* was represented by a pair of telocentrics for the long arm, the short arm being entirely deficient. Chromosome 5*B* was thus marked by structural condition that enabled its presence to be established from preparations showing either meiotic or mitotic divisions. Seeds of this genotype were soaked in 0·5 or 1·0 per cent aqueous solutions of EMS for either 16 or 24 h. However, because differences in EMS concentration or in the time of exposure resulted in no detectable modification in effect, variations in treatment can be ignored.

Immediately after treatment with EMS the seeds were thoroughly washed with water and then germinated. When the resulting plants came into ear, one spike of each plant was bagged to ensure self-pollination and other spikes were emasculated and pollinated with *S. cereale*.

Table 1. CHROMOSOME PAIRING AT FIRST METAPHASE OF MEIOSIS IN A SAMPLE OF 28-CHROMOSOME HYBRIDS FROM THE CROSS OF EMS-TREATED *T. aestivum*, DITELOCENTRIC 5*B*, BY *S. cereale*
(20 cells per plant)

Hybrid	Mean per cell Univ.	Biv.	Chiasmata
5/10	27·80	0·10	0·10
14/12	27·80	0·10	0·10
28/10	27·70	0·15	0·15
44/1	27·80	0·10	0·10
82/12	27·10	0·45	0·45

Table 2. CHROMOSOME PAIRING AT FIRST METAPHASE OF MEIOSIS IN 27-CHROMOSOME HYBRIDS, DEFICIENT FOR CHROMOSOME 5*B*, FROM THE CROSS OF EMS-TREATED *T. aestivum*, DITELOCENTRIC 5*B*, BY *S. cereale*
(20 cells per plant)

Hybrid	Mean per cell Univ.	Biv.	Triv.	Quad.	Chiasmata
9/1	15·50	3·70	1·30	0·05	7·35
72/17	19·30	2·50	0·90	—	4·60
83/11	17·30	3·85	0·60	0·05	5·60

A total of 206 hybrid plants were grown from seeds resulting from crosses between the treated plants of *T. aestivum* ditelocentric 5*B* and *S. cereale*. All these hybrids were scored at first metaphase of meiosis in order to determine the pattern of chromosome pairing. In 199 plants the majority of chromosomes were unpaired, although occasionally there was one bivalent and more rarely two (Fig. 1*a*). The mean pairing of a typical sample of five such plants is shown in Table 1. Infrequent pairing like this is characteristic of *T. aestivum* × *S. cereale* hybrids and no modification of the normal genetic regulation of meiosis was displayed in these plants.

In seven other hybrids bivalents were frequent, ranging from one to eight per cell. There were also trivalents,

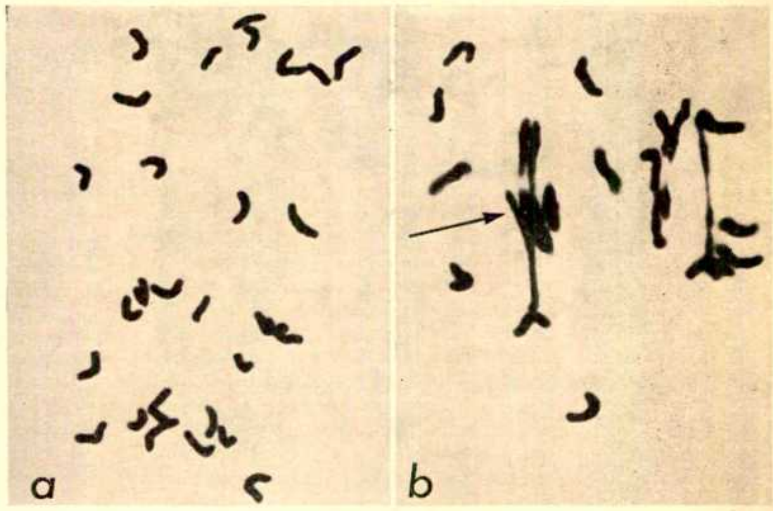


Fig. 1. Photomicrographs of first metaphase of meiosis in 28-chromosome hybrids from crosses between EMS-treated plants of *T. aestivum*, ditelocentric for chromosome 5*B*, and *S. cereale*. (*a*) Hybrid 5/10 with little chromosome pairing and therefore non-mutant, 28 univalents; (*b*) hybrid 81/15 with considerable chromosome pairing and therefore mutant, two trivalents, five bivalents and twelve univalents, the telocentric chromosome 5*B* is in a bivalent (arrowed)

Table 3. CHROMOSOME PAIRING AT FIRST METAPHASE OF MEIOSIS IN 28-CHROMOSOME HYBRIDS WITH HIGH PAIRING FROM THE CROSS OF EMS-TREATED *T. aestivum*, DITELOCENTRIC 5*B*, BY *S. cereale*
(20 cells per plant)

Hybrid	Chrom. 5*B*	Mean per cell					
		Univ.	Biv.	Triv.	Quad.	Quin.	Chiasmata
9/2	Centric fragment	19·40	3·40	0·60	—	—	4·95
5/12	Long arm telo.	21·10	2·40	0·70	—	—	3·80
8/12*	,, ,,	14·68	5·21	0·89	0·06	—	8·68
81/15	,, ,,	13·60	4·75	1·35	0·15	0·05	9·90

* 19 cells scored.

ranging from zero to six per cell, and occasionally a quadrivalent was present. The mean pairing in these hybrids is shown in Tables 2 and 3. The pattern of pairing was characteristic of that in *T. aestivum* × *S. cereale* hybrids deficient for chromosome 5*B* and it can be concluded that a change had occurred in the *T. aestivum* component of the genotype by which homoeologous pairing is normally prevented.

Of the seven plants with abnormally high pairing, three (9/1, 72/17 and 83/11) had only twenty-seven chromosomes, and were deficient for the telocentric by which the presence of chromosome 5*B* was normally marked. These plants were therefore totally deficient for chromosome 5*B* and displayed the meiotic behaviour characteristic of hybrids of this genotype (Table 2).

One other plant (9/2) had twenty-eight chromosomes, twenty-seven of which were apparently normal while one was a small centric fragment which was possibly an isochromosome. This fragment had apparently been formed by the deletion of a large terminal segment from the long arm of chromosome 5*B* because the telocentric for 5*B* was absent. Because the meiotic behaviour of this hybrid was very similar to that of those deficient for the entire chromosome, the region concerned with the prevention of homoeologous pairing must have been lost (Table 3). Consequently, this region is not immediately adjacent to the centromere.

Finally, three hybrids with high pairing at meiosis (5/12, 8/12 and 81/15) had normal chromosome complements, with twenty-eight chromosomes one of which was the 5*B* telocentric, apparently unchanged in structure. The meiotic behaviour of these plants was similar to that of those entirely deficient for chromosome 5*B* (Table 3) (Fig. 1*b*). The breakdown of the normally restricted pairing in these plants must be ascribed to gene mutation in the 5*B* system. This is not to imply that mutation had necessarily occurred at a locus on chromosome 5*B*, as the mutational origin of a suppressor of 5*B* activity elsewhere in the genotype could have given the same result.

All three hybrids displaying mutant phenotypes were derived from different EMS-treated parental plants of *T. aestivum*, so that all the mutants were of independent origin. In order to attempt to recover the mutants, seeds derived from self-pollination of the three parental plants of *T. aestivum* were grown. Eight plants, all of which had regular bivalent formation at meiosis, in the progeny of each parent were pollinated with *S. cereale*. In the resulting twenty-four families of *T. aestivum* × *S. cereale* hybrids, there were usually more than five plants and all these were checked at meiosis. A total of 159 hybrids were examined, but all had low levels of pairing comparable with that to be expected in normal *T. aestivum* × *S. cereale* hybrids. From this it was concluded that the mutants that had originated sectorially in the EMS-treated *T. aestivum* parents had not been transmitted to the progenies obtained by self-pollination. Because the *T. aestivum* × *S. cereale* hybrids with mutant phenotypes were sterile, the mutants responsible were therefore lost.

Nevertheless, important principles are displayed by this attempt to produce allelic variation in the 5*B* system. First, the origin by mutation of changes in the genetic control of chromosome behaviour is shown to be feasible. Secondly, by the use of EMS mutation can be induced with high frequency in the 5*B* system of *T. aestivum*. About 1·5 per cent of the gametes of the treated generation were mutant in the present work. In the absence of evidence as to whether the mutations had occurred on chromosome 5*B* or at suppressor loci elsewhere, nothing can be inferred about the nature of the regulation normally exercised by this chromosome. However, it is apparent that with appropriate modification of the experimental approach, further attempts to isolate and maintain mutants, giving homoeologous pairing in *T. aestivum*, are justified.

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BIOSYNTHESIS OF IMMUNOGLOBULINS. FREE LIGHT CHAIN AS AN INTERMEDIATE IN THE ASSEMBLY OF γG-MOLECULES

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THE diversity and specificity of antibody molecules make their formation a unique problem in macromolecular biosynthesis. Apart from the immunological aspects of the problem, immunoglobulins are a fascinating model for the study of the synthesis and assembly of a multichain protein with interchain disulphide bonds. (For a review of the structure see Cohen and Porter, ref. 1.)

Our present investigations, at a sub-cellular level, on the synthesis of immunoglobulins, utilize as a model system a murine plasma cell tumour (5563)[2] which produces and secretes substantial quantities of 7*S* γG-myeloma protein[3]. We have examined, in sucrose density gradients, the polyribosomes obtained from tumour cells pulse labelled with tritiated leucine, and by the use of specific antisera against parts of the γG-myeloma protein have demonstrated that different classes of polyribosomes are involved in the synthesis of light and heavy chains[4]. Synthesis of each type of chain occurs on polyribosomes of sufficient size to accommodate *m*RNA molecules coding for complete individual light or heavy chains[4].

The chains are therefore synthesized separately and then have to be assembled to form the complete molecule. This article deals with the initial steps in assembly. The results of our investigations show that completed light chains are released from ribosomes autonomously and that there is a pool of free light chains which we propose controls the release of heavy chains, and thus the balanced formation of multi-chain 7*S* γ-globulin.

Preparation and use of specific antisera. Three antisera, obtained from rabbits hyperimmunized by several courses of injections, were used in this study. (1) Anti-Fc-fragment: Rabbits were immunized with papain fragment Fc prepared after treatment of 5563 myeloma protein with papain[5]. The antiserum was absorbed with papain fragment Fab and so rendered specific for the heavy chain. After absorption the antiserum reacted with Fc-fragment and also with freshly prepared heavy chain and whole myeloma protein. It contained 3·5 mg antibody/ml. serum. (2) Anti-Fab-fragment: Rabbits were immunized with papain fragment Fab, the antiserum was absorbed with

Fc fragments and three successive times with small amounts of light chain of the myeloma protein. The light chain was prepared according to the method of Fleischman, Pain and Porter[6]. The antiserum did not bind iodine-131 labelled light chain, but still precipitated Fab-fragment and myeloma protein and contained 1·5 mg of antibody/ml. (3) Anti-light chains: Light chains were used for hyperimmunizing rabbits, and the resulting antiserum was absorbed with Fc-fragment; it still reacted with the whole myeloma protein and contained 2 mg/ml. of antibody to light chain.

For analysis of labelled proteins with these antisera, antigen/antibody equivalence points were established for each antiserum. Carrier light chain, papain fragments Fab or Fc were added to appropriate tubes and afterwards a slight excess of the selected antiserum (50–100 μl.) was also added. The mixture was incubated for 30 min at 37° C, stored overnight at +4° C. Type III pneumococcal polysaccharide antibody/antigen precipitate was then added as carrier and the precipitates were washed twice with cold saline and then with trichloroacetic acid (TCA) before counting on Oxoid membranes. Control aliquots of the samples were mixed with purified rabbit γ-globulin followed by goat anti-rabbit γ-globulin serum and then treated in the same way. The amount of radioactivity in this control precipitate was usually less than twice the background in the scintillation counter and was subtracted from radioactivity found in the specific precipitates formed by the antisera against myeloma protein or its fragments.

Pulse-labelling of proteins formed by plasma cell tumour 5563. Ascitic tumour cells (5563) were incubated *in vitro* with ^{3}H-leucine for short time intervals and then disrupted by treatment with deoxycholate. The soluble proteins were separated from the particulate cell components by layering the mixture on to a density gradient made up of 8 ml. of 30 per cent, 1 ml. of 10 per cent and 1 ml. of 4 per cent (w/v) sucrose, and centrifuging at 104,000*g* for 60 min. The top layer (2–3 ml.) was removed and, after brief dialysis in the cold against 0·14 M sodium chloride buffered with phosphate to *p*H 7·4 and then against dry 'Sephadex *G*-100' to concentrate the sample, the soluble proteins were analysed on 5–20 per cent sucrose gradients (Fig. 1*A*). Conditions were chosen which separated the myeloma protein ($S_{20}=6·6$) and the smaller molecular weight light chains ($S_{20}=2·0$). There was a clean separation of iodine-131 labelled myeloma protein and iodine-131 labelled alkylated light chain when the mixture was centrifuged overnight at 35,000 r.p.m. in a Spinco '*SW* 39' rotor (Fig. 1*B*).

Immunological identification of labelled proteins. The proteins labelled after a 5-min exposure to ^{3}H-leucine comprise a wide range of sizes (Fig. 1*A*). In order to detect labelling of myeloma protein and its precursors, equal portions of each gradient fraction were coprecipitated with rabbit antisera specific for various parts of the globulin molecule as already described. Two peaks of radioactivity specifically precipitable with antiserum were found (Fig. 1*A*); one was precipitated only by anti-light chain sera and had a sedimentation similar to free light chains; the other was precipitated by both antibody to light chain and to Fc-fragment. It corresponded in sedimentation rate to whole myeloma protein. Thus, after a 5-min pulse of ^{3}H-leucine, not only was radioactivity incorporated into whole myeloma protein, as might be expected, but also a substantial proportion of the radioactivity was in slower sedimenting material containing light chain but not the Fc portion of the myeloma protein. This material could be either free light chain or Fab-fragment. The antiserum to light chain has a low antibody/antigen ratio with myeloma protein. Since we add carrier light chain to each fraction and only a slight excess of antiserum, not all the myeloma protein is precipitated by the anti-light chain serum (Fig. 1*A*). The anti-Fc-fragment has a high antibody/antigen ratio with myeloma protein and there was sufficient antibody to bind all the myeloma protein present in the fraction.

Further characterization of both peaks of radioactivity was effected by means of an antiserum prepared against papain fragment Fab, extensively absorbed with light chains. This serum, which does not react with iodine-131 labelled light chain but still precipitates Fab fragment, precipitated exclusively the radioactivity in a single peak coinciding with the myeloma protein peak (Fig. 2). We failed to find any radioactivity which could be precipitated in the 3·5 *S* region of the gradient; under these conditions of ultracentrifugation, 3·5 *S* material would be clearly separated from 6·6 *S*; the arrow on Fig. 1*B* shows the position of iodine-131 labelled Fab-fragments on the gradient. The slower sedimenting peak of radioactivity, which could be precipitated with antibody to light chain, was precipitated neither by anti-Fc serum nor by anti-Fab serum absorbed with light chains, and for this reason the material in it clearly has the properties of labelled-free light chains.

Time dependence of labelling pattern. It was of interest next to study the soluble proteins from cells labelled for various periods of time. The labelled proteins from cells exposed to ^{3}H-leucine for 2, 5 and 10 min were analysed

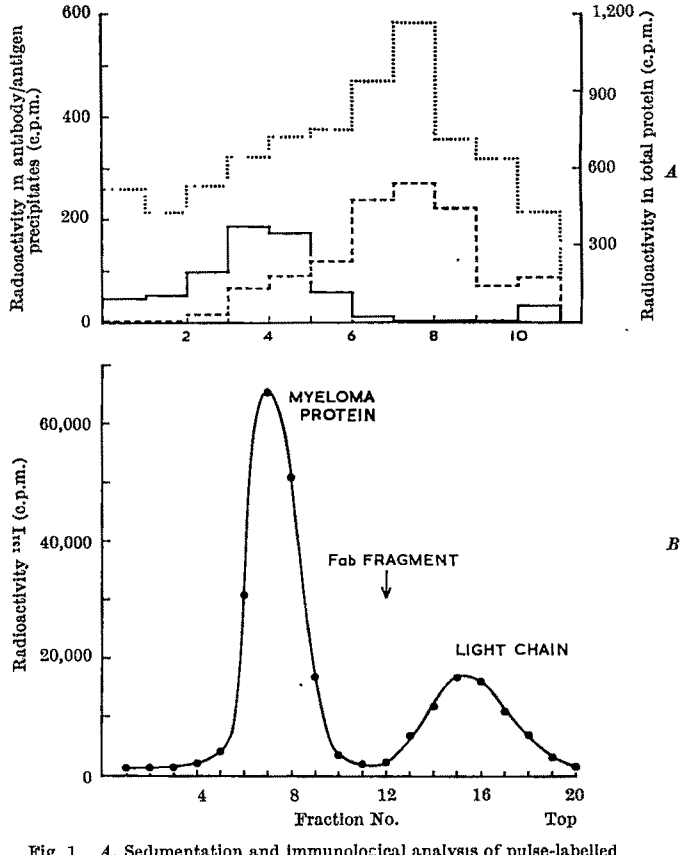


Fig. 1. *A*, Sedimentation and immunological analysis of pulse-labelled soluble proteins from 5563 ascitic tumour cells. Total radioactivity in protein, . . ; radioactivity in antibody/antigen complex precipitated with anti-Fc-fragment, ——; radioactivity in antibody/antigen complex precipitated with anti-light chain, - - - . Washed cells ($3·4\times10^8$) were incubated for 5 min at 37° C in Hanks's medium, containing 10 mg/ml bovine serum albumin, with 30 μc ^{3}H-leucine (specific activity 1·0 c/mmole) in a final volume of 4·6 ml. Cells were disrupted with deoxycholate (0·5 per cent), the soluble protein fraction isolated, and aliquots (0·5 ml.) applied to 4·8 ml. linear gradients 5–20 per cent sucrose w/v in phosphate buffer saline. After centrifugation at 35,000 r.p.m. for 16 h in Spinco '*SW* 39' rotor, fractions (thirty drops) were collected; equivalent fractions were pooled from two gradients. Equal portions of each fraction were precipitated with 5 per cent TCA and filtered on Oxoid membranes for counting in a Packard 'Tri-Carb' scintillation counter. Other portions were treated in the presence of carrier antigen with antisera specific for light chains or Fc papain fragment or, as control, with goat anti-rabbit γ-globulin serum. *B*, Sedimentation pattern of iodine-131-myeloma protein and iodine-131-light chains in a sucrose gradient. Centrifugation conditions were as already described. Fractions half the size of the fractions in Fig. 1*A* were counted for iodine-131 radioactivity directly in an Echo scintillation counter

Table 1

	Radioactivity of specific antibody/antigen precipitates (c.p.m.)	
Time of labelling:	4·5 min	10 min
Myeloma proteins*	1,307	3,305
Light chain†	953	1,360

* Myeloma protein precipitated with anti-Fc serum until no further counts are precipitated.
† Precipitation with anti-light chain following the treatment with antibody to anti-Fc-fragment which had precipitated the myeloma protein.

on sucrose gradients as already described. In each case only two peaks of specifically precipitable radioactivity were found, corresponding to myeloma protein and light chains (Fig. 3). The soluble proteins examined for each time point are derived from separate experiments and the absolute radioactivity of the peaks is not comparable from one experiment to the next. However, between 2 min and 10 min the extent of labelling of myeloma protein has been found to increase linearly (Table 1) and consequently the amount of radioactivity in the light chains compared with that in myeloma protein at each time point can be given an approximate quantitative interpretation. After exposure to ^{3}H-leucine for 2 min a considerable proportion of the radioactivity of the soluble protein fraction which can specifically be precipitated consists of free light chains, although radioactivity is already found in the whole myeloma protein peak (Fig. 3*A*). It must be emphasized that at this time most of the incorporated radioactivity is in the nascent peptide chains bound to ribosomes[4], which have already reached almost maximum specific activity. After exposure to labelled amino-acid for 5 min, radioactivity in free light chains is still of the same order as that found in myeloma protein, which implies that between 2 min and 5 min incorporation into both light chains and myeloma protein is proceeding at comparable rates. However, after 10 min exposure most of the radioactivity which can specifically be precipitated is found in 7*S* myeloma protein while only a small proportion is found in free light chains (Fig. 3*C*). We interpret this as meaning that the total radioactivity in free light chains tends towards a constant value, while incorporation into myeloma protein is still increasing linearly. This is clearly shown (Table 1) by measuring the total radioactivity of myeloma protein antibody/antigen precipitates, which increased greatly between 4·5 min and 10 min, whereas the radioactivity of the light chain showed a much smaller increase.

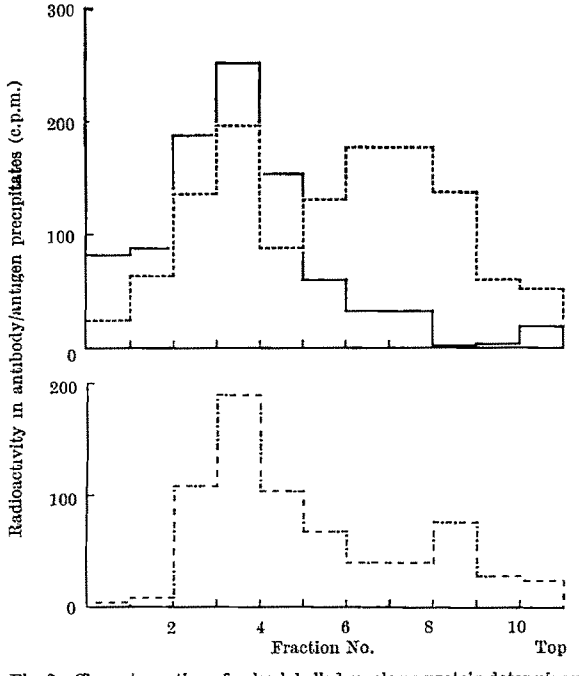


Fig. 2 Characterization of pulse-labelled myeloma protein determinants by sedimentation and immunological properties. Radioactivity in antibody/antigen precipitates on treatment of gradient fractions with antiserum to papain fragment Fc, ——; anti-light chain serum, - - - , antibody to Fab absorbed with light chain, — · — · — · —. Procedures were as described under Fig. 1, except that 3·3 × 10^6 cells were incubated with 20 μc. ^{3}H-leucine in a final volume of 2·6 ml.

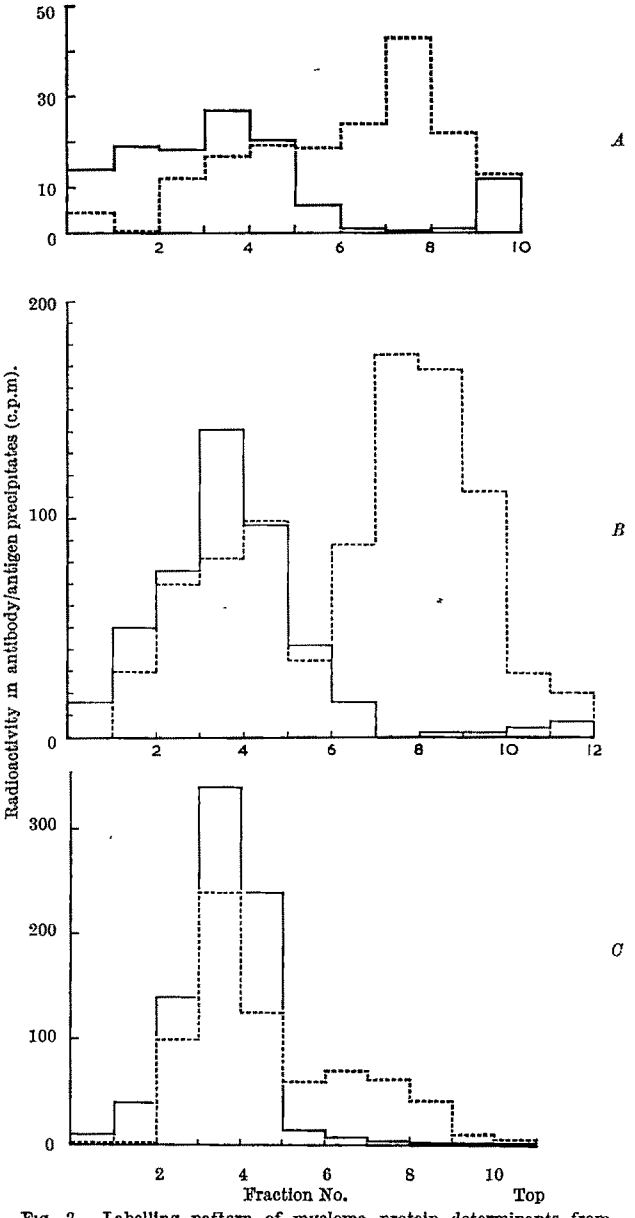


Fig. 3. Labelling pattern of myeloma protein determinants from ascitic cells incubated with ^{3}H-leucine at 37° for *A*, 2 min; *B*, 5 min, *C*, 10 min. Radioactivity antibody/antigen precipitated on reaction with antiserum to papain fragment Fc, ——; radioactivity in anti-light chain antibody/antigen precipitate, - - - - . Each time point represents a separate experiment, using incubation mixtures similar to those described in Fig 2. The volumes loaded on to the sucrose gradients were equal but represented varying proportions of the total soluble protein

It is apparent that the cell contains a small pool of free light chains in addition to completed myeloma protein. However, we would emphasize that 5563-tumour cells have been found to secrete only 7*S* myeloma protein[2] and do not produce an excess of light chains. Secretion of light chains in the serum or urine of mice bearing this tumour has not been detected. From the extent of labelling of the light-chain pool relative to that of myeloma protein at various times it would seem that free light chains are transient intermediates in the assembly of 7*S* γ-globulin. The data presented here do not permit an accurate measurement of the size of the pool of light chains, but it is small compared with the total content of intracellular

γ-globulin, which corresponds very approximately to 30 min production[7–9].

The failure to detect free heavy chains by the methods employed here does not rule out their presence, in small amounts, as heavy chains tend to aggregate and sediment in a similar position to 7*S* myeloma protein. However, the fact that antisera against both papain fragments Fab and Fc precipitated radioactivity only in the region of the myeloma protein in an identical manner, and failure to detect smaller molecular weight Fab and Fc fragments on the sucrose gradient, are highly indicative of the absence of naturally occurring Fc and Fab fragments. This suggests that these fragments are not intermediates in the biosynthesis of γ-globulin, but that the molecule is assembled directly from light and heavy chains. Light chains seem to be released autonomously from their site of synthesis and to exist for a short time as free chains before being incorporated into γ-globulin. Heavy chains, on the other hand, are either not capable of autonomous release or else are released much less efficiently than light chains, and we propose that heavy chain release is normally effected by association with light chains. On this hypothesis the pool of free light chains would control the rate of release and thus the rate of synthesis of heavy chains. Such a model provides for the balanced production of light and heavy chains under normal conditions. Moreover, the secretion of light chains (Bence Jones proteins), which occurs in some plasmacytomata, could be readily explained either by a defect in heavy chain synthesis or by an increased rate of light chain synthesis.

Our proposal of an initial assembly step, in which light chains release heavy chains, has a parallel in the mechanism of haemoglobin assembly, in which it has been postulated that free β-chains release completed α-chains from ribosomes[10]. While such an assembly mechanism for haemoglobin is supported by good evidence[10,11], no pool of labelled β-chains was found[10]. Although the model would predict the existence of a pool of free β chains, they would be in equilibrium with haemoglobin thus: $\alpha_2\beta_2 \rightleftarrows 2\alpha\beta \rightleftarrows 2\alpha + 2\beta$, as shown by Guidotti *et al.*[12]. The pool would therefore not be detected by pulse-labelling.

We thank Mr. Brian Wright and Mrs. Joyce Henderson for their excellent assistance and Dr. M. Potter, National Institutes of Health, for originally supplying the 5563 plasma cell tumour line.

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INCREASE IN PLASMA GROWTH HORMONE LEVEL IN THE MONKEY FOLLOWING THE ADMINISTRATION OF SHEEP HYPOTHALAMIC EXTRACTS

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THE control of anterior pituitary secretion is now generally accepted as being mediated by hypothalamic neurohumoral factors which are secreted into the primary plexus of the portal circulation to the anterior pituitary and which, on arrival at the anterior pituitary, promote the release of most anterior pituitary hormones. Although much work has been done on the hypothalamic releasing factors for most of the anterior pituitary hormones, it has been only very recently that such investigations have been directed to the search for a growth hormone (GH) releasing factor. Both *in vitro* and *in vivo* studies have been carried out. In the *in vitro* investigations the release of GH into a medium in which pituitary fragments are incubated and to which hypothalamic extracts have been added has been quantitated[1]. The *in vivo* studies have been carried out by assaying for residual GH in the pituitaries of animals injected with hypothalamic extracts[2]. In both cases, the GH has been assayed by the tibia test[3]. With the availability of sensitive radioimmunoassay techniques for GH (refs. 4 and 5), it seemed worth while to explore the possibility of demonstrating the release of GH into the circulation after the administration of hypothalamic extracts.

The radioimmunoassay for human growth hormone has been shown to be useful in the measurement of the plasma concentration of other primate growth hormones[5]. The method used in the present experiments for the radioimmunoassay of GH has been modified from that of Hunter and Greenwood[6]. Human growth hormone (HGH) (kindly supplied by Prof. C. H. Li, University of California, Berkeley) was used to immunize rabbits, and also labelled with iodine-125 using the chloramine-*T* method of Hunter and Greenwood[6]. The antibody-bound HGH–iodine-125 was precipitated using a goat anti-rabbit gamma globulin serum (obtained from Antibodies, Inc., Davis, California). The percentage of the labelled HGH which was bound to antibody was plotted against the logarithms of the concentrations of standard HGH (a gift from Prof. A. E. Wilhelmi, Emory University, Atlanta, Georgia. Lot No. HS503A had a specific activity of 1·7 I.U./mg, standardized in terms of the bovine GH international standard). The concentrations of monkey plasma GH were read directly off such a curve and are therefore expressed in terms of the standard HGH preparation used.

The plasma GH level has been shown to respond to many factors; insulin-induced hypoglycaemia, prolonged fasting, muscular exercise and the administration of certain amino-acids will all result in high plasma GH levels[7–9]. On the other hand, administration of glucose will depress the elevated plasma GH level of the hypoglycaemic or fasting individual[7]. In the normal human the response to insulin hypoglycaemia is quite consistent.

Male rhesus monkeys (*Macaca mulatta*) weighing between 10 and 20 lb. were used throughout the course of this work. The animals were anaesthetized with 'Nembutal' and a polyethylene cannula was placed in a branch of the saphenous vein in the lower leg. At approximately 1 h after the administration of the anaesthetic, the collection of heparinized blood samples was begun and was continued over a period of approximately 6 h. Additional 'Nembutal' was administered as needed to maintain the anaesthetic state. After a 1–2-h period, during which control blood samples were taken, the test material was injected intravenously using the cephalic vein in the forearm.

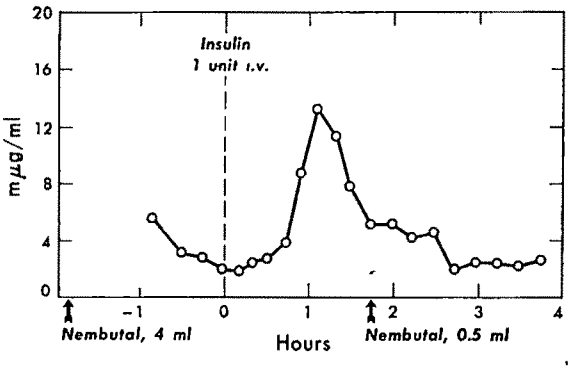


Fig. 1. Plasma growth hormone concentration in rhesus monkey after insulin hypoglycaemia

Initially, the investigations were carried out using insulin hypoglycaemia as the stimulus for GH release. Fig. 1 shows the results of one such study in which one unit of glucagon-free insulin (Lilly) was injected. The plasma GH level begins to rise at approximately 40 min, reaching a high level by 1 h. This response is not essentially different from that seen in the unanaesthetized normal human. The importance of this response, however, is that it shows that the mechanisms responsible for the reflex release of GH are intact in the nembutalized monkey and that such a preparation can be used to study the release of GH. Many of the monkeys show a high GH level at the beginning of such a study. This is perhaps due to stress or to muscular exercise expended by the monkey attempting to evade capture and restraint before the injection of the 'Nembutal'.

Extracts of sheep hypothalami were prepared by extracting acetone powder representing 200 gland (stalk-median eminence plus adjacent areas) equivalents of sheep hypothalami by homogenization with 10 ml. of 0·1 N hydrochloric acid. The homogenate was then diluted with an additional 40 ml. of acid and stirred for 30 min. The material was centrifuged for 40 min at 8,000*g*, and the residue was re-extracted with 30 ml. of 0·1 N hydrochloric acid for 30 min and then centrifuged as before. Up to this point all operations were carried out at 4° C. The supernatants were combined, heated to 90° C in a boiling water bath (approximately 5 min), chilled to 15° C, neutralized to *p*H 6·4 with 1 N sodium hydroxide and centrifuged for 20 min at 35,000*g* at 4° C. The supernatant was diluted to 100 ml. with distilled water, distributed into ten vials and frozen. The final concentration was two gland equivalents per ml. Extracts of sheep cerebral tissue were also prepared in the same manner and were diluted to a final concentration representing a weight of tissue equivalent to that of the hypothalamic extract.

The response to the intravenous injection of sheep hypothalamic extracts was investigated in eight experiments. In seven of the eight experiments a release of GH was observed following the injection. Four such studies, in which 4·5–5·0 ml. (nine or ten sheep hypothalami) of the extract was administered, are presented in Fig. 2. There was a great variation in the magnitude of the response. The reasons for this great variation are not entirely clear and at this point one can only speculate on their possible causes. It would seem possible that the state of the anterior pituitary before the administration of the extract may be a factor. The recent release of a large quantity of GH may make impossible a further large release in response to the administration of these extracts. Other factors influencing this variation may be related to possible variations in the anterior pituitary portal blood flow or to the depth of the anaesthetic state. Investigations directed to minimizing the variations in the magnitude of these responses will be necessary before the monkey can be used as an assay animal for determining GH releasing factor levels.

The responses of the plasma GH level to the injection of hypothalamic extracts are all similar, however, in their time relationships. The GH level begins to rise between 20 and 30 min and reaches peak values between 45 and 60 min after the injection. At this time GH secretion may stop rather abruptly, for the GH levels decrease thereafter at a rate consistent with the half-life found in man for HGH (ref. 10). These temporal relationships are consistent with the type of response seen following the injection of insulin.

Since hypoglycaemia appears to be a potent releaser of GH, plasma glucose determinations were made in most of these investigations. In no case was a fall in plasma glucose observed; in fact, usually a slight rise in plasma glucose was observed following the injection of the hypothalamic extract. However, in order to rule out positively the possibility of a GH release mediated by changes in plasma glucose level, in two of the hypothalamic extract studies the animals were infused, as well, with glucose. Glucose infusion, at a rate of 75 mg/min, was started 1 h before the injection and continued for 3 h. This brought the plasma glucose level to approximately 300 mg/100 ml. at the time of the injection of the hypothalamic extract, and it remained between this value and 340 mg/100 ml. throughout the remaining glucose infusion period. In both the glucose infusion experiments the hypothalamic extract caused a release of GH similar to those depicted in Fig. 2 (peak values of 12 and 36 mμg per ml.).

As a control to the investigations using hypothalamic extracts, extracts of cerebral tissue were injected. The results of four such experiments are shown in Fig. 3. In two of the experiments there was no change in the plasma GH level after the administration of the extracts. In the other two investigations there was a release of GH after the injection. This release, however, occurred much later than that following the injection of hypothalamic extracts, for a period of approximately 2 h elapsed before rises in plasma GH levels were observed.

Although these late responses are disturbing, it must be emphasized that in all four of the cerebral extract experiments no rise in plasma GH occurred during the period of time in which responses to hypothalamic extract or to insulin hypoglycaemia are evident. It is felt that these responses, although real, are not specific to a releasing factor but are reflex responses to other stimuli. In this regard, it is perhaps worth while to mention that an increase in plasma GH levels has been observed following the injection of certain amino-acids[9]. The depth of the anaesthetic state and stress must also be considered as possible contributing factors in these late GH responses.

Although several recent investigations in rats have failed to implicate vasopressin as a mediator of GH

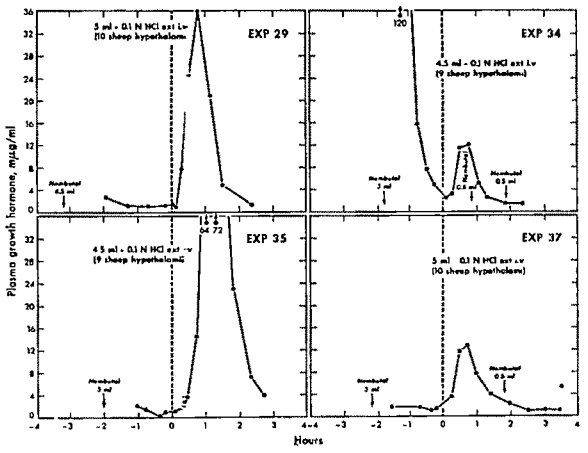


Fig 2. Plasma growth hormone concentration after administration of hypothalamic tissue extract

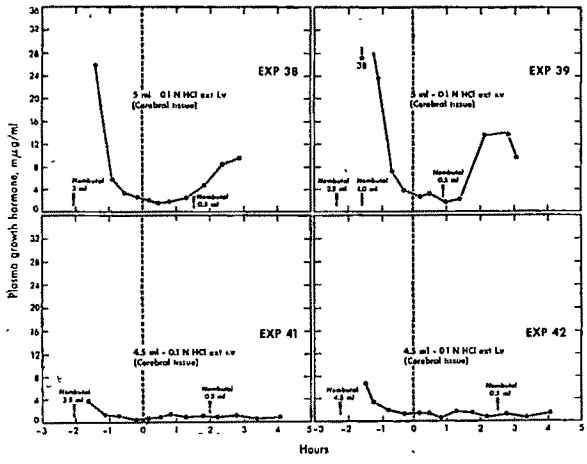


Fig. 3. Plasma growth hormone concentration after administration of cerebral tissue extract

secretion[11,12], the effect of vasopressin injection was studied in the present experiments. In our hypothalamic extract the concentration of vasopressin was 0·506 U./ml. (We thank Prof. R. Guillemin, Baylor University, Houston, Texas, for this assay.) Therefore, in the volume of hypothalamic extract injected, 2·28 or 2·53 U. of vasopressin were present. Two animals were therefore each injected with 5 U. of 'Pitressin', which resulted in a small, immediate increase in the plasma GH level. The first samples, taken about 15 min after the injection, were twice the basal level, and from this maximum the levels then decreased exponentially. Since the response to an amount of vasopressin twice that present in the hypothalamic extract was so minimal, the responses observed with the hypothalamic extract could not be due solely to its vasopressin content. The GH response to vasopressin injection is perhaps a non-specific response resulting from the well-known vasomotor effects of this hormone. In still another study the injection of 5 U. of 'Pitocin' did not result in a release of GH.

In conclusion, we feel that this preliminary work supports the concept that the secretion of anterior pituitary GH is mediated by a hypothalamic neurohumoral factor.

We thank Mrs. Patricia Smith and Mr. Robert Dewey for expert technical assistance, and Dr. J. A. Linfoot for his advice.

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Since the article was prepared for publication, a report on similar experiments by E. Knobil has appeared[13]. This investigator has found responses to the administration of porcine hypothalamic extract similar to those reported here. The results differ, however, in that the responses to the extract were more rapid than those we have observed, and the responses to the injection of vasopressin were more marked than ours, the greatest responses in any of the experiments having been obtained with vasopressin. The experiments differ in that in Knobil's experiments the monkeys were unanaesthetized but restrained, the extract injected was equivalent to only two porcine hypothalami, and the antiserum utilized for the immunoassays was developed using a highly purified simian growth hormone as antigen. Whether these differences in procedure explain the differences in response to extract or vasopressin remains to be determined.

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RESPONSE OF PINEAL SYMPATHETIC NERVE PROCESSES AND ENDINGS TO ANGIOTENSIN

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IN the past, investigators have felt that the hypertensive properties of angiotensin II were due entirely to the peripheral actions of the compound[1–3]. Recent experimental evidence has suggested that in addition to the peripheral action on the muscle, a second mechanism exists which acts centrally. The experiments of Bickerton and Buckley[4], Buckley *et al.*[5], Laverty[6], Zimmerman[7], and Benelli *et al.*[8] have stimulated interest in a nerve-mediated influence of angiotensin. The purpose of this investigation was to study the morphological effects of angiotensin on sympathetic nerve processes and endings of the rat, with particular attention given to the synaptic vesicles and their contents.

Male rats of the Holtzman strain averaging 150 g at the onset of the experiment were selected for the present investigation. They were placed on purified diets with complete salts[9]. Tap water was given for drinking purposes.

The animals were placed in groups and treated as follows: twelve intact rats weighing 170 g at autopsy received 5 per cent glucose subcutaneously without angiotensin for 12 days and were considered as a control group. Six intact rats weighing 190 g at autopsy were given 6·0 gamma/day angiotensin II (kindly donated by Ciba, Ltd.) in 5 per cent glucose administered subcutaneously for 12 days. At autopsy, the animals were decapitated and the pineal glands quickly removed and fixed in 2 per cent osmium tetroxide. The tissues were dehydrated and embedded in the epoxy resin, 'Araldite', according to the methods described by Luft[10]. Thin sections of random samples of nerve processes and endings were then examined and studied with the RCA 'Emu-3G' microscope. Fig. 1 was prepared by selecting parallel electron micrographs and comparing the number of clear vesicles with those containing osmiophilic material in both the control and angiotensin-treated rats.

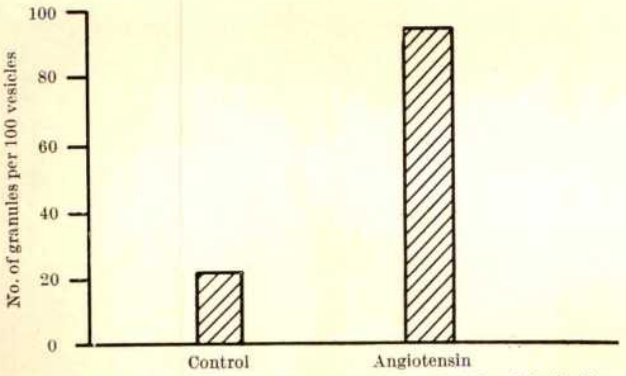


Fig. 1. Histogram comparing the number of granulated vesicles in the control and angiotensin-treated animals

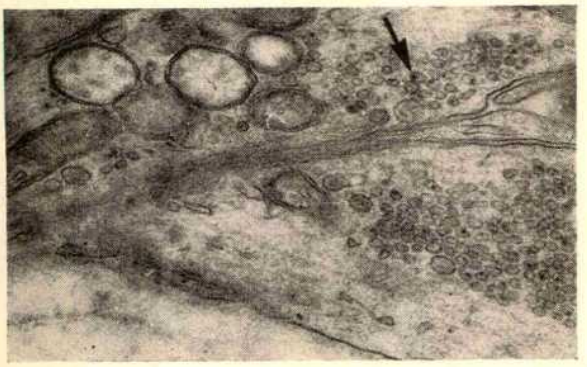


Fig. 2. Electron micrograph (× *c.* 18,000) of rat pineal gland showing a normal sympathetic nerve ending. Note the clear vesicles and vesicles containing small black (osmiophilic) granulated vesicles (arrow)

Angiotensin was administered subcutaneously in these experiments as this route of administration has been shown to be effective in reducing kidney pulse[11].

The results obtained were as follows.

Control. The nerve processes and nerve endings of the intact controls contained numerous vesicles exhibiting a heterogeneous morphology, some containing osmiophilic granules and some without. These synaptic vesicles have a definite limiting membrane and a mean diameter of 425 Å. Most of the vesicles have a clear centre, but some exhibit a dense osmium deposit about 210 Å in diameter (Fig. 2).

Angiotensin. Similar vesicles were observed in the angiotensin-treated animals; however, those containing osmiophilic granules were observed to be markedly increased when compared with sympathetic nerve endings of the control animals (Figs. 1–4).

Electron microscope studies thus established the presence of granular and agranular vesicles in many autonomic nerve endings and neurites. The granular vesicles of neurites have an average diameter of 320 Å. while those of the nerve endings are 580 Å. These granulated vesicles contain an electron-dense core 200–300 Å wide. The agranular vesicles have an average diameter of 520 Å (refs. 12–14). Recent evidence has strongly suggested that granulated vesicles contain norepinephrine[12,14]. These suggestions are based on the following circumstantial evidence: the morphological similarity of a limiting membrane and dense core observed in granulated vesicles and chromaffin cell granules[15]; the known concentration of norepinephrine in sympathetic nerves[16]; and the evidence from centrifugation studies of splenic nerve homogenates, showing that at least 20 per cent of norepinephrine is associated with "particles" somewhat similar to catecholamine containing granules from adrenal homogenates[17]. Further evidence has been presented by Wolfe *et al.*[14], who showed that circulating tritiated-norepinephrine (^{3}H-NE) was taken up into non-myelinated axons, that autonomic axons incorporating tritiated norepinephrine were adrenergic, and that the constant association of autoradiographic grains with granulated vesicles directly demonstrated the constant association of tritiated norepinephrine with granulated vesicles.

The pineal body was examined because of the richness of its sympathetic innervation[18], its known concentration of norepinephrine[19], the availability of electron microscopic descriptions of pineal autonomic nerves[13] and the



Fig. 3. Electron micrograph (× *c.* 50,000) of rat pineal gland showing sympathetic nerve ending following treatment with angiotensin. Note the marked increase in vesicles containing granules (arrows) when compared with Fig. 1

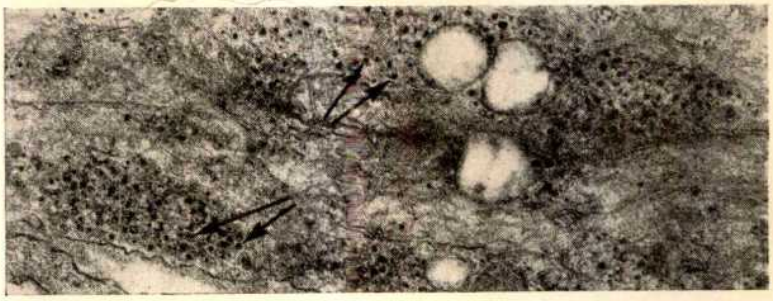


Fig. 4. Electron micrograph (× *c.* 31,000) of rat pineal gland showing sympathetic nerve processes following treatment with angiotensin. Note the marked increase in granulated vesicles (arrows)

demonstration of tritiated norepinephrine uptake in sympathetic axons of rat pineal gland[14].

Angiotensin is a humoral agent possessing powerful pressor properties. Its direct action on blood vessels has been demonstrated *in vivo* and *in vitro* and in a variety of preparations[1-3]. However, recent evidence has indicated that angiotensin may act centrally, stimulating the sympathetic nervous system. The results presented here (Figs. 1–4) would seem to support the hypothesis that angiotensin affects peripheral sympathetic transmission by promoting a greater output of noradrenaline[8]. Recent work by Bickerton and Buckley[4] and Buckley *et al.*[5] has demonstrated that angiotensin administered to the isolated perfused head of a dog caused neurogenic vasoconstriction in the trunk and limbs of the animal. Robertson and Ruben[20] reported that drugs which interfere with cholinergic transmission (ganglion blocking and anti-acetylcholine drugs as well as botulinum) are able to alter significantly the typical stimulation by angiotensin. Furthermore, Zimmerman[7] has observed that the vasopressor response to angiotensin administered extra-arterially to the perfused hindquarters of the dog was reduced significantly after acute sympathectomy. Similar results were obtained following ganglionic blockade with hexamethonium and cervical spinal transmission.

Beaulnes[21] investigated the positive inotropic and chronotropic actions of angiotensin on the isolated heart and concluded that they are at least partially due to a release of catecholamines. Similar conclusions were reached by Berry *et al.*[22] in perfusion studies of the dog. Feldberg and Lewis[23] have also reported an adrenaline-releasing effect of angiotensin on cat adrenal glands.

The results presented here would seem to provide evidence supporting the hypothesis that angiotensin, in addition to its peripheral action, may act centrally, stimulating the sympathetic nervous system. The granular vesicles of neurites and nerve endings were significantly increased in all animals treated with angiotensin. Therefore, it is possible that the pressor activities of angiotensin might be explained by the increase in adrenergic material in the endings of the sympathetic nerves. Further work is required in order to confirm that such a mechanism of action for angiotensin is possible.

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USE OF NEUTRON ACTIVATION IN THE CHARACTERIZATION OF SMALL QUANTITIES OF NUCLEIC ACIDS

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ONE approach to the characterization of nucleic acids involves the examination of maps of enzymatic digests of these substances. Such maps are produced by the application of two of a large number of separating systems at right angles on paper. The oligonucleotide spots can then be located by, among other methods, ultra-violet absorption[1] (minimum quantity detectable 1–2 μg base/cm², that is, 0·1–0·2 μg phosphorus/cm²) or the molybdate test for phosphate[2] (sensitivity about 1 μg phosphate/cm²). If it is only possible to prepare minute quantities of starting material, or if the particular system in use is subject to overloading (see below), it is customary to label the nucleotides with either carbon-14 or phosphorus-32 during biosynthesis and to detect the spots by autoradiography. In this way extremely small quantities of nucleic acid can be examined. There are occasionally circumstances where it is wished to work with such small quantities but where biosynthetic labelling is difficult or impossible. This is particularly true of materials from the higher animals, including man.

This article describes a procedure for the location of oligonucleotide and other phosphorus-containing spots by neutron activation of the completed chromatogram and subsequent autoradiography. It is most unlikely that even those oligonucleotides which have not interacted with a neutron will remain in the original chemical form. The procedure resembles that described by Benson[3] in connexion with work on phospholipids, although in the present case a considerably stronger irradiation seems to be necessary to achieve the same sensitivity.

The particular application discussed here is the mapping of digests of DNA from higher animals by the method of Sanger, Brownlee and Barrell[4]. This method, although giving a considerably greater degree of resolution than most two-dimensional systems, has not previously been used on non-radioactive material because the system overloads if more than 200 μg of digest are used. Because neutron activation gives a sensitivity of the order of 10^{-2} μg of phosphorus/cm², it is adequate for the location of spots in digests of materials which are only available in quantities of this order.

The principle is that phosphorus-31 can be converted by thermal neutrons to phosphorus-32. Thus, in the neutron flux of a typical graphite reactor, the saturated specific activity at 10^{12} neutrons/cm²/sec is about 100 mc./g of phosphorus[5]. One-quarter of this activity can be obtained after irradiation at this flux for 7 days. There is no major component of paper with a similar response to irradiation and, provided that a few days are allowed for the decay of short-lived activities, phosphorus-32 remains as the predominant substance detectable by autoradiography. This is particularly so if a thin sheet of material is interposed between the chromatogram and the film (for example, another sheet of unexposed X-ray film) in order to filter out soft radiations from contaminating activities. It is desirable to keep the papers as clean as possible before irradiation. Moisture from the fingers in particular contains sufficient sodium to interfere by virtue of its conversion to sodium-24.

Preliminary experiments were carried out in the irradiation facility in *Bepo* (Atomic Energy Research Establishment, Harwell) at a thermal neutron flux of 7×10^{12} neutrons/cm²/sec. The operating temperature (about 150° C) and the high fast neutron and gamma fluxes in this facility made the paper extremely brittle and it was impossible to unwrap rolls of paper after irradiation. However, a surface concentration of adenosine triphosphate (ATP) equivalent to 4×10^{-2} μg of phosphorus/cm² could be detected. The experiments also served to test a variety of washing procedures and a number of different types of paper. Washing was found to make very little difference to the background, which was, incidentally, considerably worse with DEAE paper than with the normal cellulose grades. Damage to the paper did not vary greatly among the common commercial grades of chromatography paper, with the exception of DEAE-paper which was affected more than the unmodified forms. Cellulose acetate ('Oxoid') melted.

Subsequent irradiations were carried out in *Herald* (Atomic Weapons Research Establishment, Aldermaston), a water-moderated reactor with a graphite thermal neutron column. A flux of $2{\cdot}5 \times 10^{11}$ neutrons/cm²/sec was used in irradiations lasting about 2 weeks. The much lower temperature of the samples and/or relative lack of fast neutrons and gamma radiation resulted in the paper remaining in fairly good condition after this time.

In an experiment designed to test the method, a mixture of unlabelled calf thymus DNA (200 μg) (Sigma Chemical Company) and ^{32}P-labelled DNA from *Escherichia coli*[6] (5 μg; 3×10^5 c.p.m.) was denatured by heat treatment and was then digested with *Neurospora crassa* endonuclease[7]. (Nucleotide maps of digests made with this enzyme were closely similar to those made with pancreatic DNase[8] (Worthington Biochemical Corporation).) The method of Sanger *et al.*[4] has provided highly reproducible nucleotide maps when applied to endonuclease digests of several independent preparations of DNA from *E. coli*[3]. An autoradiograph was made of the DEAE-paper. After storage for about 12 months, when decay of phosphorus-32 was virtually complete, the DEAE-paper was cut into strips which were interleaved with aluminium foil, rolled up, and inserted in an aluminium can for neutron irradiation. Following irradiation in *Herald* as already described, the strips were carefully unrolled, attached to a sheet of Whatman 3*MM* paper with 'Sellotape', and autoradiographed through an additional sheet of X-ray film used as a screen. The two autoradiographs are compared in Fig. 1. The initial autoradiograph (Fig. 1*A*) shows only the nucleotides derived from *E. coli* DNA. After neutron activation, all the nucleotides were radioactive, but as there was almost fifty times more calf thymus DNA present the contribution from the nucleotides from *E. coli* DNA was negligible. Thus, the second autoradiograph (Fig. 1*B*) represents only the nucleotides derived from calf thymus DNA. The nucleotides occupied an area of 222 cm² (the darkened area of Fig. 1), indicating an average surface concentration of nucleotide on the DEAE-paper of about 1 μg/cm², which is equivalent to a surface concentration of phosphorus of about 0·1 μg/cm². However, appreciable losses of material occurred in the course of preparation of the digest owing to adsorption on glass and polythene surfaces; thus the actual average surface concentration of phosphorus is probably nearer 0·05 μg/cm². It thus seems quite reasonable to claim that some of the weaker spots on the autoradiograph shown in Fig. 1*B* represent a surface concentration of phosphorus of the order of 10^{-2} μg/cm².

Although most of the spots on the two autoradiographs are in identical positions, as would be expected for the products of an enzyme with a rather broad specificity, several distinct differences are readily apparent. These differences are given in Table 1; four of the nucleotides are present in the digest of *E. coli* DNA, but are essentially absent from the digest of calf thymus DNA, while a fifth nucleotide is considerably more abundant in the digest of calf thymus DNA than in that of *E. coli* DNA. The composition of these nucleotides was determined (after elution from DEAE-paper in a separate experiment) by digestion with snake venom phosphodiesterase[8]. The observed differences in nucleotides between the two DNA digests are consistent with the relative abundance of the dinucleotides found in a pancreatic DNase digest of calf thymus DNA by Sinsheimer[9], and with the nearest neighbour nucleotide frequencies determined by Josse, Kaiser and Kornberg[10]. On the basis of the relative frequencies of the dinucleotide sequences CpG and GpC, which are given in Table 2, it might be expected that the sequence pCpG could be found in the nucleotides that were present in the digest of *E. coli* DNA but absent from the digest of calf thymus DNA (that is, nucleotides 5, 7, 12 and 15 in Fig. 1).

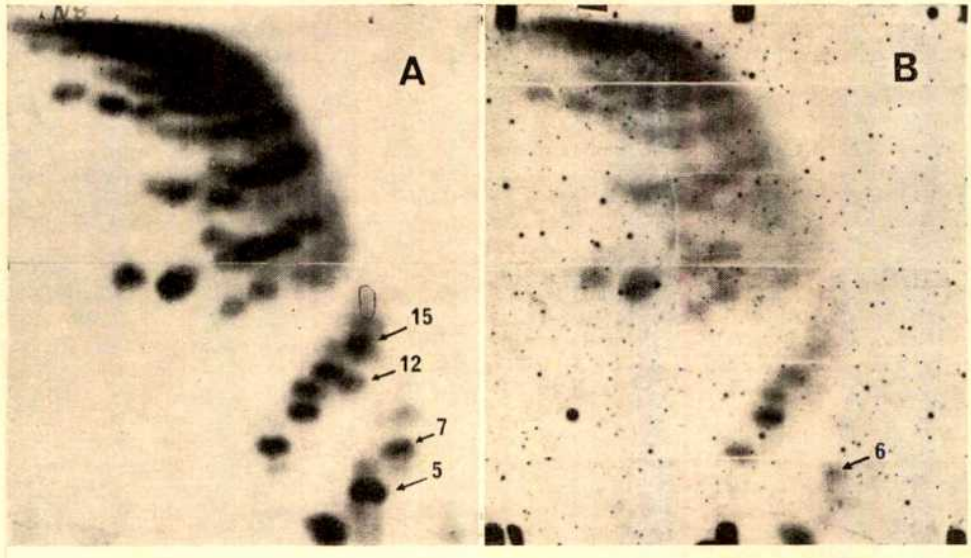


Fig. 1. Autoradiographs of the ionophoretogram of a DNase digest of a mixture of calf thymus DNA and *E. coli* DNA. *A*, Original autoradiograph of the nucleotides formed from phosphorus-32-labelled *E. coli* DNA. *B*, Autoradiograph made after a long period of storage for radioactive decay followed by neutron irradiation. The rectangular dark patches at the top and bottom of this autoradiograph are due to the activation of some component of pieces of 'Sellotape' that had remained on the DEAE-paper after the first autoradiograph. The base composition of numbered nucleotides is given in Table 1

Table 1. BASE COMPOSITION OF THE NUCLEOTIDES THAT WERE FOUND ONLY OR PREDOMINANTLY IN ONE OF THE NUCLEIC ACIDS CONTRIBUTING TO THE MIXED DIGEST

Nucleotide No.	Composition	*E. coli* DNA	Calf thymus DNA
5	CG	+	−
6	C_2T	(−)	+
7	C_2G	+	−
12	ACG	+	−
15	AC_2G	+	−

Table 2. NEAREST NEIGHBOUR FREQUENCIES OF THE CG DINUCLEOTIDES IN *E. coli* DNA AND CALF THYMUS DNA. THESE ARE VALUES PUBLISHED BY JOSSE *et al.* (ref. 10)

Dinucleotide	*E. coli* DNA	Calf thymus DNA
CpG	0·067	0·016
GpC	0·083	0·044

No other dinucleotide frequencies approach these two in the magnitude of the difference between the two species.

Thus, neutron activation of nuclease digests after two-dimensional ionophoretic separation may be used to obtain nucleotide maps on small quantities (100–200 μg) of nucleic acids from sources that cannot conveniently be labelled biosynthetically. This should facilitate a more detailed examination than has hitherto been possible of nucleic acids from large plants and higher animals (including the obvious example of tumour materials), as sufficient nucleic acid could usually be obtained from about 100 mg of tissue. Inclusion of an internal reference of phosphorus-32 labelled nucleic acid, involving the long delay for decay of its radioactivity, is not, of course, necessary in routine experiments. There seems little doubt that the method will be useful to amplify differences between nucleic acids which only differ slightly in base composition or nearest neighbour nucleotide frequency—this would be particularly so if more endonucleases of well-defined specificity became available. One enzyme that may prove valuable is spleen acid deoxyribonuclease[11]. The utility of the procedure as a fingerprinting method for nucleic acids is being investigated, and the method is also being applied in investigations of the specificity of association of different histone fractions with DNA in native deoxyribonucleoproteins. Neutron activation after ionophoresis or paper chromatography may also find limited application in protein chemistry for detection of amino-acids or peptides that contain sulphur. Although, unlike most methods of detection, it is equally applicable to compounds in which the sulphur is fully oxidized, it is in all cases a less sensitive method than for phosphorus. An additional application is the detection of serine or threonine phosphate; the method has in fact proved useful for confirmation of the presence of serine phosphate in partial acid hydrolysates of histones[12].

We thank Dr. A. Kornberg, who directed our attention to the possibility of using neutron activation for the examination of nuclease digests of unlabelled nucleic acids, and Dr. I. R. Lehman for a generous gift of *Neurospora crassa* endonuclease. We also thank Mr. M. Whiting for arranging the irradiations in *Bepo*, and Dr. D. H. Brown and his colleagues for including our materials in their schedule of work.

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N-FORMYL-METHIONYL-*s*RIBONUCLEIC ACID AND CHAIN INITIATION IN PROTEIN BIOSYNTHESIS

POLYPEPTIDE SYNTHESIS DIRECTED BY A BACTERIOPHAGE RIBONUCLEIC ACID IN A CELL-FREE SYSTEM

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THE blocking of the amino group of methionine in *N*-formyl-methionyl-*s*RNA[1] suggested that this aminoacyl-*s*RNA might act as an initiator of polypeptide chains. Experimental evidence in support of this idea has been found in bacterial cells[2–5]. Using synthetic polyribonucleotides as messengers in cell-free bacterial systems the specificity for placing methionine in the N-terminal positions of the growing polypeptide chains is carried by a methionyl-*s*RNA which is distinguished by its ability to become formylated[3]. We have now confirmed this finding, using bacteriophage RNA as a messenger in the cell-free system. We have also shown that the formyl group plays no part in locating the methionine residue at the N-terminus; its role apparently consists in increasing the rate of polypeptide chain synthesis.

Before describing our results we wish to recapitulate some of the previous findings relating to this system. The formylation of Met-*s*RNA occurs after methionine has become attached to its specific *s*RNA[1] and is catalysed by an enzyme distinct from Met-*s*RNA synthetase[6]. *s*RNA from *Escherichia coli* contains two methionine-accepting species only one of which, Met-$sRNA_F$, could be formylated[3]. The reactions leading to the formation of the formylated species, F-Met-$sRNA_F$, are summarized in Fig. 1.

The role of F-Met-*s*RNA as an initiator of protein synthesis, at least in bacteria, was first confirmed when *N*-formyl-methionine was found to be incorporated into nascent proteins made in a cell-free system directed by bacteriophage RNA[4,5]. Using a cell-free system directed by poly UG and poly UAG and fractionated Met-*s*RNA we found that Met-$sRNA_M$, the species which could not be formylated, responded only to poly UAG, whereas Met-$sRNA_F$ responded to both polymers. The interesting point was that the actual formylation of Met-$sRNA_F$ made no difference to the results: when the cell-free system was assembled so as to avoid formylation[3], the methionine attached to Met-$sRNA_F$ became incorporated almost exclusively into N-terminal positions of the newly synthesized chains, whereas methionine attached to Met-$sRNA_M$ became incorporated into internal positions[3]. We now report similar findings using bacteriophage RNA to direct polypeptide chain synthesis in a cell-free system containing negligible transformylase activity.

Fig. 2 shows an autoradiogram obtained from a chymotryptic digest of the polypeptide made in the cell-free system when *mu*2 bacteriophage RNA was used as a messenger. Peptides A and B were formed only when Met-$sRNA_M$ was used as a source of methionine. On pronase digestion both liberated methionine. No change in mobilities was seen on mild acidic hydrolysis. The use of F-Met-$sRNA_F$ yielded two peptides, F_1 and F_2. When F_1 was treated with pronase, free formyl-methionine was liberated together with a dipeptide which was identified

$$\text{Met} + s\text{RNA}^{(M,F)} \xrightarrow[\text{Met-}s\text{RNA synthetase}]{\text{ATP}} \begin{matrix}\text{Met-}s\text{RNA}_M\ (\sim 30\ \text{per cent}) \\ + \\ \text{Met-}s\text{RNA}_F\ (\sim 70\ \text{per cent})\end{matrix} \xrightarrow[\text{Met-}s\text{RNA transformylase}]{\text{Formyl-THF}} \text{F-Met-}s\text{RNA}_F$$

Fig. 1. Formyl-THF represents 10-formyl tetrahydrofolate, Met-sRNA$_M$ is Met-sRNA$_1$ of ref. 3, and Met-sRNA$_F$ is Met-sRNA$_2$ of ref. 3

as F-Met-Ala. After the same treatment, F_2 gave free formyl-methionine and a dipeptide which was tentatively identified as F-Met-Ser. In both cases free formyl-methionine was also observed, probably coming from further splitting of the respective dipeptides, because both liberated formyl-methionine on further incubation with pronase. The appearance of two different formyl-methionine-containing peptides in the chymotryptic digest of the polypeptide produced under the direction of the phage RNA suggests that at least two different proteins were synthesized under such conditions.

When unformylated Met-sRNA$_F$ was used as a source of methionine, two peptides were liberated (M_2 in rather low and variable yield compared with M_1) by chymotryptic digestion. On pronase treatment both liberated free methionine. When peptide F_1 was subjected to a very mild acidic hydrolysis to remove the formyl group (0·2 M hydrochloric acid : dioxan as 1 : 1 v/v for 24 h at room temperature) it gave a compound having a mobility identical with that of M_1. Peptide F_2 subjected to the same treatment gave a peptide having a mobility identical with that of M_2. Peptides F_1 and M_1 after limited acidic hydrolysis (6 N hydrochloric acid for 30 min at 100° C) gave rise to peptides which were identical on ionophoresis at pH 6·5. Peptides F_2 and M_2 after the same treatment yielded mainly free methionine presumably because the next amino-acid in both cases was serine. Thus we concluded that peptide F_1 corresponded to peptide M_1, and peptide F_2 probably to peptide M_2—the only difference between the respective peptides being the presence of the formyl group on the N-terminal methionine of F_1 and F_2. End-group determinations using a modification of the procedure developed by Edman[3] on the product formed when Met-sRNA$_M$ was used as a source of radioactive label revealed no N-terminal methionine. Peptides A and B must therefore be internal peptides. End-group determinations on the product formed when Met-sRNA$_F$ was used revealed that methionine was in an N-terminal position.

When F-Met-sRNA or the means of formylation are added to a cell-free system deficient in transformylase activity the rate and efficiency of the poly UG or poly UAG-directed incorporation of methionine increase[2,3]. This effect has now been examined in more detail by using the bacteriophage RNA as messenger. The results are shown in Fig. 3, where the incorporation of methionine is compared with the incorporation of leucine and valine. In the presence of F-Met-sRNA or 10-formyl-tetrahydrofolic acid, a stimulation (about four-fold) of protein biosynthesis occurs in the first few minutes of the incubation as indicated by the incorporation of the three amino-acids. Because the rate of formation of the first peptide bond is probably the rate-limiting step in the synthesis of proteins, this may be the step affected by the presence of the formylated Met-sRNA$_F$.

It is an attractive idea that F-Met-sRNA$_F$ because of its similarity with a peptidyl-sRNA (ref. 8) may directly enter the peptidyl-sRNA site on the ribosome, thereby initiating protein biosynthesis. This theory emphasizes the formylation of Met-sRNA, and allocates a critical function of the formyl-donor system in chain initiation. According to Noll[9], such a situation would permit the control of protein biosynthesis at the level of chain initiation by regulating the transformylase reaction. Contrary to his proposal, our work shows that the Met-sRNA which can be formylated has a specific structure, fitting directly into the peptidyl-sRNA site on the ribosome, whatever the state of formylation. Bretscher and Marcker's observations[10] point to the same conclusions. Thus chain initiation would occur whether or not the α-amino group of methionine is blocked. Formylation of the Met-sRNA possibly increases the rate of protein synthesis.

F-Met-sRNA has also been demonstrated in yeast, suggesting that the mechanism of chain initiation is the

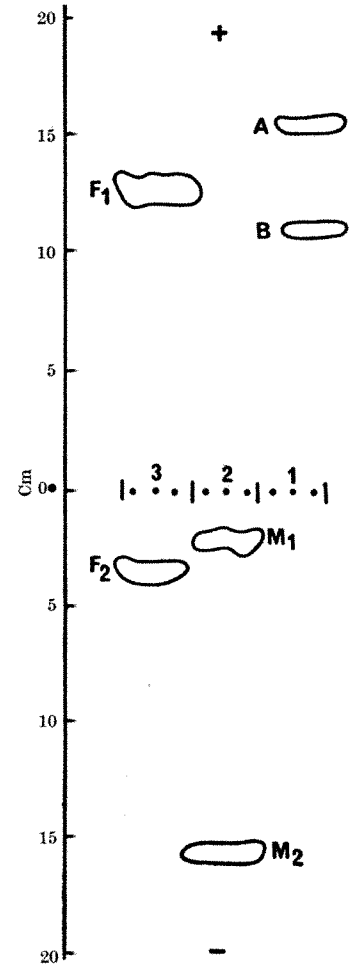


Fig. 2. Chymotryptic digestion of the polypeptide formed during incubation of an *E. coli* cell-free system with fractionated ^{35}S-Met-sRNA and *mu*2 phage RNA. The RNA was prepared from intact phage particles (a gift from Dr. G. L. Brown) by the method of Boedtker and Stumpp (ref. 7). The reaction mixture (0·5 ml.) contained 0·05 M *tris*-hydrochloric acid pH 7·4, 0·05 M ammonium acetate, 0·01 M magnesium acetate, 0·001 M adenosine triphosphate, 0·0002 M guanosine triphosphate, 0·004 M phosphoenolpyruvate, 5 μg pyruvate kinase, 0·01 M 2-mercaptoethanol, 0·005 M ^{12}C-methionine and 0·0002 M of nineteen other ^{12}C-amino-acids, 38·2 A_{260} units of MRE 600 ribosomes, 0·72 mg of supernatant protein free of formyl-donor from a DEAE-column, 8 A_{260} units unfractionated *E. coli*-sRNA, 200 μg of the phage RNA and either 3 A_{260} units of ^{35}S-Met-sRNA$_M$ (300 μμmoles ^{35}S-Met) or 1·9 A_{260} units of ^{35}S-Met-sRNA$_F$ (305 μμmoles of ^{35}S-Met) or 1·9 A_{260} units of F-^{35}S-Met-sRNA$_F$ (310 μμmoles of ^{35}S-Met)

After the reaction mixture had been incubated at 37° C for 20 min, EDTA at pH 7·0 was added to give a concentration of 0·05 M to attain the conditions for a further incubation of 10 min in the presence of 100 μg pancreatic RNase (five times crystallized, Calbiochem). The proteins were then precipitated with 10 per cent trichloroacetic acid (TCA) and the precipitate was washed five times with 5 per cent TCA containing 3 per cent casamino acids, twice with ethanol : ether (1 : 1 v/v) and finally dried with ether. Digestions with α-chymotrypsin (three-times crystallized, Worthington Biochemical Corporation) were carried out in 1 per cent ammonium bicarbonate (enzyme/protein = 1/25) at 37° C for 15 h. After the digestions the solutions of solubilized material were concentrated to permit their application to Whatman 3*MM* paper for electrophoresis at pH 6·5 for 2 h at 3 kV. The numbers refer to the applications of products from ^{35}S-Met-sRNA$_M$ at 1, ^{35}S-Met-sRNA$_F$ at 2, and F-^{35}S-Met-sRNA$_F$ at 3. A_1, B_1, M_1 and M_2 refer to ^{35}S-methionine-containing peptides. F_1 and F_2 refer to ^{35}S-formyl-methionine-containing peptides

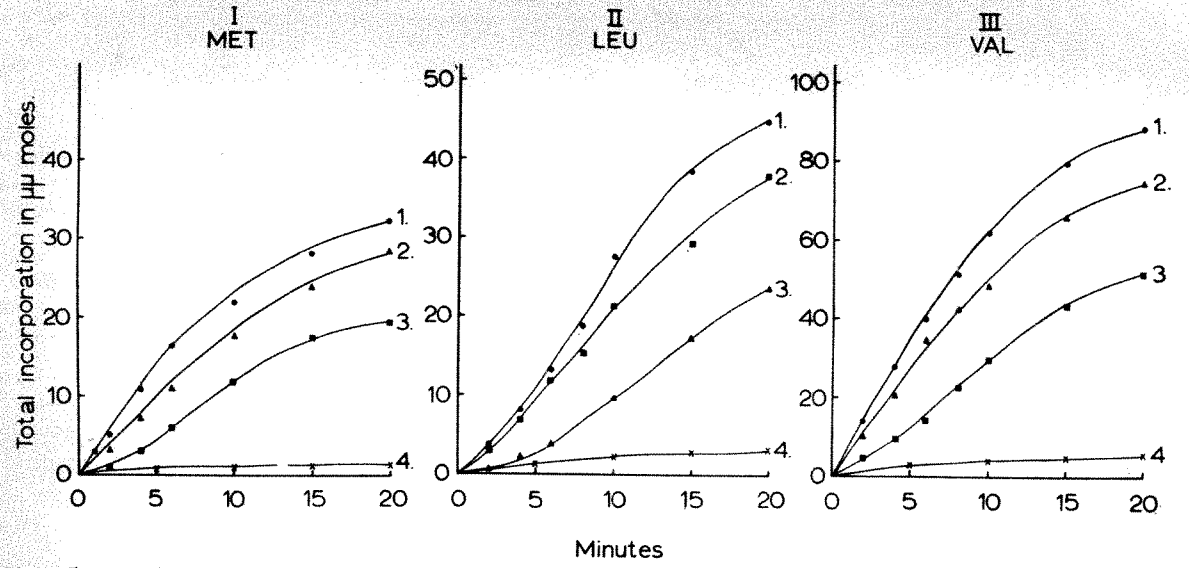


Fig. 3. Incorporation of ^{35}S-methionine, ^{14}C-leucine and ^{14}C-valine from unfractionated *s*RNA into polypeptide in a cell-free system containing no transformylase activity. The buffer and energy-providing components of the system (0·25 ml.) were the same as those described in Fig. 2. In addition the incubation mixture contained 0·0002 M of nineteen other ^{12}C-amino-acids, 22·5 A_{259} units of MRE 600 ribosomes, 0·36 mg of supernatant protein prepared as for Fig. 2, 5 A_{259} units of the charged *s*RNA and 100 μg of the phage RNA. All added *s*RNA preparations were charged in the presence of nineteen other ^{12}C-amino-acids. At the indicated time intervals during incubation at 30° C, 25 μl. samples were removed and added to tubes containing 100 μl. each of 0·5 per cent solutions of bovine serum albumin. The polypeptide product was precipitated in 5 per cent TCA and the suspended precipitate heated at 90 per cent for 15 min before being collected on a cellulose acetate filter for counting in a Nuclear Chicago thin-window gas-flow counter. The curves represent conditions in:
(I) For the addition of ^{35}S-Met-*s*RNA: (1) with 260 μμmoles of ^{35}S-Met-*s*RNA containing F^{35} S-Met-*s*RNA; (2) with 245 μμmoles of non-formylated ^{35}S-Met-*s*RNA but with the addition of 0·1 μmole of 10-formyl-THF; (3) with 245 μμmoles of non-formylated ^{35}S-Met-*s*RNA; (4) as for (3) but without the addition of the phage RNA
(II) For the addition of ^{14}C-Leu-*s*RNA: (1) with 131 μμmoles of ^{14}C-Leu-*s*RNA containing cold F-Met-*s*RNA; (2) with 141 μμmoles of ^{14}C-Leu *s*RNA containing cold non-formylated Met-*s*RNA but with the addition of 0·1 μmole 10-formyl-THF; (3) with 141 μμmoles of ^{14}C-Leu-*s*RNA containing cold non-formylated Met-*s*RNA; (4) as for (3) but without the addition of the phage RNA
(III) For the addition of ^{14}C-Val-*s*RNA: (1) with 238 μμmoles of ^{14}C-Val-*s*RNA containing cold F-Met-*s*RNA; (2) with 232 μμmoles of ^{14}C-Val-*s*RNA containing cold non-formylated Met-*s*RNA but with the addition of 0·1 μmole 10-formyl-THF; (3) with 232 μμmoles of ^{14}C-Val-*s*RNA containing cold non-formylated Met-*s*RNA; (4) as for (3) but without the addition of the phage RNA. In this experiment 18·5 A_{259} units of a different batch of MRE 600 ribosomes were used

same as in *E. coli*. On the other hand, we have so far failed to demonstrate F-Met-*s*RNA or other blocked aminoacyl-*s*RNA in mammalian systems.

We thank Dr. M. F. Perutz for his helpful suggestions about the arrangement of this manuscript.

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POLYPEPTIDYL-*s*RIBONUCLEIC ACID AND AMINO-ACYL-*s*RIBONUCLEIC ACID BINDING SITES ON RIBOSOMES

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Polypeptidyl-*s*RNA and amino-acyl-*s*RNA are bound to ribosomes in the presence of a messenger RNA (*m*RNA). We shall show here that the methionyl-RNA from *Escherichia coli* which can be formylated binds to the polypeptidyl-*s*RNA site on the ribosome, while the other methionyl-*s*RNA from *E. coli* binds to a different site, presumably the amino-acyl-*s*RNA binding site. This has been ascertained by examining the sensitivity of these methionyl-*s*RNA species to the antibiotic puromycin when bound to ribosomes.

Polypeptide chain synthesis occurs on ribosomes[1]. The detailed reactions are unresolved. It is clear, however, that the two reacting species concerned with the addition of one amino-acid residue to the growing polypeptide chain are the incoming amino-acyl-*s*RNA (ref. 2) and the growing polypeptidyl-*s*RNA (ref. 3). At least two enzymes catalyse the overall process[4], which also requires GTP* (ref. 5), *m*RNA (ref. 6) and various inorganic ions. The point here is that at any moment there must be two ribosomal sites, one capable of binding the existing polypeptidyl-*s*RNA and the other binding the correct incoming amino-acyl-*s*RNA, as directed by the *m*RNA. It is not unlikely that these two sites may have different properties (Fig. 1).

In the presence of a trinucleotide *m*RNA ribosomes bind a specific amino-acyl-*s*RNA (ref. 7); this interaction, which has been used to decipher many of the sixty-four triplets[8], occurs between the amino-acyl-*s*RNA and the trinucleotide, probably in the amino-acyl-*s*RNA binding site (the "amino-acid site") on ribosomes. One of its characteristics is the high level of magnesium ion required to obtain a stable complex (about 0·02–0·03 M Mg^{++}). Another feature is a lack of complete specificity in the recognition between the anticodon of the *s*RNA and the last nucleotide (at the 3′-OH end) of the trinucleotide, which is reflected in the form of degeneracy of the genetic code[8].

Two species of methionyl-*s*RNA exist in *E. coli*, one of which can be formylated both *in vivo* and *in vitro*[9]. The methionyl-*s*RNA which cannot be formylated (met-

* *Abbreviations:* GTP, Guanosine 5′ triphosphate; poly U, poly-uridylic acid; poly A, polyadenylic acid.

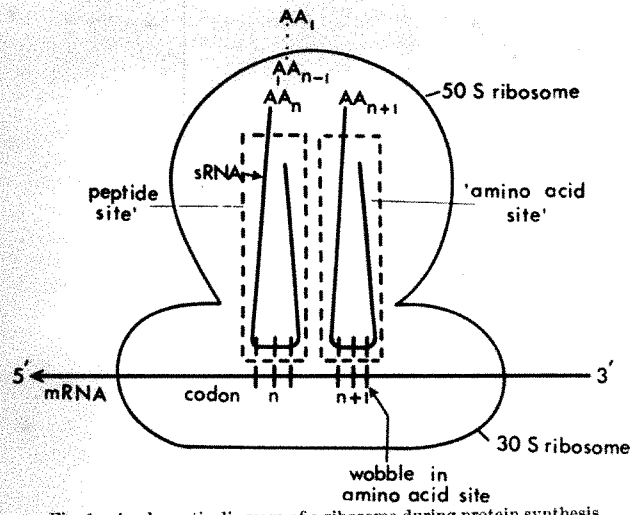
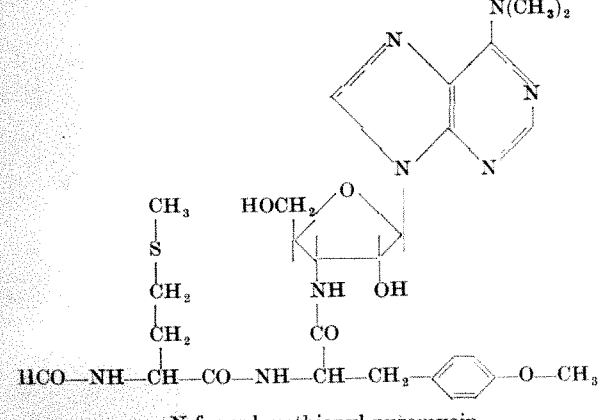


Fig. 1. A schematic diagram of a ribosome during protein synthesis

$sRNA_M$) has optimal binding conditions similar to most other amino-acyl-*s*RNAs; it binds well to ribosomes in the presence of the trinucleotide AUG in 0·02–0·03 M Mg^{++}. The methionyl-*s*RNA which can be formylated (met-$sRNA_F$), whether formylated or not, binds optimally to ribosomes in the presence of AUG at about 0·01 M Mg^{++} (ref. 10). This *s*RNA species is also bound well by GUG, UUG and less well by CUG (ref. 10), which indicates a lack of specificity in the first nucleotide residue of the trinucleotide (at the 5′-OH end). Furthermore, this met-$sRNA_F$, whether formylated or not, acts as an initiator of polypeptide chain synthesis *in vitro*[10].

We made use of the antibiotic puromycin which resembles the amino-acid of an amino-acyl-*s*RNA (ref. 11). Puromycin inhibits protein synthesis by substituting for an amino-acyl-*s*RNA and displacing the polypeptide from the peptidyl-*s*RNA as polypeptidyl-puromycin[12]. It probably enters the ribosomal structure by way of the amino-acid site and reacts with the polypeptidyl-*s*RNA bound in the polypeptidyl-*s*RNA binding site (the "peptide site").

We find that formyl-met-$sRNA_F$ and met-$sRNA_F$ are both sensitive to puromycin when bound to ribosomes with the triplet AUG. Met-$sRNA_M$ is comparatively insensitive to puromycin under identical conditions. This is taken to imply that met-$sRNA_F$, whether formylated or not, binds to the peptide site on a ribosome, while met-$sRNA_M$ binds to the amino-acid site. The product of the ribosomal reaction between formyl-met-$sRNA_F$ and puromycin is formyl-methionyl-puromycin. The product of the ribosomal reaction between met-$sRNA_F$ and puromycin is methionyl-puromycin. In a further experiment both species of methionyl-*s*RNA have been acetylated chemically. Acetyl-met-$sRNA_F$ is sensitive to puromycin yielding acetyl-methionyl-puromycin, while acetyl-met-$sRNA_M$ is comparatively insensitive to puromycin.



N-formyl-methionyl-puromycin

Our results indicate that formyl-met-$sRNA_F$ acts as an initiator of polypeptide chain synthesis by virtue of its affinity for the ribosomal site which is specific for polypeptidyl-*s*RNA. This specificity lies in the $sRNA_F$.

Ribosomes were isolated from *Escherichia coli* strain *B* (ref. 13) and washed twice in 1·0 M potassium chloride containing 0·03 M magnesium acetate in 0·02 M *tris*-hydrochloric acid, *p*H 7·5. *s*RNA from *E. coli* strain *B*, fractionated by countercurrent distribution to separate the two methionyl-*s*RNA species, was generally provided by Dr. B. F. C. Clark[10]. The met-$sRNA_F$ is uncontaminated with met-$sRNA_M$ (less than 1 per cent). The met-$sRNA_M$ contains about 8 per cent met-$sRNA_F$, determined by its ability to accept formyl groups. Both peaks of methionyl-*s*RNA were charged with ^{35}S-L-methionine of high specific activity (1,800 mc./mmole) (ref. 10), using an *E. coli* supernatant fraction free of formyl donor. For some experiments the met-$sRNA_F$ was formylated in the presence of 10-formyl tetrahydrofolate[14]. These *s*RNA fractions are the same as those used by Clark and Marcker[15]. Acetylation of both met-*s*RNAs was carried out with acetic anhydride[16]. It was found after hydrolysis at *p*H 10 and subsequent electrophoresis at *p*H 3·5 that all the radioactivity released from the *s*RNA co-electrophoresed with synthetic acetyl-methionine. The specific activities of all *s*RNA preparations were very similar, being about 4–7 × 10^6 d.p.m./mg *s*RNA. ApUpG was prepared from ApU using polynucleotide phosphorylase, GDP, and *T*1 takadiastase diesterase. The 3′-phosphate was removed with alkaline phosphatase. Puromycin dihydrochloride was purchased from Nutritional Biochemicals and was neutralized before use.

Table 1 shows the binding characteristics of the ribosome preparation used in Figs. 2–6. Formyl-met-$sRNA_F$ and met-$sRNA_F$ are both bound well in 0·01 M magnesium ions, and met-$sRNA_M$ in 0·02 M magnesium ions. In each case some 70 per cent of the added labelled *s*RNA is bound in the presence of AUG; this high level of binding is due to the excess of ribosomes used.

Figs. 2 and 3 show the rate of appearance of acid-soluble label (that is, material free from *s*RNA) when the ribosomes are incubated with ^{35}S-formyl-met-$sRNA_F$ and met-$sRNA_F$, respectively in the presence of puromycin. The initial rate of reaction in each case is stimulated some five-fold by the addition of AUG and is strongly dependent on the presence of both ribosomes and puromycin. The presence of the formyl group on the met-$sRNA_F$ increases the initial rate of reaction some five-fold.

In order to compare met-$sRNA_F$ and met-$sRNA_M$ in their sensitivity to puromycin, met-$sRNA_F$ has been incubated in 0·02 M Mg^{++} to show that higher levels of magnesium do not prevent the reaction observed in Fig. 3. Figs. 4 and 5 show the rates of appearance of acid-soluble label when met-$sRNA_F$ and met-$sRNA_M$ are incubated in the presence of puromycin, AUG and ribosomes. The label in met-$sRNA_F$ becomes acid-soluble at a rate similar to that shown in Fig. 3. In contrast, met-$sRNA_M$ has a slow rate of reaction stimulated by AUG. The difference in the two rates of reaction is about ten-fold. At least part of the observed reaction of met-$sRNA_M$ arises from the small contamination (about 8 per cent) of met-$sRNA_F$ in this *s*RNA.

Table 1

*s*RNA species	Mg^{++} molarity	C.p.m. bound to ribosomes −AUG	C.p.m. bound to ribosomes +AUG	Total c.p.m. added
(1) met-$sRNA_M$	0·01	1,840	3,770	11,000
(2) met-$sRNA_M$	0·02	2,200	8,270	11,000
(3) met-$sRNA_F$	0·01	1,600	8,080	11,000
(4) formyl-met-$sRNA_F$	0·01	2,370	11,200	13,500

Each reaction mixture (0·025 ml.) contained 0·2 mg ribosomes in 0·10 M *tris*-hydrochloric acid *p*H 7·1, 0·05 M ammonium chloride and magnesium acetate as indicated. The trinucleotide AUG was added, where indicated, at a concentration of 4 A_{260} units/ml. After incubation at 30° C for 10 min, samples were diluted and filtered on a 'Millipore' filter (ref. 7) and washed in the same buffer as that in which they were incubated. The filters were dried and counted in a scintillation counter with an efficiency of about 70 per cent. The data shown are the averages of duplicate samples.

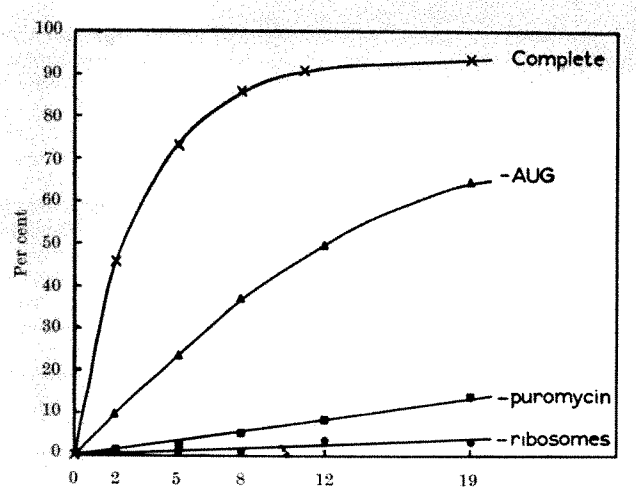


Fig. 2. The appearance of acid-soluble label with ^{35}S-formyl-met-sRNA$_F$. ^{35}S-formyl-met-sRNA$_F$ was incubated in 0·1 M *tris*-hydrochloric acid (*p*H 7·1) containing 0·010 M $MgAc_2$, 0·05 M ammonium chloride, 0·8 mM puromycin, 4 A_{260} units/ml. of the trinucleotide AUG and 8 mg/ml. ribosomes. Equal portions of the reaction mixture (0·02 ml.) were withdrawn at 0 min and at the times indicated and blown into 0·2 ml. of a solution at 0° C of 0·05 M potassium cacodylate *p*H 6·8 containing 0·01 M $MgAc_2$ and 1·5 mg/ml. bovine plasma albumin. 0·2 ml. 5 per cent perchloric acid were added; the precipitate was removed by centrifugation and the supernatant plated out on to a strip (3 cm × 4 cm) of Whatman 3 *MM* paper. This was dried, neutralized in ammonia vapour, dried and counted in a scintillation counter with an efficiency of 60 per cent. From each sample has been subtracted a small (less than 5 per cent) 0 min blank value. The total acid-precipitable material at 0 min was measured by dissolving that precipitate in 1 M ammonium hydroxide, plating this out on paper and counting as already described. The total acid-precipitable material at 0 min was 6,050 c.p.m. The effect of omitting either puromycin or AUG or ribosomes is shown

To determine what the products of reaction were, the ethanol-soluble components of incubation mixtures containing either ^{35}S-formyl-met-sRNA$_F$ or ^{35}S-met-sRNA$_F$ were subjected to electrophoresis at *p*H 3·5. An autoradiograph of the paper sheet is shown in Fig. 6. The principal product of the reaction between formyl-met-sRNA$_F$ and puromycin (sample 1, FMP) has a mobility similar to the marker formyl-methionyl-adenosine (FMA) (as would be expected of formyl-methionyl-puromycin). This labelled material was eluted and shown to co-electrophorese with chemically synthesized formyl-methionyl-puromycin, at *p*H's 1·9, 3·5 and 5·0. It is quantitatively adsorbed to 'Norite' suggesting that the formyl-methionine is attached to an aromatic base; it is not sensitive to dilute alkali (as formyl-methionyl-adenosine is), but is degraded by dilute acidic hydrolysis (0·2 M hydrochloric acid in methanol at 37° C for 90 min) yielding a compound that moves like synthetic methionyl-puromycin at *p*H's 1·9 and 3·5. We conclude that this product is formyl-methionyl-puromycin. In the absence of puromycin, formyl-met-sRNA$_F$ gives rise to a faint, unidentified band with a mobility lower than methionine (sample 3).

^{35}S met-sRNA$_F$ when bound to ribosomes in the presence of puromycin gives rise to an increase (some three-fold over the control, sample 6) of material in the methionine band (sample 4, M). Its identity with methionine (or its sulphoxide) has been checked by co-electrophoresis with authentic markers at *p*H's 1·9 and 3·5. The faint band observed in sample 4 (MP) with a mobility between methionyl-adenosine (MA) and formyl-methionyl-adenosine has been identified as methionyl-puromycin by its co-electrophoresis with synthetic methionyl-puromycin at *p*H's 1·9, 3·5 and 5·0. It is adsorbed to 'Norite' and is indistinguishable from deformylated ^{35}S-formyl-methionyl-puromycin. Both the ^{35}S-methionyl-puromycin formed from met-sRNA$_F$ and that obtained by deformylation of formyl-methionyl-puromycin appear to be unstable at low *p*H's (about *p*H 2·0), especially on elution from paper. Both give rise to two other unidentified bands on electrophoresis at *p*H 3·5, one of which is probably methionyl-0-methyl-tyrosine. The appearance of free methionine was found to be due to contamination of the ribosomes used in Fig. 6 by an amino-peptidase[17]. Another ribosome preparation which was virtually free of amino-peptidase activity yields predominantly methionyl-puromycin as the product of reaction between met-sRNA$_F$ and puromycin (Fig. 7). We therefore conclude that methionyl-sRNA$_F$, whether formylated or not, is sensitive to puromycin when bound to ribosomes, yielding the acyl-puromycin derivative.

In Figs. 8 and 9 chemically acetylated met-sRNA$_F$ and met-sRNA$_M$ have been tested for their sensitivity to puromycin. Both sRNAs were tested in 0·01 M Mg^{++}; the ribosome preparation used here was prepared as for earlier experiments but was not as active with formyl-met-sRNA$_F$. The rate of reaction of the acetyl-met-sRNA$_F$ is some twelve-fold greater than that of acetyl-met-sRNA$_M$. The product of the reaction of acetyl-met-sRNA$_F$ and puromycin has electrophoretic properties similar to formyl-methionyl-puromycin and is therefore assumed to be acetyl-methionyl-puromycin.

When puromycin is present in an incubation mixture in which poly *A* directs the synthesis of polylysine, the principal products observed are the oligolysyl-puro-

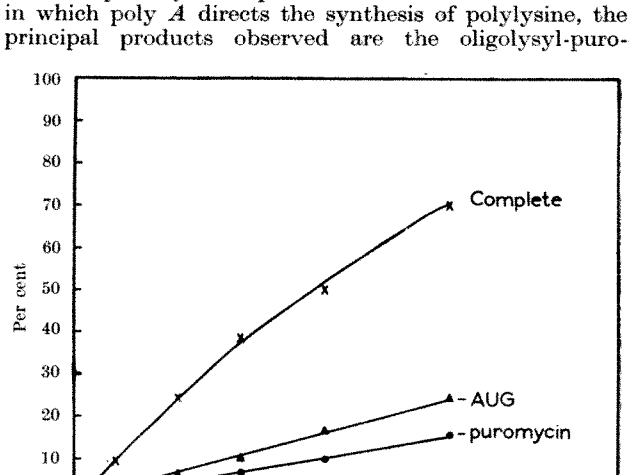


Fig. 3. The appearance of acid-soluble label with ^{35}S-met-sRNA$_F$. The conditions used were the same as in Fig. 2 except that 10^{-4} M L-methionine was present during the incubation. The total acid-precipitable material at 0 min was 5,700 c.p.m.

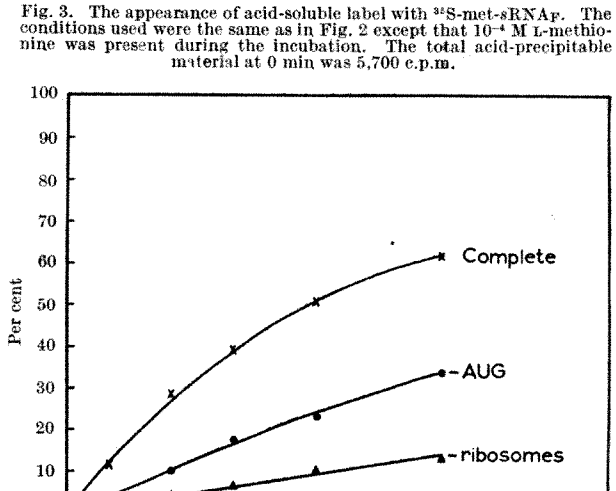
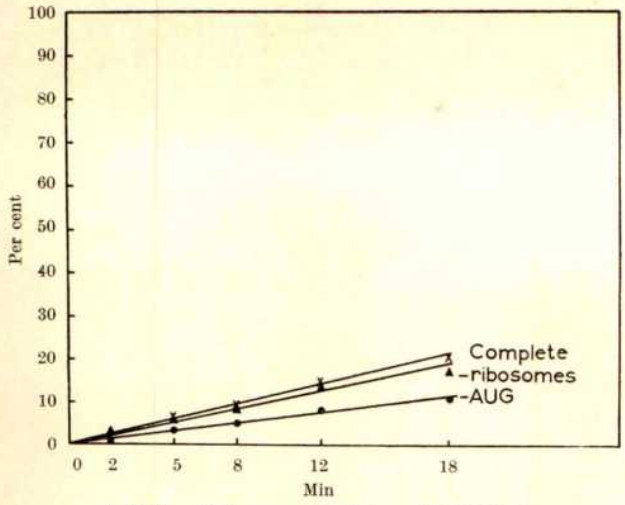


Fig. 4. The appearance of acid-soluble label with ^{35}S-met-sRNA$_F$. The conditions used were the same as in Fig. 3 except that the incubation was carried out in 0·02 M $MgAc_2$ and in 0·1 M potassium cacodylate *p*H 6·8 (instead of *tris*-hydrochloric acid, *p*H 7·1). The total acid-precipitable material at 0 min was 9,100 c.p.m.



met-$sRNA_M$, AUG, puromycin, ribosomes in 0·02 M Mg^{++}

Fig. 5. The appearance of acid-soluble label with ^{35}S-met-$sRNA_M$. The conditions used were the same as in Fig. 4. The total acid-precipitable material at 0 min was 8,900 c.p.m.

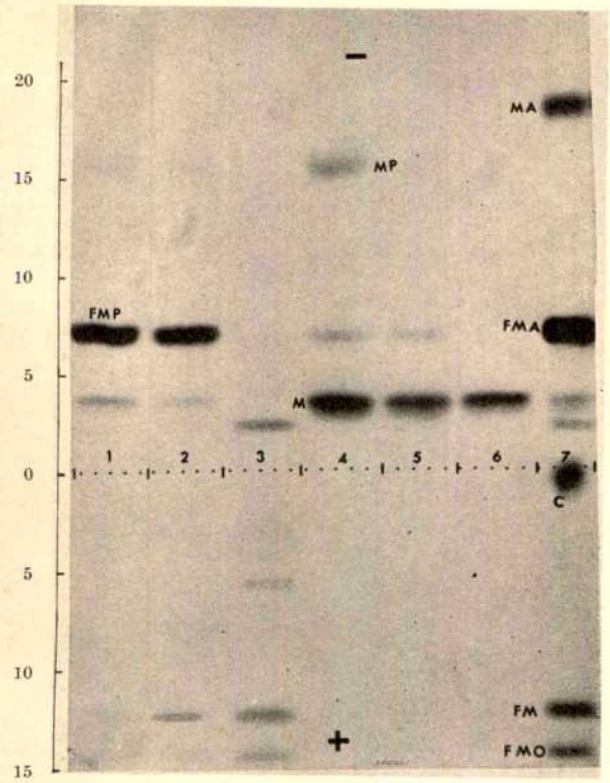


Fig. 6. Electrophoresis of reaction products at *p*H 3·5. Incubations (0·05 ml.) were carried out at 30° C for 20 min in 0·10 M potassium cacodylate *p*H 6·8 (instead of *tris*-hydrochloric acid *p*H 7·1), but otherwise as described under Fig. 3. After incubation 0·12 ml. ethanol was added to each sample, the precipitate removed, the ethanolic phase dried down and subjected to high-voltage electrophoresis on Whatman 3 *MM* paper at *p*H 3·5 (50 min at 50 V/cm). The paper was dried and left to autoradiograph with commercial X-ray paper for 4 days

Samples: (1) Complete reaction mixture using ^{35}S-formyl-met-$sRNA_F$. (2) As in sample (1) but with the omission of AUG. (3) As in sample (1) but with the omission of puromycin. (4) Complete reaction mixture using met-$sRNA_F$. (5) As in sample (4) but with the omission of AUG. (6) As in sample (4) but with the omission of puromycin. (7) This contains a pancreatic ribonuclease digest of partially formylated ^{35}S-met-*s*RNA as markers. Spots are labelled as follows: methionyl-adenosine (MA), formyl-methionyl-adenosine (FMA), methionine (M), formyl-methionyl-puromycin (FMP), methionyl-puromycin (MP), a contaminating spot picked up after electrophoresis (C), formyl-methionine (FM) and formyl-methionine sulphoxide (FMO)

A scale in centimetres is shown on the left. The radioactivity in the methionine bands in samples 4 and 6 is about 3 : 1

mycins[12]. It is also known that polyphenylalanyl-*s*RNA and polylysyl-*s*RNA are sensitive to puromycin when bound with poly U and poly A, respectively[18]. Probably only a polypeptidyl-*s*RNA which is bound in the peptide site can react with puromycin. The puromycin, on the other hand, is bound in the same environment as the amino-acyl-adenosine group of amino-acyl-*s*RNA in the amino-acid site before reaction. Ribosomes may contain enzyme systems capable of transferring a peptidyl-*s*RNA from the amino-acid site to the peptide site, but in the experimental system described here such transfer may be inefficient, because only a trinucleotide is used as *m*RNA. In the earlier experiments carried out with polypeptidyl-*s*RNAs and polynucleotide messenger, motion of the polypeptidyl-*s*RNAs between the amino-acid and peptide sites may possibly have occurred.

Our experiments indicate that $sRNA_F$ recognizes the peptide site in preference to the amino-acid site irrespective of the presence or absence of the formyl group, and that therefore the $sRNA_F$ possesses some special feature.

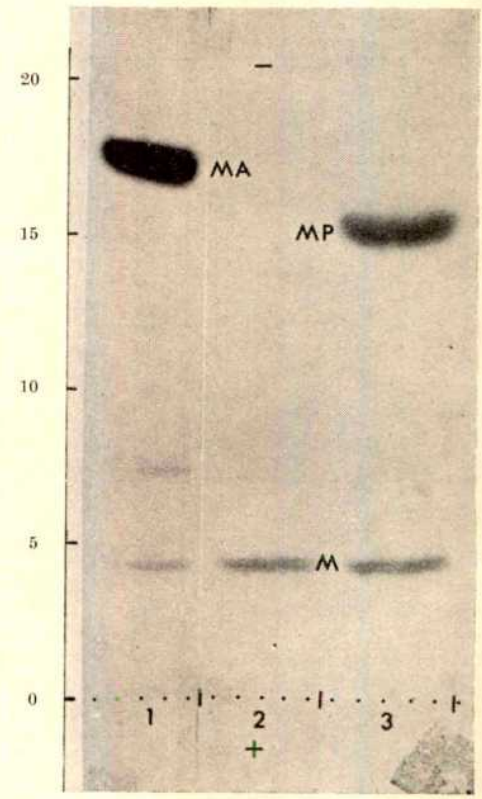
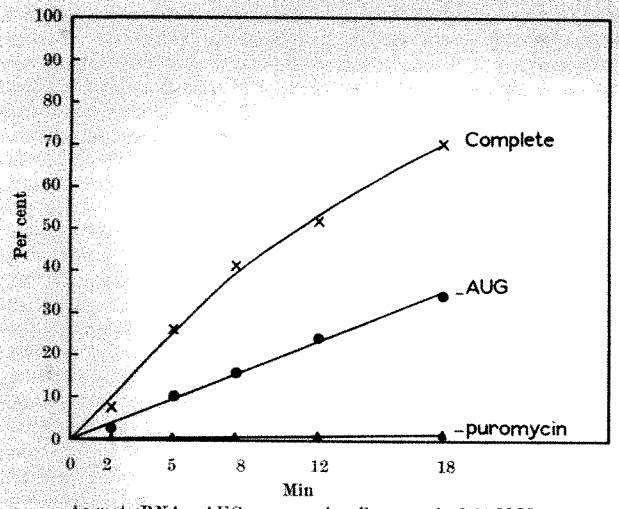


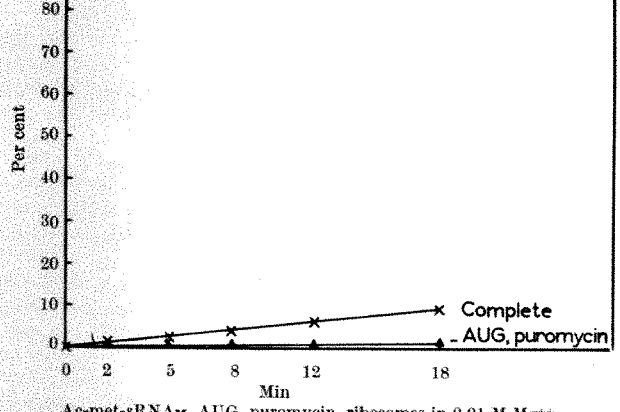
Fig. 7. Electrophoresis of reaction mixtures using met-$sRNA_F$. The experiment was as in Fig. 6. *Samples:* (1) Methionyl-adenosine from methionyl-*s*RNA. (2) Complete reaction mixture but with the omission of puromycin. (3) Complete reaction mixture

This preference for the peptide site confers on it its special function as an initiator of polypeptide chain synthesis because, whether formylated or not, this $sRNA_F$ acts as an initiator of polypeptide synthesis *in vitro*[10,15]. The specificity for binding to the peptide site and hence its ability to act as a chain initiator must reside in the $sRNA_F$. The presence of a blocked amino group does not appear to be sufficient to place an *s*RNA into the peptide site because acetyl-met-$sRNA_M$ is insensitive to puromycin under conditions where acetyl-met-$sRNA_F$ is sensitive. The presence of a formyl group on met-$sRNA_F$ simply serves to increase the rate of reaction with puromycin and thus the rate of formation of the first peptide bond. This has also been shown with the *mu*2 phage RNA in the *in vitro* system[15].



Ac-met-$sRNA_F$, AUG, puromycin, ribosomes in 0·01 M Mg^{++}

Fig. 8. The appearance of acid-soluble label with acetyl-met-$sRNA_F$. The conditions used are the same as in Fig. 6. The total acid-precipitable material at 0 min was 11,600 c.p.m.



Ac-met-$sRNA_M$, AUG, puromycin, ribosomes in 0·01 M Mg^{++}

Fig. 9. The appearance of acid-soluble label with acetyl-met-$sRNA_M$. The conditions used were the same as in Fig. 6. The total acid-precipitable material at 0 min was 9,000 c.p.m.

There may exist some subtle change in *s*RNA conformations which allows other *s*RNAs to be transferred from the amino-acid to the peptide site during protein synthesis. Whether the reversible conformation changes in *s*RNA which have recently been observed[19] are associated with different types of binding affinities is not known. The specificity of *s*RNA recognition by the *m*RNA should not be greater in the peptide site than in the amino-acid site. The binding properties of met-$sRNA_F$ suggest that the first base in the codon is either not recognized at all in the peptide site, or that A may be equivalent to G. The codon-*s*RNA relationships explored so far have, in the main, dealt with recognition in the amino-acid site. We would expect that a similar, although less specific, codon-*s*RNA recognition exists in the peptide site.

The ability to place an *s*RNA into either of two ribosomal sites, together with an assay for which site the *s*RNA is in, should be a useful tool for gaining a better understanding of ribosomal reactions. The experimental approach described here also provides a direct assay for polypeptide chain initiators.

We thank Dr. B. F. C. Clark for the generous gifts of fractionated *s*RNA species.

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NUCLEIC ACID OF INFLUENZA VIRUS

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THE isolation of infective ribonucleic acid (RNA) from tobacco mosaic virus (TMV) in 1956, by extracting the virus with either phenol[1] or sodium dodecyl sulphate[2], suggested that hereditary functions were not the exclusive property of deoxyribonucleic acid (DNA). The importance of RNA as genetic material was firmly established during the next 5-year period, when infectious material was isolated from a large number of structurally simple animal and plant viruses[3]. In these investigations, extraction of infectious RNA was based mainly on the phenol method of Gierer and Schramm[1] and the activity of the RNA was invariably destroyed by treatment with ribonuclease (RNase).

Detailed physico-chemical investigations of TMV-RNA showed that the molecular weight of the infectious RNA was about 2×10^6, and that each infectious molecule represented the complete nucleic acid complement of the virus particle[4–6].

Recently, improved extraction methods have been devised in which a combination of phenol and detergents together with RNase inhibitors is used. The nucleic acid of several larger RNA viruses has been isolated by

such methods, and shown to be of high molecular weight, as calculated from the sedimentation coefficient (*S* value)[10–13]. Tests for infectivity were not carried out, but the calculated size of the extracted material is consistent with the idea that each virus particle contains only one molecule of RNA. Listed in Table 1 are some *S* values obtained from a number of RNA viruses, together with estimates of their molecular weights.

Table 1

Virus	Sedimentation coefficient of RNA (*S*)	Mol. wt. of RNA	Reference
Polio	37	2×10^6	7
Foot and mouth disease	37	3×10^6	8
Mouse encephalitis	27·5	2×10^6	9
Newcastle disease	57	$7{\cdot}5 \times 10^6$	10
Rous sarcoma	64	$9{\cdot}5 \times 10^6$	11
Rauscher	73, 74	13×10^6	12, 13

Influenza virus has an RNA content of about 0·75 per cent–1 per cent[14,15]. The estimated molecular weight of this RNA is 2×10^6 (ref. 16). Many attempts have been made to isolate the RNA from influenza virus in an infectious form, either from whole virus or from virus-infected cells. The results reported are confusing and contradictory, as can be seen from Table 2. As there is no general agreement between these reports, it is still uncertain whether the various extraction methods used succeed in isolating the RNA from influenza virus in its naturally occurring state. Furthermore, virtually no physico-chemical data are available concerning the nature of the nucleic acid. There is some genetic evidence that the RNA may exist in the intact virus particle as several separate pieces[26]. On the other hand, if the RNA is a single large molecule, as is the case for other RNA viruses, the confusion apparent in Table 2 might reflect the inadequacies of the extraction procedures used. The purpose of the experiments described in this article was to investigate this problem further, in an attempt to determine the size and nature of the RNA of influenza viruses.

The influenza A_2 strain *A*/Singapore/1/57 was used throughout. It was prepared as follows:

Eleven-day-old embryonated eggs were inoculated with 0·1 ml. of infected allantoic fluid diluted 10^{-6}, incubated at 37° C for 36 h and then collected. For unlabelled virus, yields of allantoic fluid were obtained from two separate batches of 5,000 eggs. To obtain virus labelled with phosphorus-32, batches of twelve eggs were inoculated with 0·4 mc. of phosphorus-32 (as phosphate buffer) per egg, 24 h before virus infection.

Both labelled and unlabelled virus was purified in the same way. Freshly washed fowl red cells were added to the allantoic fluid to a final concentration of 0·5 per cent and kept at 4° C for 1 h with occasional shaking. The red cells were deposited by centrifugation and washed once in ice-cold phosphate buffered saline. Virus was eluted by stirring the red cells in 0·9 per cent sodium chloride at 37° C for 2 h. The eluate was then treated with 5 μg/ml. DNase and 5 μg/ml. RNase for 20 min at 37° C and immediately centrifuged on a linear 15–60 per cent sucrose density gradient at 20,000 r.p.m. for 1 h in a Spinco *SW*25 rotor. 3 ml. fractions were collected through a hole pierced in the bottom of the centrifuge tube. 0·1 ml. amounts of each fraction were used to measure haemagglutinin content and an equal amount was used for radioactivity measurements. The level of radioactivity was counted in a Packard 'Tricarb' liquid scintillation counter, series 4,000. The scintillation fluid used was prepared according to Bray[27].

The results of a typical experiment using labelled virus are shown in Fig. 1. Most of the virus present was confined to fractions 12, 13 and 14 of the gradient. Most of the phosphorus-32, on the other hand, was found at the top of the gradient. However, considerable radioactivity was associated with the virus. From any such gradient, only the two or three fractions (such as Nos. 12, 13 and 14 in Fig. 1) in which the haemagglutinin peak occurred were kept and processed further. These fractions were pooled and dialysed overnight at 4° C against 0·9 per cent sodium chloride. The dialysed virus was then deposited by centrifugation at 20,000 r.p.m. for 1 h in a Spinco model *L* ultracentrifuge; the pellet was resuspended in a small volume of 0·1 M sodium chloride–0·001 M ethylenediamine tetraacetate (EDTA) and the resulting suspension was treated for 1 min with an M.S.E. ultrasonic disintegrator. This treatment breaks apart any aggregates of virus caused by centrifugation but has no effect on the biological properties of influenza virus.

Extraction of RNA was carried out in the following way. Sodium dodecyl sulphate (SDS) was added to the purified virus preparation to give a final concentration of 1 per cent. The virus was then deproteinized by the addition of an equal volume of water-saturated, freshly redistilled phenol, *p*H 7·5. Extraction with phenol was carried out in two ways: (1) all extractions performed at 4° C; (2) the first phenol extraction performed at 52° C, then all subsequent extractions at 4° C. Identical results were obtained in both cases.

The aqueous and phenol phases were separated by centrifugation and the aqueous phase re-extracted with phenol until all traces of an interface had disappeared. RNA was precipitated from aqueous phase by addition of 2·5 volumes of absolute alcohol and 0·1 volumes of 20 per cent sodium acetate, the mixture then being stored at −20° C for at least 2 h. The precipitate was washed twice with absolute alcohol, once with ether and resuspended in 0·5–1 ml. of 0·01 M acetate buffer *p*H 5·3, containing 0·1 M sodium chloride and 0·001 M EDTA. The last traces of ether were blown off with compressed nitrogen.

A batch of unlabelled influenza virus, consisting of 100 mg of purified virus, was subjected to the extraction procedure outlined here. A total of 630 μg of nucleic acid was recovered; the ultra-violet absorption spectrum of this material is shown in Fig. 2.

A diphenylamine test for deoxyribose was negative. To investigate the physical nature of this RNA, about 30 μg was layered on to a 5–20 per cent linear sucrose density gradient and centrifuged at 37,000 r.p.m. for 3 h in a Spinco rotor *SW* 39. A second sample of the same RNA was treated with RNase (0·5 μg/ml.) for 30 min

Table 2

Virus	Extraction procedure	Result of infectivity test	Properties of extracted material	Reference
Fowl plague	Phenol	−ve	Heterogeneous in size 8–14*S*	17, 18
MEL, WSE, NEURO-WS, LEE, NDV	Cold phenol	−ve		19
Influenza *T*	Ether, cold phenol	+ve	Orcinol +ve; amino-acid −ve	20
Influenza/A_2/Sing.	Ether, cold phenol	−ve	Typical nucleic acid spectrum. Susceptible to RNase	21
Influenza *A*	EDTA, ether, cold phenol	+ve	Typical nucleic acid spectrum. Susceptible to RNase	22, 23
Influenza	Chloroform, cold phenol	+ve	Susceptible to RNase and immune gamma globulin	24
Influenza	Sodium deoxycholate, phenol at various temperatures	not tested	Largest sedimentation coefficient = 11. Single stranded RNA	25

at room temperature, before centrifugation. The distribution of nucleic acid in the fractionated gradients was determined by optical density measurements. The results are shown in Fig. 3. Most of the untreated RNA banded in a single broad peak rather less than half way down the tube. The remainder was diffusely scattered throughout the low-density region of the gradient. The RNA was completely degraded by pretreatment with RNase. Those samples which contained the untreated RNA peak (fractions 12–16) were pooled, the RNA reprecipitated with alcohol and sodium acetate and redissolved in 1 ml. acetate buffer for analysis in the analytical ultracentrifuge. During centrifugation the RNA began to break down, making an accurate estimation of the *S* coefficient difficult. However, an average value was calculated to be 16*S*.

In this experiment, it took a fortnight to purify and to concentrate virus from about 100 l. of infected allantoic fluid. It seemed likely that the low *S* value obtained arose from breakdown of larger molecules during this lengthy process. However, by using much smaller quantities of phosphorus-32 labelled virus, the time involved in purification and extraction could be reduced to 24 h.

Several separate batches of phosphorus-32 labelled virus were prepared and the RNA extracted. Fig. 4 shows a typical sedimentation pattern for phosphorus-32-labelled RNA. Two distinct components were present. Pre-treatment with RNase converted all the RNA to slowly sedimenting material. It can be seen that about 57 per cent of the radioactivity bands in the faster sedimenting peak–fractions 17–21 of the gradient. This is considered to be intact virus RNA; the remainder of the radioactivity is distributed at the top of the gradient and is presumably RNA which has been degraded during the extraction process. This figure of 57 per cent compares well with the data of Duesberg and Robinson[10], who found that a maximum of only 21 per cent of NDV-RNA bands in the faster sedimenting peak. Identical results have been obtained using several different batches of phosphorus-32-labelled virus. By comparing the sedimentation rate of virus RNA with that of chick embryo fibroblast RNA spun under identical conditions, it can be seen that the faster sedimenting component of the influenza virus RNA coincides exactly with the 18*S* ribosomal RNA component. This is shown in Fig. 5. The fact that the majority of influenza RNA forms a band in the leading peak, and that this peak is homogeneous in nature, suggests that the bulk of the virus RNA has been extracted in an undegraded state.

To test the possibility that the extraction method itself was breaking down what was really a high molecular

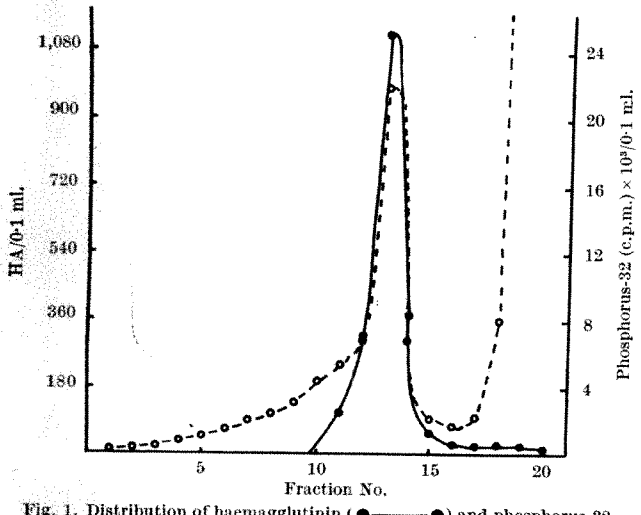


Fig. 1. Distribution of haemagglutinin (●——●) and phosphorus-32 (○- - -○) after velocity sedimentation of purified phosphorus-32 labelled influenza virus in a 15–60 per cent linear sucrose gradient. Centrifugation at 20,000 r.p.m. for 1 h at room temperature in a Spinco *SW* 25 rotor

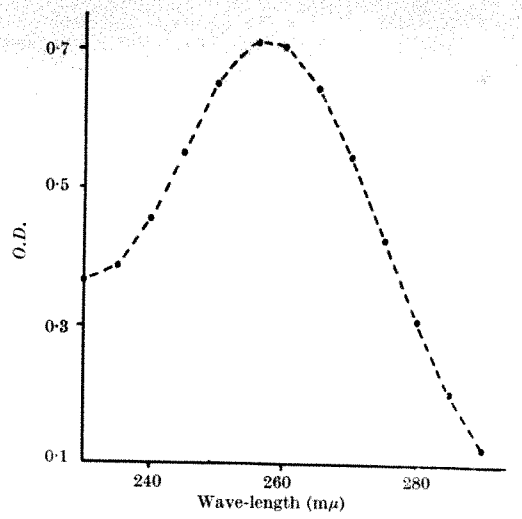


Fig. 2. Ultra-violet absorption spectrum of a 10^{-1} dilution of RNA extracted from purified influenza virus

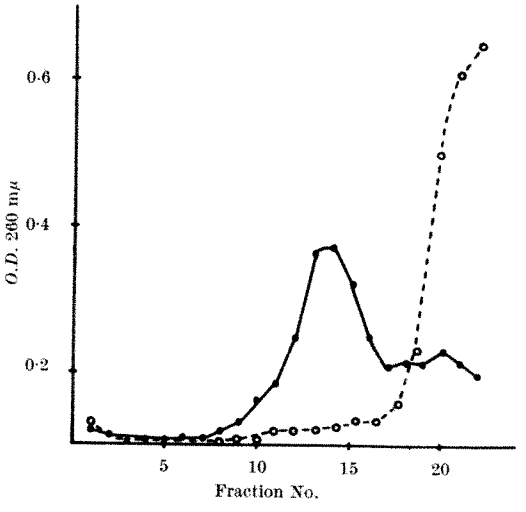


Fig. 3. Sedimentation pattern of influenza RNA (●——●) and RNase treated RNA (○- - -○). 0·5 μg/ml. RNase at room temperature for 30 min. 5–20 per cent linear sucrose gradient in 0·01 M acetate buffer, *p*H 5·3, containing 0·1 M sodium chloride, 0·001 M EDTA. Centrifugation at 37,000 r.p.m. for 3 h, at 4° C, Spinco *SW* 39 rotor

weight RNA into regular pieces having an approximate *S* value of 18, the nucleic acid of TMV was extracted and examined in the same way. Purified TMV (17 mg) was extracted, and 342 μg of RNA were obtained. Analysis in the analytical ultracentrifuge of a 50 μg/ml. solution in acetate buffer *p*H 5·3, 0·10 M sodium chloride, and 0·001 M EDTA gave a sharp boundary, and an *S* value of 31 was calculated from the densitometer tracing. This value corresponds exactly with that obtained by Spirin[28] and indicates that an undegraded molecule of molecular weight 2×10^6 has been obtained. The successful isolation of TMV-RNA by the combined phenol-SDS extraction method suggests that the homogeneous 18*S* material extracted from influenza virus is actually the RNA as it occurs in the intact particle.

It is generally agreed[28] that 18*S* ribosomal RNA has a molecular weight of about 500,000. As the extracted nucleic acid of influenza virus is about the same size, it seems that four such molecules would be required to provide a full molecular weight complement of 2×10^6.

From a consideration of the genetics of influenza virus, and the existence of incomplete virus, Hirst[26] has developed a case for the existence of separate pieces of RNA in the intact influenza virus particle. Similarly, experiments on the multiplicity reactivation of this virus[29] indicate that there could be six separate functional pieces. The experi-

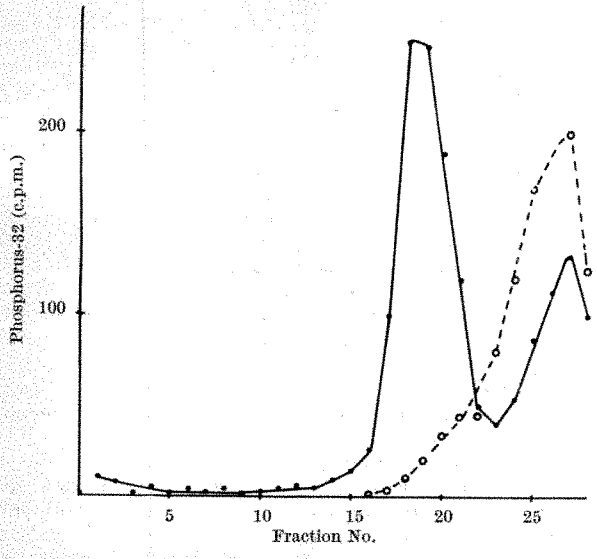
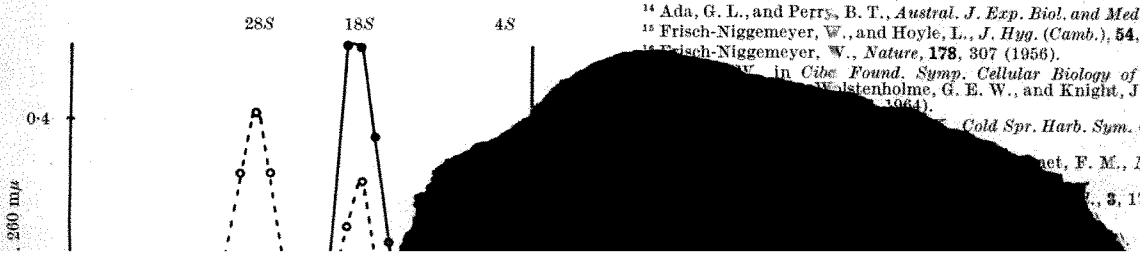


Fig. 4. Sedimentation pattern of phosphorus-32-labelled influenza RNA (●——●) and RNase treated RNA (○ - - - ○), in a 5–20 per cent linear sucrose gradient. Conditions as described for Fig. 3

ments reported here support the idea that the nucleic acid influenza virus occurs in separate pieces, and because the virus RNA appears to be of similar size to ribosomal RNA, there is the possibility that it is a variety of ribosomal RNA. In support of this idea is the fact that the synthesis of both virus RNA[30] and ribosomal RNA[31] occurs in the nucleus of the cell. Furthermore, inhibition of the synthesis of both types of RNA by actinomycin *D*[32,33] suggests that they are both made on a DNA template.



Despite the homogeneity of the extracted influenza RNA, there is still a chance that it represents broken-down molecules. Burgi *et al.*[34] have recently described preferred breakage points in the DNA molecule of *T5* bacteriophage, when subjected to shear. The effect of such shearing is to produce two or three pieces of different size, which can be readily distinguished as separate bands in a sucrose density gradient. Unless influenza RNA has three preferred breakage points, evenly distributed along the length of a single molecule, any random breakdown of virus RNA should be detectable in a sucrose gradient. Thus, the results reported here suggest that RNA occurs in the intact influenza particle in pieces with molecular weight of about 500,000.

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The action of DMBA seems to be related to the presence of corticosterone in the adrenal, and it has been suggested that the induction of necrosis is dependent on synthesis of this hormone[3]. Currie *et al.*[4] reported that 2-methyl-1,2-*bis*-(3-pyridyl)-1-propanone (metyrapone), which inhibits 11β hydroxylation and therefore interferes with the synthesis of cortisol and corticosterone[5], protected the adrenal glands from DMBA. Dao and Tanaka[6], in addition to confirming this finding, showed that 3-methylcholanthrene, which also inhibits corticosterone synthesis[7], also had a protective action, and furthermore, that a number of other polycyclic hydrocarbons—including DMBA itself in a small dose—were also effective[8]. Recently Wattenberg and Leong[9] reported that substances such as phenothiazine, which enhance detoxification mechanisms of polycyclic hydrocarbons non-specifically, can protect the adrenal gland from DMBA. Protection may have been achieved by speeding up the removal of DMBA from the body, but, on the other hand, it has been known for some time that DMBA, like other carcinogenic polycyclic hydrocarbons, can be metabolized by the rat and it is possible that DMBA exerts its action only after conversion to an active derivative[10].

We have investigated the effect of impairment of liver function by carbon tetrachloride and by partial hepatectomy on DMBA-induced adrenal necrosis.

Female Sprague–Dawley rats were obtained commercially at 40 ± 1 days of age from Oxford Laboratory Animal Colonies, Oxford. In the first experiment, groups of rats (see Table 1) were treated intraperitoneally with carbon tetrachloride (0·3 ml. of a 50 per cent v/v solution in olive oil) or olive oil alone when 49 ± 1 days old. In the second experiment, partial hepatectomy was performed under ether anaesthesia. The median and left lateral lobes of the liver were excised after the method of Higgins and Anderson[11]—from our previous experience about 65 per cent of the liver is removed. Control groups included sham hepatectomy and unilateral nephrectomy (see Table 3). DMBA in 15 per cent lipid emulsion (3 mg in 0·6 ml. emulsion) was injected intra[illegible] 24 h after treatment with carbon tet[illegible] operation. In control groups [illegible] emulsion containing no [illegible] stunning 3 days af[illegible] were removed [illegible] formaldehyde. [illegible] were similarl[illegible]

laris); + +, focal necrosis; + + +, severe necrosis involving > 90 per cent of the zona fasciculata and zona reticularis. On no occasion was there disagreement on categorization, and in almost all cases the two adrenals from the same rat showed the same histology. In the rats treated with carbon tetrachloride, liver damage was also assessed independently without knowledge of the previous treatment. Eighty-five to ninety per cent of the rats treated with carbon tetrachloride developed centrilobular liver necrosis (Fig. 1).

Carbon tetrachloride. The results for the first experiment are summarized in Table 1. The majority of adrenal glands in rats given DMBA 24 h after carbon tetrachloride showed little or no damage. For ease of comparison we have grouped together the glands showing massive necrosis (+ + and + + +); the glands in the other grades (− and +) show little or no damage (Table 2). Pretreatment of rats with carbon tetrachloride gives a high degree of adrenal protection against DMBA; in four of the seven rats in which carbon tetrachloride did not do so, only minimal liver damage had been produced. Adrenal necrosis was not seen in rats given only carbon tetrachloride. There was no histological difference between the livers of rats treated with both carbon tetrachloride and DMBA and those of the animals given only carbon tetrachloride.

Table 1. EFFECT OF CARBON TETRACHLORIDE ON ADRENAL NECROSIS INDUCED BY DMBA

Group	Pretreatment—intraperitoneal injection of	Challenge (intravenous)	No. of rats	No. rats surviving expt.	Adrenal grading −	+	+ +	+ + +
1	Carbon tetrachloride	3 mg DMBA	55	41	17	17	—	7
2	Olive oil	3 mg DMBA	20	19	3	2	1	13
3	Carbon tetrachloride	Control emulsion	10	8	7	1	—	—
4	Olive oil	Control emulsion	10	10	10	—	—	—

Table 2. ADRENAL GLANDS SHOWING MASSIVE NECROSIS

	Carbon tetrachloride + DMBA	Vehicle + DMBA
[illegible]ssive adrenal necrosis	7/41	14/19

[illegible]tween the two groups is highly significant ($P < 0{\cdot}001$).

[illegible]ARTIAL HEPATECTOMY ON DMBA-INDUCED ADRENAL NECROSIS

that in this group—as also after sham hepatectomy—DMBA caused massive adrenal necrosis in all the rats. Eight out of ten rats given only DMBA (group 4) developed adrenal necrosis: this is in accord with our previous experience that 3 mg DMBA causes massive adrenal necrosis in 70–80 per cent of the rats.

It is quite clear that when liver function is impaired either by the treatment with carbon tetrachloride or by partial hepatectomy the adrenal glands are protected from the action of DMBA. It seems likely that DMBA is metabolized in the liver to a derivative that is adrenocorticolytic. Boyland, Sims and Huggins[10] have shown not only that DMBA can be metabolized by rats but also that the derivative 7-hydroxymethyl-12-methylbenz(*a*)-anthracene (American terminology), given in smaller doses than DMBA, produces severe adrenal necrosis. Metyrapone, which inhibits the adrenal enzyme 11β-hydroxylase, protects the adrenals[4], and it is possible that it may also act on liver hydroxylases, thereby preventing the metabolism of DMBA.

The interval between administration of carbon tetrachloride and the challenge with DMBA is important: with the dose used by us, treatment with carbon tetrachloride 24 h before DMBA gives the highest degree of protection. Tanaka and Dao[12] recently reported that carbon tetrachloride has no effect on DMBA-induced adrenal necrosis. In our earlier experiments, with doses of carbon tetrachloride similar to those used by these workers and a similar vehicle (mineral oil), we found little or no liver damage and no evidence of adrenal protection.

The 'stress' of treatment with carbon tetrachloride or of partial hepatectomy is likely to cause increased production and secretion of corticosterone and, furthermore, if DMBA is metabolized less efficiently because of impaired liver function, the adrenal glands will be exposed to circulating DMBA for longer than controls. Despite these two factors—which might be expected to cause a greater incidence of adrenal necrosis—the adrenal glands are protected from the action of DMBA. This is all the more remarkable because operative stress, as in the sham hepatectomy and nephrectomy groups, apparently increases the susceptibility of the adrenal glands to DMBA.

Thus, in summary, pretreatment of female Sprague–Dawley rats with 0·3 ml. of a 50 per cent v/v solution of carbon tetrachloride in olive oil intraperitoneally 24 h before challenge with DMBA protected the adrenal glands from the necrotizing effect of DMBA. This dose of carbon tetrachloride caused centrilobular necrosis of the liver in 85–90 per cent of the rats. Protection was also achieved by partial hepatectomy 24 h before challenge with DMBA. It is concluded that DMBA itself is not adrenocorticolytic and that it is metabolized in the liver to a substance that causes massive adrenal necrosis.

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DEFICITS IN PRIMITIVE PERCEPTUAL AND THINKING SKILLS IN SCHIZOPHRENIA

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Traditionally, though not perhaps with complete justification, the diagnosis of the complex of conditions known as schizophrenia has been the responsibility of the medical speciality of psychiatry. While logical doubts remain whether this condition of psychological abnormalities is classifiable as an illness in the sense that anaemia or tuberculosis is an illness, the major treatment of choice has been for at least 30 years a variety of medically prescribed biochemical or electrical agents. This direction of responsibility, interest and treatment has derived basic support not only from the history of medicine but from a variety of genetical assumptions founded on a variety of evidence concerning the familial incidence of the condition, thus leading to specific as well as general assumptions concerning the physiological or neurological origin of schizophrenia.

By contrast, in a recent article Huxley, Mayr and Osmond[1] have made a case for considering the solution of the problem of schizophrenic behaviour as a wide interdisciplinary concern to which *inter alia* biochemistry and psychology may be expected to make valid contributions. Baldessarini and Snyder[2] have appropriately taken issue with Huxley *et al.* on the degree of incautious certainty with which the latter have put forward their views (*a*) concerning the genetical determination of schizophrenia and (*b*) concerning the mechanisms of genetical transmission. It is not my purpose to enter what is primarily a geneticist's province. From the psychological point of view it is less important to know that biochemical or morphological mechanisms may be genetically determined by one form of transmission or another, or even that these mechanisms may determine early abnormal developmental trends in children, than that behavioural predispositions to develop one or the other form of what is termed schizophrenia may originate early in life, and that the start of schizophrenic processes may be found in inadequacies and idiosyncrasies of perception and thought which have their roots in this early stage of individual development. This hypothesis, so far still not proved, ceased to be original a long time ago: one only has to cite the somewhat unpopular psycho-analytic theory concerning inadequate ego processes, reality testing and ill-defined and permeable ego boundaries, which in terms of this particular theory usually develop in early childhood. While this and other aspects of psycho-analytic theory have been castigated for lack of precision, for their fancifulness and untestability, testable alternative propositions concerning the possible early developmental roots of schizophrenic forms of behaviour have been slow in coming forward. There seem to have been six main

reasons for this omission. In the first place, psychiatry appears to have been rather jealous of its traditional position with respect to a human condition which has been classified as an illness, and has frequently been reluctant to invite the co-operation of non-medical personnel in the study of all the so-called mental illnesses. Secondly, and largely because of this, psychological hypotheses, both relevant and testable, concerning the genesis of schizophrenic perception and thought are only rarely formulated and even less frequently submitted to tests of falsification. Thirdly, a fully developed condition of schizophrenic behaviour in many cases rules out those types of experimental psychological investigations which require alert, rational and sometimes prolonged participation by the patient. Fourthly, the condition of schizophrenia, despite many attempts at meaningful sub-classification, and despite considerable evidence concerning the heterogeneous nature of its individual characteristics, frequently continues to be conceptualized as an internally consistent, homogeneous state. Fifthly, because the diagnosis of the condition of schizophrenia is made in most cases on overt behavioural, that is to say phenotypical grounds, its diagnosis is subject to the notorious errors of the unreliability and invalidity of subjective inspection, and furthermore the eclectic psychiatric definition itself of the condition contains more than the suspicion of a tautological error. Finally, the clinician as well as the experimental psychologist is faced in the patient with the end result of a long developmental history and not usually with the early stages of the abnormal processes. Under these conditions the testing of explanatory hypotheses is patently not appropriate because it would depend excessively on retrospective inference, while the required experimentally controlled longitudinal studies of a sufficiently large sample of children beginning at an early age have not yet been undertaken. Yet this degree of persistence will eventually be required in order to say whether a state of disordered perception and thinking in a fully developed schizophrenic condition represents the outcome of an early state of maldevelopment which has been continuous and of increasing implication for maladaptation of the individual, or whether the abnormal end state represents the outcome of an inhibition of culturally normal and acceptable processes and functions possibly as the result of a single traumatic experience or a series of such experiences, or whether a conjunction of both possibilities must be considered. Any investigation, therefore, showing deficits of rather basic skills of perception and thought in schizophrenia is subject to this important limitation.

It is commonly agreed that there are three major deficits in people diagnosed and classified as representing schizophrenic states: disorders of cognition, impoverishment of affect and diminution of motivation and motility. At present psychological theory based on experimentation is best qualified to offer models of explanation in the field of cognition. These models may be at different genotypical levels: they may be in terms of a restricted environmental field being available to a schizophrenic from which to draw appropriate cues for perception and thought, or they may concentrate on unusual or excessive use of cues giving rise to cognitive over-inclusion (see, for example, ref. 3). Alternatively, a more general model may be based on a reinforcement-type of learning theory, in which, for reasons as yet inadequately understood—but possibly through anxiety reduction—culturally inappropriate behaviour will be rewarded with progressive frequency (see, for example, ref. 4).

From 1959 until 1963 I was engaged in studying the effects of a variety of rehabilitation techniques and re-activating and re-motivating devices which might lead to an increase in the discharge rate and in the re-integration into the general social and occupational environment of schizophrenics who had been hospitalized for many years[5–7]. In the process of observing the patients concerned while engaged in actual or quasi-industrial assembly tasks, and while observing them and listening to them during the associated periods of group discussions, it was possible to obtain many examples of the operation of delusional, irrational and inconsequential thinking. In the present case this observational evidence suggested, because of its lack of everyday logic, because of the primitiveness of its cause and effect thinking (quite apart from the content of any delusional verbalizations), that it might be possible to find in schizophrenics evidence of an impairment at levels of thinking operations which are usually fully established in children of about 11 years of age possessing normal intelligence*. The kind of processes which were thought to be impaired were a sample of those cognitive skills which Piaget and his Geneva school of developmental psychology consider to occupy a fairly lowly and early, though essential, position in the hierarchical development of adult rational thought and perception. The aim of brevity of exposition forbids an account here of Piaget's conceptualizations concerning the development of propositional, logical thinking operations by way of earlier and developmentally more primitive stages of so-termed "sensori-motor, pre-operational, intuitive and concrete thinking". Apart from the many books and monographs by Piaget and his associates, eminently readable summaries of the relevant theories are available[8–10]. While Piaget's own work and particularly his earlier studies have been criticized because of an absence of experimental controls, a number of English workers have more recently produced experimental evidence that the hierarchical conception of the development of more and more complex, abstract and analytical thinking operations appears to be generally valid[11–15]. This means *inter alia* that children who on educational grounds are considered retarded and who also have a low intelligence quotient do not perform at the level of more normal children with tasks requiring abstraction, generalization or hypothetico-deductive forms of reasoning, and furthermore that children capable of logical/deductive operations are capable of them by virtue of the fact that developmentally earlier and more basic operations have been successfully assimilated. If it is held that delusional and other thinking abnormalities in schizophrenia suggest a breakdown of higher level logic in relation to certain events or situations impinging on an individual, it becomes relevant to enquire if lower level cognitive skills have remained unimpaired. Such an enquiry would involve the application of certain tasks under standard conditions which, according to Piaget's theories, are said to reflect the availability of such lower level skills.

In the experiment reported here, three cognitive skills usually fully or near fully developed in children of 11 years of age were selected to test the hypothesis that pre-operational, pre-logical thinking and perception are impaired in patients diagnosed as schizophrenic. The skills chosen were those of conservation of quantity and volume, the skill of seriation, and knowledge of parameters entering into perspective which requires both the skills of conservation and of seriation. In a task aimed at the availability of the conservation skill, subjects are required to make a comparison statement about quantities or volumes objectively equal in size or amount despite the presence of misleading spatial arrangements or distributions. Two tasks were given to test the availability of this ability. In the first, the investigator counted out two heaps of twelve dried beans from a jar and in the presence of the subject laid out the beans in two rows, 1 in. apart in one row and roughly 3 in. apart in the other row. To obtain a maximum score, subjects had to state without qualification that there were the same number of beans in each row. For the second conservation task, subjects were presented with two glass beakers filled to the same level with dried beans, pointing out that both beakers contained the same amount of

* This possibility was also suggested by, for example, Freeman *et al.*[16].

beans. The amount of beans in the first and then the second of these containers was then poured into two or more taller or shorter, narrower or wider glass beakers in a standard procedure. To obtain the maximum score subjects had to state without qualification that the amounts being compared were equal despite their varied distribution, difference of levels in glasses, number of glasses and apparent differences in volume.

The skill of seriation consists at its lower levels of the ability to order a set of material according to some principle of classification, which in the present investigation was order of size of randomly presented objects. Here, subjects were required to place ten differently coloured sticks 1 cm square varying in length from 1 to 10 cm, in order of size on a board with a straight horizontal bar to provide a level base for the series, after first indicating verbally and by pointing the order in which the sticks ought to be placed. For a fully adequate performance obtaining maximum score, anticipation and construction of the series had to be correct in detail without overt trials.

Knowledge of perspective requires availability of the knowledge that removal in distance does not affect the actual size of the object removed, only its apparent size. It also requires the ability to be aware of and profit from ordering of stimuli according to real or apparent size. For the assessment of present awareness of parameters operating in perspective, subjects were presented with a perspective drawing of a "Stately Home . . . with a long drive leading from the gate to the house . . .", as well as three sets of eight cardboard cut-outs shaped and painted to represent trees, which had to be placed ". . . at marked points along the drive as they would appear to us as we look from the gate along the drive to the house . . .". The trees were proportional in size to the perspective angle of the lines representing the drive in the drawing. Subjects received no further instructions and were thus free to use the same size trees for all positions or any combination of sizes in distance reducing or non-reducing series, placed in the true vertical position or perpendicular to the line demarcating the pathway. To obtain maximum score for this task, subjects had to place the cut-outs in the true vertical, size reducing series in correct sequence.

The system of scoring adopted here was simple and objective: 3 for a fully correct response, 1 for an incorrect response or a number of errors exceeding an operationally defined maximum, 2 for an intermediate response. It is indeed a rough-and-ready scoring system, though it is of a type frequently applied to the scoring of intelligence test items. For subsequent analysis responses were appropriately classified according to the degree to which the underlying cognitive principle was differentially present, where fully correct responses indicated presence of the full principle, and incorrect responses no principle present.

The investigation was carried out on an experimental group of twenty-five clinically diagnosed non-paranoid schizophrenic men aged 32–48 years ($\bar{X}$ = 40·8), detained in hospital 1–20 years ($\bar{X}$ = 11·9), with progressive matrices intelligence quotients 74–133 ($\bar{X}$ = 95·3) who participated in the conservation and seriation experiments only. A second group of ten schizophrenic men who participated only in the perspective experiment possessed the following basic characteristics: age 26–49 ($\bar{X}$ = 41·8), intelligence quotients 81–115 ($\bar{X}$ = 95·6), hospitalization 1–24 years ($\bar{X}$ = 9·1). The patients were receiving various maintenance doses of a variety of tranquillizers and almost all were employed either in rehabilitation workshops or on hospital service duties. The control group consisted of twenty secondary modern school boys aged 11·5–12·8 years ($\bar{X}$ = 12·2) with Moray House verbal reasoning quotients of 82–112 ($\bar{X}$ = 95·2), selected on the basis of their availability in relation to the school time-table.

The results of the investigation are shown in Fig. 1. They indicate that the skills pertaining particularly to conservation and knowledge of perspective parameters are impaired in the schizophrenic subjects compared with the much younger children, although the groups were well equated in terms of performance on (albeit different) intelligence tests. The statistical probability of the differences was assessed by χ^2 tests comparing for both groups the availability and non-availability of the full principle of the skill. For conservation $\chi^2 = 4{\cdot}06$, $P < 0{\cdot}05$; for seriation $\chi^2 = 0{\cdot}13$, not significant; for perspective $\chi^2 = 1{\cdot}27$, $P < 0{\cdot}001$. The main reason for the non-significant difference for seriation appeared to be the greater haste of the sample of children which led them into errors, which were scored as such, but which were in almost all cases quickly corrected on second inspection by the subjects themselves. A similar, though slightly smoothed pattern of differences was obtained, when comparing the control group performance with that of a sub-sample of the schizophrenic group, none of whom had an intelligence quotient lower than any intelligence quotient in the control group. Furthermore, when considering a schizophrenic sub-sample consisting only of subjects with an intelligence quotient of 100 or more, the sample of children still performed better. Additional evidence concerning the relationship between the skills assessed here and the status of schizophrenia was present when comparing the performance of schizophrenic subgroups with high and low ratings on a rating scale aimed at obtaining an index of degree of impairment or "illness" using consistent and relatively objective rating criteria[5]. This comparison showed that patients with the greater amount of clinical impairment produced less frequently a fully or partially adequate reply to the test questions in the conservation and seriation tasks. None of the differences, however, was statistically significant. This analysis could be carried out only in respect of patients belonging to the larger group on whom, furthermore, knowledge of perspective was not assessed.

It is clear that these findings provide no evidence concerning their possible role in the development of schizophrenic perception and thinking, or whether deficiencies in these cognitive skills antedate or post-date the incidence of the diagnosed condition. Without further careful investigations it is also not possible to exclude the possibility that the obtained deficits are the result of faulty verbalization, idiosyncratic interpretation of the task instructions or temporary psychotic pre-occupation. Close observation of the subjects, however, suggests that these explanations are less plausible than the conclusion that the patient sample had real difficulties with the tasks, and that their performance indicates deficiencies in

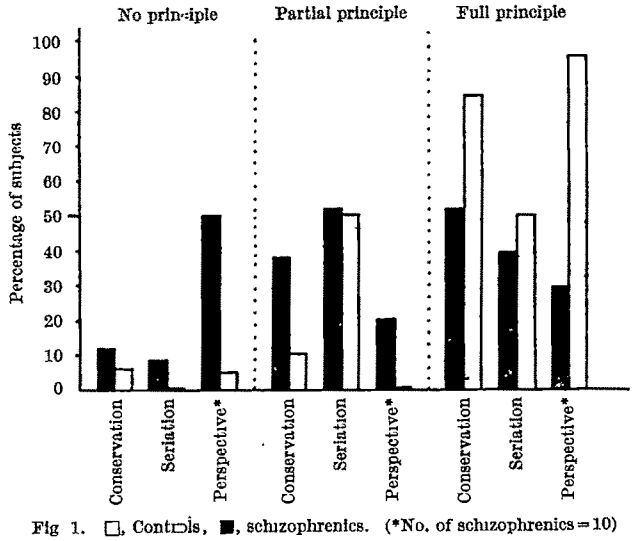


Fig 1. □, Controls, ■, schizophrenics. (*No. of schizophrenics = 10)

primitive reasoning and perceptual skills the intactness of which is usually considered an essential pre-requisite for higher level adult cognitive capacities. Given the presence of such deficits and their exacerbation with more severe psychological abnormality, their relationship to all kinds of aspects of schizophrenic behaviour will have to be more extensively investigated.

Contrary to the impression conveyed by Huxley *et al.*[1], it is not at all easy to make a psychological differential diagnosis between neurosis, psychosis and normality, partly for reasons given earlier and partly because the development of experiment-derived psychological tests and techniques of sufficient sensitivity, reliability, validity and relevance has been seriously retarded. One of the main reasons for this has been the lack of research funds in sufficient amounts and of sufficient duration to attract the experienced investigator most likely to make a breakthrough contribution. In parenthesis it might be added that the references cited in this connexion by Huxley *et al.* do not appear to exist or are irrelevant to this point.

Two further matters raised in the article by Huxley *et al.* seem to deserve some comment. First, the somewhat uncritical acceptance of some evidence suggesting that future schizophrenics may be differentiated from their sibs at a relatively early age, differences which could not be associated with environmental influences or early trauma. It is perhaps worth noting that the article cited to support this contention has a clinical/subjective bias, is retrospective and not in any way based on scientifically acceptable evidence. The only acceptable evidence would be of the kind that could emerge from fairly continuous longitudinal studies with closely controlled techniques of a sufficiently large sample of children which would eventually contain a large enough sample of schizophrenics about whose developmental antecedents meaningful conclusions could be drawn.

A final point may be made concerning the thinking characteristics of the parents of schizophrenic patients which is indirectly referred to by Huxley *et al.* Experiments by McConaghy[16] and by Lidz[17] have tended to show that on a so-called object sorting test, the scores of the parents of schizophrenics were closer to the scores frequently obtained from schizophrenics than the scores of parents who had no schizophrenic offsprings. It is implicit in the article by Huxley *et al.* that they accept this as evidence of the genetic transmission of schizophrenia (as does McConaghy himself) because it supports their hypothesis of "schizoproneness" or undeveloped schizophrenic predispositions which have become openly expressed only as the result of the low penetrance of a dominant gene in their offsprings. While this kind of evidence is necessary for a genetic theory of schizophrenia, it is by no means sufficient. None of the experiments has succeeded in controlling the environmental component which through intellectual, social or occupational learning might yield forms of conceptual thinking which are sufficiently infrequent to be comparable with the performances of schizophrenics on this kind of test. Moreover, anxiety is known to affect the efficiency of conceptual thinking, so that when comparing samples of parents with or without schizophrenic offsprings, the anxious involvement of the former is likely to affect their performance perhaps in the direction obtained. Quite apart from these possibilities, however, the presence of rare, unusual or intellectually less efficient forms of thinking in the parents of schizophrenics may be of very great importance for the development of later-appearing forms of thinking in their children which society classifies as being schizophrenic. As the result of imitation or other forms of social learning, inappropriate and irrational forms of thinking may be established in successive generations with a similar degree of predictability as would apply to the transmission of, say, racial or class prejudice. These forms of thinking would in turn affect the degree of veridicality with which such an individual will perceive his environment, with the result that perception that is class-, group- or family-specific will develop. Under these conditions, the development of progressively more logical modes of thinking may also be inhibited, and thus intellectual parentogenic influences might conceivably result in the kinds of deficits in primitive cognitive skills which have been described here. This, however, is most likely to happen only under conditions in which a child feels parent-bound by excessive demands for parent-dependence and obedience, and where, through non-compliance, the child may feel over-exposed to real or implied threats of punishment. The anticipatory sensitivity of schizophrenics to punishment has been experimentally demonstrated by Rodnick and Garmezy[4], who also describe the results of experiments on the possible effects on the development of schizophrenia of an over-protective mother whose punitive control of her child is only partially hidden by her overt expressions of love and affection. In other words, systems of communication between parents and children could develop from which the child would have the greatest difficulty in extracting a veridical and unequivocal message to which a response is required. This situation is likely to be fraught with considerable anxiety for the child because the presence of meta-messages, or "meta-meta-messages", as they have been called[18], is likely to hinder the development of certainty with respect to stimulus or response or both. In this "double-bind" situation, as it has been termed by its authors, patterns of cognitive as well as emotional responses considered as normal and appropriate by society are thus less likely to develop, either because a set of stimuli to this child does not mean what it means to the vast majority of his peers, or because interaction with other individuals becomes so stressful because of the absence of certainty and predictability that he will attempt to remove himself from the influence of such an environment. This isolation is likely to increase the degree of subjectivity in the individual's thinking and perception; it will reduce opportunities for feed-back and self-correction so that primitive processes develop less readily into more complex and rational ones, and a basis for adult processes which by their irrationality are considered abnormal will have been laid. A child's statement that he is afraid that the Moon might enter his bedroom at night and hurt him reflects the present developmental state of his reasoning ability. An adult's statement that lunar forces are injurious to him and are determining his behaviour towards others, and who habitually proceeds to act on this statement, may be indicative of schizophrenia and an impairment of average logic. It has been the purpose of the investigation reported here to show that a variety of behavioural deficits classified as schizophrenia by virtue of their irrationality and illogicality may be found in conjunction with impaired pre-logical cognitive skills which are normally established adequately in pre-adolescent children.

I thank Miss B. Bambridge for carrying out parts of the experimental work.

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LETTERS TO THE EDITOR

ASTRONOMY

Solar Eclipse of May 20, 1966, observed by the *Solrad* 8 Satellite in X-ray and Ultra-violet Bands

THE solar eclipse of May 20, 1966, was observed by the U.S. Naval Research Laboratory's 1965 93 *A* satellite and telemetered at Arcetri. This observation, so far as we know, is the first one performed by an orbiting device.

The photocells mounted aboard the satellite operated in the bands 0·5–3, 1–8, 8–16, 1080–1350 and 1225–1350 Å (further information on the satellite and its measuring system is given elsewhere[1]).

Fig. 1 shows: (*a*) the projection of the orbit of the satellite from 10h 06m 30s U.T. to 10h 15m 00s U.T. on May 20; (*b*) the projection of the umbral and penumbral cone of the Moon at 10h 10m 00s U.T. It can be seen that during these few minutes the orbit of the satellite crossed only the penumbral zone and therefore the photocells carried by the satellite observed a partial eclipse. This fact is confirmed by the telemetered signals which are graphically shown in Fig. 2 (the abscissae are the times of observation and the ordinates the solar flux in arbitrary units of three photocells). The behaviour of the signal

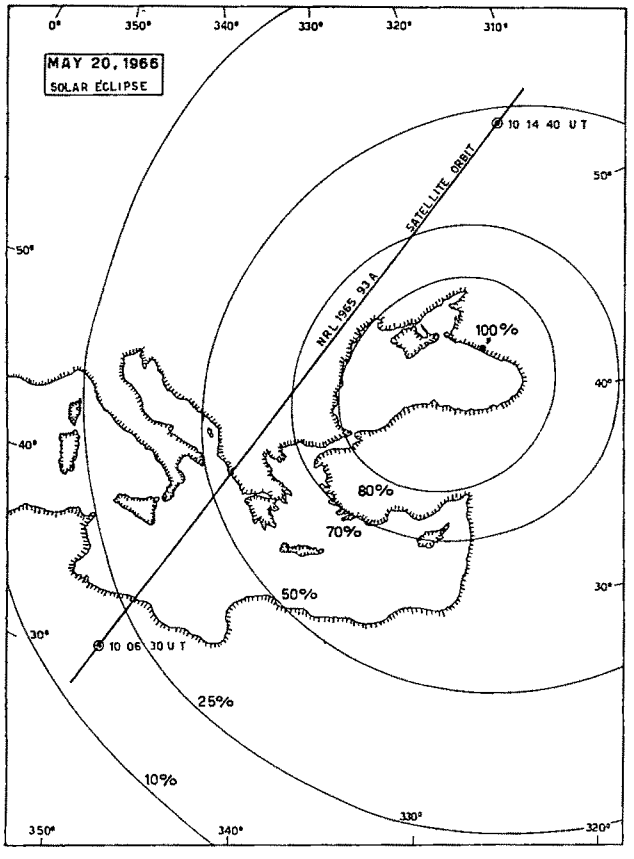


Fig. 1. Map of Eastern Europe showing the satellite orbit during the maximum phase of the solar eclipse of May 20, 1966. The circles labelled with percentages give an approximate picture of the various fractions of the occulted solar disk at the altitude of the satellite at 10 10 U.T.

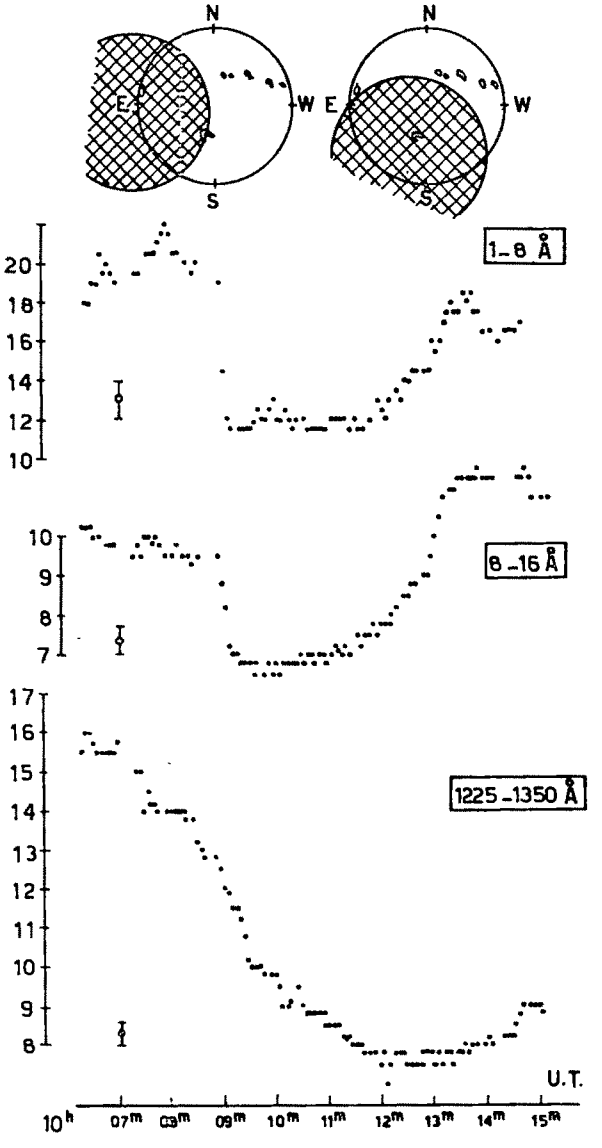


Fig. 2 Solar eclipse of May 20, 1966, observed in some X-ray and ultra-violet bands. The variations of signal (arbitrary units) for the bands 1–8, 8–16 and 1225–1350 Å are plotted against the time between 10 07 and 10.15 U.T. The symbol Φ indicates the reading error for each plot. At the top of the figure the Moon–Sun reciprocal positions, as viewed from the satellite, are shown for the time 10.09 U.T. (occultation of the central source) and 10.13 U T. (appearance of the eastern limb source), respectively

occultation for the ultra-violet band is very different from that for the X-ray band.

The difference is mainly concerned with the time of the minimum, the value of the minimum and the shape of the decreasing phase of the signal. Such behaviour can be interpreted by assuming the presence of active regions on the solar disk: these would contribute in a different way to the emission of the X-radiation and ultra-violet radiation. On May 20 there were three main sources on the disk. One remained uncovered during the whole eclipse. At the beginning of the observation the east limb source

was already occulted. It can be seen from the maps at the top of Fig. 2 that the occultation of the central active region occurred at about 10h 09m U.T.; at the same time the X-ray flux dropped abruptly. The occultation of the central source was complete within about 13 sec for the 8–16 Å band and within 9 sec for the 1–8 Å band: these values correspond to angular dimensions of 50″ and 35″, respectively. The spatial resolution along the direction of the lunar motion was 15″ of arc, which is lower than the best resolution so far obtained for pictures of the Sun in the X-ray and ultra-violet regions[2].

At about 10h 13m U.T. the source on the east limb was uncovered and the X-ray flux increased again. The ultra-violet radiation does not show any sharp variation, which indicates that the emission of ultra-violet light by the Sun is far less dependent on the localized sources.

A more detailed investigation is being carried out and will be reported later.

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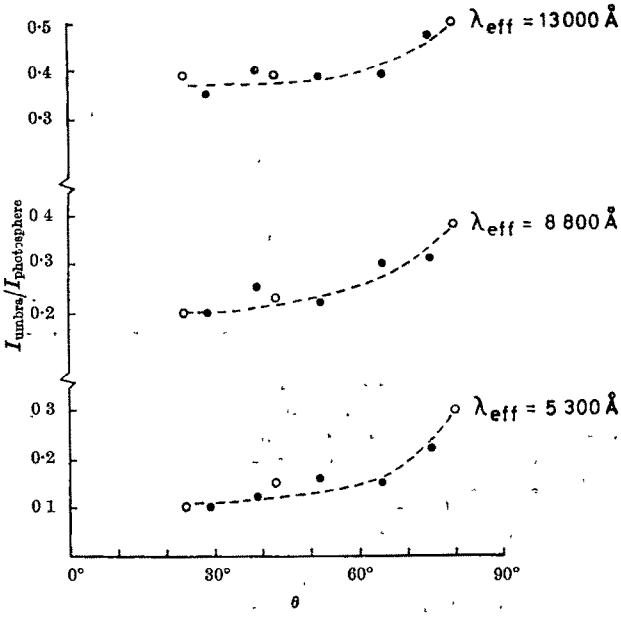


Fig. 1 Centre-to-limb variation of the intensity ratio I_u/I_{ph} for three wave-length regions. ○, Eastern hemisphere; ●, western hemisphere. The abscissa denotes heliocentric distance

Umbra Intensity of a Large Sunspot

OBSERVATIONS of the relative umbra intensity as function of the position of sunspots on the solar disk can provide information concerning the temperature distribution within the sunspots. Unfortunately, earlier workers in this field usually combined observations of different spots at several positions on the solar disk. As the umbra intensity can change considerably from one spot to another it is possible that serious errors may have been introduced. Therefore observations of the same spot on several days during its passage across the solar disk are likely to give data which are more reliable.

The large sunspot (umbra area about 150×10^{-6} solar hemisphere) which was visible during March 15–27, 1966, was observed in several positions on the solar disk at the Solar Observatory, Harestua. Intensity profiles of the spot were obtained by a pinhole photometer in three wave-length regions. The instrument used consists of a metal screen with three pinhole apertures. Just behind each hole is placed the following combination of filters (Schott u. Genossen) and detectors (Siemens):

Filter	*BG*18/2	*RG*8/2	Silicon-window
Detector	Silicon	Silicon	Germanium
Half-width	200 Å	300 Å	1400 Å
Effective wave-length	5300 Å	8800 Å	13,000 Å

The detected signals are amplified in three transistor amplifiers connected to Sanborn high-speed recorders. The image of the Sun (27·25 cm in diameter) is focused on to the screen and the scan performed by stopping the coelo-stat driving mechanism, thus causing the image to drift across the screen at a speed of 15 sec of arc per sec. Each time the spot passes the pinholes a picture is taken by a camera placed in front of the screen (slightly off-axis) and simultaneously a marker pen on the recorder indicates the time. This makes it easy to compare individual scans and to make isophote maps of the spot. After each registration, the instrument is calibrated by a step filter in front of the pinhole apertures. Possible drifts in the amplifiers are checked by blocking the holes at regular intervals. The theoretical angular resolution is set by the dimensions of the pinholes and is 1, 1.75 and 2.4 sec of arc at wave-lengths λ_{eff} 5300 Å, λ_{eff} 8800 Å and λ_{eff} 13,000 Å, respectively.

Intensity profiles of the spot were obtained on 10 days during the passage of the spot across the solar disk. On two days the observations were seriously hampered by haze and these registrations are not included. The centre-to-limb variation of the ratio $\varphi_\lambda = I_u(\lambda)/I_{ph}(\lambda)$, where I_u is the umbra intensity and I_{ph} the intensity of the photosphere, is shown in Fig. 1. The variation with position angle is pronounced on all wave-lengths, but is greatest in the blue region where $\varphi_{5300\text{Å}} = 0{\cdot}10$ at $\theta = 29°$ and $= 0{\cdot}30$ at $\theta = 80°$ (θ denotes the heliocentric angle). A comparison of the observations in the eastern and the western hemispheres reveals that the umbra intensity is constant with time; however, the spot showed certain changes in topology during the period. The scatter of individual scans through the central parts of the umbra is small. The lowest values are plotted in Fig. 1.

The scatter in previous observations of the ratio $\varphi_\lambda(\theta)$ is so great that it is difficult to be certain of the true mean centre-to-limb variation. It is often assumed that the entire variation is due to the increasing influence of scattered light toward the solar limb, and this may be the case for smaller spots.

Because the present spot is so large, the scattered light has minor influence. As seen from the tracings in Fig. 2, the umbra intensity profiles are remarkably flat even for spot positions close to the solar limb, thus indicating that possible corrections for scattered light must be insignificant. This is evident (especially in the near- and infra-red regions) from the intensity profiles at $\theta = 75°$, where the scan direction is perpendicular to the solar limb so the gradients in the spot profiles can easily be compared with the limb gradients. Corrections for scattered light can also be neglected for the observations at $\theta = 29°$.

One of the controversial questions about present sunspot models is concerned with the transparency of the umbra. Michard[1] used the observed centre-to-limb variation of $\varphi_\lambda(\theta)$ in order to determine a temperature distribution. From the equivalent widths of certain iron and titanium lines he found the electron pressure and obtained a transparent umbra model ($\tau = 1{\cdot}0$ at 2,000 km depth). Mattig[2], using the same temperature distribution, but assuming hydrostatic equilibrium in deriving the electron pressure, obtained a less transparent model with nearly the same transparency as the photosphere. Makita and Morimoto[3] also determined a temperature

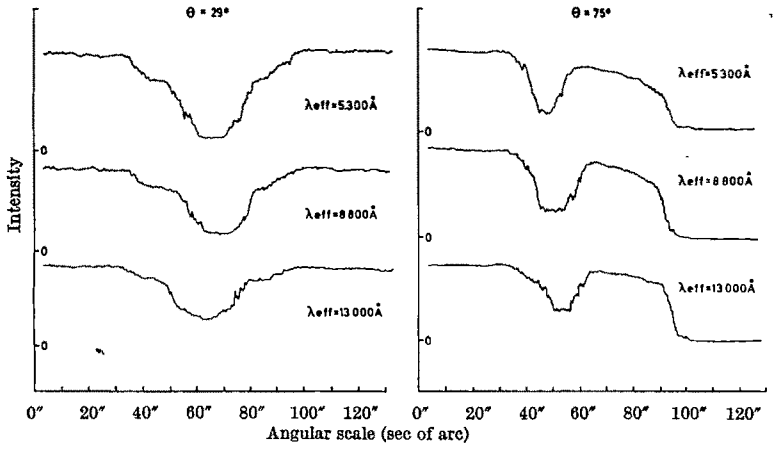


Fig. 2. Recorder-tracings showing the intensity profiles at two positions on the solar disk. The intensity scale is linear and the zeros indicate the dark level

distribution from the observed $\varphi_\lambda(\theta)$-curves at three wave-lengths. Several precautions were taken (in their observations) to avoid scattered light. They obtained a temperature distribution considerably below Michard's for spots of nearly identical size (mean umbra area in Michard's and Makita and Morimoto's measurements is 80×10^{-6} and 70×10^{-6} solar hemisphere, respectively). The difference increases with increasing optical depth and amounts to over 700° K at $\tau = 2{\cdot}0$. As was pointed out by Makita and Morimoto, the reduced temperature would solve the discrepancy in Michard's and Mattig's models if the lower temperature was used in the derivation of the electron pressure assuming hydrostatic equilibrium.

Previous observational data concerning the θ-dependence of I_u/I_{ph} consist of measurements of a number of spots at various heliocentric angles, while the present observations are for the same—rather stable—spot during its passage across the solar disk. Thus the latter should yield a more reliable temperature distribution than the earlier ones, mainly because the uncertainty due to the intrinsic scatter of umbra intensities is eliminated.

I have compared my observations at $\lambda_{eff} = 5300$ Å with Michard's and Makita and Morimoto's at λ 5452 Å and λ 5451 Å, respectively. The present observations fit well the measurements of Makita and Morimoto (toward the disk centre these give about half the values of Michard's intensity). Because the present spot is much larger than the mean spot in Michard's observations, the deviation could arise from a size-dependence of umbra temperatures. However, as the agreement with Makita and Morimoto's observations is very good, it is more likely that the deviations are caused by the different influence of scattered light, and of the intrinsic scatter of individual umbra intensities in the earlier measurements.

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Infra-red Stars

MUCH interest has been aroused by the discovery of stars which radiate most of their energy at wave-lengths of several microns[1].

It has been suggested that these are newly formed stars in the process of gravitational contraction to the main sequence, but the time scale of the contraction is so short that the objects should be much less numerous than they are, unless contraction is slowed down as a result of the proto-star experiencing difficulty in getting rid of its angular momentum[2].

Multi-colour photometry has shown that all the infra-red stars appear to be heavily reddened by interstellar dust clouds, and that the spectral types of the stars themselves fall into two widely separated groups, intrinsically hot early-type stars, such as Cygnus II No. 12, and cool late *M* stars[3–6].

Measurements of the obscuration by interstellar dust of young hot stars in stellar associations have shown that in some associations the obscuration is highest for the most luminous stars[7,8]. Recent observations of the Cygnus II association[9] have provided a striking example of a strong correlation between luminosity and obscuration for some 400 *OB* stars, leading to the conclusion that the individual stars are still surrounded by parts of the clouds of dust and gas out of which they formed, and that these clouds have diameters of not more than a few tenths of a parsec each, with masses about equal to the masses of the stars embedded in them. One of the brightest and most heavily dust-reddened of these stars is Cygnus II No. 12.

No substantial HII radiation is observed from the Cygnus II association; it is not known whether this is because of high obscuration, very high cloud densities, a lack of hydrogen with the dust in the clouds, or extreme youth. It is significant that the association is at a minimum in the thermal radio emission from the region[10], indicating that the absence of visible Hα radiation is not the result of high obscuration by interstellar dust.

The hot *OB* stars will heat up the clouds in which they are embedded, from ~100° K to several thousand °K. Two questions arise: Will the dust be evaporated, and how far will the clouds expand?

The chemical composition of the dust is still uncertain; present observations are best accounted for by the theory of graphite grains[11], which have an evaporation temperature ~2,400° K. The kinetic temperatures in HII regions vary widely[12] and may well be as low as this in parts under extreme conditions. On the other hand, the presence of large quantities of interstellar dust embedded in large, bright, well-developed HII regions, such as in the clusters *NGC* 6611 and *IC* 5146, indicates that it is not easily evaporated and the evaporation temperature may be considerably higher than 2,400° K. In addition, there is the possibility that dust is formed in the denser regions behind shock fronts as the HII regions expand.

The extent of the expansion may be limited by the elasticity of the surrounding interstellar medium. Evidence of non-gravitational forces acting on the interstellar gas[13,14] suggests the existence of substantial pressures, possibly produced by strong magnetic fields, in regions of very low gas density outside the clouds. Thus the heated clouds surrounding *OB* stars may be contained by the surrounding medium.

The lifetimes of these *OB* stars are very short, probably much shorter than current predictions now that the theory of evolution by mass loss has received support from the discovery of strong emission lines in ultra-violet spectra of early-type stars obtained from rocket flights[15]. After not more than a million, and perhaps only a few hundred thousand, years the *O* stars will evolve into *M* supergiants. This evolution occurs relatively quickly. The sharp reduction in the temperatures of the stars in the expanded clouds will result in de-ionization and cooling of the gas. The gas pressure will fall and the clouds will be re-compressed around the stars by the surrounding medium in a time probably comparable with that in which the original expansion occurred. Thus the *M* star will become surrounded by an obscuring cloud and will suffer considerable interstellar reddening.

It is therefore proposed that the infra-red M stars have evolved from O stars surrounded by the remnants of the interstellar clouds out of which they formed. If this is so, two predictions can be made: the spatial and velocity distributions of the infra-red M stars should be similar to that of the O stars, and they should be highly luminous supergiants.

If these objects occur in expanding associations, their distances from the centres of the associations should be rather larger than those of the O stars on account of their slightly greater ages; on the other hand, if they are contracting stars they will be younger than the O stars and should be nearer the centres; this may be used as a criterion for deciding between the alternative theories of contracting stars and evolved stars.

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PLANETARY SCIENCE

Implications of the Revised Mars Radius

Kliore, Cain and Levy[1], in an analysis of the trajectory of the *Mariner* IV vehicle across Mars in July 1965, have presented revised values for the radius of Mars. For the radii measured from the centre of mass, they give 3,384 ± 3 km at the entrance occultation (50·5° S. lat.) and 3,379 ± 4 km at the exit (60° N. lat.). These figures formally yield 3,392 km for the mean radius R, but give a rather greater value for the ellipticity of figure ε than the usual value (near 1/192) inferred from dynamical observations. If the usual value of ε is used the yielded value of R is nearer 3,388 km. If one accepts the authors' estimates of the observational uncertainties, it would therefore appear safe to assume that the observations yield 3,390 ± 6 km.

This result is a substantial increase from earlier estimates over the past 10–15 years, and has interesting implications on the internal structure of Mars. In 1949, I formally arrived[2], on certain assumptions, at an estimate of 3,391 km for R. Later, the observational evidence appeared to favour a value of order 3,330 km (see, for example, ref. 3), and this was used as a principal argument by many authors for rejecting one or more of the assumptions made in 1949. The return to 3,390 km, in removing this objection, means that the assumptions can now be rejected only on other grounds.

The assumptions are, essentially, that (*a*) Mars has the same overall composition as the Earth model B (ref. 4); (*b*) the changes at the Earth's mantle-core boundary are associated with change of phase rather than with change of chemical composition, in line with the postulate of Ramsey[6] and myself[8].

As regards (*a*), the crucial property of model B in this context is the comparatively high density reached fairly high up in the mantle, as compared with most Earth models. Some difficulties have arisen in sustaining this high density, but the question is by no means closed. Recent work by Lyttleton[7] takes, in effect, a slight variant of (*a*) (see ref. 8).

I am under no misapprehension as to the difficulties associated with (*b*), which involves a phase change at a pressure of 1·3 million atmospheres, accompanied by a density jump in a ratio of the order of 1·7. But again the matter is not closed, and several writers, including Lyttleton[9], continue to follow the phase-change postulate. Al'tshuler and Kormer[10] point out that an argument from shock waves (see ref. 11) that has been used to refute the postulate is not conclusive.

An essential point in the revised Mars radius is that it does shift the probabilities somewhat on the matters mentioned, and carries with it the need for a measure of re-assessment of previous theories and models.

The revision, if sustained, is also valuable in enabling theories on the internal constitution of Mars to be somewhat narrowed. For example, it removes the necessity for considering Mars models of the type constructed by me in 1957[3], and it rules out the type of structure examined by Kovach and Anderson[12]. These authors had shown the feasibility of an overall common composition for Mars and the Earth without the need for resorting to the phase-change postulate, but subject to the proviso that R does not much exceed 3,310 km. The new Mars radius would also entail some revision of calculations by Urey[13].

It may be added that the *Mariner* IV observations also yield a slightly reduced value for the mass M of Mars. The revisions of R and M thus both act in the direction of reducing the estimated mean density of Mars and are of interest in relation to curves for planetary models connecting masses and mean densities (see, for example, ref. 14).

I thank Dr. E. A. Gaugler of the National Aeronautics and Space Administration, Washington, and Mr. D. L. Cain of the Jet Propulsion Laboratory, Pasadena, for kindly amplifying the *Mariner* IV results to me in personal communications.

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Atmospheric Pressure Wave from an Explosion

PRESSURE waves were obtained from one of two explosions set off at White Sands Missile Range (33° 27′ N., 106° 34′ W.; elevation 4,900 ft. above mean sea-level), New Mexico. The first explosion occurred at 1100 p.m. M.S.T., October 28, 1965, and the second at 1100 a.m. M.S.T., November 2, 1965. These explosions were identical shots of 10 tons of TNT. Pressure waves from the first explosion

were recorded at the Socorro Campus of the New Mexico Institute of Mining and Technology (34° 4′ N., 106° 54′ W.; elevation 4,617 ft. above mean sea-level) and at the Institute's Irving Langmuir Mountain Laboratory (33° 59′ N., 107° 11′ W.; elevation 10,630 ft. above mean sea-level). No pressure waves were recorded from the second event.

At Socorro, the pressure disturbance was recorded with two hot-wire pressure transducers and also with three seismographs. The seismographs were not compensated for pressure changes and thus were not of much interest to this report. At the Mountain Laboratory, the pressure wave was recorded with a third hot-wire pressure transducer. The hot-wire pressure transducer consisted of two grids in a metal tube connected to a reference volume. The two grids were connected to a bridge circuit which detected differential variations in their resistances. The grids were cooled differentially by the air passing through the transducer into or out of the reference volume. The resistance of the two grids varied as their temperature, and the differential output of the bridge was recorded.

Meteorological soundings obtained from White Sands for the approximate times of the two explosions showed significant differences. The sounding for October 28 showed a ground-level temperature inversion with a height of 300 ft. At this time the ground temperature was 10° C and the temperature at the top of the inversion was 21° C. The sounding for November 2 gave no indication of such an inversion. No surface wind was reported on October 28. A north-east surface wind of 4·1 knots was reported on November 2.

Fig. 1 shows the pressure wave as recorded at Socorro and at the Langmuir Laboratory. The wind speed at both locations was very small. Both records continue for about 40 sec with small fluctuations. The Mountain Laboratory record shows a marked change in character after the third overpressure peak when compared with the Socorro record. The maximum overpressure at Socorro is 82 μbars and at the Langmuir Laboratory 11 μbars. The distance from the explosion site to Socorro and Mountain Laboratory is 47·5 and 50·5 miles, respectively. The difference in observed amplitudes at the two stations can be explained by considering the propagation inside and outside the temperature inversion layer. Within the layer the energy decays as the inverse of the distance. Outside the layer the energy falls off as the inverse square of the distance. Coupled with these considerations, if Snell's law is taken into consideration the calculated ratio of amplitudes turns out to be about twice the observed value. This is understandable, as the effects of wind and the leakage of energy at the boundary layer are neglected. It is anticipated that, if these two factors are properly considered, the calculated value should equal the observed one. The unfavourable wind at the explosion site and the absence of a temperature inversion were probably responsible for the failure to record the wave on November 2.

Fig. 2 shows the observed dispersion for the two records of Fig. 1, by the method of Ewing and Press[1]. The slopes of the linear portions are 14·4 ft./sec² and 13·0 ft./sec² for Socorro and Mountain respectively. The variation in group velocity for these pressure waves is from 1,134 ft./sec to 1,106 ft./sec. The signal velocity or group velocity of the first overpressure peak can be interpreted because of an average temperature over the travel path. For Socorro the signal velocity is 1,142 ft./sec which corresponds to a temperature of 27° C. For Mountain the signal velocity is 1,130 ft./sec which corresponds to 20° C.

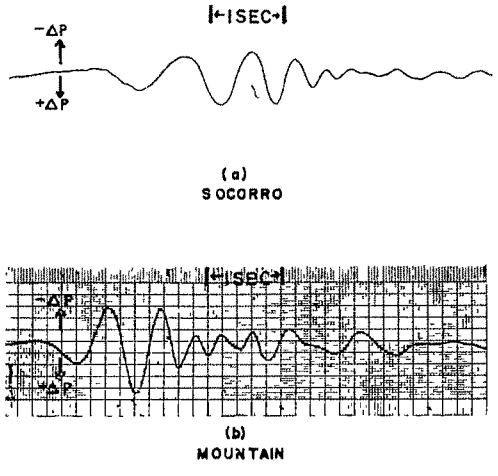


Fig. 1. Atmospheric pressure waves from a 10-ton TNT explosion

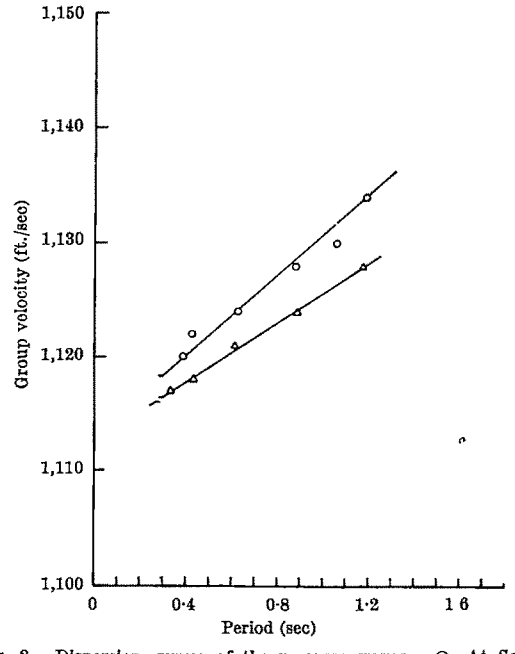


Fig 2. Dispersion curves of the pressure waves. ○, At Socorro, △, at Mountain

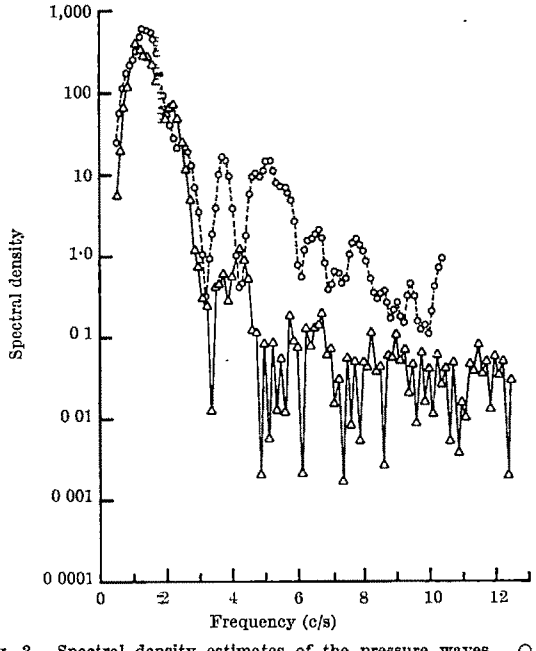


Fig. 3. Spectral density estimates of the pressure waves. ○, At Socorro, △, at Mountain

Fig. 3 shows the results of a power spectral analysis of the records shown in Fig. 1. Forty seconds of each record were digitized and the spectral density was computed on an IBM-1620 computer. The computational procedure has been described by Blackman and Tukey[2]. The Nyguist frequency is taken to be 10·4 c/s for the Socorro record and 12·5 c/s for the Mountain record. For Socorro, the maximum energy is found at 1·35 c/s with smaller maxima at 3·74 and 5·20 c/s. For the Mountain Laboratory, the maximum energy is at 1·13 c/s with a smaller maximum at 4·25 c/s.

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Noctilucent Clouds observed in Antarctica

FOGLE[1] recently directed attention to the paucity of observations of noctilucent clouds in the southern hemisphere; in fact he was able to cite only three observations of doubtful reliability and concluded that "the question of whether noctilucent clouds do occur in the southern hemisphere is still an open one". However, he himself has since answered this question with observations and photographs of a display over Punta Arenas[2].

We kept a watch at the Australian National Antarctic Research Expedition's station, Mawson (67·6° S., 62·9° E.), on every evening and morning in the periods October 19–November 6, 1964, and February 4–February 23, 1965, while the solar depression angle was greater than 6°. Noctilucent clouds were observed on three occasions.

On October 30, 1964, from 1900 U.T. to 1930 U.T. the display consisted of two moderately bright bands to the south-west. On February 8, 1965, the display lasted from the commencement of observations at 1830 U.T. until 2030 U.T. It consisted initially of bands of moderate brightness developing into bright bands and waves, then waning over a period of an hour as bands and veil. The display extended from azimuth 152° to 193° east of north between elevations 7·4° and 25·2°; colour photographs were obtained. On February 12, 1965, between 2030 U.T. and 2115 U.T., veil and moderately bright bands were observed and photographed to the south-east.

Towards the end of the display of February 8, extremely thin, bright filaments, not visible to the naked eye, were observed through a theodolite telescope of × 6 magnification. This structure is probably similar to that reported by Paton[3].

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Ancient Continental Configurations

DR. K. M. CREER's attempt[1] to superimpose the palaeomagnetically derived polar wandering paths of the various continents is a promising new method which may eventually help to solve the problem of the former continental configurations. At present, however, many of the palaeomagnetic results are still too doubtful, as Van Hilten[2] pointed out, to eliminate the guess-work in the construction of the paths of polar wanderings. Van Hilten's acceptance of Creer's paths of polar wanderings for Africa and South America makes it desirable to point out some of the uncertainties and discrepancies contained in Creer's data:

(1) *The Silurian pole.* Neither the Silurian age of the Urucum beds nor that of the Table Mountain Series has been proved by fossils. The Silurian pole, as derived from the Urucum beds, seems to lie too far from the thick glacial deposits of the Silurian Zapla beds[3] to fit the geological evidence.

(2) *The Devonian pole.* The reconstruction shows the Devonian pole situated on northern Africa. Such a position is contradicted by the extensive development of Devonian coral reefs in West Africa[4]. The only well-documented Palaeozoic glacial deposit in northern Africa is of Ordovician age[5]. This discrepancy between the geological facts and the palaeomagnetic measurements casts doubt on the age of the magnetization of the South American Devonian red-beds as used by Creer. The fact that Devonian glacial deposits have been reported from Argentina[6] and from Brazil[7,8], though the evidence is perhaps not completely conclusive, strengthens this suspicion. No evidence for a Devonian pole has as yet been derived from African rocks.

(3) *The Pennsylvanian pole.* The pole position used for the reconstruction of the polar wandering path would harmonize with the geological observations, but the palaeomagnetic data cannot be regarded as reliable according to Irving's list of palaeomagnetic pole positions[9].

The conclusion seems unavoidable that the polar wandering paths of Africa and South America have not yet been satisfactorily established. In Africa the almost complete Palaeozoic succession, which exists in northern Africa, should offer opportunities for the much-needed additional palaeomagnetic studies.

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Nomenclature of the Keuper–Lias Facies of the Bristol Channel Region

WHILE working on the Keuper, Rhaetic and Lower Lias strata of the Cowbridge district in the Vale of Glamorgan, which are typical of those of the Bristol Channel region, it was felt that the terms in present use for the two main facies developed in these strata were inadequate. Some of the terms previously used are misleading and may suggest palaeoecological or palaeogeographical relationships which may not exist.

Buckland and Conybeare[1] considered the Dolomitic Conglomerate of the Keuper to represent screes accumulated on the sides of steep hills. This may be so for part of the Dolomitic Conglomerate, but it does not account for the majority of occurrences which are horizontally bedded. This interpretation does not agree with the evidence from the Rhaetic and the Lias.

De la Beche[2] attributed a beach origin to the same conglomerate. Although ahead of his time, his conception is not supported by evidence from these rocks which indicates accumulation under continental, and possibly sub-aerial, conditions.

The term 'abnormal deposits' was introduced by Moore[3] when referring to the coarse-grained facies. He used 'normal deposits' for the fine-grained facies. Both

terms have much to recommend them since neither qualifies genesis or palaeogeography. However, they do suggest a relative state of 'normality' which is impossible to assess.

Strahan and Cantrill[4,5] used the terms 'littoral' and 'normal' deposits. The former conveys a palaeoecological concept which is difficult to prove for the Rhaetic and Lias and which is definitely out of place for the continental Keuper. The latter fails on the same count as does Moore's 'normal'.

'Near-shore' and 'off-shore' facies have been introduced more recently[6]. These terms suggest a palaeogeographical relationship which cannot be proved at all times. So-called 'near-shore' facies may develop around an inlying mass of older strata projecting above the depositional interface but not necessarily projecting above the surface of the body of water in which deposition occurred.

Because the inferences of the terms mentioned here cannot be verified, are ambiguous or are misleading, new terms must be introduced. Terms conveying purely geometrical relationships must be considered. These terms should best be defined in relation to the distribution of the facies on one depositional interface. Two possible terms are: peripheral and periphractic for the coarse- and fine-grained facies, respectively. These terms are cumbersome and almost homophones and therefore do not seem practical. 'Proximal' and 'distal' are more familiar and like peripheral and periphractic do not have any palaeoecological or palaeogeographical connotations.

'Proximal' facies have accumulated along the periphery of one depositional interface and are closely associated with masses of older rock projecting above the interface immaterial as to whether these masses occur as submerged reefs or land. The sediments in this facies are on the whole coarse-grained, often containing much rudaceous material. 'Distal' facies are those general to the parts of the same depositional interface at some distance from the margins of the interface. This facies is generally fine-grained.

Regarding the two main facies of the Keuper to Lias strata of the Bristol Channel region, I should like to propose that the term 'proximal' be used for the generally coarse-grained facies which has developed close to the masses of older rock projecting above the depositional interface, and 'distal' for the generally fine-grained facies which has developed at a distance from these masses.

Part of this work was done while I was in the Department of Geology, University College, Cardiff, and while I was holding a D.S.I.R. research scholarship.

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Origin of Pillow Structure in Lavas

SINCE pillow structures were first observed, their origin has been controversial and diverse suggestions have been put forward (for excellent summaries see refs. 1 and 2). Despite a number of fairly recent papers favouring a sub-aerial origin, the consensus of opinion falls heavily on an aqueous environment for pillow formation. This conclusion is based mainly on field association, and also on the idea that the pillows are akin to emulsions, with the magma dispersed in the aqueous phase[1,3,4]. Fuller[3], and Snyder and Fraser[4], briefly mention the role of surface tension. Fuller suggests that "As in all emulsions, the dispersal of the fluid with the higher surface tension may have been attained either by the agitation of the two phases or by its entry as detached units". Snyder and Fraser, however, suggested that "some force akin to surface tension is instrumental in the formation of the Unalaskan pillows".

I suggest that the two major factors in the formation of pillows are: (i) the surface tension of the molten magma; (ii) the density of the medium in which the pillows develop.

When lava erupts into any medium, it will tend to reduce its surface area to a minimum, that is, form spheres. If the density of the magma equalled that of the medium, a sphere would form and grow indefinitely, provided magma continued to be supplied. As it grew, the force required to enlarge the sphere (P) would decrease, because $P = 2\gamma/r$, where γ = surface tension and r = radius of the sphere. However, where the density contrast between medium and magma is great, gravitational forces would prevent growth of a sphere and cause the magma to spread out as a sheet. The surface tension of basaltic magma in air is probably in the region of 300 dynes/cm (ref. 5) and this force is insufficient to maintain a sphere in air. However, in water the situation is much more favourable because the density contrast between magma and medium is reduced from, say, 1,500/1 in air to 1·5/1 in water. The contrast is still further reduced if the magma enters unconsolidated mud or tuff. The fact that many of the best developed pillows have formed in muds and unconsolidated water-laden tuffs supports this conclusion[4,6]. It is likely that most of these pillows are essentially intrusive in that they never reach the main sediment-water interface.

Gravitational forces tend to put an upper limit on the size of stable spheres, depending on the density contrast, and near this limit pillows tend to neck or bud. In some cases, the chilled skin developing on the pillow may retard or prevent further pillow growth, as was pointed out by Rittmann[6]. However, chilled skins may thicken quite slowly if convection in the surrounding poorly-conducting water is inhibited, as it might be in mud or tuff, and it is clear that many pillows and their skins remain at sufficiently low viscosity to undergo considerable plastic deformation due to accumulating overburden. Thus the effect of the chilled skin on growth may often be small.

In conditions of fairly rapid cooling, a high rate of lava emission would presumably favour formation of large pillows.

Several other factors are likely to affect the tendency of a magma to form pillows. For example, a relatively high viscosity would make the surface tension effect negligible, and this, combined with the effect of the high viscosity on the explosiveness of the magmas, is probably why there are only a few examples known of rhyolitic or andesitic pillows.

The size of the orifice through which the magma emerges must also be an important factor, and it seems reasonable that relatively small orifices (for example, cracks on the edges and surface of a flow) will favour pillow development.

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A Proposed Correlation between Elastic Strain Rebound Increments and Isostatic Gravity Anomalies

ELASTIC strain rebound increments have been extensively considered by Benioff and others[1–6]. The results of their investigations indicate that strain rebound increments vary from region to region. Their characteristics are

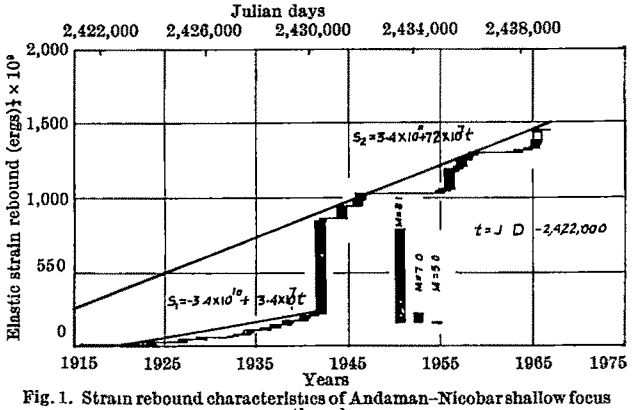


Fig. 1. Strain rebound characteristics of Andaman–Nicobar shallow focus earthquakes

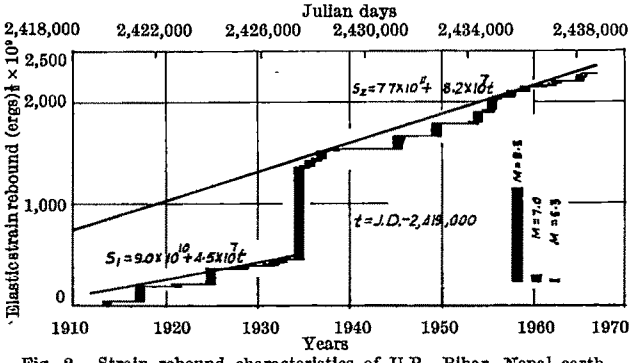


Fig. 2. Strain rebound characteristics of U.P., Bihar, Nepal earthquakes (shallow)

found to be linear and non-linear, tending to asymptotic and irregular. Such elastic strain rebound increments are simply a plot of the cumulative strain factor, $J^{\frac{1}{2}}$, versus time, where J is the seismic wave energy and can be calculated from Gutenberg–Richter's relation[7] as used by Benioff:

$$\text{Log } J = 9{\cdot}0 + 1{\cdot}8M$$

where M is the magnitude of the earthquake.

The elastic strain rebound increments of the Andaman Nicobar[5] and Bihar Nepal[5] regions shown in Figs. 1 and 2 are very similar. Both consist of two linear segments of the $S = A + bt$, where S is the strain rebound increment and where A and B are constants and where t is the time in Julian days. The activities and phases of strain recovery are also similar. The isostatic gravity anomalies given by the Survey of India and by Woollard[8] show that negative gravity anomalies of the order of -40 to -60 mgal prevail in both regions. This correlation is at first surprising, but an investigation of the elastic strain rebound increments has led me to enquire whether other similarities exist in these regions. The earthquakes used in plotting the elastic strain rebound increments are shallow focus shocks and thus the foci of all the earthquakes are located more or less in the crust. For this reason, I feel that some other similarities may exist in these regions.

In this type of correlation the magnitude of the range should be about the same. Other regions which show similar elastic strain rebound increments are Dhubri[5], Kashmir[5] and North Japan[4], where they consist of a single segment of the form $S' = a + b \log t$, where S' is the elastic strain rebound increment, a and b are constants, and t is the time in Julian days. These three regions show identical earthquake activity, and the fact that the rate of strain release decreases with time indicates that creep occurs. Here the isostatic gravity anomalies are of the order of -60 to -80 mgal and thus fit the correlation. It would seem that a definite relation exists between the elastic strain rebound increments and the isostatic gravity anomalies.

Applying this correlation to the Sadiya[5] and Tonga Kermadec[4] regions, where only shallow shocks are used in the construction of elastic strain rebound increments, it is observed that the elastic strain rebounds are of a similar nature and that they are very irregular. If this correlation is valid, it can be said that the isostatic gravity anomalies prevailing in these two areas must be similar in size and sign.

This investigation has for the main been confined to India, but such a correlation can also be established for other regions of the world. I thank Dr. V. K. Gaur for his help and Dr. Jai Krishna, director, School of Research and Training, for providing facilities for this work.

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PHYSICS

Modulation of Diffracted Neutrons with a Piezoelectric Crystal

THE production of pulses of neutrons from a reactor beam by means of a rotating scatter is a well-known technique[1]. We have investigated the possibility of using a

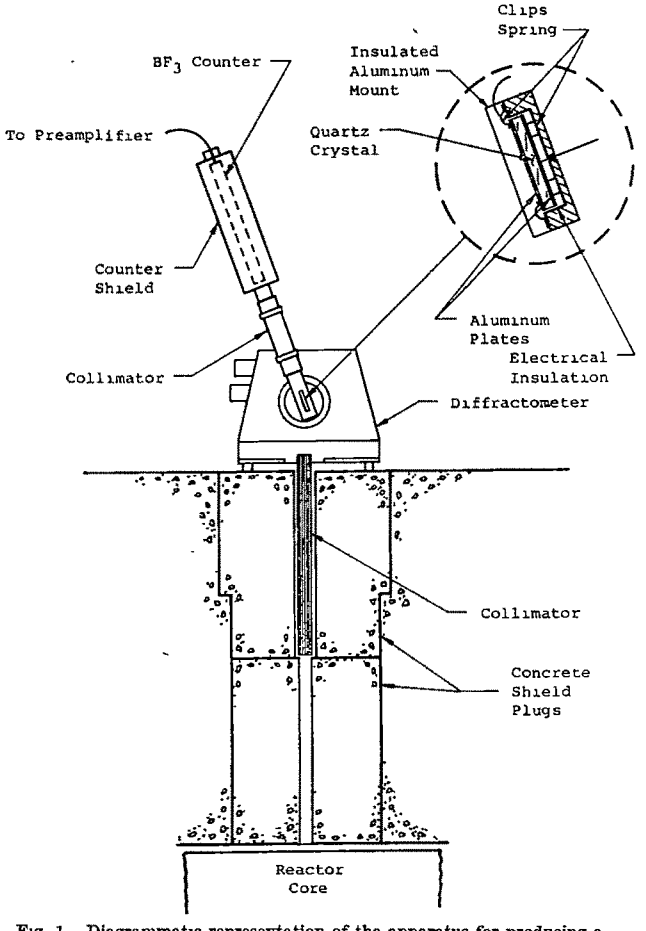


Fig. 1. Diagrammatic representation of the apparatus for producing a modulated neutron beam

quartz piezoelectric crystal as a means of modulating a beam of neutrons. It was believed that in this way it might be possible to obtain a variable frequency pulsed or sine-modulated source of mono-energetic neutrons.

A diagrammatic representation of the apparatus is shown in Fig. 1. An X-cut 500 kc/s quartz crystal 2 in. × 2 in. × 0·25 in. was used. The crystal was mounted in an aluminium holder designed to be used with a diffractometer (Picker Model No. 3527). Care was taken to eliminate all hydrogenous material from the crystal holder. Aluminium plates covered each face of the crystal and the crystal and plates were clamped in the holder with springs.

The oscillator circuit was driven by a signal generator and amplifier. The voltage across the crystal was measured with an oscilloscope which indicated when the crystal was in parallel resonance.

To restrict the divergence of the neutron beam, a Sollar collimator was used in the centre vertical port of the University of Florida Training Reactor. The angular divergence of the collimator was about 13·0 min. A second collimator was used between the crystal and the borontrifluoride detector (Reuter-Stokes model No. *RSN*-1805).

The first results we obtained were rocking curves with the crystal set for a neutron energy of about 0·03 eV; the data are plotted in Fig. 2. Here the rocking curve with the vibrating crystal is much more intense than that for the stationary crystal. The full width at half maximum for both cases is about 0·30 and the maximum counting rates differ by a factor of about two. This ratio was observed to vary directly as the power dissipated in the crystal.

To investigate whether the effect is energy dependent, we next scanned the spectrum of neutrons from the reactor with the crystal and detector fixed to maintain the Bragg condition. The factor of two in intensity between the vibrating and static crystal was essentially constant for all neutron energies.

Finally, we measured the time dependence of the detector output with a fast time analyser. Typical results are shown in Fig. 3; it appears that the neutron beam is modulated at 500 kc/s.

Possible explanations for this effect include: (1) Focusing effect of the quartz crystal when it is vibrating. (2) Change in the effective crystal plane spacing, *d*, which permits a larger 'bandwidth' of neutrons to impinge on the detector. (3) Polarization of the scattering nuclei,

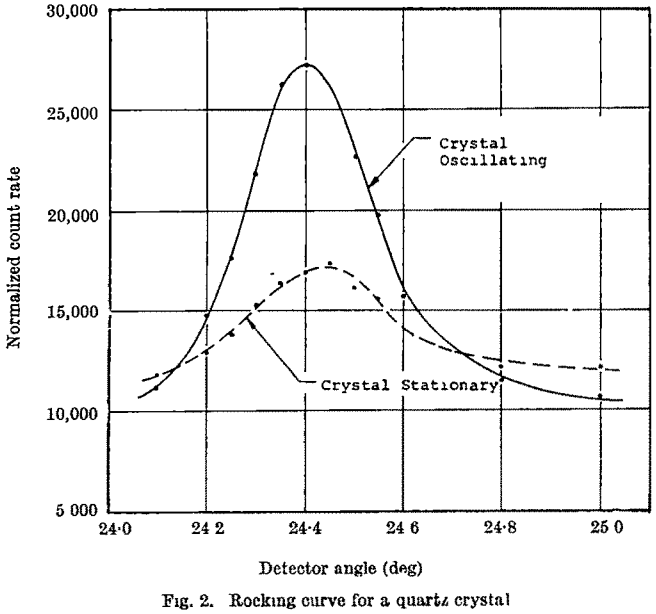


Fig. 2. Rocking curve for a quartz crystal

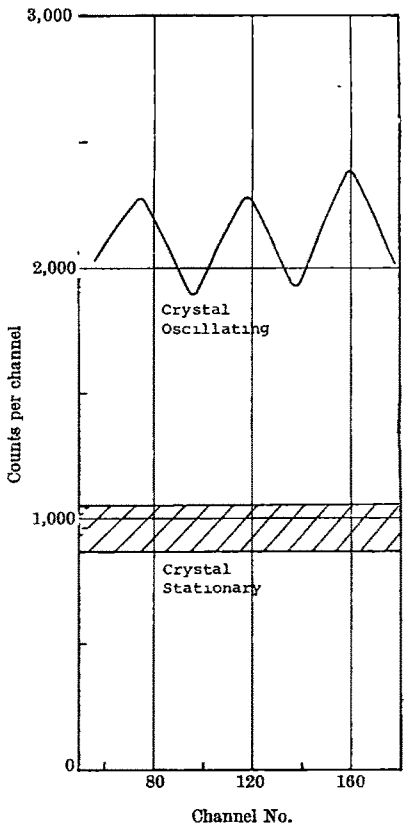


Fig. 3. Time dependence of modulated beam

which decreases the randomness of vibration and enhances the diffracted neutron intensity.

Whatever the explanation for the effect, it seems to offer a unique method for producing modulation of mono-energetic low-energy neutrons.

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Photoelastic Behaviour of Sodium Nitrate

Experimental investigations of the photoelastic behaviour of sodium nitrate are of particular interest because the single crystal of sodium nitrate is highly birefringent but very soft and brittle. It has a cleavage plane parallel to face *r* (ref. 1) which can be cleaved very easily. This sets a limit to the maximum stress that can be borne by the crystal. Unfortunately, this maximum stress is too small to produce additional birefringence measurable with the usual optical devices. All soft crystals such as alums and alkali halides require a stress ranging from 0·5 to 0·8 kg/mm² for photoelastic studies. A part of the load (about 0·2–0·3 kg/mm²) is used to make the crystal prism sink a little into the thin lead washers and its orientation is then maintained steady at all subsequent loads. This is particularly necessary in the case of naturally birefringent crystals like barite and quartz, for the slightest tilt of the prism is sufficient to vitiate the result. The birefringence of sodium nitrate is about twelve times that of quartz or barite and still greater attention has to be paid to the correct orientation of the crystal prisms. Several trials on prisms of sodium nitrate for different directions of stress showed that the maximum stress that

could be carried was about 0·35 kg/mm². The major portion of this stress was necessary for maintaining steady the orientation of the prism. Hence the small additional birefringence that might be produced as a result of the safest additional load was too small to be detected by the usual optical devices. It has been found that if any information could be sought at all on the photoelastic behaviour of this crystal, it was only by the ultrasonic method which I have developed[2].

Because of the existence of a very easily cleavable plane and considerable brittleness of the crystal, the cutting, grinding and optical polishing of the raw sodium nitrate crystal to give the necessary rectangular prisms for the ultrasonic experiments proved very difficult. Cutting with a jeweller's saw had to be abandoned. Grinding carborundum powder and cast iron base proved ineffective. The final method adopted was as follows: the raw crystal (grown in these laboratories by the evaporation of an aqueous solution) was rubbed on the moist surface of a soft and uniformly worn piece of cotton cloth stretched on a plane glass block. The water content on the cloth slowly dissolved the unwanted material of the crystal and the crystal was thus reduced approximately to the desired shape and size. The prism was then ground on the polished surface of a glass block using high-grade emery powder, with kerosene oil as lubricant. Immense patience and care were necessary during this stage, otherwise the crystal cleaved. The faces of the prism were then polished on a freshly prepared polishing tool of putty powder. The crystals keep remarkably well in dry weather.

The results obtained in the ultrasonic experiments are as follows: $p_{12}/p_{11} = 3{\cdot}19$; $p_{31}/p_{11} = 0{\cdot}41$; and $p_{13}/p_{33} = 1{\cdot}02$. It is interesting to note the high photoelastic anisotropy of sodium nitrate for ultrasonic excitation along the X-axis and the observations along Y and Z axes respectively[3].

I thank Dr. S. Bhagavantam under whose guidance the work was carried out in the Physical Laboratories, Osmania University.

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Stress Relaxation of Bitumen and Bituminous Mixtures

THE rheological properties of bitumens and bituminous mixtures are well known and standard procedures for testing them have long been established. Relations between fundamental rheological parameters and quantities measured in routine tests have been reported by several authors. A study of creep and response to dynamic loading has been published by Van der Poel[1]. In a recent investigation on stress relaxation, Kubát[2] found that the normalized inflexion slope of the relaxation curve $\frac{1}{\Delta\sigma}\left(\frac{d\sigma}{d \ln t}\right)_{\text{Inflexion}}$ (where t is the time, σ is the stress, and $\Delta\sigma$ is the stress reduction due to relaxation) was the same for a wide variety of materials, including plastics, rubbers and metals—the last-mentioned also in the form of single crystals. The numerical value was $0{\cdot}1 \pm 0{\cdot}01$. This relationship applied provided the samples were free from internal stress.

I have found that the same expression applies for petroleum bitumen and bituminous mixtures, and gives a value close to 0·1. In these experiments the samples were compressed in an 'Alwetron' universal testing machine at 25° C. The temperature of the testing atmosphere was kept constant at $\pm 0{\cdot}1$° C and the stress relaxation, after compression had been discontinued, was recorded until a constant stress value was obtained. For bituminous mixtures, this required several days. The samples of bituminous mixtures, with a bitumen content of 6·7 per cent, were compacted in moulds for a Marshall stability test. Samples of pure bitumen were cast in the same moulds and during the test they were either kept in these or removed and submerged in a water thermostat. The upper pressure plate in the tester had a diameter of 60 mm and the lower one 76 mm. In neither case, therefore, was the test one of pure compression. All samples contained a 250 pen. straight-run petroleum bitumen, and the aggregate was crushed moraine (largest size 4 mm). The following parameters were varied: void fraction, rate of compression and compressive force at the start of relaxation (Table 1). The normalized inflexion slope varied between 0·1 and 0·16, and apparently was independent of the experimental parameters. It can be concluded that for bitumen and bituminous mixtures, after compressive stress, the inflexion slope of the relaxation curve is similar to that for the substances examined by Kubát even though they are fundamentally of a different nature. The comparatively large values for the pure bitumens may be due to the fact that they were compressed considerably more than the bituminous mixtures before relaxation.

Table 1. NORMALIZED INFLEXION SLOPE OF RELAXATION CURVES OF BITUMEN AND BITUMINOUS MIXTURES UNDER COMPRESSION

Void fraction (per cent)	Rate of compression (mm/min)	Maximum pressure (kg)	Inflexion slope
4·4	1	200	0·12
4·1	10	250	0·13
4·0	100	250	0·12
15·3	10	90	0·16
2·6	10	250	0·13
4·8	1	190	0·12
2·9	1	180	0·10
2·6	1	175	0·15
2·5	1	175	0·14
2·5	1	500	0·12
2·5	10	500	0·14
Bitumen sample in water bath	10	8	0·16
,,	10	10	0·16
Bitumen sample tested in mould	10	50	0·14
,,	10	40	0·16

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MINERALOGY

Pyrrhotite: a Common Inclusion in South African Diamonds

LARGE batches of industrial diamonds from several South African localities were carefully examined and any that showed large dark regions were weighed and then crushed by a sharp blow in a stainless steel mortar. The crushed fragments were then examined under a binocular microscope (× 15). Any particle which did not appear to be diamond was transferred to a drop of ethyl acetate on a glass microscope slide. After the drop had evaporated, a rubber ball mount of the grain was prepared[1], and the mount cemented to the end of a small plastic brush fibre ready for mounting in a 57·54 mm diameter Debye–Scherrer powder camera. Each specimen was X-rayed for 3 h using cobalt radiation ($\lambda = 1{\cdot}7902$ Å) with an iron filter. If the resulting pattern was weak, the exposure was repeated for 9 h.

The X-ray powder photographs showed, except for one specimen of pentlandite (DP-3, $a = 10{\cdot}108 \pm 0{\cdot}01$ Å),

Table 1. LATTICE PARAMETERS OF PYRRHOTITES RECOVERED FROM CRUSHED SOUTH AFRICAN DIAMONDS

Specimen No.*	Original diamond: Mass (g)	Original diamond: Shape	Cell-parameters of pyrrhotite (Å): a	Cell-parameters of pyrrhotite (Å): c	Calculated d_{102}	Other phases intergrown with pyrrhotite
DP-4	0·01814	Rounded dodecahedral	3·434 ± 0·01	5·683 ± 0·01	2·055	Pentlandite
SH-2	0·03540	Rounded tetrahexahedral	3·430	5·671	2·051	Pentlandite
SH-4	0·02175	Rounded tetrahexahedral	3·436	5·675	2·053	Pentlandite + pyrite
SH-6	0·02711	Rounded dodecahedral	3·435	5·667	2·052	Pentlandite
SH-8	0·01835	Octahedral	3·437	5·684	2·055	Pentlandite
CA-?	–	–	3·434	5·684	2·055	–
J-4	0·01722	Irregular fragment	3·439	5·870	2·090	Pentlandite
J-20	0·02189	Cleavage flat	3·444	5·877	2·093	Cohenite
J-5	0·01043	Irregular fragment	3·435	5·690	2·056	Pentlandite
J-29	0·01712	Irregular fragment	3·435	5·675	2·053	Pentlandite
P-43	0·01591	Octahedral	3·429	5·674	2·051	–
P-44	0·02025	Rounded tetrahexahedral	3·446	5·758	2·072	Pentlandite
P-46	0·01552	Rounded dodecahedral	3·443	5·733	2·066	Pentlandite + pyrite

* *DP*, De Beer's Pool (mixed Wesselton, Bultfontein, and Dutoitspan); *SH*, Stadium Heap (mixed Kimberley, De Beer's and De Beer's Pool); *CA*, Congo alluvial; *J*, Jagersfontein Mine; *P*, Premier Mine.

that all the brassy coloured particles picked out from among the diamond fragments were pyrrhotite. The d-spacings were then measured using diamond ($a = 3{\cdot}567$ Å) as an internal standard. The lattice constants were calculated by Cohen's method of least squares by assuming an NiAs type of cell. The final results are given in Table 1 together with the calculated d_{102}, which can be used to infer the composition[2]. Eleven of these specimens are iron deficient and can be classed as ordinary pyrrhotite; the remaining two (*J*-4, *J*-20) are nearly stoichimetric and can be considered to be troilite.

From a total of eighty-nine South African diamonds specially selected for detailed examination, twenty were definitely coloured, and of these, only five had inclusions that could be X-rayed, whereas of the uncoloured, forty-six had inclusions that could be X-rayed. Eleven of the twelve pyrrhotites and the single specimen of pentlandite were recovered from uncoloured diamonds. Pyrrhotite was thus found in 13 per cent of all diamonds examined and in 24 per cent of the diamonds containing inclusions that could be X-rayed. Furthermore, pyrrhotite was found in diamonds from all localities that were checked. This included specimens from the Congo, one of which is included in Table 1 for comparison.

The X-ray patterns of the pyrrhotite showed that the specimens were commonly intergrown with other phases. The most common was pentlandite ($a = 10{\cdot}05 \pm 0{\cdot}02$ Å), which occurred in ten of the thirteen pyrrhotite specimens listed. In specimens *SH*-2 and *P*-46, additional lines were found which are best attributed to pyrite ($a = 5{\cdot}403 \pm 0{\cdot}02$ Å and $a = 5{\cdot}381 \pm 0{\cdot}02$ Å, respectively). The assemblages of pyrrhotite + pentlandite, and pyrrhotite + pentlandite + pyrite are probably the result of exsolution from a former high-temperature phase included at the time of diamond formation. Specimen *J*-20 was very unusual because it consisted of an intergrowth of cohenite with troilite. The cell dimensions determined on the cohenite were: $a = 4{\cdot}516 \pm 0{\cdot}01$ Å, $b = 5{\cdot}072 \pm 0{\cdot}01$Å and $c = 6{\cdot}742 \pm 0{\cdot}01$ Å.

In the past, iron pyrite has been reported as an inclusion in diamond[3–6]. Although pyrite is reported here, no specimens were found in which pyrite occurred as an original inclusion. The question is greatly confused by the occasional occurrence of irregular broken diamonds with masses of pyrite attached. I have recently examined a 2-carat stone of this kind from the Premier Mine, and am of the opinion that the pyrite is a secondary growth on the diamond which was probably formed at the same time as the other well-formed pyrite crystals frequently occurring in the blue ground of the Premier Mine. I therefore believe that, at present, there are no verifiable reports of pyrite as an original inclusion in diamond.

The results suggested by this study are: (1) that pyrrhotite is a common inclusion in diamond; (2) that it is more than coincidence that pyrrhotite (troilite) should be found so closely associated first with diamonds in meteorites[7] and now with diamonds from kimberlite pipes.

I thank the Mineralogy Section of the Anglo-American Research Laboratory, and Mrs. Eckert in particular, for helping me to X-ray the samples and to develop the films.

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METALLURGY

Stress-corrosion Cracking of Aluminium

ALUMINIUM forging alloys used in aerospace technology have, on occasion, suffered catastrophic failure as a result of stress-corrosion cracking. Study of this problem by thin-film transmission electron microscopy has disclosed

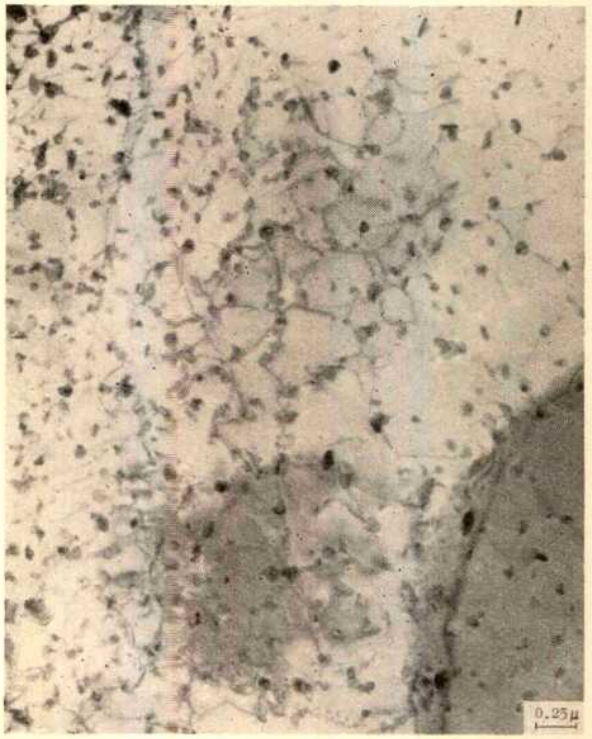


Fig. 1. Alloy 7075 *T*6 highly susceptible to stress-corrosion cracking. Note dislocation lines—many are associated with $MgZn_2$ precipitates



Fig. 2. Alloy 7075-*T*73 immune to stress-corrosion cracking. Note absence of dislocations. Coarse and fine precipitation is $MgZn_2$

a fundamental relationship between those aluminium structures within the same alloy (achieved by heat treating) that are highly sensitive to stress-corrosion cracking and those that are immune.

Specifically, the Al–Zn–Mg–Cu 7075 alloy (a typical composition is, in per cent, 5·5 zinc, 2·2 magnesium, 1·2 copper, 0·18 chromium, balance aluminium) is susceptible to stress-corrosion failure in the $-T6$ temper (highest strength condition), but is completely immune to cracking in the $-T73$ temper (low strength condition). The $-T6$ structure contains dislocation networks (Fig. 1) which are absent in the $-T73$ structure (Fig. 2).

Because pitting proceeds identically in the $-T6$ and $-T73$ tempers, namely, by the dissolution of large $MgZn_2$ precipitate particles in the grain boundaries, it is concluded that the critical role of dislocations is to assist in the nucleation of cracks of appropriate geometry for propagation.

These new findings possibly apply to any precipitation hardenable alloys that develop dislocations during the quenching operation. Similar situations have been found to exist in the 2014 and 2024 alloys.

These findings will be of practical use in the identification of structures susceptible to stress-corrosion, and may also be useful in the development of heat treatments that will give alloys of high strength and no tendency toward cracking by stress-corrosion.

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CHEMISTRY

Infra-red Luminescence (Fluorescence) and Reflectance of Coals, Coal Derivatives and Graphite

WE have investigated the infra-red luminescence (fluorescence) of coal and coal derivatives. Little, if any, visible luminescence is shown by coal except (1) in the presence of fluorescing minerals, and (2) in solutions of coal derivatives. Substances containing presumably large amounts of polynuclear aromatic compounds as well as large amounts of free radicals should exhibit appreciable visible luminescence. The lack of luminescence in solid coals or solid derivatives, such as coal-hydrogenation asphaltenes and pyridine extracts, can be explained on the basis of quenching due to various factors. But in the dissolution of asphaltene or coal extract, an intense light blue luminescence develops. A corresponding increase in specific absorption with dilution of coal-hydrogenation-asphaltene solutions was previously found and was correlated with an increase in free radicals, presumably formed by dissociation[1]. The free radicals existing in solid coal may be of such a large size that luminescence missing from the visible could possibly exist beyond the visible range. With the development of a new method for determining infra-red luminescence[2], it has become possible to check coal and coal derivatives for luminescence in this region. Infra-red luminescence has now been observed.

Experimental procedures used are described in detail in ref. 2; excitation in the blue-green region was used, and luminescence in the near infra-red region was detected photographically. Two other infra-red luminescence investigations have been reported[3]. Fig. 1*a* shows the infra-red reflectances of the eight samples examined together with a reference piece of greenockite (native cadmium sulphide); Fig. 1*b* gives the infra-red luminescences. The results obtained are shown in Table 1.

Table 1

No.	Sample	Infra-red luminescence	Infra-red reflectance
(1)	Anthracite, Glen Burn, Pa.	None	Strong
(2)	Asphaltene from 400° C hydrogenation, Pittsburgh coal	Med. strong	Med. strong
(3)	Coal (*hvab*), Pittsburgh, Pa.	Weak	Med. strong
(4)	Vitrain (*hvab*), Pittsburgh, Pa.	Very weak	Strong
(5)	Lignite, Velva, North Dakota	None	Strong
(6)	Pyridine extract from Pittsburgh coal	Weak	Weak
(7)	Graphite, spectral grade	None	Med. strong
(8)	Solution of asphaltene in benzene	Med. strong	Very weak
	Greenockite (control)	Strong	Very strong

Luminescence is observed in the infra-red in decreasing order: Coal-hydrogenation asphaltene dissolved in benzene > solid asphaltene > Pittsburgh coal = extract from Pittsburgh coal > Pittsburgh vitrain.

Part of the top surface of the asphaltene sample was scraped in order to produce a fresh unoxidized surface. The luminescence of this fresh surface is appreciably greater than that of the rest of the sample. Pittsburgh coal shows stronger luminescence than the vitrain. This can probably be explained by the fact that the coal contains other petrographic constituents which may luminesce to a greater extent than vitrain. The stronger luminescence of the coal cannot be attributed to mineral matter, for anthracite, which contains much larger amounts of similar minerals, would show an even greater luminescence. But anthracite shows no luminescence at all. Also no luminescence is exhibited by lignite; infra-red luminescence was found only for the medium rank coal. Graphite also showed no luminescence. In both anthracite and graphite, luminescence may occur at even longer wave-lengths than can be attained at present.

It is doubtful whether the infra-red luminescence observed in coal and coal products can be attributed to polynuclear aromatics; aromatics even as large as ovalene, with ten condensed rings, exhibit luminescence in the visible region of the spectrum and it is probable that they do not exhibit luminescence in the infra-red region. It would seem that infra-red luminescence of coal can be attributed to free radicals, which generally exhibit luminescence at longer wave-lengths than non-radicals of similar structure and molecular weight. It is also possible that charge-transfer complexes may be involved.

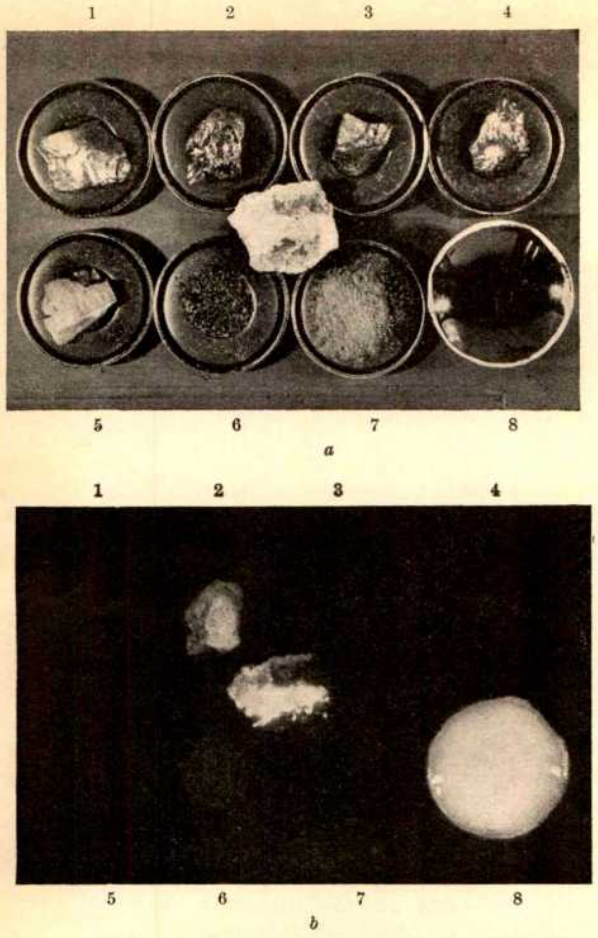


Fig. 1. *a*, Infra-red reflectance of various coals and coal derivatives. (Numbers as in Table 1. Reference piece of greenockite in the centre.) *b*, Infra-red luminescence of the same as *a*

Comparisons of infra-red reflectances and of reflectance versus luminescence are of interest. Infra-red reflectance is intense for Pittsburgh vitrain, anthracite, and lignite. Pittsburgh coal, solid asphaltene and powdered graphite have appreciable reflectances. Asphaltene in solution and pyridine extract have low reflectances. The infra-red reflectances of anthracite, lignite and graphite given in Fig. 1*a* are reflectances only; they do not involve emission of luminescence as the luminescence observations were negative (Fig. 1*b*). In general, the highest infra-red reflectances are observed for the weakest luminescing samples; and the strongest infra-red luminescence is found for the weakest reflectors.

Petrographic constituents of coals with fresh and oxidized surfaces will also be investigated. Other work will cover solutions of pyridine extract at various concentrations, residues from pyridine extractions, known free radicals, charge-transfer complexes, and polynuclear condensed aromatic compounds.

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Diffusion of Triplet Excitons produced by Ionizing Radiation in Aromatic Materials

THIS report demonstrates that the analysis of experimental radioluminescence decay curves provides estimates of the diffusion coefficient of triplet excitons produced by ionizing particles in aromatic materials; the values obtained are in accordance with the hopping model calculation.

The diffusion of triplet excitons in molecular crystals has been the subject of several recent publications[1-6]. A treatment of triplet energy transfer has been developed by Jortner *et al.*[1] and is based on a hopping model; according to these authors, their calculated diffusion coefficient ($D_T \simeq 5 \times 10^{-6}$ cm^2 sec^{-1}) in anthracene is in good agreement with measured triplet–triplet annihilation rate constant[2,3] and exciton diffusion lengths[4]. More recently, Singh *et al.*[5] and Kepler and Switendick[6] reported that the diffusion coefficient of Jortner *et al.* in anthracene seemed to be between two and four orders of magnitude too small and that a band model would be more appropriate to describe triplet exciton transfer in this crystal.

In a study of the time dependence of scintillation intensity in aromatic materials[7], a theoretical model has been developed to describe the origin of the delayed scintillation component[8] and excellent agreement with experimental results has been obtained. The delayed component was attributed to emission from singlet states ('delayed' singlets) formed during the encounter of two triplets in the particle track.

During the retardation of a charged particle in the organic medium, slow secondary electrons are produced, which in turn induce molecular ionized and high excited singlet states, located in small, approximately spherical volumes, referred to as 'spurs' and 'blobs' in a recent work[9]. Within these highly activated areas, a great triplet density results from the major processes of ion recombination and direct excitation by slow electrons; the local concentration of singlet states is reduced by fast quenching effects which concern mainly the primary upper singlet levels ('ionization quenching')[7]; intersystem crossing from the lowest singlet states is not expected to influence to any great extent the triplet state population. In the case of heavy particles with high specific energy loss (for example, 5-MeV α-particles) the distance of successive spurs is so small that the activated species are distributed along the particle path within a cylindrical track; the initial triplet distribution function can then be considered in cylindrical symmetry as:

$$c_T(r,0) = \frac{n_T(0)}{\pi r_0^2} \exp\left(-r^2/r_0^2\right) \qquad (1)$$

Here $c_T(r,0)$ denotes the triplet density at time $t = 0$ and at a distance r from the particle path and $n_T(0)$ the total number of triplet excited states produced per unit path-length; the length r_0 characterizes the lateral distribution of triplet states and should be comparable with the dimensions of the elementary blobs. With these conditions, the following kinetic-diffusion equations for excited triplet and delayed singlet states were resolved:

$$\frac{\partial}{\partial t} c_T(r,t) = D_T \nabla_r^2 c_T(r,t) - \frac{1}{\tau_T} c_T(r,t) - \gamma_{tt} c_T^2(r,t) \qquad (2)$$

$$\frac{\partial}{\partial t} c'_s(r,t) = D_s \nabla_r^2 c'_s(r,t) - \frac{1}{\tau_s} c'_s(r,t) + k_{tt} c_T^2(r,t) \qquad (3)$$

where $c'_s(r,t)$ is the density of delayed singlets, D_s the singlet state diffusion coefficient, τ_s and τ_T the fluorescence and phosphorescence decay times respectively, γ_{tt} the bimolecular annihilation rate constant, and k_{tt} the rate constant for singlet state production resulting from the bimolecular reaction between triplet states.

For times when $t \gtrsim 10\ \tau_s$, the light intensity as a function of time was found to be given by:

$$I(t) = M\,[1 + A \ln (1 + t/t_d)]^{-2}\,(1 + t/t_d)^{-1} \qquad (4)$$

where M and A are constants and $t_d = r_0 2/4D_T$.

Experimentally, using a single photo-electron technique[10,11], we have measured the luminescence decay pulse shapes for liquid scintillators excited by ionizing radiation. In Fig. 1, two typical decay curves are shown, obtained with deoxygenated toluene solutions irradiated by 5·3-MeV α-particles. Taking $A = 1{\cdot}0$ and $t_d = 5$ nsec, it is seen that the shape of the delayed component is well described by relation (4). This equation also accounts accurately for the slow-scintillation decay in crystals and, by comparison with experimental curves published by Bollinger and Thomas[10] and Wasson[12], we obtained $t_d = 40$ nsec for a crystal of anthracene and $t_d = 80$ nsec in the case of *t*-stilbene.

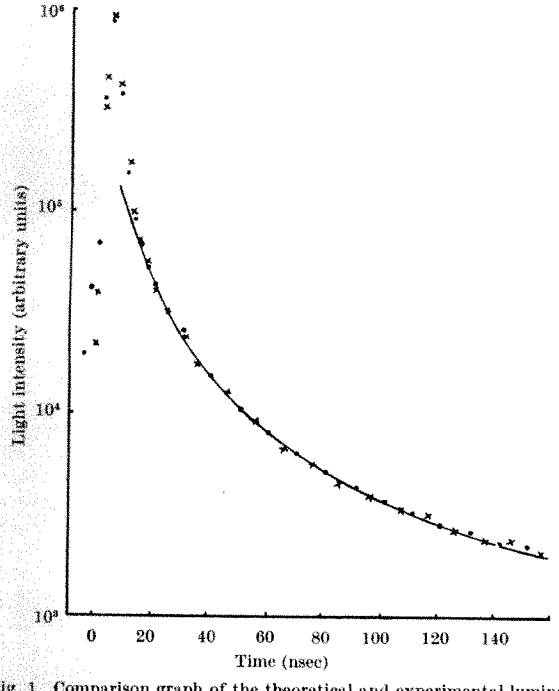


Fig. 1. Comparison graph of the theoretical and experimental luminescence decay curves for liquid scintillators (2-phenyl 5-bi-phenyloxadiazole (PBD) and terphenyl (TP) in toluene) excited by 5·3 MeV α-particles. Experimental points: ●, PBD (10 g/l.) in toluene; ×, TP (7 g/l.) in toluene. ———, Theoretical curve ($A = 1{\cdot}0$, $t_d = 5$ nsec.)

Analysis of the values of t_d measured with liquid toluene, in conjunction with those obtained for crystalline anthracene and stilbene, provides estimates of the length r_0 and of the triplet state diffusion coefficients in the crystals. In liquids, D_T is close to the material diffusion coefficient; so that, for the two scintillator solutions shown in Fig. 1 having toluene as a solvent, $D_T = 2{\cdot}2 \times 10^{-5}$ cm² sec⁻¹; with $t_d = 5$ nsec, we then obtain $r_0 = (4D_T t_d)^{1/2} = 7 \times 10^{-7}$ cm. This value is in agreement with $r_0 = 5 \times 10^{-7}$ cm previously used for an aromatic crystal[13]. Also it is comparable with the predicted dimensions of the blobs generated by slow secondary electrons[9], the ranges of which are not expected to differ notably in the aromatic liquids and crystals. Assuming, therefore, a similar value for r_0 to be valid for the crystalline scintillators and using the measured values of t_d, we obtain $D_T = 3{\cdot}0 \times 10^{-6}$ cm² sec⁻¹ for anthracene and $D_T = 1{\cdot}5 \times 10^{-6}$ cm² sec⁻¹ for *t*-stilbene.

These triplet state diffusion coefficients are in good agreement with those calculated using the hopping model. It should be noted, however, that analysis of the delayed scintillation component decay—which is essentially controlled by triplet diffusion out of the particle track, where the crystal lattice is possibly perturbed by radiation effects—may give values of D_T which are slightly different from the actual value corresponding to diffusion in the perfect lattice. The possible extent of this difference, the validity of the assumptions regarding r_0 in crystals together with the exactitude with which the theoretical equations fit the available experimental data, all require consideration for an assessment of the accuracy of the derived values in this work. These values are expected to be valid to within an order of magnitude, but further experimental work should allow a more precise comparison.

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Retardation of the Crystallization of Calcium Sulphate Dihydrate

CONCENTRATED aqueous suspensions of calcium sulphate hemihydrate generally convert into a solid mass within 20–40 min owing to the crystallization of dihydrate ($CaSO_4{\cdot}0{\cdot}5H_2O + 1{\cdot}5H_2O \rightarrow CaSO_4{\cdot}2H_2O$). It is common practice to increase this time to about 4–6 h by addition of water-soluble substances in amounts of 0·1–1 per cent. These 'retarders' are usually hydrolysates of protein, although a variety of widely different materials such as phosphates, borates, citrates and tartrates are also effective[1].

Investigations of materials that retard the crystallization of dihydrate reported so far have been carried out without consideration of the structure and the functional groups of the retarder molecules[2,3]. Thus, an investigation was undertaken of the mechanism of retardation and special emphasis placed on the types of functional groups of aliphatic α-amino-acids present in protein hydrolysates. Using equal molar concentrations, it was found that the retarding potency is considerably higher in hydrolysates containing a large percentage of aspartic acid, glutamic acid or cysteine, which have carboxyl or thio groups at the end of the aliphatic side chain in addition to carboxyl groups in the α-position. The presence of hydroxyl, amino or amide groups in place of terminal carboxyl or thio groups decreases the retarding potency. Poor retarders are obtained when the amino-acids have largely straight or branched aliphatic side chains, without functional groups. The effect of molecular size of the hydrolysates is negligible provided they can be made to dissolve in water.

To study the role of the carboxyl groups, a number of aliphatic amino-acids have been condensed with formalde-

hyde. This technique produces molecules which differ from protein hydrolysates in that the amino-acids are linked together with repeating $-CH_2NR-$ groups instead of $-CH-CO-NH-$ groups (R = amino-acid rest). Therefore, carboxyl groups instead of amide groups are present in condensates, and these can be located near the main chain, at the end of the aliphatic side chains or in both positions depending on the type of amino-acid used. The results show that the retarding potency of condensates is always greater than that of protein hydrolysates; this is particularly so for aspartic acid, glutamic acid and all amino-acids where the carboxyl groups are located at the end of the side chains. Carboxyl groups are present near the main chain when α-amino-acids are used, which results in potent retarders only if the aliphatic side chain is very short or absent as in the case of glycine.

A series of polyamino carboxylic acids has been synthesized to examine the relationship between amino-groups and carboxyl-groups with respect to the chelation of calcium ions. It was found that one of the most potent of the chelating agents, ethylenediamine tetraacetic acid $(HOCOCH_2)_2N(CH_2)_n\ N(CH_2COOH)_2$, $n = 2$, is a poor retarder. Its homologues with $n = 3$–6, which form incomplete chelates with calcium ions are considerably more potent. Carboxymethylated and carboxyethylated polymers of ethylenediamine or propylenediamine are excellent retarders provided the ratio of amino-groups to carboxyl-groups is 1 : 1 or smaller. The difference in performance between these polyamino polycarboxylic acids is insufficient to contribute to the advancement of a theory for a mechanism of retardation. It seems that the complete chelation of calcium ions in solution does not explain the delay of the crystallization of dihydrate. However, it is evident that complex formation plays an important part.

The relative importance of carboxyl-groups, amino-groups and thio-groups was studied with a number of aliphatic polycarboxylic acids. In the course of this investigation, it was observed that retardation is possible only when at least two carboxyl groups are present in a molecule. The distance between them should be equivalent to 2 or 5 carbon atoms in the aliphatic chain of the molecule. Thus succinic and pimelic acid are more powerful retarders than the neighbouring members of the same dicarboxylic acid series with a smaller or greater number of carbon atoms separating the carboxyl groups. The preferred distance would seem to be five carbon atoms.

Although polyamines do not retard the crystallization of dihydrate, the presence of an amino-group in a dicarboxylic acid always increases its retarding potency. For example, α-amino pimelic acid is a more potent retarder than pimelic acid. The presence of a thio-group instead of an amino-group in a dicarboxylic acid results in an equal or greater increase of the retarding potency. Other functional groups are not effective with the exception of phosphoric acid-groups.

Based on these observations, retardation of the crystallization of dihydrate is considered to be due to chemisorption of the retarder molecule by complex formation with more than one calcium ion across the a- and c-axis of the growing dihydrate crystal. The distances of the calcium ions at the a- or c-axis of the dihydrate crystal correspond to an equivalent distance of five or two carbon atoms respectively[4]. The proposed mechanism is akin to the threshold effect of phosphates preventing the precipitation of calcite.

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Surface Tensions of Molten Mixtures and Thermodynamic Ideality

ONLY a few of the techniques available for studying the thermodynamic properties of ionic melt mixtures[1] can be used to study mixtures of technological interest above 1,000° C. Volumes of mixing of the sodium *meta*phosphate: potassium *meta*phosphate system have been measured by one of us[2], and this technique could be used to study phosphate–silicate melts of interest to steelmakers. The thermodynamic ideality of a molten system can also be tested by measuring the surface tension of the molten mixtures and comparing these with the predictions of Guggenheim's ideal equation[3,4]. We have used this method for studying molten phosphate mixtures and shall here describe the results for the sodium *meta*phosphate: potassium *meta*phosphate system.

The *meta*phosphates were prepared by heating the respective dihydrogen phosphates at 1,100° C for 2 h, and were analysed by the method of Boltz[5]. Surface tensions were measured with an apparatus similar in design to that described by Bradbury[6], and depending on the "Maximum pull on a cylinder". A platinum-10 per cent rhodium cylinder was suspended from the arm of a balance into the melt held in a platinum crucible heated in a molybdenum wire-wound furnace controlled to ± 1° C at 1,000° C. The apparatus was calibrated using carbon tetrachloride, water, benzene and nitrobenzene by the method of Harkins and Jordan described by Bradbury[6]. The measured surface tensions were estimated to be accurate to within 0·5 dynes/cm.

Measurements were obtained in the range 850° C–1,200° C and some results are shown in Fig. 1. The variation of surface tension with temperature was of the order of 0·045–0·050 dynes/cm/° C. The experimental results were fitted to Guggenheim's formula:

$$\exp(-\gamma a/kT) = x_1 \exp(-\gamma_1 a/kT) + x_2 \exp(-\gamma_2 a/kT)$$

where T = temperature (° K), x_1 and x_2 are the mole fractions of components 1 and 2, γ is the surface tension of the mixture while γ_1 and γ_2 are the surface tensions of pure components 1 and 2, k is Boltzmann's constant and a is an adjustable parameter.

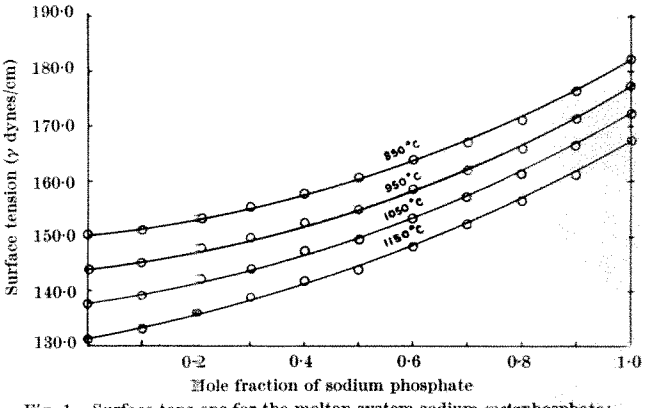


Fig. 1. Surface tensions for the molten system sodium *meta*phosphate: potassium *meta*phosphate

A value of $a = 50·0$ Å²/molecule gives very close agreement for the calculated surface tension of the mixture (γ) for the temperatures 850°, 950°, 1,050°, 1,150° C. For the three highest temperatures, indeed, the deviation of the calculated γ is within the range ± 0·6 dynes/cm for all but two of the twenty-seven compositions studied. For the results at 850° C, and with the same value of the constant a, the maximum deviation is 0·8–0·9 dynes/cm. Simple calculation shows that at the composition $x_1 = 0·4995$, $x_2 = 0·5005$, a variation of 10 Å²/molecule causes a variation of 1·0 dynes/cm in the calculated value of γ.

These results would thus seem to be consistent with the view that the molten system sodium *meta*phosphate:

potassium *meta*phosphate is thermodynamically ideal. Similar results have been obtained for the systems lithium *meta*phosphate : potassium *meta*phosphate and lithium *meta*phosphate : sodium *meta*phosphate, and will be reported in greater detail elsewhere.

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Reactivity of Excited Water Molecules in Photochemical Carboxylation

WE have shown that carbon dioxide in aqueous solution can be reduced and converted into simple organic substances under the influence of ultra-violet light[1–3]. The reduction process is the result of the action of hydrogen atoms produced by the photolysis of water and also of excited molecules of water (H_2O^*) (refs. 2 and 3):

$$H_2O \rightsquigarrow H_2O^* \rightarrow H + OH \qquad (1)$$

for $\lambda = 184{\cdot}9$ nm the quantum yield for H and OH is $\Phi = 0{\cdot}6$ (ref. 4).

Subsequent reactions are:

$$CO_2 + H \rightarrow COOH \qquad (2)$$

$$k_2 < 8 \times 10^6 \text{ moles}^{-1}.\text{sec}^{-1} \text{ (ref. 5)}$$

$$(CO_2.H_2O) \rightsquigarrow (CO_2.H_2O)^* \rightarrow COOH + OH \qquad (3)$$

and:

$$(CO_2.H_2O)^* \rightarrow CO_2^- + H_2O^+ \qquad (4)$$

For simplicity, instead of reactions (3) and (4), the following reaction can be written:

$$CO_2 + H_2O^* \rightarrow COOH + OH \qquad (5)$$

In the presence of aqueous bicarbonate the excited water molecules can also lead to the formation of CO_2^- radical ions, according to the reaction (6) (ref. 6):

$$HCO_3^- + H_2O^* \rightarrow CO_2^- + OH + H_2O \qquad (6)$$

In addition we have been able to carboxylate a number of organic substances in aqueous solutions by ultra-violet irradiation[3,6,7]. In an investigation of the carboxylation process of methanol we found a possible method for calculating the rate constants of reactions (5) and (6). Besides reactions (2), (5) and (6), reactions (7) and (8) are also responsible for the formation of glycolic acid, which is the carboxylation product of methanol:

$$CH_3OH + OH \rightarrow CH_2OH + H_2O \qquad (7)$$

$$k_7 = 3 \times 10^8 \text{ moles}^{-1}.\text{sec}^{-1} \text{ (ref. 5)}$$

$$CH_3OH + H \rightarrow CH_2OH + H_2 \qquad (8)$$

$$k_8 = 2 \times 10^6 \text{ mole}^{-1}.\text{sec}^{-1} \text{ (ref. 8)}$$

Hence:

$$CH_2OH + COOH \rightarrow CH_2(OH).COOH \qquad (9)$$

$$CH_2OH + CO_2^- \rightarrow CH_2(OH).COO^- \qquad (9a)$$

$$k_{9a} = 5 \times 10^8 \text{ mole}^{-1}.\text{sec}^{-1} \text{ (ref. 5)}$$

The yield of glycolic acid as a function of the concentration of methanol at two different *p*H values is shown in Fig. 1. Both curves pass through a maximum at 4×10^{-1} moles methanol/l. The yield of the glycolic acid formed achieves a maximum only when the concentration of both radicals—CH_2OH and COOH (or CO_2^-)—is equal. Below the concentration of 4×10^{-1} moles methanol/l. solution the COOH (or CO_2^-) radicals are in excess. In this case they lead as a result of dimerization to the formation of oxalic acid[6]:

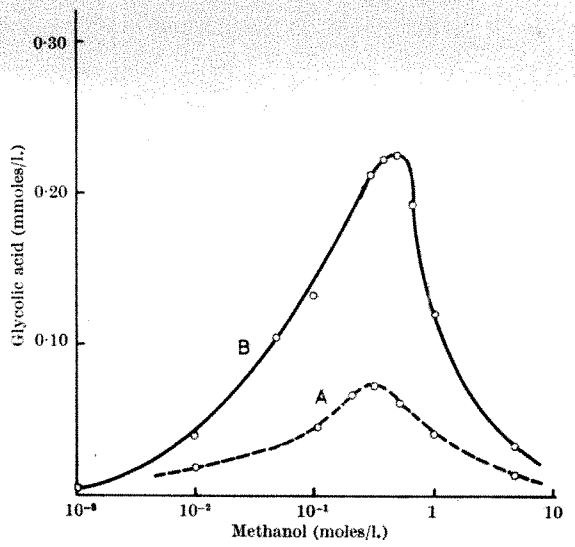


Fig. 1. Yield of glycolic acid as a function of methanol concentration. Irradiation time, 3 h; ultra-violet source: Hanau lamp, type *NK*6/20. *A*, 0·1 M sodium bicarbonate solution, saturated with carbon dioxide gas, *p*H 6·7; *B*, 0·1 M sodium bicarbonate solution, *p*H 8·5

$$COOH + COOH \rightarrow H_2C_2O_4 \qquad (10)$$

or:

$$CO_2^- + CO_2^- \rightarrow C_2O_4^{--} \qquad (10a)$$

At a concentration above 4×10^{-1} moles of methanol/l., glycol is produced according to:

$$CH_2OH + CH_2OH \rightarrow C_2H_4(OH)_2 \qquad (11)$$

For the formation of glycolic acid at *p*H = 6·7 reactions (2), (5), (7) and (8) prevail and one can calculate the rate constant k_5 for the maximum of the curve *A* as follows:

$$k_5 = \frac{C_{CH_3OH}.(2k_7 + k_8) - k_2.C_{CO_2}}{2.C_{CO_2}} \qquad (12)$$

In this case we assume: $C_{H_2O^*} \simeq C_{OH}$, since $\Phi = 0{\cdot}6$ (ref. 4). By substitution of $C_{CH_3OH} = 4 \times 10^{-1}$ moles/l., $C_{CO_2} = 1{\cdot}5 \times 10^{-2}$ moles/l. and the *k*-values for reactions (2), (7) and (8) given here in equation (12) are obtained as:

$$k_5 = k_{CO_2+H_2O^*} = 8 \times 10^9 \text{ moles}^{-1}.\text{sec}^{-1} \qquad (13)$$

In basic media (*p*H 8·5) glycolic acid is produced at three times the yield (see Fig. 1). This can be explained, as already mentioned, as being due to the action of excited water molecules on bicarbonate with the formation of CO_2^- radical ions by way of reaction (6). In this case, reaction (14) need not be taken into consideration:

$$H + OH^-_{aq} \rightarrow e^-_{aq} \qquad (14)$$

$$k_{14} = 2{\cdot}4 \times 10^7 \text{ moles}^{-1}.\text{sec}^{-1} \text{ (ref. 9)}$$

This becomes important only at higher *p*H values. As a consequence of this, reaction (15) also plays a negligible part:

$$CO_2 + e^-_{aq} \rightarrow CO_2^- + nH_2O \qquad (15)$$

$$k_{15} = 0{\cdot}77 \times 10^{10} \text{ moles}^{-1}.\text{sec}^{-1} \text{ (ref. 10)}$$

By a process similar to the one just applied to curve *A* we can now determine the k_6-value from the maximum of curve *B*. Under these conditions (for $C_{HCO_3^-} = 10^{-1}$ moles/l.) one can estimate:

$$k_6 = k_{HCO_3^- + H_2O^*} = 1{\cdot}2 \times 10^9 \text{ moles}^{-1}.\text{sec}^{-1} \qquad (16)$$

Although the k_6-value is lower than the k_5-value, the higher yields of glycolic acid at *p*H 8·5 (curve *B*) can be explained as a *p*H effect and because k_{9a} is relatively high. Similar results have been obtained also by the carboxylation of methanol induced by cobalt-60 gamma-rays[5,11].

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Analysis of the Major Constituents of Fuel Gases by Gas Chromatography

RECENTLY, Hollis[1] has reported retention data for the separation of methane, carbon dioxide, and polar materials on porous polymer beads. In these laboratories a polymeric bead column packing commercially available in the United Kingdom[2] has been investigated, and has been found to have considerable advantages over conventional adsorption columns for the analysis of fuel gases which contain hydrogen, carbon monoxide, methane, carbon dioxide, and the C_2-hydrocarbons as major constituents.

Hitherto the gas chromatographic analysis of fuel gas has necessitated the use of two adsorption columns. Usually a type 5*A* or 13*X* molecular sieve column is preferred for the separation of methane and carbon monoxide, but because this material strongly absorbs carbon dioxide, which eventually poisons it, a second column of silica gel must be used to separate this gas and the C_2-hydrocarbons[3]. It has now been found possible using a single column to analyse a fuel gas for these components including carbon dioxide.

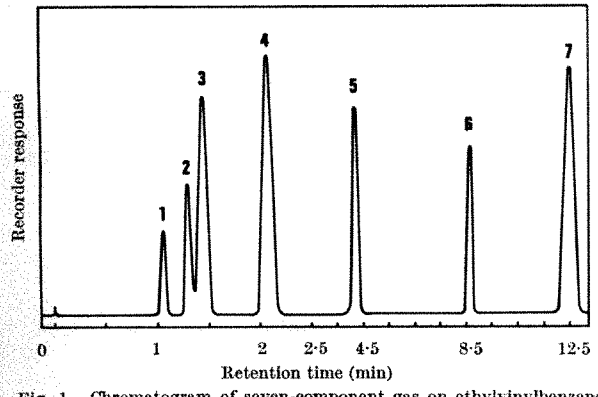


Fig. 1. Chromatogram of seven-component gas on ethylvinylbenzene polymer at 30° C. (1) Hydrogen; (2) air; (3) carbon monoxide; (4) methane; (5) carbon dioxide; (6) ethylene and acetylene; (7) ethane

The chromatogram in Fig. 1 was produced using a Perkin–Elmer model 451 gas chromatograph. A methane-rich gas containing 3 per cent carbon monoxide was analysed at 30° C using a column 10 ft. long and 3/16 in. internal diameter, packed with 80–100 mesh beads of an ethylvinylbenzene polymer. This was conditioned before use by heating to 230° C for 1 h as recommended by the manufacturer. Helium was used as carrier gas at an inlet pressure of 15 lb./in.^{2} gauge and a flow rate of 40 ml./min, and the components were detected using a thermistor katharometer. On this column carbon monoxide and methane were well separated, and carbon dioxide eluted within 5 min of injection. The column separated unsaturated C_2-hydrocarbons from ethane under the same conditions.

When ethylvinylbenzene polymer was used as column packing, rapid analyses were obtained with good separation and without the 'tailing' peaks associated with other adsorption columns. In this laboratory it has enabled a gas to be analysed for seven components in 30 min with a single injection on one column, the sample size being adjusted according to the recommendations of Pauschmann[4] so as to obtain a nearly linear response for hydrogen using helium as carrier gas.

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Sensitivity to Oxygen of an Ionization Detector with Helium and Neon as the Carrier Gases

WE are interested in the analysis by chromatography of trace (v.p.m.) mixtures of oxygen in gases, and the Lovelock radioactive ionization detector[1] was selected as the most appropriate for the purpose. This detector, originally developed for sensitive detection of organic compounds in a carrier stream of argon, is not very sensitive to permanent gases in argon because the metastable energy of argon (11·6 eV) is slightly less than the ionization potentials, 12–16 eV, of most of the permanent gases. Berry[2] suggested that helium which has a metastable energy of 19·8 eV would be a suitable carrier gas. We have carried out tests first with helium[3] and later with neon, the metastable energy of which is 16·5 eV.

In these tests we determined the sensitivity of a Lovelock strontium-90 detector (made by W. G. Pye, Ltd., Cambridge) to helium and neon containing constant (v.p.m.) amounts of added oxygen, for different voltages applied to the detector. In each case, the flow was maintained at 85 cm^3 (N.T.P.)/min and the original carrier gas was purified to less than 0·1 v.p.m. oxygen. The sensitivity was found to increase with detector voltage up to 1,300 V for helium and 850 V for neon, above which voltages spark discharge occurred.

Table 1

Carrier gas	Detector voltage (V)	Standing current (amp)	Sensitivity amp/v.p.m. oxygen	Noise-level (amp)
Helium	1,300	5×10^{-9}	3×10^{-9}	5×10^{-11}
Neon	850	5×10^{-9}	5×10^{-9}	5×10^{-11}

Using these voltages, we determined the standing current and the noise-level for this current with carrier gas containing no added oxygen, and the change in current with added oxygen. The variation in current with added oxygen is linear up to at least 1·0 v.p.m.

The results are given in Table 1, from which it can be seen that the minimum detectable addition of oxygen, defined as that producing a change in detector current of twice the noise level, is 0·03 v.p.m. oxygen for helium and 0·02 v.p.m. oxygen for neon.

In practice, neon with its much higher cost would scarcely be chosen in preference to helium unless there were some special reason, for example if the gases to be detected were already mixed with neon, or if a recirculation system were used, the gases being recirculated through the purifier which is needed in any event when

the detection of trace amounts of permanent gases is attempted.

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Observation of the Nitrene in the Flash-photolysis of 1-Azidoanthracene

NITRENES have been postulated as intermediates in the decomposition of organic azides, iminoxides, nitro- and nitroso-compounds[1–3]. Most of the evidence in support of this is convincing but circumstantial[4–9]. Direct proof of the existence of nitrenes is based on the electron spin resonance spectra of triplet species in the photolysis products of several aromatic azides in a solid matrix at 77° K (ref. 10). Several alkyl-nitrenes have also been discovered in the even more extreme conditions of an organic matrix at 4° K (ref. 11). Recently we recorded the absorption spectra of several aromatic nitrenes[12] produced by photolysis in organic glasses at 77° K and were able to detect the nitrene intermediate in the photocyclization of 2-azidobiphenyl[13].

All these observations relate to solid-state systems at very low temperatures, and their bearing on what happens in the more usual liquid conditions at normal temperature is uncertain. Lwowski *et al.*[14] looked for nitrenes in the flash-photolysis of methyl and ethyl azidoformate but could only detect the spectrum of the fragment NCO.

This communication reports a preliminary experiment which demonstrates the appearance of 1-anthrylnitrene as a short-lived intermediate at normal temperature. A solution of 1-azidoanthracene in ethanol (5×10^{-4} M) was exposed to a photolytic flash of 170 J in a flash-photolysis unit. The spectrum of the system was recorded photographically with an analytical flash 4 μsec after excitation (the half-life of the photolytic flash being 3 μsec). Curve (*a*) in Fig. 1 is a densitometer trace of a photograph of the spectrum before photolysis, curve (*b*) is the trace for the system 4 μsec after excitation, and curve (*c*) was obtained without excitation after the sample had received two photolytic exposures. The absorption bands on the curves are identified by comparison with the low-temperature spectra of 1-azidoanthracene and its nitrene (EPA matrix at 77° K), Fig. 1 (*d*) and (*e*) respectively. The appearance of the characteristic peak of the nitrene at 342 mμ is clearly visible in curve (*b*). The half-life of the nitrene in these conditions is estimated provisionally to be between 3 and 10 μsec.

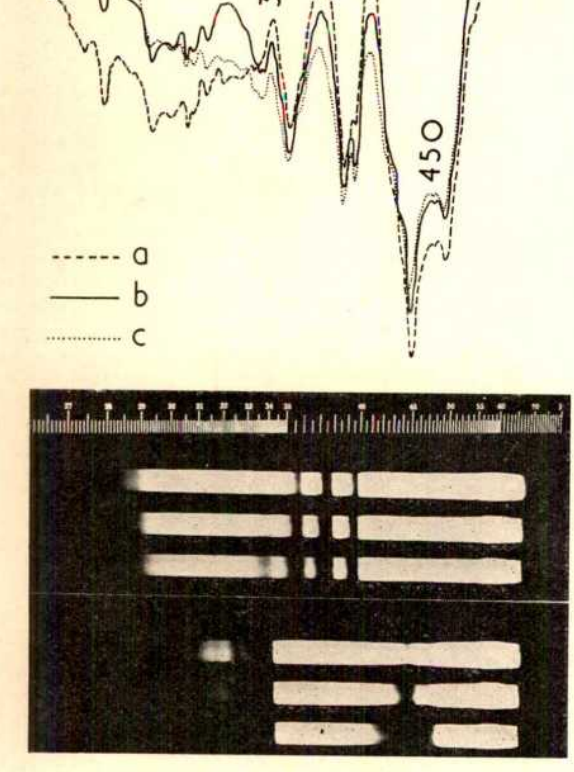


Fig. 1. Spectra of 1-azidoanthracene

A more detailed investigation of nitrenes by flash-photolysis is at present under way.

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Effect of Deuterium Substitution on Radiation-less Decay of Anthracene Triplet

ALTHOUGH radiation-less transitions in aromatic molecules have been investigated experimentally for more than 10 years, it is only recently that any real understanding has been achieved. The main reason for this is that interpretation of the experimental results has been limited by the difficulty of distinguishing between temperature and viscosity effects in systems where temperature and viscosity are dependent variables[1]. Concomitant theoretical developments have contributed towards the resolution of some of the experimental problems, approaching the subject from the point of view of both intramolecular and intermolecular perturbations.

In the Robinson–Frosch theory[2,3], the radiation-less conversion probability is considered to depend largely on intramolecular vibrational perturbations characteristic of the molecule which is being investigated. The transition probability for radiation-less conversion between the lowest triplet T and the ground singlet G is given by:

$$P \propto [< \varphi_G | H_{s\cdot o\cdot} | \varphi_T > \pi_r < \theta_{G,n} | \theta_{T,O} >]^2 \qquad (1)$$

Here the first factor is the matrix element for the spin-orbit perturbation coupling the lowest triplet and the ground singlet, and the second factor is the vibrational overlap integral (Franck–Condon factor) of the 'zero' vibrational wave-function of the triplet with nearby (excited) vibrational wave-functions n of the ground state, taken over all normal modes r. The theory explains, *inter alia*, the reason why the observed lifetime of anthracene triplet, for example, is small compared with

benzene triplet (the vibrational overlap integral for the low-lying anthracene triplet being large compared with that for benzene), and the deuterium isotope effect[4,5] whereby deuterium substitution in benzene and naphthalene results in a marked increase in the phosphorescence lifetime of these compounds. The product over r in equation (1) includes C—H as well as C—C modes and substitution of hydrogen by deuterium is responsible for a decrease in the transition probability owing to reduction of the overlap integral by the lower energy C—D modes. In addition, the theory predicts that there will be no marked temperature effect on the transition probability and that as the triplet-ground singlet energy gap becomes small, deuterium substitution will not affect the transition probability.

Anthracene is of particular interest because its triplet state lies less than five vibrational quanta above the ground state and may already be so low-lying that there will be a vanishing deuterium effect in this molecule. The decay kinetics of both perprotonated and perdeuterated triplet anthracene in room temperature and 77° K glasses were monitored by triplet → triplet* absorption at 4250 Å using an E.M.I. 9592*B* multiplier phototube d.c. coupled to a wideband pre-amplifier and displayed on a calibrated 'Tektronix 535' oscilloscope. Samples had a path-length of 10 cm and were solid-solutions of the hydrocarbon in polymethylmethacrylate prepared from the thoroughly degassed monomer using 0·001 per cent *N*,*N*′-azodiisobutyronitrile to initiate polymerization. The exciting radiation was a 10-μsec 300-J xenon flash passing into a horizontal quartz Dewar system through Schott–Jena *WG*3 windows (2 mm thick) of transmission:

$$T(\lambda) < 10^{-5} \text{ for } \lambda < 3200 \text{ Å}$$
$$T(\lambda') > 0{\cdot}5 \text{ for } \lambda' > 3400 \text{ Å}$$

Decays of the transients were strictly exponential and no difference in transient lifetime was observed over many successive flashes. The mean lifetimes for the triplets of perprotonated and perdeuterated anthracene at room temperature and 77° K are shown in Table 1.

Table 1. MEAN LIFETIMES (k^{-1} VALUES) OF PERPROTONATED (τ_P) AND PERDEUTERATED (τ_D) ANTHRACENE TRIPLETS IN LUCITE

Temperature	τ_P(sec)	τ_D(sec)
298° K	0·025	0·073
77° K	0·048	0·153

It can be seen from Table 1 that there is a definite deuterium effect in anthracene, and that deuterium substitution causes a threefold reduction in the transition probability for radiation-less decay of the triplet. The effect is, however, far smaller than the effect in other polynuclear hydrocarbons which have higher triplet energies, deuterium substitution in naphthalene increasing the phosphorescence lifetime by an order of magnitude[4], and in pyrene[6] by a similar factor. Such comparisons are, of course, only valid if vibrational perturbations in the polynuclear hydrocarbons do not affect the radiative transition probability. Consideration of the nuclear co-ordinate dependence of the transition moment for the phosphorescence process:

$$\vec{M}_{TG} \propto < T_{\pi,\pi^*}|H_{s\text{-}o}|S_{\sigma,\pi^*} > < S_{\sigma,\pi^*}|\vec{r}|G >$$

shows that vibronic coupling between triplet and ground state can in fact occur, and in benzene may be relatively important[7]. In polynuclear hydrocarbons, however, vibronic coupling between triplet and ground state is of secondary importance: the phosphorescence emission polarization is pure out-of-plane throughout the spectrum[8], which indicates that spin–orbit coupling with upper σ,π^* singlets is the primary mechanism for the radiative transition.

The results also show that in the rigid matrix used, the radiation-less transition probability is virtually independent of temperature and that the effect of deuterium substitution is completely independent of temperature. The difference between the value of 48 msec for the lifetime of the protonated anthracene triplet in lucite at 77° K and the value of 95 msec quoted by Hilpern, Porter and Stief[1] for the same triplet in isopentane at 77° K is most likely a medium effect, as the viscosity of lucite is about 10^{15} poise at room temperature—thus bimolecular quenching processes at 77° K will therefore be negligible. It would in fact be surprising if the radiation-less mechanism were completely intramolecular and not subject also to solvent perturbation.

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Chromogenicity of Sakaguchi-reactive Compounds, Monosubstituted Guanidines and Histidine

THE chemistry of the Sakaguchi reaction—between arginine, *l*-naphthol and alkaline hypochlorite—is not known. As originally described 40 years ago[1], this sensitive reaction fortuitously revealed itself as a red coloration which slowly developed and faded. By substituting hypobromite[2] for hypochlorite, almost instantaneous colour development was effected, and more recently Szilagyi and Szabo[3] achieved an improvement in the reproducibility of the reaction with the aid of *N*-bromosuccinimide (BSI)—a more stable source of available bromine.

Sakaguchi[4] also observed that the *l*-naphthol could be replaced by 8-hydroxy-quinoline (OHQ), in which case an orange-yellow colour is produced, and Satake and Luck[5], using OHQ, were able to develop reasonably stable colours from arginine. However, I have found that at normal laboratory temperatures a reagent blank reaction slowly develops, which compensates for the slow disappearance of the Sakaguchi chromogen.

In the absence of Sakaguchi-reactive compounds, bromination of *l*-naphthol or OHQ in N sodium hydroxide results in a yellow coloration. This reaction is inhibited by an increase in the concentration of bromide, a decrease in *l*-naphthol concentration or a low temperature. If a strongly alkaline solution of OHQ (2·8 μmoles) and aqueous BSI (5·6 μmoles) are mixed together and allowed to stand for a few seconds, no colour is obtained on adding the mixture to an arginine solution, although the addition of more BSI will result in a pink colour. Apparently OHQ (or *l*-naphthol) competes with the guanidine derivative for bromide, and the higher the temperature the more successfully does it do so (see Table 1). For this reason, a fairly stable and low temperature (0–5° C) is essential for accurate and reproducible arginine analysis. This was stipulated by Sakaguchi[1,4], although the condition has been ignored in more recent attempts[5,6] to adapt the reaction for quantitative analysis.

Amino-acids have been shown to inhibit the Sakaguchi reaction[7]. It can be demonstrated, however, that small amounts (0·05–0·2 μmoles) of many amino-acids intensify the colour reaction developed from 1 ml. 0·01 mM arginine with *l*-naphthol (2 ml., 0·25 mM) and BSI (1 ml., 2·5 mM)

Table 1. EXTINCTION COEFFICIENTS OF SAKAGUCHI REACTION MIXTURES

	Arginine (μmoles)				
	0	0·025	0·05	0·10	0·15
Method *A*					
Temperature (° C)		Readings at 5 min			
4	3	70	178	350	520
18	40	103	179	338	500
23	69	124	190	350	490
30	80	139	198	325	482
Method *B*					
Temperature (° C)					
4	0	141	278	550	780
19	30	166	276	540	740
30	81	190	288	475	615

A, Arginine solution (1 ml.): 1·0 mM OHQ (2 ml.); 5·0 mM BSI (1 ml.).
B, Arginine solution (1 ml.): 0·25 mM *l*-naphthol (2 ml.); 2·5 mM BSI (1 ml.).

or with OHQ (2 ml., 1·4 mM[5]) and BSI (1 ml., 5 mM). The increase in colour occurs with glycine and with taurine, and is most marked with lysine and ornithine. It is also found with ethylene diamine. No such effect on the arginine reaction is seen with similar small amounts of cystine, glutamine, citrulline, glucosamine, creatinine, urea or EDTA. The group responsible for the colour-enhancing effect appears to be $-CH_2.NH_2$. A larger amount (1–5 μmoles) of any amino-acid and also of glutamine, cytosine, guanidine and bilirubin may remove bromine and thereby decrease colour production from 1 ml. 0·01 mM arginine; adenine, EDTA and haematin are without influence.

Histidine also reacts under similar conditions with alkaline *l*-naphthol and BSI to give a rose-red colour (λ_{max} : 495 nm). This reaction appears not to have been previously reported. It is about twenty times less sensitive than the arginine reaction. It occurs when hypochlorite is used as the oxidizing agent, as in the original technique of Sakaguchi[1]. It does not occur in the absence of *l*-naphthol (or alternatively OHQ), and thus it differs from the Knoop reaction[8] in which bromination of histidine at *p*H 1 produces a similar coloured product. The histidine colour, but not the arginine colour, is intensified by the addition of a few μmoles of a cupric salt. Neither urocanic acid nor histamine, which gives a positive Knoop reaction[9], yields colour in the Sakaguchi reaction.

With high concentrations of OHQ (for example, 2·8 moles per 5 ml. reaction mixture) lower blank colours develop than with similar concentrations of *l*-naphthol[5]. However, with smaller amounts of *l*-naphthol (0·25–0·5 moles), that is, about one-fifth that used by previous workers, I find there is no blank colour. Furthermore, at the lower *l*-naphthol reagent levels the pink arginine colour is always more intense than the yellow colour given by OHQ for the same concentration of arginine, and a two- to three-fold increase in the sensitivity of the original reaction may readily be achieved.

The monosubstituted guanidine compounds, methyl guanidine, *l*-guanidino acetate, *l*-guanidino butyrate and octopine (*N*-(*l*-carboxyethyl)arginine), are known to show a similar colour reaction to arginine[1,2]. The molar chromogenicity of these and some other compounds is indicated in Table 2. Not all monosubstituted guanidine derivatives are 'Sakaguchi-reactive'. Canavanine, for example, yields no coloured product. Under optimum reaction conditions, methyl guanidine yields the most

Table 2. CHROMOGENICITY OF SAKAGUCHI-REACTIVE COMPOUNDS

Compound	No. of guanidine groups	No. of nano-moles compound required to give colour*
Albumin, human	24	1·5
Salmine (mol. wt., 4,000)	20	2·8
Methyl guanidine	1	11
Arginine	1	18
l-Guanidino acetate	1	36
l-Guanidino butyrate	1	36
Octopine	1	36
Streptomycin	2	40
Dicyandiamidine	1	140
Histidine	0	440

* Optical density of 0·100 at 510 nm, 1·5 cm given by arginine solution (2 ml.), alkaline *l*-naphthol solution (2 ml.), bromosuccinamide solution (1 ml.) at 5° C.

colour, while the Sakaguchi product of human albumin has a molecular extinction per guanidine group equal to that of *l*-guanidino acetate and is not increased by trace amounts of copper.

These observations were made at the Regional Urological Centre, Liverpool, during the course of a study of blood serum amidine transferases, full details of which will be published elsewhere. The work was supported by a grant from the Department of Surgery, University of Liverpool.

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BIOCHEMISTRY

Infra-red Spectroscopy of Tissues in the 700–400 cm^{-1} Region

As part of the study of the far-infra-red spectroscopy of biological materials, the spectra of various tissues were examined to determine if any unique absorption bands were present. Previous investigations of the infra-red spectroscopy of tissues[1] did not include this region. These results represent the extension of infra-red studies on tissue into the region of 700–400 cm^{-1}. The tissue examined included samples of bone, various rat tissues, and human cerebrospinal fluid.

The samples of bones were obtained from human femoral diaphysis and were treated in various ways: sample 1 was heated to 650° C for 8 h; sample 2 was treated in an autoclave[2]; sample 3 was treated with glycol[3]; sample 4 was treated with ethylene diamine[4]. A sample of ossein was prepared by decalcification of this bone sample with 2 per cent nitric acid for 24 h. The rat tissues were prepared by lyophilization of homogenates of the tissue immediately after removal from the animal. They were then stored until they were used in a desiccator as dry powders. The cerebrospinal fluid was removed from seven human patients with various neurological diseases. Immediately on receipt, the fluids were lyophilized without removing the salts normally present.

All samples were examined as caesium bromide disks. The caesium bromide was prepared as suggested by Bentley *et al.*[5]. The disks were prepared by mixing the sample and the caesium bromide in 'Wig-*L*-Bug' for 15 sec. In general, the disks contained about 10 mg per 380 mg of the bromide. The mixture was then pressed for 2–4 min under vacuum. The infra-red spectra of the disks were run on a Perkin Elmer model 21 infra-red spectrophotometer equipped with a caesium bromide prism. It was flushed with nitrogen to remove carbon dioxide and water and calibrated using carbon dioxide and the water bands in this region.

The bone samples show absorption in this spectral region that would be expected for inorganic materials (Fig. 1). Comparison with the data of Miller *et al.*[6] indicates that the bands at 700, 600 and 560 cm^{-1} can be ascribed to the inorganic components of bone, phosphate and calcium carbonate (Table 1). The various samples of bone all showed the same spectrum, which indicates that

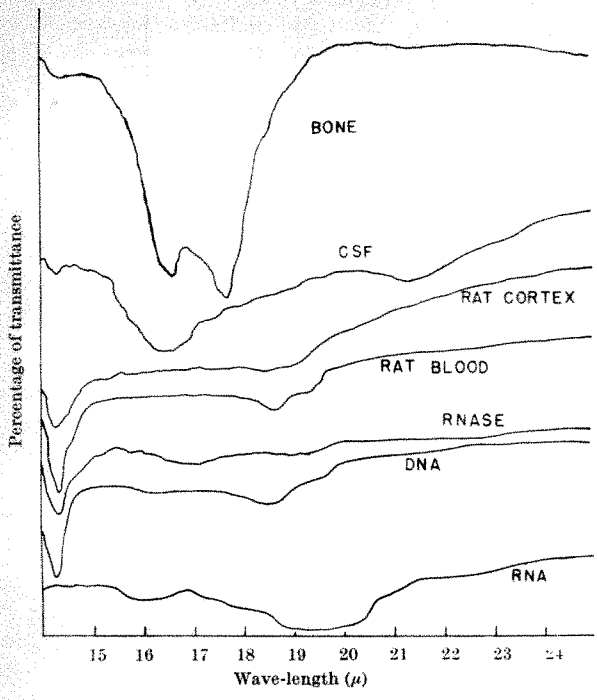


Fig. 1. Infra-red spectra of representative tissues and constituents in the 700–400 cm^{-1} region. All in caesium bromide disks

any change in bone due to treatment is not reflected in this spectral region. Ossein shows one large absorption band at 700 cm^{-1}, which is normally found in protein material.

Table 1. INFRA-RED ABSORPTION OF VARIOUS TISSUES AND ASSIGNMENTS*

Tissue	Absorption bands (cm^{-1})		
Bone	700*w* ($CO_3^=$)	600*s* ($PO_4^{\equiv}$)	560*s*
Spinal fluid	700*w* (protein)	600*s* ($PO_4^{\equiv}$)	465*m* ($PO_4^{\equiv}$)
Cerebral tissues†	700*m*		535*w*
Non-cerebral tissue‡	700*m*	676*sh*	535*w*
Ossein	700*m* (protein, DNA)		DNA

* *s*, Strong; *m*, medium; *w*, weak; *sh*, shoulder.
† Cortex, medulla and white matter.
‡ Blood, spinal cord and spleen.

An examination of the spectra of the rat tissues indicates that they fall into two groups (Fig. 1). The only difference between the two groups is a shoulder that occurs at 676 cm^{-1} (Table 1). For comparison, the spectra of a protein (ribonuclease), ribonucleic acid, and deoxyribonucleic acid are included. The tissue band at 700 cm^{-1} is found in protein, and the bands at 535 and 700 cm^{-1} are also found in deoxyribonucleic acid. Thus, the tissue band at 700 cm^{-1} results from the absorption by proteins and deoxyribonucleic acid, and the band at 535 cm^{-1} from deoxyribonucleic acid only. The nature of the 676 cm^{-1} band is still obscure.

The spectra of the lyophilized cerebrospinal fluid generally show bands at 600 and 465 cm^{-1}, and a band occurs at 700 cm^{-1} in some of the samples (Fig. 1). The bands at 600 and 465 cm^{-1} are associated with the inorganic components of the fluid (phosphate). The band at 700 cm^{-1} is related to the protein concentration in the fluid. To test this, several samples were redissolved in water, and the ultra-violet absorption measured. The specimens in which a peak appears at 700 cm^{-1} also show absorption in the ultra-violet at 280 mμ. This clearly confirms that the 700 cm^{-1} band is related to the protein concentration.

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Isolation of β-Naphthol from Urine after its Application to Skin

LOCAL applications of β-naphthol to the skin have been used in dermatology since its introduction by Ludwig and Kaposi in 1881. Although it has been known for many years that toxic effects have followed the application of β-naphthol to the skin of children[1], there has previously been no definite evidence for the absorption of this compound through the skin.

A β-naphthol peeling paste formed part of the treatment in a series of fourteen cases with acne vulgaris in whom urinary steroid excretion was being investigated. Considerable interference was observed in these steroid assays. Further investigation showed that this was associated with the presence of β-naphthol in the urine. A relatively large quantity of this phenol was isolated and identified in the urine of a young man after the application of β-naphthol to his skin. It was estimated that about 3–4 g of the compound was applied to about 20 per cent of the surface area of the patient[2] for 12 h. After this treatment a complete 48-h specimen of urine was collected.

The urine was extracted once with an equal volume of benzene and then hydrolysed with a β-glucuronidase preparation. After incubation at 37° C overnight, the urine was then extracted with ether and the acidic fraction removed by the method of Brown[3]. These extracts contained one main component when small portions were chromatographed on paper and thin layers of silica gel. This compound behaved chromatographically like β-naphthol and produced a blue colour with Folin and Ciocalteau's reagent. The residues obtained from the extracts were chromatographed on 10 × 1-cm columns of alumina with a Brockman activity of 1, to which 0·1 ml. of water per gram of alumina had been added. The columns were eluted with benzene; the 10-ml. fractions of the eluate containing phenolic material according to Folin and Ciocalteau's reagent were then pooled and evaporated to dryness. The residues were then crystallized from benzene : light petroleum (90 : 10 by volume) and recrystallized from benzene : light petroleum (10 : 90 by volume). A total of 0·3759 g of white crystals was obtained. The melting point corresponded to that of β-naphthol 122°–123° C (uncorrected) and was not depressed by admixture with authentic β-naphthol. The infra-red absorption spectrum in the region 625–4,000 cm^{-1} (16·0–2·5μ) was determined in carbon disulphide on a Perkin–Elmer 237 infra-red spectrophotometer; the absorption spectrum was identical with that of authentic β-naphthol. From all this evidence it was concluded that the material was indeed β-naphthol.

Some interference with steroid assays was observed in all fourteen patients who had been treated with β-naphthol. This interference was removed by thorough alkaline extraction of the phenol in the initial urinary extract. In order to remove this compound from diethyl ether, benzene and toluene, two extractions with one volume of N sodium hydroxide were required. When one wash of the solvent with 0·1 volume of 0·25 N sulphuric acid was used after

the alkaline extraction, only two washes with 0·1 volume distilled water were necessary to obtain neutral washings. Addition of this procedure to urinary neutral steroid methods with less efficient removal of acids and phenols[4,5] abolished the interference associated with the presence of β-naphthol in the urine.

We thank Miss Brenda Shanks for her technical assistance during this work.

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Nuclear Magnetic Resonance Spectrum Changes accompanying Rigor Mortis

INFORMATION on the behaviour of water in biological cells is essential to developing an understanding of the roles of that ubiquitous molecule in biological systems. This communication reports observations of nuclear magnetic resonance spectra of cod muscle over a period of time from shortly after death to the cessation of rigor mortis, which suggests that cellular water suffers a loss in structure and an increase in mobility during this time.

Two codfish (*Gadus morhua*), each weighing approximately 4 lb., were filleted within 10 min of death. Three core samples of the dorsal muscle fillet were obtained using a modified core cutting nuclear magnetic resonance sample tube. Nuclear magnetic resonance spectra were run in a 'Varian *A*-60' high resolution nuclear magnetic resonance spectrometer (14·092 kG field) adjusted for optimum field homogeneity, and 'ringing' with distilled water. The probe temperature was +15° C. Spectra of the three samples were taken at approximately 45, 280 and 380 min after death.

At 150 min after death the fillets from which samples were removed were stiffened and had a rough, hardened texture. Rigor mortis subsided 5·5 h after death, at which time the fillet tissue had softened and was exuding clear liquid.

The muscle spectra all consist of a single symmetrical water peak which is four to eight times broader than that produced by pure water. The peak width, taken at 0·6 of the peak maximum, decreases by 14–22 per cent in approximately 6 h, as shown in Fig. 1. Peak area does not change appreciably.

Broadening of the nuclear magnetic resonance absorption peak of water in the presence of proteins, cellular material, starches and other materials has been previously reported[1–5]. Broadening is evidence of a decrease in transverse relaxation time ($T_2 = f[1/\text{peak width}]$), and reduction in proton mobility such as might result from adsorption or bonding of water by cell protein[1,6]. Bratton *et al.*[4] have reported that a reversible narrowing of water peak width (and increase in T_2) accompanies the contraction of living frog muscle, and suggest an explanation in terms of change in extent of the 'bound water' phase. Reduction in the water-holding capacity of muscle in rigor mortis is well established[7].

The core-cutting sample tube is essential for producing geometrically uniform samples and reproducible spectra, and may be useful to other nuclear magnetic resonance investigations of semi-solid materials. The tubes are made from 'Varian' 5-mm nuclear magnetic resonance sample tubes, by cutting off the sealed end and grinding a scalloped cutting edge in its place. The tube cuts a uniform cylindrical core when screwed into fish muscle. The core remains in the tube, the lower end of which is then sealed with a 5-mm plug of putty. The water contents of sample cores have been estimated at 81 ± 2 per cent by comparing their absorption peak areas to samples of known water content.

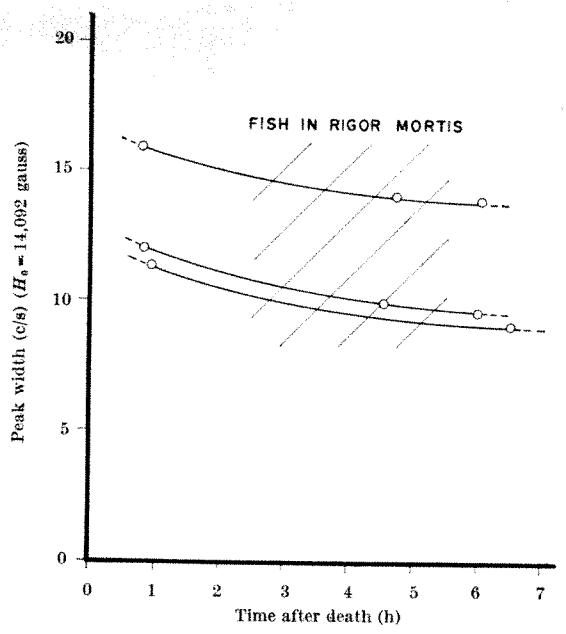


Fig. 1. Nuclear magnetic resonance peak width plotted against time after death

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Two-dimensional Thin-layer Chromatography of the Major Mononucleotides of Ribonucleic Acid

THIN-LAYER chromatography, by virtue of its convenience, sensitivity, and short developing time, is useful for the separation and quantitation of many types of nucleic acid derivatives[1–3]. This communication describes the method used by this laboratory for the separation of the products of alkaline hydrolysis of ribonucleic acid.

Preparation of thin-layer plates: 15 g of '*MN*-cellulose powder 300*G*' (Macherey and Nagel, Düren, Germany) is mixed with 90 ml. of distilled water by shaking for 1 min. Clean 20 × 20 cm glass plates are coated with the use of a Desaga-Brinkmann 'Fixed-Thickness Applicator'. The plates are dried in air for 30 min and the cellulose is activated by heating for 10 min at 110° C. The plates are washed with water and air-dried before they are used.

Chromatography procedures: RNA is hydrolysed by incubation either for 18 h at 37° C in 0·3 N potassium hydroxide, or for 48 h at 60° C in 10 per cent piperidine. The potassium is removed by precipitation with perchloric

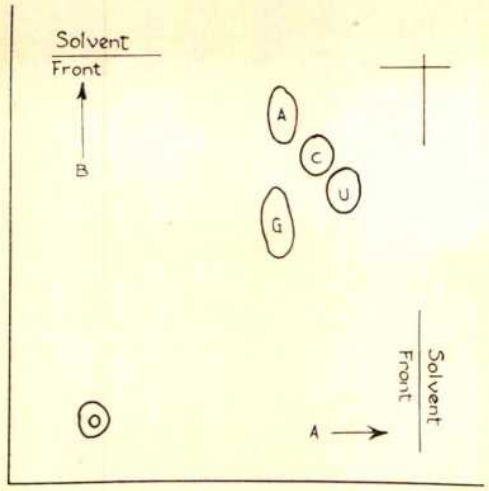


Fig. 1. Diagram of two-dimensional separation of nucleotides in solvents *A* and *B* as described in the text. *A*, AMP-2′,3′; *C*, CMP-2′,3′; *U*, UMP-2′,3′; *G*, *GMP*-2′,3′; O, origin

acid; the piperidine being volatile does not have to be removed. The nucleotide solution is applied with a micropipette to a spot 3 cm from two adjacent sides. The following solvents are used: *A*, Isopropanol/hydrochloric acid/water (85·0 : 20·5 : 19·5, v/v). *B*, Isobutyric acid/ammonium hydroxide/water (58·0 : 3·8 : 38·5, v/v). Development times at room temperature are 2·5 h for solvent *A* and 1·5 h for solvent *B*. The plates are thoroughly dried in air between developments.

The spots are located by their ultra-violet absorption (Fig. 1), and the nucleotides are eluted from the powder in 0·01 N hydrochloric acid. The R_F values under these conditions are given in Table 1.

Table 1. R_F VALUES OF NUCLEOTIDES

	Solvent *A* Isopropanol–hydrochloric acid	Solvent *B* Isobutyric–ammonia
AMP-2′,3′	0·59	0·84
CMP-2′,3′	0·68	0·75
UMP-2′,3′	0·77	0·63
GMP-2′,3′	0·55	0·54

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Fractionation of Porcine Macroglobulins

PORCINE serum is particularly rich in macroglobulins: we have found it to contain 8 per cent of components with sedimentation coefficients up to approximately 17 *S* (Fig. 1). Gel filtration with 'Sephadex *G*200' offers a useful means for the preparation of macroglobulins[1–3] and preparative zone electrophoresis allows a good separation of the $\gamma_1 M$ and $\alpha_2 M$ globulins.

Serum samples were taken from slaughterhouses. 1 g of the 'globulin' fraction, obtained by precipitation with 2 M ammonium sulphate, was fractionated in a column (5 × 110 cm) containing approximately 70 g of 'Sephadex *G*200' (Pharmacia, Lot No. *To* 3016). Elution buffer consisted of 0·02 M sodium phosphate–0·08 M sodium chloride, *p*H 7·5. Protein appeared in the eluent in two main peaks (Fig. 2*a*); the localization of $\gamma_1 M$ and $\alpha_2 M$

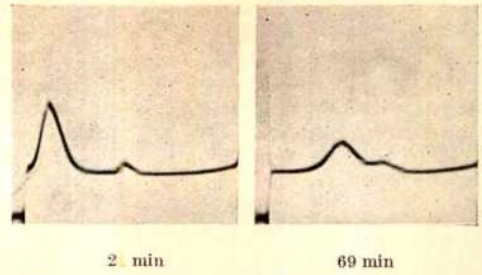


Fig. 1. Ultracentrifuge patterns of porcine serum. Pictures were taken after 21 and 69 min at 59,780 r.p.m. Peaks move from left to right on sedimentation with coefficients of 4·2 *S* (75 per cent), 6·6 *S* (17 per cent) and 17 *S* (8 per cent)

globulins in peak I was confirmed by immunoelectrophoresis, performed according to the micro-modification of the method of Scheidegger[4] with rabbit anti-porcine serum. The fractions making up this peak were pooled and precipitated with ammonium sulphate. The precipitate was dissolved in 0·05 M barbital buffer to make 20 ml.; 4 ml. were applied to a trough cut in a 'Pevikon' block (35 × 30 × 1·2 cm), 7 cm from the cathodal end, for electrophoresis fractionation. Electrophoresis was allowed to proceed at 500 V for 30 h at 0°–2° C in a 'Pherograph' apparatus.

After electrophoresis, the block was sliced transversely into 1-cm segments; each segment was eluted with 15 ml. of 0·9 per cent sodium chloride and the fractions were analysed for protein content by the method of Lowry[5].

The electrophoresis pattern was characterized by two peaks (Fig. 2*b*). It can be seen that a good separation of

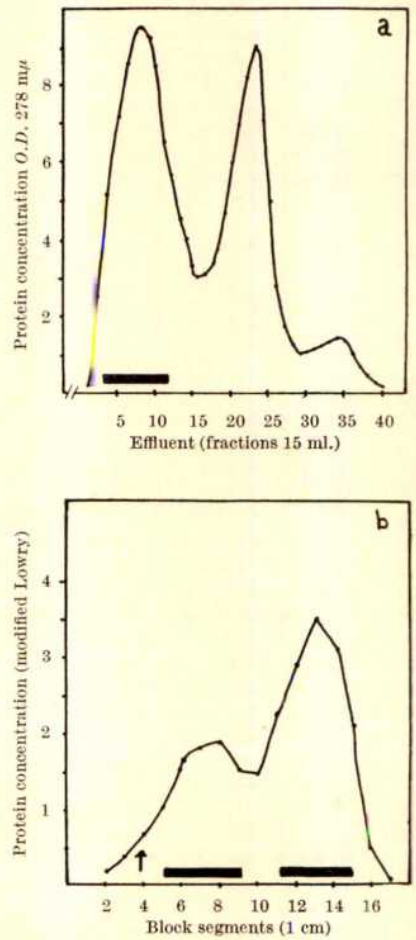


Fig. 2. (*a*) Gel filtration chromatogram of porcine serum globulins. (*b*) Zone electrophoresis in 'Pevikon' of proteins eluted as peak I from gel filtration

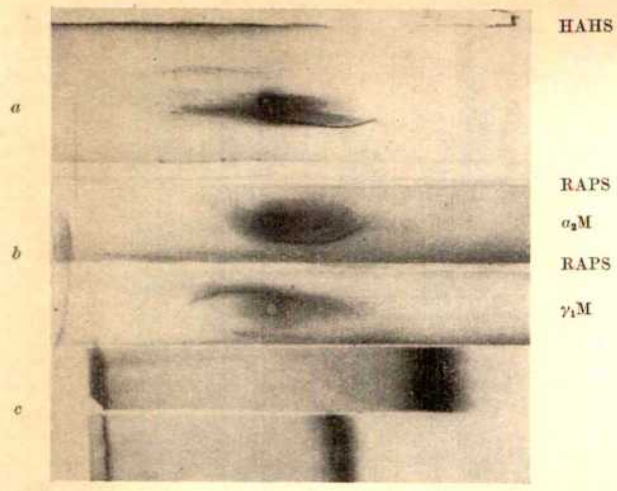


Fig. 3. Immunoelectrophoresis of peak I from (*a*) gel filtration and (*b*) fractions from the electrophoretic separation. RAPS, rabbit anti-porcine serum; HAHS, horse anti-human serum. (*c*) Cellulose acetate electrophoresis of fractions 8 and 13 from zone electrophoresis

the two macroglobulins had been accomplished: the γ_1M (segments 5–9) and the α_2M globulin (segments 11–15) appeared to be homogeneous (Fig. 3*b*). These fractions, examined in the analytical ultracentrifuge, were found to contain components with sedimentation coefficients of approximately 18 *S*. Immunoelectrophoresis of these porcine serum macroglobulins with horse anti-whole human serum (Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, No. 2116) showed that porcine γ_1M globulin cross-reacted antigenically with human γ_1M globulin (Fig. 3*a*).

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Changes in Muscular Glycogen Synthetase Activity following Denervation

Hines and Knowlton[1,2] have demonstrated a decrease of muscular glycogen levels after denervation. The biochemical mechanism underlying this phenomenon, however, is not clearly understood. It does not seem to be explained by an activation of phosphorolytic processes, as phosphorylase was shown by Huls and Leonard[3] to decrease in the skeletal muscle after nerve transection.

Bass[4] has reported a lower incorporation of glucose-^{14}C into the glycogen of denervated muscular tissue, which suggests that it might be possible to explain the loss in glycogen by an impairment of the biosynthetic processes.

In order to investigate further the turnover of glycogen in denervated muscle, we studied the activity of glycogen synthetase (uridindiphosphoglucose-glycogen transglucosylase) at various intervals after nerve section. Because Villar-Palasi and Larner[5] were able to show that glycogen synthetase is rate-limiting in glycogen synthesis, it might be possible to account for the loss of glycogen observed by a lowering of its activity.

Denervation was obtained by cutting the right sciatic nerve of white rats weighing about 250 g under light ether anaesthesia. The animals were killed by decapitation at various time intervals. Both recti femuri were excised and immediately chilled until determinations were performed. Spinal cord transection was performed under ether anaesthesia at the C6 level; fasted unoperated rats were used as controls.

Glycogen synthetase activity was determined according to the method developed by Leloir and Goldemberg[6], and non-collagen nitrogen according to that by Johnson[7]. The results obtained after denervation are presented in Table 1. Starting a few hours after cutting the nerve, the enzymatic activity steadily decreased until it reached about 30 per cent of the control level after 2 months.

After transection of the spinal cord (Table 2), a fall of glycogen synthetase activity was observed 24 h after surgery: eventually the enzymatic levels return to normal. Other experiments (not reported here) have shown that in this case the course of enzymatic variations is much more variable and may depend on the strain of the animals used.

Table 1

Time after denervation	No. animals	Glycogen synthetase*
—	20	2,031 ± 30·4
2 h	3	1,568 ± 79·7
24 h	4	1,250 ± 59·9
3 days	3	1,029 ± 4·6
5 days	5	991 ± 32·1
10 days	5	974 ± 33·1
15 days	5	910 ± 45·6
30 days	4	900 ± 20·6
60 days	3	696 ± 10·3

* Expressed as γ of UDP formed per 1 mg of nitrogen in 15 min at 37° C ± *S.E.* of the mean.

Table 2

Time after cordotomy (h)	No. of animals	Glycogen synthetase*	Per cent
—	20	2,031 ± 30·4	—
24 h	3	1,359 ± 17·7	−33
48 h	3	1,775 ± 44·6	−12·5
72 h	2	1,742 ± 96	−14

* Expressed as γ of UDP formed by 1 mg of nitrogen in 15 min ± *S.E.* of the mean.

It should be pointed out that, from a biochemical point of view, only form *D* (according to Rosell-Perez *et al.*[8–10]), which is fully active in the presence of glucose-6-phosphate, was determined in our experiments; no attention was paid to the relationships existing between this and the *I* form—that is, the one acting in the absence of glucose-6-phosphate. It would be advisable to undertake an investigation of the rate between the *I* and *D* forms, and of the process of interconversion $I \leftrightarrow D$ under our experimental conditions (denervation and spinal transection) because, as Danforth[11] has recently pointed out, this process in muscular tissue seems to be influenced by several experimental conditions such as nerve stimulation or administration of epinephrine.

From a biological point of view, our results would seem to suggest the existence of a nervous control of the muscular levels of glycogen synthetase. Although its exact mechanism is not understood, it is evident that a fully efficient peripheral innervation is required for the maintenance of normal levels of enzymatic activity in muscle. The results obtained with cordotomized animals indicate that spinal centres are probably independent of higher nervous control in regulating the muscular glycogen synthetizing activity. The short-lived fall of enzymatic activity observed immediately after spinal transection could be ascribed to the spinal shock following surgery.

This hypothesis is, at the present state of our research, only a tentative one aimed at interpreting our own experimental findings. Further investigations of the physiological and biochemical factors underlying the

phenomena observed should throw some light on the validity of this hypothesis.

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Gigartinine: a New Amino-acid in Red Algae

A STUDY of free amino-acids and related compounds in a red alga, *Gymnogongrus flabelliformis*, revealed the presence of two different substances, both containing a guanylurea moiety. One was afterwards identified as 1 - amidino - 3 - (3 - carboxypropyl)urea or γ - guanylureido)butyric acid (I)[1], and we propose here to call it 'gongrine'. It is now reported that the other compound has been designated 'gigartinine' and shown to be N^5-(amidinocarbamoyl)-L-ornithine or L-α-amino-γ-(guanylureido)valeric acid (II).

After removing gongrine as precipitate from aqueous ethanolic extracts of the alga, the filtrate was passed through a column of 'Dowex 50 *X*-8'. The basic amino-acids were eluted from the cation exchange resin with ammonium hydroxide and the gigartinine was freed from the small quantity of arginine present by repeated recrystallization of its nitrate from 50 per cent aqueous ethanol. The nitrate was obtained as thin plates, melting at 197° C (uncorrected). Elementary analysis gave: carbon, 30·23 per cent; hydrogen, 5·70 per cent; nitrogen, 29·73 per cent. $C_7H_{16}O_6N_6$ requires: carbon, 30·00 per cent; hydrogen, 5·76 per cent; nitrogen, 29·99 per cent. $[\alpha]_D^{22}$ + 7·5° (c = 2·0 in water).

The ultra-violet spectrum (in 0·1 N sodium hydroxide) has a maximum at 225 mμ (ε = 23,000). The infra-red spectrum (in 'Nujol') shows bands at 1,530, 1,610, 1,680 and 1,725 cm^{-1}, in the region of 1,500 ~ 1,800 cm^{-1}.

Gigartinine gave an orange reaction with Sakaguchi's reagent, unlike the orange-red given by arginine, and with ninhydrin the typical violet colour, the intensity of which was 0·94 in leucine equivalent[2]. Carbon dioxide, guanidine and L-ornithine were obtained on degradation at 120° C either in distilled water or in 3 N hydrochloric acid; guanylurea and succinic acid on oxidation with potassium permanganate; citrulline and ornithine on hydrolysis with barium hydroxide. Each degradation product except carbon dioxide was isolated in appropriate forms and identified by melting point, elementary analysis, infra-red spectrum and other properties, respectively.

All these properties are in agreement with formula II.



(I)



(II)

A paper chromatographic survey on the distribution of these guanidino compounds in algae was carried out by using the basic fraction of aqueous ethanolic extracts, partially purified by ion exchange chromatography. The R_F values were 0·24, 0·32 and 0·70, respectively, for arginine, gigartinine and gongrine in a solvent system consisting of butanol/acetic acid/pyridine/water (4 : 1 : 1 : 2 by volume). Among fifteen species of red algae tested, gigartinine was found in the following seven species: *Gelidium amansii*, *Grateloupia livida*, *Polyopes polyideoides*, *Carpopeltis flabellata*, *Hypnea japonica*, *Gracilaria textorii* and *Gymnogongrus flabelliformis*. An appreciable quantity of gongrine was recognized only in the last species. These guanylureido compounds, however, were detected neither in brown algae (seven species) nor in green algae (three species) examined.

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Hyperin and Nonacosane Constituents of Tutsan

I HAVE extended my investigations on tutsan (*Hypericum androsaemum*)[1] and have obtained from its leaves samples of hyperin (3-D(+) galactosidylquercetin)[2-4], and from its berries nonacosane[5].

Hyperin. The dried leaves were successively extracted with petroleum ether (80°–100° C), diethyl ether and methanol. The methanolic extract, when evaporated under reduced pressure, yielded a yellow solid which on repeated crystallization from ethanol furnished a yellow crystalline material, m.p. 235° C (*d*). An infra-red absorption spectrum[6] was obtained of the anhydrous vacuum-dried material and was found to be identical with that of an authentic specimen of hyperin (3-D(+) galactosidylquercetin), the latter also showing no depression of melt when admixed with the extracted solid.

Before these comparisons were made, the glycoside was hydrolysed in 2 per cent aqueous sulphuric acid to yield a mixture of quercetin and D(+) galactose. Quercetin was identified by acetylation to the penta-acetyl derivative, m.p. 197° C, which showed no depression of melt when admixed with an authentic specimen of penta-acetylquercetin. Both samples had identical infra-red absorption spectra. D(+) galactose was isolated from the aqueous solution by forming the yellow crystalline phenylosazone, m.p. 201° C, which crystallized from aqueous ethanol in an identical manner to that of an authentic specimen of D(+) galactose phenylosazone. The two had identical infra-red absorption spectra and there was no depression of melt when they were mixed.

Nonacosane. De-seeded unripe berries were distilled at oil bath temperature 220° C/12 mm for 5 h. A wax-like solid remained in the aqueous distillate. On crystallization from aqueous ethanol, this solid furnished a colourless solid, m.p. 62·5° C. An infra-red absorption spectrum of the sample (potassium bromide disk) showed absorption bands, which indicated methyl and methylene vibrations, together with two bands at 720 cm^{-1} and 730 cm^{-1}, which indicated the presence of —$(CH_2)_n$—. Thus it was concluded that the extracted material was a straight-chain hydrocarbon of high molecular weight. The hydrocarbon was subjected to mass spectroscopy, the resulting scan showing that the C_{29} molecular ion was intense although small abundances of C_{30}, C_{31} and C_{32} were present; however, the C_{29} peak was the major component at high mass and thus the chief constituent of the extract was nonacosane, $C_{29}H_{60}$. The small impurities of other hydrocarbons may account for the observed melting point being slightly lower than that cited (64° C) in reference sources.

I thank the Associated Electrical Industries, Ltd., for kindly effecting the mass spectroscopy, and Dr. E. Von Rudloff, Prairie Laboratory, Saskatoon, Canada, for supplying me with an authentic specimen of hyperin.

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Detection and Estimation of Ochratoxin *A*

THE recent isolation[1] of ochratoxin *A* from *Aspergillus ochraceus* Wilh., which has a widespread natural distribution, made the development of a rapid method for the detection and estimation of the mycotoxin desirable. The following methods have been used during screening of fungal strains and media for toxin production.

A 20-g sample of mouldy material in a 100-ml. Soxhlet apparatus is extracted with 1 : 1 chloroform–methanol for 12 h. The extract is diluted with an equal volume of chloroform, extracted with 0·1 M aqueous sodium bicarbonate, and the toxin extracted with chloroform from the aqueous phase after acidification with 2 M hydrochloric acid. After removal of the solvent *in vacuo*, the residue is dissolved in 5 ml. chloroform and a 20-μl. portion spotted on a silica thin-layer chromatoplate together with a standard sample of pure ochratoxin *A*. The chromatoplate is developed in 3 : 1 benzene–acetic acid and inspected under ultra-violet light. Ochratoxin *A* appears as a green fluorescent spot at R_F 0·5. Blue-green and pale green fluorescent spots at R_F 0·35 and 0·65 indicate the presence of ochratoxins *B* and *C*, the dechloro and the ethyl ester derivatives of ochratoxin *A* (ref. 2). On spraying with 0·1 N aqueous sodium hydroxide, ochratoxins *A*, *B* and *C* exhibit a bright blue fluorescence. The ochratoxin *A* content of the original material can be estimated by comparing the size of the observed spot with that from a standard solution of ochratoxin *A*. In this manner 0·1 p.p.m. ochratoxin *A* added to maize meal could be detected.

In cases where unknown substances interfere with this method, or where additional proof is desirable, the results can be verified by thin-layer chromatography on silica using 12 : 2 : 1 benzene–methanol–acetic acid (giving a green fluorescent spot at R_F 0·6) or paper chromatography on Whatman No. 1 paper with the system 3 : 1 propanol–3 N aqueous ammonium carbonate (giving a blue fluorescent spot at R_F 0·65).

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Elevation of Alkaline Phosphatase Activity in the Intestine of the Chick Embryo by Actinomycin *D*

DURING the development of the mouse, alkaline phosphatase activity in the duodenum rises sharply before birth, falls back slightly, and then increases twenty-fold or more between 15 and 20 days[1]. This second rise is accelerated rather than retarded by inhibitors of RNA and protein synthesis[2,3]. Injection of actinomycin *D*, an inhibitor of DNA-dependent RNA synthesis[4], into 9-day-old mice more than doubles their phosphatase activity within a 54 h period[3], and the enhanced activity shows the normal increased preference for phenylphosphate over beta-glycerophosphate. Because these effects were obtained at a period of development when a considerable amount of phosphatase activity is already present, we thought it would be interesting to investigate the effect of actinomycin at a time when phosphatase activity first appears.

In the duodenum of the chick embryo, alkaline phosphatase activity is first found in the brush border of the epithelial cells at about 17 days[5,6]. By 21 days the adult level of phosphatase activity is reached—the enzyme activity has undergone a 100-fold increase after its initial appearance[5]. The effect of actinomycin *D* on this increase has been investigated during the 16–19-day interval, during which the enzyme activity increases about ten-fold. It has been found that phosphatase activity in the chick embryo duodenum in these stages is elevated two to three times above the normal levels by actinomycin injection at 16 days.

Single 5 μg doses of actinomycin *D* (obtained from Merck, Sharp and Dohme) dissolved in 0·1 ml. of 0·9 per cent saline were injected into a vein in the chorio-allantoic membranes of 16-day-old chick embryos; controls received an equal volume of saline alone, or were not injected. Embryos were killed after 1 or 3 days (that is, at 17 and 19 days of incubation); the duodenal loop was removed, weighed, and homogenized in iced distilled water with a 'Ten Broeck' grinder. Homogenates were assayed for protein content[7] and for enzyme activity on phenylphosphate (PhP) and beta-glycerophosphate (BGP) as described by Kato[8], except that 0·2 M sodium carbonate–sodium bicarbonate buffer was used in the incubation mixture.

Table 1. EFFECT OF ACTINOMYCIN *D* INJECTED AT 16 DAYS ON ALKALINE PHOSPHATASE ACTIVITY OF THE DUODENUM OF THE CHICK EMBRYO

Injection	Day of killing	No.	Specific activity* (mean and range)	PhP† / BGP	Protein concentration (μg/mg fresh weight)
Saline	17	5	45·1 (13·5–84·6)	2·62	97·5
Actinomycin	17	7	159·7‡ (77·1–266)	6·59	97·5
None	19	7	402·6 (267–543)	7·98	90·7
Saline	19	14	372·0 (141–600)	7·39	89·4
Actinomycin	19	11	947‡ (301–2,203)	8·25	94·8

* μg phosphorus released from PhP per mg protein per 30 min.
† Ratio of the amount of phosphorus released from PhP to amount of phosphorus released from BGP.
‡ For actinomycin injected versus saline control, $P < 0·001$ as determined by Student's *t* test (ref. 10).

The results of these experiments are given in Table 1. In 17-day-old embryos which received actinomycin 24 h earlier, the duodenal phosphatase activity is more than three and a half times that of saline-injected controls. Even at 19 days the enzyme activity is more than twice the normal level for that stage. The increases in both 17- and 19-day-old embryos are highly significant. Histochemical investigations of duodena from embryos into which actinomycin had been injected show that the elevated phosphatase activity is localized in the brush border, where it is normally found[5]. Actinomycin treatment elevates phosphatase activity on both substrates tested, but a relatively greater enhancement of activity on PhP was observed, as indicated by the increased PhP/BGP ratio in 17-day-old embryos. This ratio normally rises at the time when phosphatase activity first appears in the apical border of the epithelial cells[8]. The protein content of the duodenum was not lowered by treatment with actinomycin; body-weight was not significantly affected, nor did development appear to be retarded.

The effects demonstrate that the paradoxical effect that actinomycin *D* exerts on the phosphatase activity of the duodenum of the nursling mouse may be elicited also in the chick embryo at a stage before the enzyme has normally begun to appear in the striated border. In the mouse, in which the low activity, low ratio form of the enzyme in the infant intestine is normally replaced during the third week by forms of higher activity and PhP/BGP ratio[9], the enhancing effect of actinomycin and other inhibitors of protein synthesis has been attributed to the inhibition of the synthesis of a repressor protein that is believed to restrain the shift from one form of phosphatase to another[3]. The present results suggest that a similar restraining mechanism may be operating even at a time when histochemical methods reveal no phosphatase in the striated border[5,6]. If this were so, it would seem that in the duodenum of the 16-day-old chick embryo, the enzyme is present in an inactive form, which may be activated later. To examine further this possibility, it is necessary to determine whether direct inhibitors of protein synthesis, such as *cyclo*heximide, would produce the same result as actinomycin *D*, and also to examine the effects of these antibiotics at earlier stages, both *in vivo* and *in vitro*. Such investigations are now in progress.

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Production of Carbonyl Compounds by Tomato Fruit Tissue in the Presence of Aliphatic Alcohols

IT is well established that, in the absence of oxygen, many types of plant tissue produce ethanol. Under normal conditions, ethanol, other alcohols and various aldehydes are produced in small amounts by ripening fruits and other plant organs[1–3]. The question whether ethanol is metabolized by plant tissues has, on the other hand, been a source of controversy. In recent years Phillips[4], working with barley seedlings, Wager[5] with peas and Lowe and James[6] with mature carrot disks have found no evidence for appreciable utilization of ethanol. However, Cossins and Turner[7–9] showed that ethanol was consumed by the tissue in germinating peas and was converted initially to acetaldehyde and acetone, followed by the production of organic acids and amino-acids. Homogenates of a variety of germinating seeds were shown[10], by redox indicator, to have alcohol dehydrogenase activity when the normal C_2^-, C_3^- and C_4^- aliphatic alcohols were added as substrates. Less activity was evident in the presence of the normal C_1^- or C_5^-, secondary C_3^- or iso-C_4^-, and tertiary C_4^- or C_5^- alcohols. Recently, Cossins and Beevers[11] showed that carrot disks, corn and pea shoots, potato disks and castor bean endosperm rapidly metabolized carbon-14-labelled ethanol, while apple tissue consumed it very slowly. Their results suggested that acetyl coenzyme-*A* was derived from ethanol and converted to lipids, amino-acids, organic acids and sugars by known pathways.

During a study of ethylene production by tomato fruit tissue kept under sterile conditions (Meigh and Pratt, unpublished data) we observed that ethanol and acetaldehyde were present in the culture tubes. This phenomenon was investigated in greater detail to determine whether these substances arose because of the wound factor introduced in preparation of the tissue portions. However, the rings of tomato pericarp tissue had been prepared with the usual sterile techniques which included the use of alcohol in sterilizing the fruit, tools and the working surfaces in the sterile laboratory. As a result, alcohol vapour inevitably got into the culture medium. When the preparative sterile technique was modified to exclude alcohol from all stages of the procedure, subsequent analysis showed that both ethanol and acetaldehyde were absent from the culture tubes. In this respect, therefore, wounding of the tissue seemed to have no effect, and the production of ethanol and acetaldehyde were artefacts of technique.

In further experiments in which accidental alcohol addition was carefully excluded, but various alcohols were deliberately added, addition of ethanol to tomato tissue resulted in the production of acetaldehyde but no detectable acetone. Addition of *n*-propanol or iso-propanol yielded propionaldehyde or acetone respectively but no other carbonyl compounds. After methanol or *n*-butanol were added, carbonyl products could not be detected.

The identification of the carbonyl compounds was based (*a*) on analysis of the atmospheres in the culture tubes by gas chromatography, comparing the retention times with those of authentic compounds on three columns with stationary phases of varied polarity (silicone oil, tritolyl phosphate and polyethylene glycol 400), and (*b*) on separation by paper chromatography[2] of the 2,4-dinitrophenylhydrazones prepared by addition of an aqueous hydrochloric acid solution of the reagent to the culture solution, followed by extraction of the derivatives with carbonyl-free[12] carbon tetrachloride.

Table 1. PRODUCTION OF CARBONYL COMPOUNDS BY RINGS OF TOMATO FRUIT PERICARP TISSUE*

Substance added†	Sample	Compound identified in gas phase	Estimated concentration (mM) in the culture solution after the designated period of post-treatment gas accumulation‡ 0–16 h	40–56 h
(Ethanol used in sterile procedure)				
Water	Tomato tissue	Ethanol	4·6	3·8
		Acetaldehyde	0·3	0·3
(No ethanol in sterile procedure)				
Ethanol (0·08 M)	Control	Ethanol	22·2	20·4
		Acetaldehyde	0·0	0·0
	Tomato tissue	Ethanol	15·7	14·7
		Acetaldehyde	0·7	0·8
n-Propanol (0·08 M)	Control	*n*-Propanol	24·5	23·8
		Propionaldehyde	0·0	0·0
	Tomato tissue	*n*-Propanol	13·6	11·8
		Propionaldehyde	0·3	0·3
iso-Propanol (0·08 M)	Control	iso-Propanol	26·4	25·7
		Acetone	0·0	0·0
	Tomato tissue	iso-Propanol	16·3	15·4
		Acetone	0·1	0·6

* Each ring of tissue (average weight 1·3 g) was inserted under sterile conditions into a culture tube (19 × 150 mm) containing 1·5 ml. of culture solution (0·25 M glucose, 0·0002 M magnesium sulphate, 0·005 M potassium dihydrogen phosphate and a micromineral supplement based on White's (ref. 13) recommendations) initially adjusted to *p*H 4·5 with potassium hydroxide. The cultures were capped with filter paper and stored in a tube roller for 11 days at 20° C. For analysis of the gas phase, the culture tubes were placed inside larger tubes (29 × 200 mm) closed with soft rubber bungs (net gas phase volume *c.* 80 ml.).

† 0·5 ml. of test solution was added to each culture using sterile technique.

‡ The concentrations in the culture solutions were estimated from the concentrations in the gas phase after allowing about 16 h for accumulation and equilibration; the results from four tubes were averaged. A 1·0 ml. sample of the atmosphere was withdrawn with a hypodermic syringe inserted through the rubber bung and was analysed on a polyethylene glycol 400 column (15 per cent on 60–85 mesh 'Celite' : 4 mm × 184 cm long) at 70° C. For each compound a calibration curve was prepared, relating concentration in the culture solution to the peak height obtained under the standard analytical conditions.

Table 1 shows the results of a typical experiment in which a quantitative estimate of the course of the reaction was obtained. Concurrent determination of the carbon dioxide evolved by the tissue (using gas chromatography

with a silica-gel column) did not indicate any significant difference between the samples.

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CYTOLOGY

Variability of Ribosomal Aggregation in Lymphocytes

WE have been studying the fine structure of the bovine lymphocyte as part of an investigation into the effects of prolonged lymphocyte depletion[1]. In the course of this work it was observed that large lymphocytes, which make up most of the cellular component of thoracic duct lymph in the lymphocyte-depleted calf, can be divided rather distinctly into two populations according to the distributions of their ribosomes.

One class of cells contains large numbers of membrane-free ribosomes which are dispersed more or less evenly throughout the cytoplasm, whereas the other class shows prominent aggregation of ribosomes into congeries of five to eight sub-units. This is illustrated in Fig. 1, which shows portions of cytoplasm of two adjacent lymphocytes, one of each type. These cells were obtained from thoracic duct fluid of a lymphocyte-depleted calf which was 7 months old and which had received no deliberate antigenic stimulation. For 16 days this animal was subjected to continuous removal of cells from the thoracic duct lymph by means of a refrigerated, continuous-flow centrifuge, cell-free lymph being returned to the venous system. Specimens of sedimented cells were fixed in glutaraldehyde followed by osmic acid, and sections were stained by lead citrate and uranyl acetate. The great majority of cells present were large lymphocytes, and about a quarter of them contained aggregated ribosomes and three-quarters dispersed ribosomes.

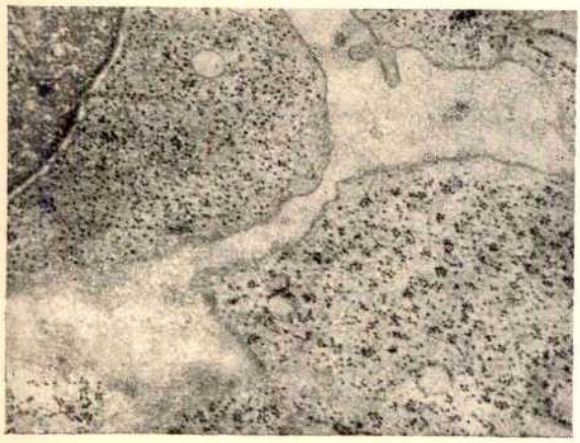


Fig. 1. A thin section showing cytoplasm of two adjacent large lymphocytes from the thoracic duct of a lymphocyte-depleted calf. The ribosomes of one cell are aggregated. (×24,000)

The two classes of cells seem to be morphologically identical in other respects. They have large, irregular nuclei characteristically divided into two lobes by a V-shaped notch. At the mouth of this notch a Golgi body is typically present. The cytoplasm of these cells is abundant and contains many spheroid mitochondria. The endoplasmic reticulum is inconspicuous, but a few scattered, non-parallel cisternae are present in most sections, and the membranes of these are lined by ribosomes which are apparently fixed.

Since it was first shown that haemoglobin synthesis occurs on polyribosomes of the rabbit reticulocyte[2], the association of ribosomal aggregates with protein synthesis has been established in many systems. The implication is that the two classes of large lymphocytes described here are either synthesizing protein at different rates or synthesizing protein molecules of different sizes. It is possible that this indicates a variation in the growth rates of morphologically similar lymphocytes.

A more interesting possibility is that these ribosomal aggregates represent the 'immune aggregates' found by Manner and Gould[3] in the ribosomal fraction of rat lymph nodes after stimulation by *Salmonella* antigen. These aggregates are associated with the synthesis of gamma-globulin, and differ from the usual polysomes in that they are disaggregated by mild treatment with trypsin rather than by mild treatment with ribonuclease. If the polysomes in bovine large lymphocytes are 'immune aggregates', then the all-or-none nature of this aggregation among various cells indicates that antigenic stimulation affects some cells and not others, thus providing a morphological marker for the 'immunologically committed' lymphocyte.

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Form and Function of Lysosomes in Oral Epithelium

MUCH of the acid hydrolytic enzyme activity of many tissues is believed to reside in membrane-bound bodies termed lysosomes[1,2]. The cells of the oral epithelium, during their migration from the basal layer to the free surface, lose organelles such as mitochondria, melanin granules and probably keratohyalin granules[3]. This loss raises questions similar to those posed for the cells of skin by Riley[4].

Jarrett and Spearman[5] have suggested that this lysis of organelles in epidermal cells during keratinization is mediated by lysosomes deposited in the keratinocytes by cytocrine transfer[6] from high-level melanocytes (Langerhans cells). We have observed ultrastructural bodies having the commonly accepted morphology[2] of lysosomes

in Langerhans cells, but not in the keratinocytes, of human oral epithelium.

Our histochemical investigations confirm the presence of acid phosphatase in keratinizing and non-keratinizing oral epithelium, which has been reported by several workers[7,8]. The distribution of acid phosphatase and other acid hydrolases is similar in keratinizing oral epithelium to that in skin, where Diengdoh[9] has interpreted aggregates of acid phosphatase as lysosomes. We have preliminary electron histochemical evidence for the presence of acid phosphatase in Langerhans cells. Riley[4] found acid phosphatase in certain high-level dendritic cells by an azo-couple technique. In keratinocytes of oral epithelium we have detected some localization of acid phosphatase on, or near, the plasma membrane. This observation has been made using both the Gomori lead nitrate technique and an azo-couple method[10], and by electron histochemical techniques[11].

We suggest that these deposits represent small bodies containing acid phosphatase which are to be regarded as lysosomes and which may correspond to the small granular bodies commonly seen at, or adjacent to, the plasma membrane in oral epithelial keratinocytes[12,13].

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HAEMATOLOGY

Haemagglutinating Properties of Deoxyribonucleic Acid

Ishiyama has described[1] a phenomenon of 'direct haemagglutination' by the nucleic acids (DNA and RNA), and has shown that it is produced in a fixed range of *p*H (*p*H 2–6) and that it is inhibited by human, rabbit, sheep and guinea-pig sera. The present communication reports some results obtained in the investigation of this phenomenon.

In my experiments, sheep erythrocytes (2·5 per cent in physiological saline) were used. 0·1 per cent DNA solution was prepared with 'DNA Ex-herring Sperm' (Light and Co.) in physiological saline at *p*H 5·4. The possibility of there being any contamination with proteins was tested by ethanol and heparin precipitation. The tests were negative. Erythrocyte agglutination by DNA at *p*H 5·4 is reversible if the agglutinates are treated with a saline buffer at *p*H > 6.

Neither thermal denaturation of the DNA (when the solution is heated at 100° C for 10 min and then immersed in an ice-cold bath) nor ultra-violet denaturation (when 2 mm thick layers of the DNA solution were exposed for 45 min, 24 cm from a 450 W, type *Q*-99 ultra-violet lamp) destroys the haemagglutinating capacity of the DNA (ultra-violet denaturation was controlled spectrophotometrically—Hitachi recording spectrophotometer—at 260 mμ). The fact that both thermal and ultra-violet denaturation failed to destroy the haemagglutinating capacity of the DNA would seem to indicate that this property is not related to the structural integrity of the DNA macromolecule. Thermal denaturation results in a breakage of the hydrogen bonds and is responsible for the change-over from double-stranded to single-stranded structure[2,3]; ultra-violet denaturation modifies the helical configuration by breaking the hydrogen bonds into a minimum of ten bonds[4]. It is possible that during thermal denaturation a number of thermoresistant molecules persists in the solution, or that during its cooling a degree of renaturation of the DNA macromolecules occurs[3].

DNA haemagglutination is blocked by 0·01 M EDTA ('EDTA dinatriumsalz', Merck). The blockage is effected by treating the DNA solution with EDTA only. Previous treatment of the erythrocytes with EDTA does not destroy their susceptibility to the haemagglutinating effect of the DNA. It is probable that this effect is also related to the existence of some bivalent cations associated with the DNA macromolecule and that it plays a part in the agglutination phenomenon. Data have been reported on the association of Mg^{++} with DNA macromolecules and the part that it plays in the biological activity of this nucleic acid[5].

If the erythrocytes are treated for 30 min at 37° C with 0·002 M sodium metaperiodate the tendency for them to be agglutinated by DNA disappears. The trypsin (NBC 1 : 300), 1 per cent in buffered saline (*p*H 7·3), restores their susceptibility to agglutinate on treatment with DNA. The same can also be observed after erythrocytes which cannot be agglutinated by serum adsorption are treated with trypsin. It is known that the metaperiodate destroys the receptors for the influenza virus[6,7] and that trypsin restores the haemagglutinating capacity lost after treatment with metaperiodate[8]. These facts would seem to be consistent with the suggestion made by Ishiyama[1] that erythrocyte receptors for DNA intervene in the haemagglutination induced by some viruses.

The DNA haemagglutination is also inhibited by lecithin ('Lecithin ex ovo', Merck) at a final concentration of 1/20,000, but is not inhibited by cholesterol. In this respect, DNA haemagglutination differs from that produced by some bacteria (*Escherichia coli*), as in the latter case the haemagglutination is inhibited by both lecithin and cholesterol[9]. Lecithin acts at the DNA macromolecule level, and only treatment of the DNA solution by lecithin blocks the haemagglutination; even after previous treatment, the erythrocytes cannot be agglutinated.

Investigations are in progress in an endeavour to throw some light on the mechanism of interaction between DNA and cellular surfaces.

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HISTOCHEMISTRY

Mast Cell Mucopolysaccharides

THERE has been a great deal of controversy in the past few years as to whether mucopolysaccharides other than heparin are present in mast cells. Although the biochemical analysis of normal rat peritoneal mast cells has only revealed heparin[1,2], some histochemical evidence strongly suggests that additional mucopolysaccharides may be present in rat mast cells[3,4]. Recently, the dual staining of mast cell granules in an alcian blue–safranin sequence was described by Worthington and Bailey[5]. They found, using alcian blue at a low *p*H with safranin as a counterstain, that even within a single mast cell some granules stain blue and others stain orange-red. It could be postulated that such dual staining arises from the partial removal of an alcian blue staining mucopolysaccharide, which is not heparin as the granules retain their metachromasia.

To test this hypothesis, mast cells in rat mesentery and loose areolar tissue from the dorsum of the foot were examined. Mesenteric biopsies were taken using spring clips with opposing loops at one end and the biopsies were fixed and stained intact. Sections of skin from the foot were examined from blocks which had been fixed and embedded in paraffin wax. In addition, frozen sections were cut from the loose areolar tissue beneath the subcutaneous fat and were stained immediately or post-fixed. Sections were stained with 3 per cent alcian blue in 2 N sulphuric acid for 30 min and counterstained with 1 per cent aqueous safranin for 1 min.

After fixation of the mesentery for 30 min in 10 per cent buffered formalin, the mast cells showed the characteristic dual staining described by Worthington and Bailey[5]. However, after fixation in methanol the mast cells stained uniformly blue. As the same staining reactions were obtained with extracellular granules following degranulation with compound 48/80, it would seem that alcian blue reactivity is a property of the granules alone and that both heparin and the alcian blue staining mucopolysaccharide are retained when the granules are discharged from the cell.

In paraffin-embedded sections of skin fixed in aqueous formalin and methanol, the mast cells stain orange-red with alcian blue–safranin. However, they stain blue in fresh frozen sections post-fixed for 24 h in 10 per cent and 20 per cent formalin, methanol, Bouin's fluid, Zenker's fixative and Carnoy's fixative. But they stain orange-red after post-fixation in 40 per cent formalin. The effect of concentrated formalin is not due to acidity, as alcian blue reactivity is retained after treatment with buffer solutions ranging from *p*H 1·0 to *p*H 7·0 for 24 h. This property of formalin was further studied and it was found that after fixation in 10 per cent formalin the mast cells in fresh frozen sections of skin from the foot also lost their alcian blue reactivity when the sections were later heated at 56° C for 2 h. Methanol-fixed sections retained their reactivity in this test.

Other procedures were investigated in order to determine whether the dual staining could be reproduced. After treatment with testicular hyaluronidase at *p*H 4·9 and *p*H 7·0 the mast cells all stained blue. However, the dual staining was produced when unfixed frozen sections were previously treated with 'tetracycline' (400 mg/100 ml. saline) or vitamin C (>1 mg/ml.) for 24 h, or with ultra-violet light for periods of more than 30 min. In all instances the metachromasia of the granules to safranin was retained and could be confirmed with toluidine blue.

From this study, therefore, it would appear that mast cells do contain a mucopolysaccharide in addition to heparin although the nature of this mucopolysaccharide is unknown. Because the mucopolysaccharide is completely removed by embedding in paraffin wax it is distinct from the mucopolysaccharides stained by other procedures[3,4]. The strong possibility that there are in fact a number of mucopolysaccharides in the mast cell granules should be reconsidered.

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IMMUNOLOGY

Circulating Antigens in Cattle infected with *Babesia bigemina* or *B. argentina*

SOLUBLE antigens have been found in the serum of monkeys infected with *Plasmodium knowlesi*; these antigens fixed complement with homologous antiserum but had no immunogenic activity[1]. However, antigens found in the serum of rats and mice infected with *Trypanosoma brucei* have been shown to be effective immunizing agents[2,3].

This communication reports the occurrence of soluble antigens in the serum of cattle heavily infected with *Babesia argentina* or *B. bigemina*. In the preparation of antigens for use in complement fixation tests for the diagnosis of infection with these organisms, heavy parasitaemia was produced by serial passage of the protozoa through splenectomized calves[4]. Plasma from these infected calves was lyophilized and afterwards reconstituted to one-third of its original volume. These concentrated materials gave two precipitin lines in gel-diffusion tests with serum obtained from cattle 2–4 weeks after recovery from the clinical effects of infection with the homologous organism. As control tests with serum from uninfected calves and with extracts of normal bovine erythrocyte stroma were negative, these antigen–antibody reactions were assumed to be associated with babesial infection.

Antisera to serum from calves infected with *B. argentina* or *B. bigemina* were prepared in rabbits, and antibodies to the constituents of normal bovine serum were removed from the respective rabbit sera by absorption. In gel-diffusion tests with sera from infected calves and homologous absorbed rabbit antiserum, three precipitin lines were detected in the case of *B. argentina* and two lines in the case of *B. bigemina*. Only one line was common to both systems. The differences between the two sets of plates are taken as an indication that some reacting precipitinogens were not normal cattle or rabbit serum components but originated from specific protozoan antigens in the cattle sera inoculated into the rabbits.

Further investigations of the antigens of *Babesia* in the serum of infected cattle are being undertaken, particularly with regard to their isolation and to the assessment of their significance in the production of antibodies related to a state of acquired immunity.

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Adenovirus Antibody and Antibody to 'T' Antigen (Neoantigen) Location in Gamma-globulin

THE properties and possible functions of the complement-fixing tumour and 'T' antigens (neoantigens) of adenoviruses and papovaviruses as related to virus infection and transformation have already been described[1-6]. The present communication reports the first of a series of investigations dealing with the location of antibodies to the 'T' antigen and to the virion of various adenoviruses.

Using sucrose density gradient separation[7], we separated the 19*S* and 7*S* fractions of human and hamster sera. The serum was diluted 1 : 2 with phosphate buffered saline and a portion was saved as control serum. 1 ml. of this material was layered over 4 ml. of a sucrose density gradient (12 per cent to 37 per cent) and centrifuged at 35,000 r.p.m. for 18 h. The 7*S* fraction was located just below the albumin fraction (stained with bromphenol blue for identification) and the 19*S* fraction was in the lowest millilitre of the gradient. The 7*S* fractions, removed by the 'side withdrawal' method[8] and the 19*S* fractions, removed by bottom puncture[7], were identified by electrophoresis. Paper electrophoretic investigations[9] revealed no differences between the patterns of sera from normal hamsters and those with tumours. The 7*S* component appeared mainly as γ-globulin and the 19*S* fraction migrated in the range of β-globulins. The adenovirus type 4 convalescent human sera gave a normal electrophoretic pattern. Although the same general appearance was noted for both human and hamster serum, the 7*S* fraction of human serum contained a small quantity of β- and γ_2-globulins. Each sample was investigated for antigenic titres in complement-fixation tests[10].

A pool of adenovirus type 12 hamster sera selected for its 'narrow' reactivity, that is to say, for its specific reaction with tumour antigens but not with virion antigens[4], was separated into 19*S* and 7*S* fractions. Antibody to adenovirus type 12 tumour antigen was located in the 7*S* fraction as shown in Table 1. The 7*S* fraction was also tested against two types of 'T' antigen preparations obtained from adenovirus 12-infected *HEK* cells. One of the 'T' antigens was produced in *HEK* test-tube cultures in the presence of cytosine arabinoside which blocked the formation of virion complement-fixing antigen and infectious virus while permitting the formation of 'T' antigens[6,11]. The other 'T' antigen was isolated from the supernatant of a 55 h collection of adenovirus 12-infected *HEK* cultures by a two-phase partition separation method[12]. Because the 'T' and tumour antigen complex has at least one heat labile and one heat stable component[1,4,11], the quantitative discrepancies observed between the reactivities of the 7*S* fraction with tumour and 'T' antigens from two different sources were not surprising.

Several sera from individual hamsters carrying adenovirus type 12 tumours were also separated into 19*S* and 7*S* components. The complement-fixing antibodies to adenovirus type 12 tumour antigen (Table 2) were located almost entirely in the 7*S* fraction of each serum.

A serum, from an adult volunteer two weeks after infection with adenovirus type 4, was obtained from Dr. R. M. Chanock and similarly fractionated into 19*S* and 7*S* fractions. This serum, which was devoid of any reaction with adenovirus 12 hamster tumour or 'T' antigens, revealed high-titred antibodies to virion mostly located in the 7*S* fraction; however, some antibody appeared in the 19*S* fraction (Table 3). This reactivity may be characteristic for adenoviruses in general, as almost identical results were obtained when the fractions were tested with the purified group antigen (A antigen) isolated from adenovirus type 12-infected cell culture fluids[13] (Table 3).

Three serial sera specimens were obtained from a child before and after infection with adenovirus 31 through the courtesy of Dr. A. Vargosko of the Children's Hospital in Washington, D.C. Density gradient separation investigations showed that the antibodies to virion were present in the 7*S* fraction (Table 4).

In conclusion, a preliminary investigation indicated that complement-fixing antibodies to the non-virion

Table 1. LOCATION OF COMPLEMENT-FIXING ANTIBODIES TO ADENOVIRUS 12 TUMOUR AND 'T' ANTIGENS IN THE γ-GLOBULIN FRACTION OF A POOLED HAMSTER ANTISERUM

Serum	Antigen	Antiserum dilutions 1 : 10	1 : 20	1 : 40	1 : 80	Titre
Unfractionated serum	Tumour	4	4	4	3	1 : 80
7*S*	Tumour	4	4	4	4	1 : 80 or >
19*S*	Tumour	0	0	0	0	< 10
7*S*	'T' CA-inhibited*	4	4	2	0	1 : 20
7*S*	'T' dextran-separated†	4	4	0	0	1 : 20

* Virion-free 'T' antigen—4 units used.
† Virion-free 'T' antigen—2 units used.

Table 3. LOCATION OF ANTIBODY TO ADENOVIRUS GROUP-SPECIFIC ANTIGEN IN SERUM OF A HUMAN CONVALESCENT FROM ADENOVIRUS TYPE 4 INFECTION

	Antibody titres: Adenovirus 2*				Adenovirus 12 'A' antigen†			
γ-Globulin fractions	1 : 10	1 : 20	1 : 40	1 : 80	1 : 10	1 : 20	1 : 40	1 : 80
19*S* fraction	4	0	0	0	4	4	0	0
7*S* fraction	4	4	4	4	4	4	4	4
Control original sera	4	4	4	4	4	4	4	4

* Adenovirus type 2 antigen used at 4–8 units.
† Adenovirus 12 'A' antigen used at 8–16 units based on titration versus 'A' antigen rabbit antisera.

Table 2. LOCATION OF COMPLEMENT-FIXING ANTIBODY TO ADENOVIRUS TYPE 12 TUMOUR ANTIGEN* IN THE γ-GLOBULIN FRACTIONS OF SERA TAKEN FROM INDIVIDUAL HAMSTERS CARRYING TRANSPLANTED TUMOURS

Hamster antisera	Inoculum	Unfractionated antibody titre	Days after transplantation	Days after tumour appeared	γ-Globulin fraction	Antibody titres 1 : 10	1 : 20	1 : 40	1 : 80
H 56204	New-born transplant (first passage)	1 : 80	96	22	19*S*	1	0		
					7*S*	4	4	4	4
H 56205	New-born transplant (first passage)	1 : 80	96	22	19*S*	1	0		
					7*S*	4	4	4	2
H 56203	New-born transplant (first passage)	1 : 40	96	22	19*S*	0			
					7*S*	4	4	0	
H 57395	Weanling transplant (third passage)	1 : 80	38	19	19*S*	2	0		
					7*S*	4	4	4	0
H 57908	Weanling transplant (third passage)	1 : 40	54	35	19*S*	3	1	0	
					7*S*	4	4	1	0
H 58003	Weanling transplant (fourth passage)	1 : 40	27	11	19*S*	1	0		
					7*S*	4	4	3	0

* Adenovirus type 12 tumour antigen used at 4–8 units.

Table 4. LOCATION OF ANTI-VIRION COMPLEMENT-FIXING ANTIBODY IN THE γ-GLOBULIN FRACTION OF SERA OBTAINED FROM A CHILD AT THREE DIFFERENT TIME PERIODS BEFORE AND AFTER INFECTION WITH ADENOVIRUS TYPE 31

	Antibody titres							
	Adenovirus 31				Adenovirus 12 'A' antigen			
	1:8	1:16	1:32	1:64	1:8	1:16	1:32	1:64
6 months								
19S fraction	0				0			
7S fraction	0				0			
Control original sera	0				0			
2 years								
19S fraction	0				0			
7S fraction	4	4	3	0	4	4	tr	0
Control original sera	4	4	4	1−	4	4	3	0
2 years, 6 months								
19S fraction	0				0			
7S fraction	4	4	4	tr	4	4	0	0
Control original sera	4	4	4	2	4	4	4	0

tumour and 'T' antigens (neoantigens) found in sera of hamsters bearing adenovirus 12 tumours were located in the 7*S* γ-globulin fraction. Antivirion complement-fixing antibodies resulting from infection with adenovirus type 31 were also found entirely in the 7*S* fraction. Separation of a convalescent human serum taken from an adult patient with adenovirus type 4, 2 weeks after infection, revealed some antivirion antibody in the 19*S* fraction. However, at least four times as much antibody was demonstrated in the 7*S* fraction.

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Cells producing IgD Immunoglobulins in Human Spleen

A FOURTH class of immunoglobulins has recently been identified in human sera by Rowe and Fahey[1,2] and has been named IgD. This class is characterized by the unique antigenic and physico-chemical properties of the *H* chain; its concentration has been found to vary between wide limits in different sera, but it seldom exceeds 1 per cent of all immunoglobulins.

As it has been ascertained that, as a rule, the different classes of immunoglobulins are produced by different cells in human lymphoid tissues[3,4], it was of interest to investigate if this was also the case for IgD, as well as to determine the number and disposition of IgD-containing cells.

This has been done by conjugating the gamma globulin fraction of a specific anti-IgD antiserum, with fluorescein isothiocyanate, prepared as indicated by Rowe and Fahey[2], and by staining sections from three different human spleens obtained at necropsy from adult individuals with this conjugated antiserum. The specificity of the staining for IgD was checked by inhibition through previous treatment of the sections with unconjugated anti-IgD antiserum.

Adjacent sections were alternately stained with a fluorescein-conjugated anti-IgG antiserum. A typical result is shown in Figs. 1 and 2: it is clear that the anti-IgD antiserum stains a minority of the cells of the plasma cell line in the human spleen; this staining is as brilliant as that obtained, on a much larger number of cells, by means of the anti-IgG antiserum.

When the anti-IgD (fluorescein-conjugated) and the anti-IgG (rhodamine conjugated) antisera were applied in sequence to the same section, the cytoplasm of a few

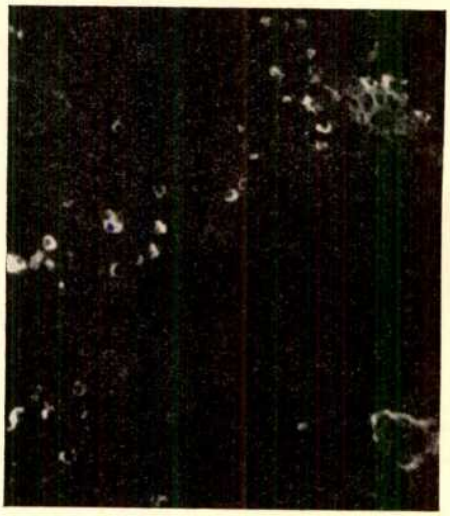


Fig. 1. Section of human spleen stained for IgG; many plasma cells and plasmablasts are visible in the peripheral zone of a periarteriolar sheath. The small artery is visible in the lower right corner, marked by its autofluorescence. (×250)



Fig. 2. Section adjacent to Fig. 1, stained for IgD; only one mature plasma cell containing IgD is present in this field. (×250)

cells showed a brilliant green fluorescence, while that of the majority of cells was stained by rhodamine. It can be concluded that the IgD globulins are produced by separate cells.

As already stated the number of these cells is very small, compared with that of cells producing IgG globulins. To estimate this ratio, all the IgD-positive cells present in a section of splenic tissue about 1 cm² were counted, and their number compared with that of all the IgG-positive cells visible in an adjacent section; the ratio obtained was found to be reproducible in different pairs of sections from different areas of the same spleen; on the other hand, significant differences were found among the three individual spleens examined, the values obtained being 0·13, 0·30 and 0·45 IgD cells per 100 IgG cells, respectively.

These values fall within the range of ratios of IgD to IgG molecules that exist in the majority of normal individuals, calculated on the basis of the concentrations of IgD observed by Rowe and Fahey and on the basis of a normal concentration of IgG of 12·63 mg/ml.[5]. It may be that the concentration of IgD in the serum depends on the number of IgD-producing cells in the lymphoid tissues.

With regard to the morphological characteristics of the IgD-producing cells, it can be said that they appeared similar to those elements positive for IgG; in fact many had the appearance of mature plasma cells, but IgD globulins were also found in the cytoplasm of 'immature' cells of the plasma cell line and in the cytoplasm of elements with a large nucleus and a high nuclear/cytoplasmic ratio (the so-called 'large lymphocytes').

The distribution of the IgD-positive cells in the human spleen appeared to be essentially random inasmuch as most of the cells appeared scattered throughout the tissue; clusters of a few IgD-positive elements were, however, seen in some instances. It seems likely that these clusters were derived from the recent cellular division of one element already differentiated towards the production of IgD; on the other hand, the fact that most of the cells were scattered might indicate that either the IgD-positive cells themselves or their precursors are endowed with considerable capacity for displacement, a property that is presumably possessed also by the cells involved in the production of other immunoglobulins.

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PHYSIOLOGY

A Smooth Muscle Contracting Substance in Extracts of Human Umbilical Cord

At the birth of most mammals the umbilical cord is not ligatured and serious haemorrhage seldom occurs[1]. In man active constriction of the umbilical blood vessels follows delivery.

The umbilical blood vessels have been shown to constrict in response to a variety of stimuli. Haselhorst[2] has shown that the walls of cord vessels are sensitive to temperature and to irritation from handling. Schmitt[3] showed that umbilical vessels contracted when the oxygen content of perfusing fluid was increased. Umbilical vasoconstriction is also produced by the catecholamines, histamine, posterior pituitary extract and 5-hydroxytryptamine[4]. These facts suggested that a chemical substance may be released which will act on the umbilical blood vessels after birth.

Aqueous extracts of human umbilical cords were shown to contain a substance which stimulated smooth muscle. Preliminary investigations involved extraction of different parts of the umbilical cord and the placenta. The active substance seemed to be present only in the walls of the umbilical arteries and vein and was absent from the substance of Wharton's jelly, from extracts of placenta and from umbilical arterial and venous blood.

Purification of crude aqueous extracts was attempted and the active substance was shown to behave as a weak acid on partition between water and ether; it was retained in the aqueous phase at alkaline or neutral *p*H, but at acid *p*H it passed into the ether phase. It migrated towards the anode on paper electrophoresis.

From pharmacological investigations undertaken so far, it would seem that the smooth muscle-stimulating action of the umbilical cord extracts results from a hitherto unknown substance. The substance has been distinguished from active substances which are found in mammalian tissues namely esters of choline, histamine, 5-hydroxytryptamine, bradykinin, angiotonin, substance *P*, oxytocin, vasopressin and other polypeptides.

It was shown that the substance caused contraction of preparations of rat colon, guinea-pig small and large intestine, rat uterus and rabbit ileum. Guinea-pig proximal colon was used for the routine assay of the extracts of umbilical blood vessels as this preparation had been found to be the most sensitive. The purified extract also caused contraction of isolated umbilical artery preparation and isolated pregnant human myometrium.

The pharmacological, physical and chemical properties of the active substance, so far as they have been investigated, indicate that it is a lipid-soluble unsaturated hydroxy acid.

Its physiological significance is a matter for speculation, but it provides a possible explanation of the contraction of the cord vessels at birth.

Further experiments aimed at establishing the chemical structure of the active substance and its possible role in foetal placental circulation are in progress.

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Effect of Muscle Loading on the Behaviour of Interneurones in the Lumbar Spinal Cord

In an earlier paper[1] we showed that when a muscle was loaded there was an increase in the synaptic activation noise recorded from within motoneurones and that this increase had two peaks, one as the load was being applied, the "on" peak, and one as it was released, the "off" peak. There would not seem to be any previous account of a muscle receptor the behaviour of which could explain the origin of this "off" peak. We speculated in our paper that the "off" peak may have its origin in the activity of spinal interneurones, and the results of an investigation of the behaviour of spinal interneurones during muscle pull are presented here.

The experiments were carried out on cats anaesthetized with pentobarbitone ('Nembutal', 35 mg/kg), and im-

mobilized with gallamine triethiodide ('Flaxedil', 20 mg/kg). Glass micropipettes filled with 2 M potassium citrate and having a resistance of 7–15 Mohms were inserted into the lumbar spinal cord which had been previously exposed by laminectomy. The extracellular spikes picked up by these electrodes were recorded on magnetic tape. The tendons of the tibialis anticus, gastrocnemius-soleus and semitendinosus muscles were dissected free and severed from their insertions and were loaded by weights hung on threads tied to their tendons[2].

The results are from thirteen spontaneously firing units located in and adjacent to the ventral horn of the spinal cord. All the units which showed a change during loading of gastrocnemius-soleus and semitendinosus except one showed no change during loading of tibialis anticus. In five of the six cases where units changed their firing rate during loading of gastrocnemius-soleus and semitendinosus but not with loading of tibialis anticus, the change with both loads was similar; in four of the five units there was increase in firing rate and in the other unit a decrease. One unit showed an increase with gastrocnemius-soleus loading and a decrease with semitendinosus loading. In one case a unit increased its rate with loading of semitendinosus and tibialis anticus but loading gastrocnemius-soleus had no effect. There were three units which responded to loading of only one muscle; those which responded to loading of gastrocnemius-soleus and tibialis anticus increased their rate of firing while loading of semitendinosus decreased the rate of firing of one unit. Six of the ten units which changed their firing rate during loading did so at "off" and in all cases there was an increase in rate.

Two examples of unit behaviour are shown: in Fig. 1 there is an increase in the firing rate both at "on" and "off"; in Fig. 2 there is a decrease in the firing rate at "on", and at "off" there is an increase above the spontaneous rate.

The behaviour of these cells contrasts markedly with that of seven units encountered more dorsally and medially in the cord which we have recorded from, with similar electrodes inserted so as to explore the dorsal horn. These cells, while showing an increase in firing rate during loading of the muscles, showed two types of behaviour when the load was released. One group reverted to the spontaneous firing rate without a period of increased firing whereas the other group showed a period of decreased firing and in some cases complete silence (Fig. 3). From these results it is suggested that the interneurones of the ventral horn, the behaviour of which we have described, could by their increased firing rate during the release of tension cause the "off" peak in the synaptic activation noise of the motoneurone.

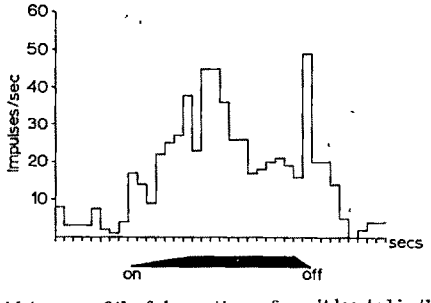


Fig. 1. A histogram of the firing pattern of a unit located in the ventral horn showing the effect of loading the semitendinosus muscle with 500 g

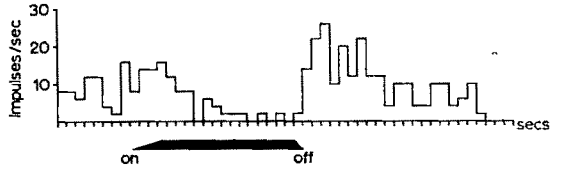


Fig. 2 A histogram of the firing pattern of another unit located in the ventral horn showing the effect of loading the semitendinosus muscle with 500 g

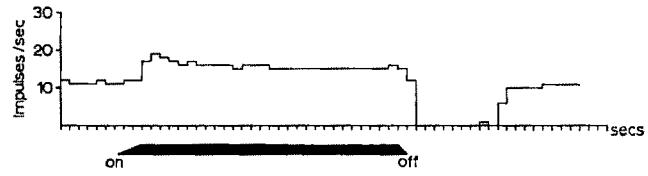


Fig. 3. A histogram of the firing pattern of a unit located in the dorsal horn showing the effect of loading the gastrocnemius-soleus muscle with 100 g

Work is now continuing to investigate whether the behaviour of these two groups of interneurones in response to muscle loading is related and also the part they play in regulating motoneurone function.

We are grateful to the Nuffield Foundation for supporting this work.

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Stretchings and Yawnings induced by Adrenocorticotrophic Hormone

NATURAL crude or purified preparation of adrenocorticotrophic hormone (ACTH) and melanotrophin (MSH) can induce stretching and yawning motions in various species of animals when injected intracisternally. This property is also shared by synthetic polypeptides endowed with MSH and/or ACTH activity[1–4].

Experiments were performed to establish whether or not synthetic $\beta^{(1-24)}$ACTH can induce stretching and yawning motions. This polypeptide has an ACTH potency of 106 I.U./mg (ref. 5).

The experiments were performed on unanaesthetized adult mongrel dogs, cats and vervet monkeys (*Cercopithecus aethiops*). The animals, which had been fasting for 12 h, were injected intracisternally with various doses of the drug being tested. The drugs were dissolved in 0·5 ml. of saline and 0·5 ml. of liquor was withdrawn for each injection. The stretching potency of $\beta^{(1-24)}$ACTH was assessed in dogs by determining the minimal dose which produced the 'stretching crisis'. (It was considered that this had been reached when the dogs showed, at intervals not exceeding 30 min, long periods of stretching each of which began immediately after the preceding one.)

The results of experiments performed on dogs are shown in Table 1. They indicate that $\beta^{(1-24)}$ACTH is as potent as pure natural α_s ACTH in producing stretching motions. The symptoms induced by $\beta^{(1-24)}$ACTH are the same as those induced by the natural hormone: that is, after a delay of about 1–2 h, the dogs start to stretch and yawn in the way they usually do when wakening from physiological sleep. The intervals between successive stretching acts become shorter and shorter until eventually the stretching crisis is reached.

Table 1. STRETCHING ACTIVITY OF $\beta^{(1-24)}$ACTH IN DOGS

Peptide	γ/kg intracisternally	No. of animals with stretching crisis/No. of animals treated
α_s ACTH	3	18/20
	1·5	8/20
$\beta^{(1-24)}$ACTH	3	21/25
	1·5	12/20

$\beta^{(1-24)}$ACTH induces in cats repeated, isolated stretchings in a similar fashion to the natural hormone, but with only a few yawns. These symptoms are accompanied by marked drowsiness.

In monkeys, $\beta^{(1-24)}$ACTH mostly induces repeated yawning. (It should be remembered, however, that yawning in monkeys may be an emotional behaviour[6].) The periods of stretching are very limited in number. Furthermore, the yawnings and stretchings are accompanied by an obvious drowsiness.

The stretching activity persists for about 24–72 h in dogs, and 6–12 h in cats and monkeys; it ends with a complete return to normal behaviour.

We do not know whether the phenomenon described here has any physiological meaning. At present, we are investigating the effect of micro-injections of ACTH in different areas of the central nervous system.

We thank Dr. R. Schwytzer of Ciba, Basle, for providing supplies of $\beta^{(1-24)}$ corticotropin, and Dr. A. Lerner of the Section of Dermatology, Department of Medicine, Yale University, New Haven, for supplies of α_s ACTH.

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PATHOLOGY

Comparison of Direct and Indirect Effects of Alkylating Agents and Radiation on Murine Leukaemia Cells *in vitro*

Reports have recently appeared concerning the quantitative assessment of cytocidal effects of alkylating agents and radiation on several mammalian cell lines *in vitro*. Alexander and Mikulski[1,2] have obtained dose-survival curves of murine leukaemic lymphoblasts (*L*5178*Y*) *in vitro* after treatment with alkylating agents and X-rays. Levis[3], using the technique of Puck *et al.*[4] in which macroscopic colonies are grown *in vitro* from single mammalian cells, compared the dose-survival curves of guinea-pig cells after treatment with nitrogen mustard and with X-rays. Berry[5] reported on the effects of several chemotherapeutic agents and X-rays on HeLa cells and mouse fibroblasts *in vitro*. We have recently compared the effects of nitrogen mustard and dimethyl myleran on *L*5178*Y* cell lines of different radiosensitivity *in vitro*[6].

A simple but fundamental question, common to all these studies, arises but has not yet been answered: what fraction, if any, of the cytocidal effects observed following alkylating agents or ionizing radiation represents an indirect effect exerted on the medium as distinct from a direct effect on the cell? From a previous investigation[6] it appeared that only a small fraction of the alkylating agent actually reacted with the cell, the vast majority being "wasted" by alkylation of constituents in the medium. Reactions of this sort conceivably could produce cytocidal effects by alteration of essential nutrients in the medium or by formation of toxic metabolites. Similarly, indirect effects of ionizing radiation could also be postulated.

In this study the direct effect of dimethyl myleran (DMM), nitrogen mustard (HN2) and ionizing radiation (cobalt-60) on murine leukaemic lymphoblasts (*L*5178*Y*) *in vitro* has been compared with their indirect effect on the tissue culture medium. Medium was treated with increasing doses of dimethyl myleran, nitrogen mustard and ionizing radiation. *L*5178*Y* cells were added to DMM-treated medium at 240 min, to HN2-treated medium at 300 min and to irradiated medium at both 2 min and 240 min after treatment. The purpose of these delays was to create a situation in which the direct cellular effect of alkylating agents could be separated from their indirect effect on medium. The half-life in tissue culture medium at 37° C is about 30 min for DMM[7] and 75 min for HN2[8]. Therefore, at the time cells are introduced into the treated medium (eight half-lives for DMM and four for HN2), a minimal residue of alkylating activity will remain to affect cells directly, and the dominant cytocidal effect will be indirect, achieved by reactions with constituents in the medium that may occur. The early (2 min) and late (240 min) delays after irradiation of the medium were arbitrarily selected. Control flasks with medium containing cells were treated at zero time with identical doses of DMM, HN2 and radiation. Radiation was administered from a radioactive cobalt-60 source at a dose rate of 79·5 rads/min. Cell number was determined by a haemocytometer count and growth curves of control and treated flasks plotted on a semilogarithmic scale with log of cell concentration on the ordinate against time on the abscissa. Dose-survival curves illustrating the effects of radiation were obtained by the method of Alexander and Mikulski[1,2].

The cytocidal effects observed when murine leukaemic lymphoblasts (*L*5178*Y*) were treated at zero time with DMM and HN2 are depicted by the growth curves shown in Figs. 1*A* and 2*A*. No cytocidal effects were observed

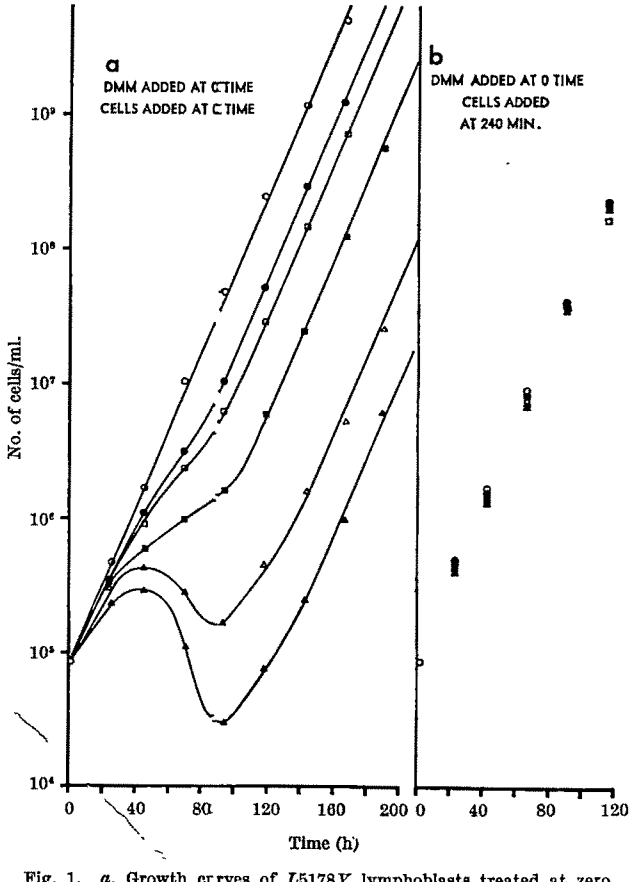


Fig. 1. *a*, Growth curves of *L*5178*Y* lymphoblasts treated at zero time with DMM at the following dose-levels: ○, none; ●, 5 μg/ml.; □, 10 μg/ml.; ■, 20 μg/ml.; △, 30 μg/ml.; ▲ 35 μg/ml. *b*, Growth curves of *L*5178*Y* lymphoblasts introduced into tissue culture medium, 240 min after the medium was treated with DMM, at 37° C, at the same dose-levels indicated in *a*

when the cells were introduced into the medium 240 min after DMM (Fig. 1*B*) and 300 min after HN2 (Fig. 2*B*). A complete absence of cytocidal action was also observed when cells were added to medium 2 and 240 min after irradiation as demonstrated by the dose-survival curves shown in Fig. 3. It would appear from this study that the cytocidal effect observed following treatment with DMM, HN2 and radiation represents a direct effect on the cell and does not involve any sustained indirect effect on the cell by alteration of constituents in the medium. The formation of cytocidal metabolites of very short half-life has not been excluded by this investigation.

I thank Mr. J. L. Everett of the Chester Beatty Research Institute for kindly supplying the dimethyl myleran and the Merck, Sharp and Dohme drug company for supplying the nitrogen mustard ('Mustargen'), and Miss S. Shniderman for technical assistance. This work was supported by a grant from the National Cancer Institute of Canada.

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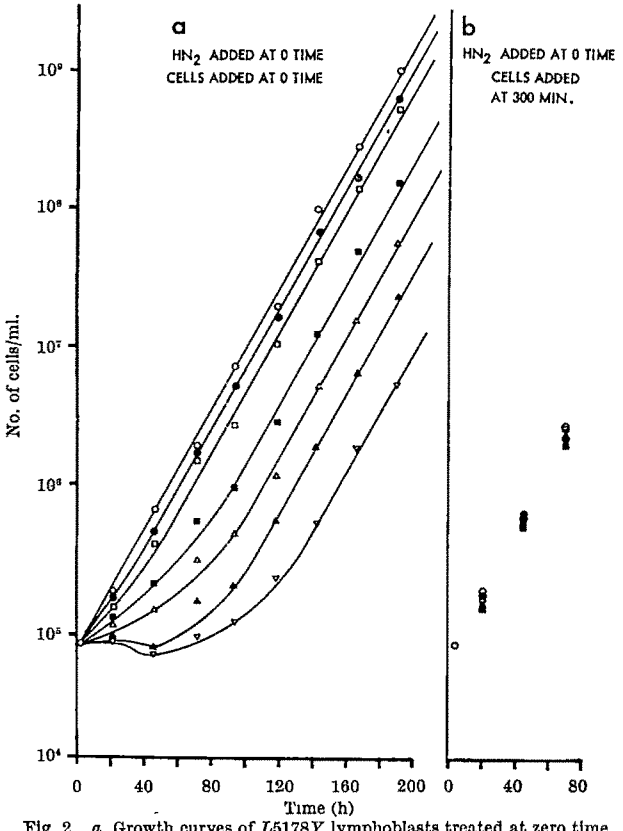


Fig. 2. *a*, Growth curves of *L*5178*Y* lymphoblasts treated at zero time with HN2 at the following dose-levels. ○, none; ●, 0·05 μg/ml.; □, 0·1 μg/ml.; ■, 0·2 μg/ml.; △, 0·25 μg/ml.; ▲, 0·3 μg/ml.; ▽, 0·4 μg/ml. *b*, Growth curves of *L*5178*Y* lymphoblasts introduced into tissue culture medium, 300 min after the medium was treated with HN2, at 37° C, at the same dose-levels indicated in *a*

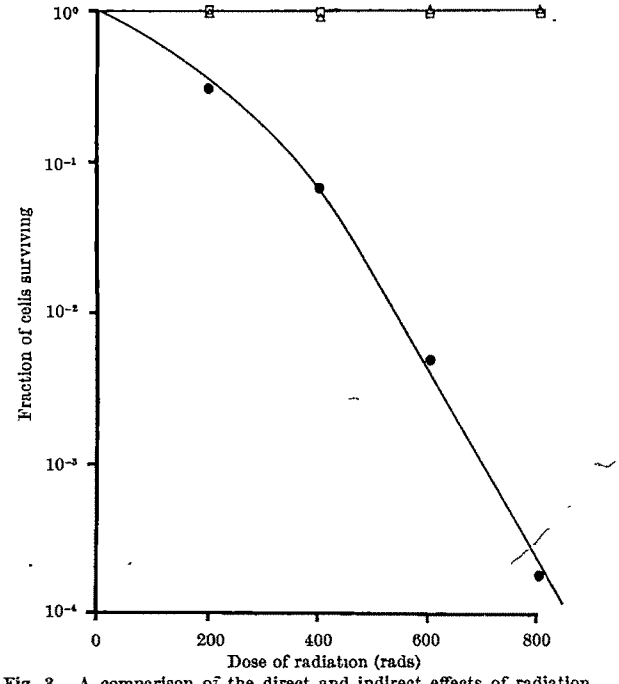


Fig. 3. A comparison of the direct and indirect effects of radiation (cobalt-60) as measured by the dose-survival curves of *L*5178*Y* lymphoblasts. All samples were radiated at 0 time ·●; Cells added at 0 time, □, cells added at 2 min, △, cells added at 240 min

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Toxic Effects of 7,12-Dimethylbenz(a)-anthracene in New-born and Adult Swiss Mice

DURING previous investigations of chemical carcinogenesis, we repeatedly found that new-born mice injected once with 7,12-dimethylbenz(*a*)anthracene (DMBA) died at a certain time after the treatment. On this basis we decided to investigate, in an exact manner, the toxicity of a carcinogenic dose of DMBA in new-born mice. Furthermore, we included in this investigation an adult group of mice in order to compare the toxicity rate of this carcinogen in two age groups of animals.

Randomly bred Swiss mice from a colony at our laboratory were used in the experiments. The DMBA used was obtained from Eastman Organic Chemicals and purified by chromatography on magnesia/'Celite'. For purposes of injection, it was dissolved in olive oil (Fisher Scientific Co.), and animals of both age groups received subcutaneous injections in the interscapular area. Siblings were weighed on a 'Gram-atic' balance (E. Mettler, Zurich), and only those which weighed 1·3, 1·4, 1·5 and 1·6 g and were less than 24 h old were selected for the treatment. Adult mice were weighed on a 'Shadowgraph' balance (The Exact Weight Scale Co., Columbus), and those weighing 19·5 g (5 weeks old) were used in the experiments. In the new-born group, only five siblings were allowed to remain with each mother. In the adult group the mice were separated according to sex and kept in groups of ten per cage. All animals were housed in clear plastic cages with sterilized granular cellulose bedding and were given Rockland diet in pellets and tap water *ad libitum*.

The experiment was divided into four groups as shown in Table 1. After treatment or, in the case of the control group, from birth, all groups were carefully checked each day for 1 month. Necropsies were performed on all dead animals and sections were routinely taken for microscopical examination. The treatment, toxicity rates, and percentage of mortality on the thirtieth day are presented in Table 1.

Epilation, hyperkeratosis and ulceration were observed at the sites of injection of most of the animals treated with DMBA. At autopsy, marked cellular depletion of the haematopoietic and myelopoietic systems, including bone marrow, thymus, liver and spleen, were readily discerned, as previously described[1,2]. A few of the mice which did not receive injections and those injected with

Table 1. MORTALITY RATE OF DMBA-TREATED NEW-BORN AND ADULT SWISS MICE AND OLIVE-OIL INJECTED AND UNTREATED CONTROLS

Group	Treatment	No. and sex of mice	No. of dead after beginning of experiment on day: 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	Percentage of mortality on the 30th day
1	New-born mice injected subcutaneously with 100 μg/1 5 g bodyweight of DMBA in 0 05 ml olive oil	100	2	3	4	3	5	2	1	–	1	–	2	2	11	9	10	3	5	1	–	2	–	–	–	–	1	1	–	–	–	1	69
2	Adult mice injected subcutaneously with 100 μg/1 5 g bodyweight of DMBA in 0 5 ml. olive oil	50♀	–	–	–	–	–	–	–	–	–	–	–	1	–	2	1	–	–	–	–	–	–	–	–	–	–	1	–	–	–	23
		50♂	–	–	–	–	–	–	–	–	1	–	1	–	–	2	5	2	–	3	1	1	–	1	–	–	–	–	–	–	–	1	
3	New-born mice injected subcutaneously with 0 05 ml olive oil	100	4	10	1	1	–	–	–	–	–	–	–	–	1	1	1	1	–	1	–	–	1	–	–	–	–	1	1	1	–	1	26
4	Untreated controls observed from birth	100	–	4	3	–	–	1	–	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	9

olive oil died of diarrhoea; however, the cause of death in other members of these groups was not established.

The present findings clearly show that when a carcinogenic dose of DMBA is administered on a strictly bodyweight basis to two age groups of animals, it is more toxic to new-born than to adult Swiss mice.

Considerable interest has recently been attached to the differences in susceptibility to carcinogenesis between new-born and adult animals. This has been particularly so for viral carcinogenesis[3], but additionally so in investigations of chemical[4–7] and radiation[8] carcinogenesis. In the present investigation it has once again been found that new-born animals are more responsive than adults—this time to the acute toxic administration of a chemical carcinogen. The reason for the difference has not been completely explained in any instance. Various suggestions have been put forward, including a difference in the immunological response, and more recently, differences in the rate of metabolic breakdown[9–11]. The present findings with DMBA are in keeping with suggestions that this carcinogen is metabolized far more slowly in new-born mice than in adult ones. It would seem likely, therefore, that the toxic action of this compound arises from the intact molecule rather than a metabolite.

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Chronic Fluorosis in the Guinea-pig

DEATHS often reaching epidemic proportion occurred in at least eight major guinea-pig breeding units in Sydney and Canberra during 1964 and early 1965. Affected animals were characterized by loss of weight, depression, inappetence, a marked slobbering causing saturation and often excoriation of the chin, neck and venter, and ultimate death. All ages were affected, but newly-weaned animals and pregnant or post-parturient females appeared especially susceptible. Decrease in weight was evident for 1 to several weeks until increasing loss of fluid from the oral cavity presaged death within 5–20 days.

Autopsy revealed loss of body fat, lack of food in the anterior gastrointestinal tract and, occasionally, lung consolidation and increase in peritoneal fluid. Striking changes were found in the oral cavity. The lower molars were usually overgrown in a lingual direction so as to form an arch over the fixed part of the tongue, while the upper molars were correspondingly worn down to the level of the hard palate. Frequently there was a buccal elongation of the anterior upper molars and a wearing down of the anterior lower molars. Most molar teeth were coated with a tartar encrustation, and histopathological examination of them revealed imperfect calcification and hypoplasia of the enamel. The dentine and odontoblast layer showed changes characteristic of sub-acute hypovitaminosis C. Calcification of bone appeared to be impaired and resorption to be increased. The only other significant changes were in the kidney, in which corticomedullary calcareous deposits occurred in every case and distension of the tubules in some.

Aspects of the pathology suggested fluorine toxicosis, and diets used in the breeding units were investigated. Proprietary pelleted feed was used in all but one laboratory, which relied entirely on green feed and hay. No case of "slobbers" was recorded from the last unit. When bone flour had been difficult to obtain, Christmas Island rock phosphate had been substituted as a mineral source in the pellets. The rate of incorporation of the crude phosphate into the pellets varied from 0·8 to 2·0 per cent. The fluoride content of the Christmas Island phosphate dust has risen as quarrying has progressed from less than 1·5 to about 2·2 per cent. This would mean a fluorine content in the pellet in the range 60–440 p.p.m.

Samples of pellets from a colony in which only sporadic cases occurred had the lowest fluorine content (130 p.p.m.). The fluorine content of other samples from affected colonies varied from 190 to 400 p.p.m. High mortality, up to 100 per cent on occasion, was strongly associated with increasing levels of fluorine in the pellets used. The teeth and jaw-bones of a limited number of affected guinea-pigs showed fluorine contents of 3,940–6,700 p.p.m., the range in normal animals being 100–160 p.p.m.

The disease was reproduced with identical histopathology by administering calcium fluoride to healthy guinea-pigs, resulting in a 100 per cent mortality within 25 weeks. All cases showed characteristic molar changes and 90 per cent developed the symptom of slobbering.

The present communication is designed to bring immediate attention to the potential danger of incorporating certain crude phosphates in proprietary pellets. This investigation demonstrates one possible cause of a disease syndrome which has been known in the guinea-

pig, but little understood, for many years. Aspects of this syndrome have been reported by a number of authors[1-4].

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RADIOBIOLOGY

Sensitivity of the Phage and Complementary Strands of the Replicative Form of Phage φ X 174 Deoxyribonucleic Acid to Ultra-violet Light

PHOTOPRODUCTS of pyrimidines are known to play an important part in the inactivation of nucleic acids by ultra-violet light. The replicative form (RF) of phage φ *X* 174 DNA is a convenient material to use for an investigation of the dependence of DNA ultra-violet sensitivity on base composition. The properties of both strands of RF DNA are similar, yet their nucleotide composition differs: the complementary strand contains 8·2 per cent less thymine and 5·6 per cent more cytosine than the phage strand[1,2]. The present investigation was undertaken to compare the ultra-violet inactivation of the infectivity of both chains of RF φ *X* 174 DNA.

The complementary chain was isolated from RF RNA. The replicative form was heated at 100° C for 10 min and the fraction of denatured DNA was separated on a MAK column[3]. The complementary chains present in this fraction were fixed in 'renaturation' with a 50–100-fold excess of ultra-violet inactivated φ *X* DNA. Renatured double-stranded DNA was eluted from a MAK column by 0·7 M sodium chloride and denatured. The infectivity of this preparation was due to the complementary strands. DNA from phage φ *X* 174 served as the phage-type strands. Infectivity of DNA was measured by the number of infected protoplasts of *E. coli C* (ref. 4). The concentration of DNA during irradiation was 0·005–0·05 μg/ml. Ultra-violet irradiation was carried out using a low-pressure *BUW*-15 lamp which predominantly emitted at 254 mμ.

Fig. 1 shows the kinetics of ultra-violet inactivation of both the RF DNA strands. The complementary chain is more resistant than the phage strand. If DNA inactivation arises mainly from thymine dimerization and if both strands of RF DNA have similar thymine–thymine nearest neighbour frequencies, then the sensitivity of the phage and the complementary strands to ultra-violet light should differ and depend on their thymine contents:

$$\frac{D_{37}^{\text{phage}}}{D_{37}^{\text{compl.}}} = \frac{N^{\text{compl.}}}{N^{\text{phage}}}$$

where D_{37} is the dose of ultra-violet light required to inactivate to a survival of 37 per cent (one hit), and N the number of thymines in the DNA strand. According to our measurements, the value of D_{37} for φ *X* DNA is 78 ergs/mm². As N is 1790 and 1342 for the phage and the complementary chains, respectively, then D_{37} for the complementary chain will be 104 ergs/mm². As can be seen in Fig. 1, the theoretical survival curve for the complementary chain constructed in accordance with the computed dose is close to the curve for the experimentally obtained results. This demonstrates that thymine plays an active part in the formation of lethal ultra-violet lesions. The sensitivity of the two polynucleotide chains in the RF DNA molecule to ultra-violet irradiation correlates well with their thymine contents. Correlation between the composition of a nucleotide and its sensitivity to ultra-violet light is not revealed when different phages are compared[6]. Here additional factors, including some of a genetical nature rather than base composition, determine the actual sensitivity to ultra-violet light.

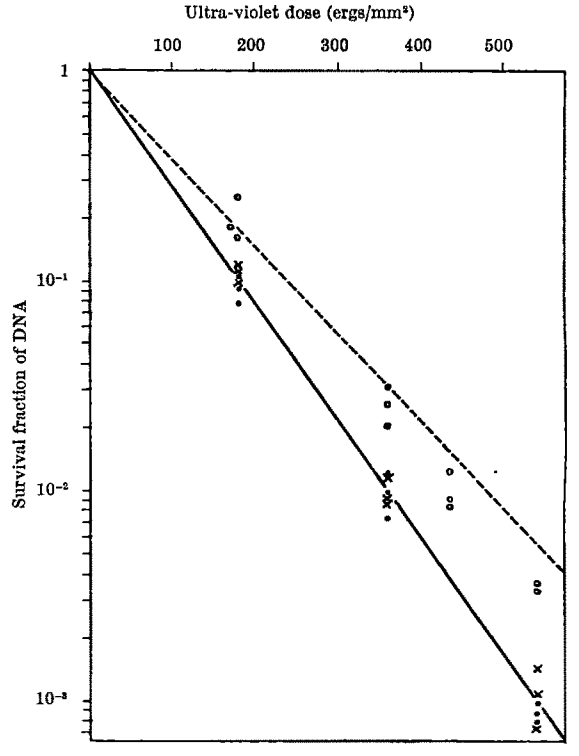


Fig. 1. Ultra-violet survival curves for φ *X* 174 DNA and complementary strand of RF DNA. ●, φ *X* 174 DNA; ×, φ *X* 174 DNA after reactivation; O, complementary strand of RF DNA; - - - -, theoretical curve for inactivation of complementary strand

Despite the approximate correlation between the sensitivity to ultra-violet light and thymine content, only about 40 per cent of lethal lesions result from thymine dimerization in φ *X* 174 DNA[5,6]. Apparently, the reaction of cytosine photohydration does not produce a lethal effect. Such cytosine photoproducts as 5-hydro-6-hydroxycytosine are unstable and at increased temperature in the dark revert to cytosine. As is shown in Fig. 1, the incubation of ultra-violet deactivated φ *X* 174 DNA at 37° and 42° C in the dark for 2–20 h is not accompanied by recovery of infectivity. Nevertheless, in addition to thymine dimers other lethal photoproducts exist in φ *X* 174 DNA.

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Cysteine Incorporation and Radio-protection in *Saccharomyces*

THIS communication reports some data on the time dependence of cysteine incorporation and the increase of radio-protection in cells of a diploid *Saccharomyces* strain in two different physiological conditions.

There are many reports dealing with the radio-protective effects of cysteine and other SH-compounds in various organisms, but there is still no general theory about the mechanisms of radio-protection by sulphydryl compounds. It has been shown[1] that under *in vitro* conditions, that is in cell-free solutions of organic macromolecules, cysteine may act as radio-protector owing to its great reaction velocity with primary radiation products ("competition effect"). However, under conditions *in vivo*, that is in cells in aqueous milieu containing cysteine, the reactions leading to radio-protection may be more complicated[2].

In many experiments it has been shown that cysteine can only act as a radio-protector if it is present during irradiation[3]. Several theories have been put forward to explain the protection effect[4]. Under *in vivo* conditions, the penetration of cysteine from the surrounding medium into the cells is generally considered to be a prerequisite for protection. It was therefore considered to be of interest to gain more information concerning the correlation between cysteine incorporation in cells and the radio-protection effect.

Cells of the diploid *Saccharomyces* strain 211-1*a*/2 from our strain collection were used, and were taken either from the stationary growth phase (*s*-cells) or from the logarithmic growth phase (*l*-cells) in liquid nutrient medium (1 per cent Difco yeast extract, 0·25 per cent glucose) aerated by agitation. Before irradiation the cells were washed three times in M/20 potassium dihydrogen-phosphate buffer. The proportion of budding cells was reduced to about 10 per cent by fractional centrifugation, and the cell suspension diluted in buffer to an appropriate cell titre. During irradiation, the cells were either suspended in buffer or in a solution of buffer containing in addition 0·01 M cysteine (cysteine solution).

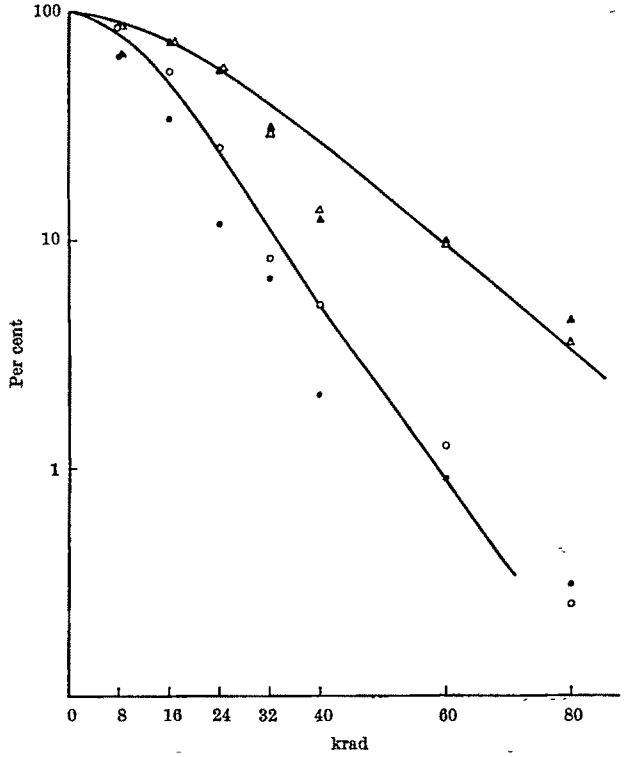


Fig. 1. Inactivation of the colony-forming ability of diploid *Saccharomyces* cells by 50-kV X-rays. The radiation source was a Machlett tube with beryllium window without additional filter. △, *s*-cells, and ▲, *l*-cells, incubated for 20 min in a 0·01 M cysteine solution before irradiation. ○, *s*-cells, and ●, *l*-cells, without cysteine treatment

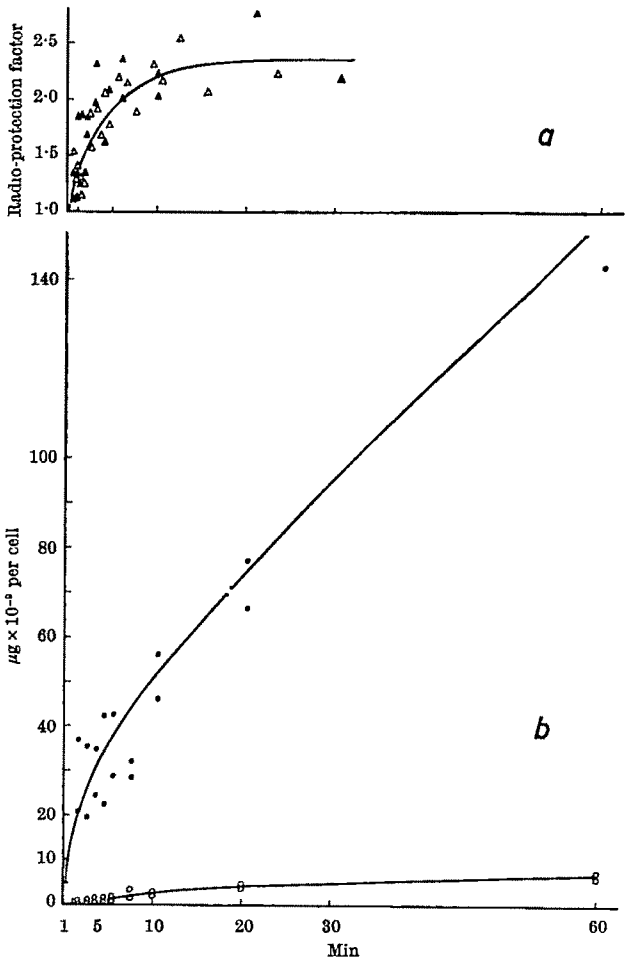


Fig. 2. *a*, Dependence of radio-protection of diploid *Saccharomyces* cells on the time of incubation in a 0·01 M cysteine solution. The absorbed dose of 50-kV X-rays was kept constant at 24 krads. Irradiation time 30 sec. The radio-protection factor is calculated from the quotient: $\frac{\text{percentage of colony-forming cysteine-treated cells}}{\text{percentage of colony-forming untreated control cells}}$. The time marked on the abscissa includes the time necessary for irradiation (30 sec). △, *s*-cells; ▲, *l*-cells. *b*, Dependence of cysteine-incorporation in diploid *Saccharomyces* cells on the time of incubation in a 0·01 M cysteine-^{35}S-solution. The time marked on the abscissa includes the time necessary for centrifugation. ○, *s*-cells; ●, *l*-cells

When *s*-cells or *l*-cells are suspended in cysteine solution for 20 min and then irradiated with 50-kV X-rays both types of cells exhibit various degrees of radio-protection, as can be seen from a comparison with controls treated in the same way but irradiated in the absence of cysteine (Fig. 1). The inability of the cells to form macroscopic colonies after incubation for 4 days at 30° C on nutrient agar (1 per cent Difco yeast extract, 0·5 per cent peptone, 2 per cent glucose 2 per cent Difco bacto-agar) was taken as an indication of radiation damage. As can be seen in Fig. 1, the X-ray dose necessary for an inactivation of 90 per cent of the cells (10 per cent survival) is nearly twice as large for cysteine-treated cells as for untreated controls. There may be a tendency for greater radio-protection in *l*-cells than in *s*-cells; however, a statistically significant difference in the degree of protection between both types of cells has never been observed.

The dependence of the effectiveness of radio-protection on the period of cell exposure to cysteine can be seen in Fig. 2*a*. The cells were exposed for different periods to a solution of 0·01 M cysteine in buffer at room temperature and then irradiated. An optimal radio-protection of *s*-cells and *l*-cells was achieved after exposure to cysteine for about 10 min. After this period, the percentage of cysteine-

treated cells of both types surviving an X-ray dose of 24 krads was about twice as great as that for the surviving untreated controls.

The dependence of cysteine-incorporation in *s*-cells and *l*-cells on the period of exposure was determined by means of labelling the cysteine with sulphur-35. The cells grown in the specified liquid medium were washed three times in buffer and suspended in solutions of buffer containing in addition 0·01 M cysteine-^{35}S (specific activity 3·3 mc./mM) for various intervals of time at room temperature. Afterwards the cells were washed three times in inactive 0·01 M cysteine-buffer solution. The radioactivity of the cells thus treated was measured with a Geiger–Müller counter *FHZ* 15*b* (Friesecke and Hoepfner) and the amount of radioactivity per cell calculated. The percentage of budding cells was kept approximately constant in all experiments with radioactive cysteine. As can be seen in Fig. 2*b*, there is a significant difference in the amount of cysteine incorporated in the *l*-cells from the *s*-cells. Saturation in the incorporation of cysteine was not achieved with *l*-cells during an exposure time of 60 min. In *s*-cells, however, the incorporation of cysteine reached a limit after about 20 min of exposure.

These experiments show that an optimal radioprotection is only achieved after a certain quantity of cysteine has penetrated into the cell. The minimal quantity of cysteine necessary for a maximal radioprotection of *s*-cells is reached after about 10 min and amounts to about 10^{-15} g per cell. This value corresponds to about 0·01 per cent of the total quantity of amino-acids per cell or to about 1 per cent of the total quantity of cysteine and cystine per cell. The considerably larger amount of cysteine incorporated in *l*-cells within the same period of exposure does not, however, result in a significant increase in radio-protection. Furthermore, radioprotection does not increase in *l*-cells during the continued cysteine-incorporation in an additional exposure period of 50 min. These observations can be explained if it is assumed that part of the incorporated cysteine is rapidly used in protein synthesis and consequently loses its radio-protective effectiveness. This assumption implies that only the pool of free cysteine acts as radio-protector. That the same degree of maximal radio-protection is observed in *s*-cells and *l*-cells may indicate that the content of free cysteine is about the same in both types of cell after exposure for 10 min to a 0·01 M cysteine solution. Synthesis of protein, however, as an effective consumer of free cysteine, should be abundant in *l*-cells, but should be strongly reduced or absent in *s*-cells. Further investigations concerning the presence of free and protein-bound cysteine in yeast cells are now under way.

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GENETICS

Chiasma Frequencies and the Potential Genetic Variability of *Lolium* Populations

RECENT investigations in *Lolium* show that annual and other short-lived populations have higher chiasma frequencies than more perennial populations[1]. The variation in chiasma frequency was inferred to be adaptive. It was also suggested, however, that the variation in chiasma frequency was not directly but only indirectly dependent on the longevity of the population. The basis for this suggestion was, briefly, as follows: (1) It is well established that inbreeding species, the members of which on average carry low potential genetic variability, have higher chiasma frequencies than related outbreeders[2–4]. The high chiasma frequencies, which promote a high rate of release of variability whenever hybridization does occur, may be regarded as compensating for the low potential variability[2,3]. (2) There was some evidence in *Lolium* to indicate that the shorter-lived rye grass populations contain less potential variability than the longer-lived populations. As in the inbreeders already referred to, the inference is that their high chiasma frequencies compensate for this relatively low potential variability.

On this view the chiasma frequencies are adaptive in direct relation to the potential variability of populations and only obliquely in relation to longevity. A critical test for this view would be to survey the chiasma frequencies in populations for which data on both longevity and potential variability were available. Estimates of the latter are, however, particularly difficult to obtain. Fortunately the selection work of Cooper[5] and the more recent work of Edwards and Cooper[6] provide such information with respect to four *Lolium* populations, namely, the varieties *Kent*, *Irish*, *Italian* and *Hunsballe*. The information on potential variability is adduced from the degree of response to selection. For example, with selection for leaf size the greatest response was shown by *Irish*, then *Italian* and, last, *Hunsballe*. From this we may conclude that *Irish* has the highest potential genetic variability, then *Italian*, *Hunsballe* having the lowest potential variability. In other experiments where selection was practised for ear emergence, *Kent* rye grass gave a much greater response than *Irish*. While it is unfortunate that the selection data involve only a few characters, the results nevertheless give a reasonable indication of the order of magnitude of potential variability in the four populations. In descending order the sequence is *Kent*, *Irish*, *Italian*, *Hunsballe*. If, as is proposed, the chiasma frequencies are inversely related to the degree of potential variability of the populations, the expectation is that the order of increasing chiasma frequencies will be *Kent*, *Irish*, *Italian* and *Hunsballe*.

Fig. 1 shows the chiasma frequencies of the four populations plotted against the order of potential vari-

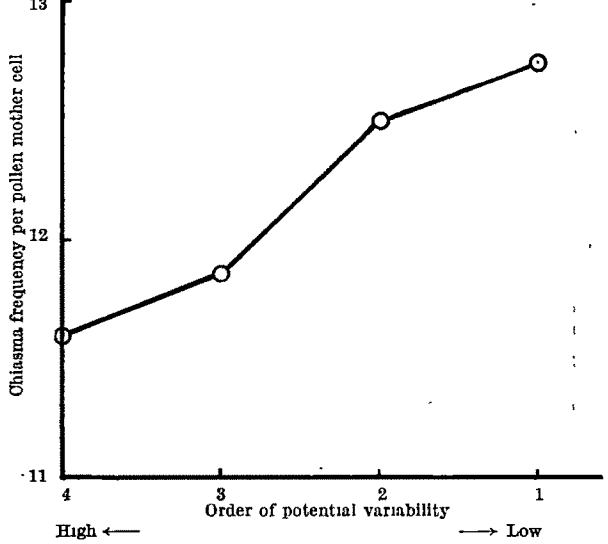


Fig. 1. Average plant chiasma frequencies of *Lolium* populations plotted against the order of potential genetic variability of the populations. Chiasma frequencies (expressed as chiasmata per cell) were estimated from counts in twenty pollen mother cells in each of ten plants in each population

ability. As will be seen, the chiasma frequency variation agrees completely with expectation of an inverse correlation with potential variability.

Turning to longevity, *Kent* and *Hunsballe* are both highly perennial; *Irish* is next in order of perenniality and *Italian* the least perennial. *Kent* and *Hunsballe* are of particular interest in that they show the same high degree of perenniality but differ with respect to potential variability. If population chiasma frequencies relate directly to longevity, the chiasma frequencies of *Kent* and *Hunsballe* should be alike at one end of the scale with *Italian* at the other. In fact, the chiasma frequencies of *Hunsballe* and *Kent* are significantly different ($P < 0{\cdot}01$) and are highest ($12{\cdot}79 \pm 0{\cdot}20$) and lowest ($11{\cdot}58 \pm 0{\cdot}31$), respectively. These chiasma frequencies vary quite independently of longevity but are completely correlated with variation in potential variability, as already mentioned.

From these comparisons it appears that variation in chiasma frequency is inversely related to the degree of potential genetic variability of populations. Finally the correlation between chiasma frequencies with longevity in many of the *Lolium* populations investigated[1] follows as an inevitable consequence of the correlation between longevity and potential genetic variability generally, but not invariably, found in *Lolium* populations.

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BIOLOGY

Effect of Magnetic Fields on the Respiration of Malignant, Embryonic and Adult Tissue

WITH the development of stronger magnetic fields, a renewed impetus has been given to the investigation of the effect of these fields on biological material. Already proposed theories, elaborately expressed mathematically, appear to be far in advance of the facts.

Because energy must be involved in the effect of a magnetic field on biological material, it is natural to suppose that it is on a molecular level, and that it involves enzymes and other biochemical constituents of the cell. It has been suggested by Valentinuzzi[1] that aspects of chemical kinetics, closely related to the velocity of reactions, might significantly fit into magnetobiological phenomena. Theoretical investigations, according to Valentinuzzi, take into account that the effect due to modification of the rotational diffusion of paramagnetic molecules caused by magnetic fields could explain some of the experimental observations. It is held by Valentinuzzi (see also Setlow and Pollard[2] and Barnothy[3]) that, "If such molecules with specific reactive sites are paramagnetic, there exists the possibility that an externally applied magnetic field may either slow or stop their rotation, a fact which would imply a decrease of the probability of effective collisions, and therefore a retardation of the corresponding biochemical reaction".

Whatever the fundamental mechanism, it has been observed that either a stimulatory or inhibitory effect can be induced by exposure to a magnetic field, the plus or minus deviation from the normal depending on the kind of tissue and its metabolic or proliferative status.

Reno and Nutini[4] were perhaps the first to investigate the effect of magnetic fields on the respiration of normal, neoplastic and embryonic tissues. They observed that the respiration of cancer and embryonic tissues was depressed when they were exposed to a magnetic field, while normal adult tissues showed no significant response.

It would seem, from a review of the literature, that most, if not all, of the experiments conducted used constant fields applied to the test material over various periods of time. This procedure resulted in effects which were usually on the borderline of significance and predictability. It occurred to us that a more pronounced effect would be realized if the field could be intermittently applied so that, in effect, the tissue or cells would receive a series of pulses analogous to building up and collapsing the magnetic field in order to do work. Two methods were used: (1) a quadruple permanent magnet (6 kG) mounted on a dolly so as to bring the field to and away from the specimen; (2) an electromagnet (10 kG) which permitted the field to be turned on and off at predetermined intervals.

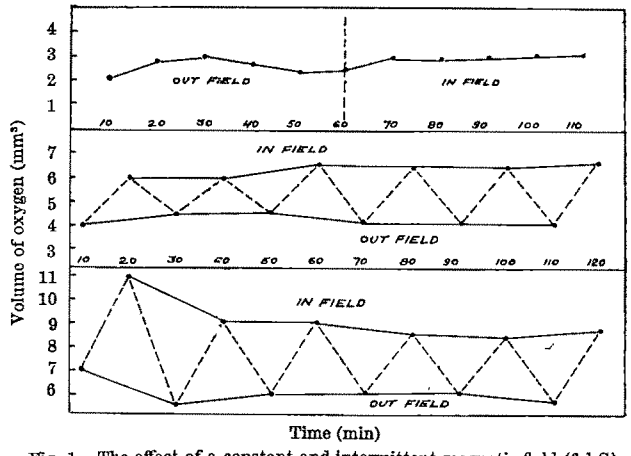


Fig. 1. The effect of a constant and intermittent magnetic field (6 kG) on the respiration of adult mouse kidney tissue

Measurements of respiration were made with a specially designed microrespirometer similar to that used by Reno and Nutini[5]. Fig. 1 (adult mouse kidney tissue) is representative of the results obtained, clearly demonstrating no effect in a constant field, whereas in experiments with an intermittent field a definite stimulatory effect was noted. The broken line, connecting the 'in-field' and 'out-field' solid lines, indicates the 10-min intervals during which the specimen was in the field and out of the field, respectively. Other tissues and cells exposed to constant and alternating fields gave comparable results. Whether or not the magnetic field stimulated the respiration depended on the kind of tissue. For example, it was found that yeast and *Bryophyllum* were stimulated, while sarcoma 37 cells, embryo heart, liver and intestine were depressed.

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New Records of the Shrimps *Hymenopenaeus tropicalis* and *Parapenaeus americanus* from Brazil

A SMALL number of unknown shrimps were among the material obtained from one station (24° 25′ S., 44° 40′ W.), about 40 miles south-east of Punta do Boi, São Sebastião Island, in June 1962, on a muddy bottom and at a depth of 130 m. These were kindly identified by Dr. F. A. Chace, jun., of the Smithsonian Institute, as *Hymenopenaeus tropicalis* (Bouvier) and *Parapenaeus americanus* Rathbun(?). According to Dr. Chace the latter could be an undescribed species, on the basis of apparent thelycal differences, but no final statement could be made before some adult specimens were examined. The specimens of *P. americanus* (one male and two females) had a total length of 47 and 48 mm respectively. So far as can be ascertained, both species have not previously been encountered from Brazilian waters.

The occurrence of some lesser-known shrimps along the coastal waters of South America has already been described by Mistakidis and Neiva[1]. Among the species referred to were *Sicyonia dorsalis* Kingsley and *Trachypeneus constrictus* (Stimpson). Both species were obtained from hauls made near Santos (about 24° S.), and were considered as first records from Brazil.

Since then the material collected during the exploratory survey of January–February 1963 along the Santa Catarina coast has been re-examined and it is of interest to observe that the distribution of these species extended farther south than Santos.

A single female *S. dorsalis* (45 mm total length) and seven specimens of *T. constrictus* (two males and five females, 52–63 mm total length) were found in material obtained from one station (26° 53′ S., 48° 33′ W.) a few miles off the coast. The haul contained also one *Sicyonia typica*, three *Hymenopenaeus mulleri*, twenty-eight *Penaeus aztecus* and thirty-four *Xiphopeneus kroyeri*. Three female *T. constrictus* were impregnated, and the spermatophores of one male were well developed (February 1). The bottom was mud grading to sand and the depth was 21 m. Another impregnated female *T. constrictus* (50 mm total length) was obtained from another haul made about 12 miles south of this station, also on a muddy bottom, but in shallower water. In addition to the accompanying fauna the 30-min trawl haul contained three *Penaeus schmitti* and 430 *X. kroyeri*. More detailed information on the distribution and abundance of these and other species obtained during the survey has been given in a mimeographed report published by the Departamento Estadual de Caça e Pesca de Santa Catarina[2].

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Records of *Macrobrachium carcinus* (Linnaeus) and *Macrobrachium acanthurus* (Wiegmann), from Santa Catarina, Brazil

AMONG unidentified material kept at the Fish and Wildlife Service Laboratory at Florianopolis, capital of the State of Santa Catarina, were several specimens of the freshwater prawn *Macrobrachium*. These were identified as *M. carcinus* and *M. acanthurus*. The two specimens of *M. carcinus*, of 200 and 210 mm total length (excluding the second pair of pereiopods, which measured 230 mm), were from Lagoa da Conceição, a brackish to freshwater lagoon on the Santa Catarina Island. The eight specimens of *M. acanthurus*, of 80–140 mm total length (excluding the second pair of pereiopods, which measured up to 120 mm in length), were from Rio Pinheira (27° 50′ S.), a small river on the mainland, about 3 miles south of the southernmost tip of S. Catarina Island. Local fishermen stated that *M. carcinus* had occasionally been taken from Rio Pinheira, but this could not be verified.

According to Holthius[1,2] the distribution of both species extends from Georgia and Florida in the United States, to South Brazil, including the West Indies. Holthius[1] lists several records of *M. carcinus* from Brazil, including the State of Santa Catarina, the southernmost locality being the Rio Itajai. As the Lagoa da Conceição is approximately 40 miles south of Itajai the distribution appears to extend farther south than was previously known.

The distribution of *M. acanthurus* extends farther south than *M. carcinus*, and it has been recorded as far south and west as Itaqui, on the River Uruguay, and from São Lourenço, Rio Grande do Sul, Brazil's southernmost state. However, the species had not previously been recorded from Rio Pinheira.

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Plasminogen Activator in Saliva of the Vampire Bat *Desmodus rotundus*

IT was generally accepted that vampire bats were blood-sucking animals until 1932 when Dunn[1] observed that these animals use their tongues to lap blood from freely bleeding wounds inflicted by means of razor-sharp superior incisors. This observation was confirmed by Ditmars and Greenhall[2] and by many subsequent investigators, and it has been noticed that bites inflicted by vampire bats not only bleed very freely when the wound is first made, but also continue to bleed for several hours after the bat has ceased to feed[3]. It is therefore of interest to investigate the mechanism by which vampire bat saliva brings about this degree of interference with the haemostatic mechanism of the host animal.

The presence of an anticoagulant in saliva of *Desmodus rotundus* has been suggested by several workers including Romana[4] and Mann[3], and dissolution of blood clots containing *D. rotundus* saliva was noted by Bier[5]. DiSanto[6] reported that addition of a saline suspension of the principal submaxillary gland to fibrin brought about hydrolysis and suggested that a proteolytic substance in the gland was responsible for this. In the present investigation the effect of saliva from *D. rotundus* on platelet adhesiveness and aggregation, blood coagulation, fibrinolysis and vasoconstriction has been investigated and it has been demonstrated that the saliva increases vascular permeability and contains a potent plasminogen activator. Preliminary tests for inhibition of platelet adhesiveness to glass surfaces and aggregation of platelets by adenosine diphosphate have given negative results and tests for anticoagulant activity have been inconclusive. Some characteristics of the plasminogen activator are reported here.

Tests have been carried out on two specimens of *D. rotundus* in captivity at the London Zoological Gardens. A small drop of pilocarpine hydrochloride was applied to the buccal mucosa with a fine polythene Pasteur pipette and approximately 2 min later it was possible to collect saliva by means of a second polythene pipette. This process could be carried out without handling the animals. Saliva was obtained in a similar manner from normal laboratory mice for comparative tests. The saliva was

placed in an ice bath immediately and tested without delay.

Dilutions of saliva were prepared in 0·9 per cent saline and 0·03-ml. volumes of each dilution were incubated in triplicate on standard[7] and heated[8] bovine fibrin plates for 20 h at 37° C. No lysis occurred on heated (plasminogen-free) fibrin plates, excluding the presence of a direct fibrinolytic enzyme. On standard (plasminogen-rich) fibrin plates, vampire bat saliva produced large areas of lysis (Table 1) suggesting that a potent plasminogen activator was present. This was confirmed by testing mixtures of saliva and human plasminogen (Kabi Pharmaceuticals) on heated plates.

Table 1. FIBRIN PLATE TESTS

Source of saliva	Area of lysis/mm² Standard plates	Heated plates
Bat 1, undiluted	1,089	0
Bat 2, undiluted	2,200	0
1/10	868	0
1/100	310	0
1/1,000	38	0
1/10,000	0	0
Mouse 1	64	0
Mouse 2	0	0

Plates prepared from bovine plasma fraction I (Armour Pharmaceutical Co.) and bovine thrombin (Leo Laboratories). Results are the average of three readings.

Table 2. LYSIS OF HUMAN PLASMA CLOTS BY VAMPIRE BAT SALIVA

Source of saliva	Lysis time by saliva diluted Undiluted	1/10	1/100	1/1,000
Vampire bat 1	1·5 min	6·5 min	2 h	24 h
Vampire bat 2	4 min	28 min	4 h	24 h
Vampire bat 2 while feeding	—	2 min	4 min	25 min
Mouse	No lysis apparent after 24 h			

Clots consisted of human plasma 0·1 ml., saliva 0·05 ml., thrombin (50 N.I.H. units/ml.) 0·05 ml. Lysis was measured at 37° C.

Further experiments were carried out in which dilutions of saliva were mixed with human plasma before clotting with thrombin. Rapid lysis occurred with undiluted saliva collected at intervals between feeding a bat with defibrinated ox blood; the potency of the activator was markedly increased (Table 2). No lysis occurred of clots containing mouse saliva.

Lysis of human plasma clots by vampire bat saliva is inhibited by the presence of ε-amino-caproic acid at concentrations of 10^{-4} M and greater. The activator is stable for at least 2 weeks at −35° C, 24 h at 4° C, 3 h at 37° C and 30 min at 56° C. It is destroyed by boiling. Preliminary experiments have indicated that animals of different species vary greatly in susceptibility to the activator. Reactivity does not depend on the presence of proactivator, however, as lysis of fibrin prepared from bovine fibrinogen and thrombin takes place (Table 1).

It is not yet known if the potent plasminogen activator present in vampire bat saliva is solely responsible for the prolonged bleeding which takes place from bites inflicted by these animals. Further experiments are planned to determine if vampire bats can feed successfully from animals in which the plasminogen is not activated by vampire bat saliva.

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Eleven-month "Embryonic Diapause" in a Marsupial

THE tammar, *Protemnodon eugenii* (Desm.), is included in the marsupial family Macropodidae in which the majority of species so far studied exhibit lactation-controlled delayed implantation[1]. Tyndale-Biscoe[2] pointed out that, unlike eutherian mammals which have delayed implantation, the marsupial blastocyst does not implant as soon as development recommences, and he suggested that the term "embryonic diapause" be used to describe the marsupial type of discontinuous development of the embryo.

Embryonic diapause in a marsupial was first described by Sharman[3] in the quokka, *Setonix brachyurus*. Female quokkas fertilized at post-partum oestrus carried an unimplanted dormant blastocyst in one uterus while suckling a young in the pouch. Premature removal of that young during the breeding season (approximately January–August) was followed by resumption of development by the blastocyst. On the other hand, the dormant blastocysts of the continuously breeding red kangaroo, *Megaleia rufa*, completed development following removal of pouch young at all times of the year and at the end of pouch life of the young[4]. Most female quokkas entered anoestrus while carrying a pouch young, and Sharman[3] thought that the blastocyst then degenerated. This view was supported by Shield and Woolley[5], who demonstrated a progressive decrease in the percentage of quokkas giving birth following removal of pouch young as the breeding season progressed and a total failure of the blastocyst to develop during anoestrus.

Sharman[6] reported the occurrence of embryonic diapause in the tammar, and Poole and Pilton[7] gave the gestation period as 29 days. The mean length of the gestation period of tammars studied in this investigation was $28{\cdot}4 \pm 0{\cdot}2$ days ($N = 12$) and a breeding season extending from January to July was indicated for both wild and domestic tammars.

A sample of fifty wild tammars was shot on Kangaroo Island, South Australia, in October 1964. Most females were carrying advanced pouch young (213–245 days old) or were suckling young which had left the pouch. None was in active pregnancy but 68 per cent had dormant blastocysts in one or other uterus. The ovary corresponding to the uterus which contained the blastocyst had a resting corpus luteum similar in size and histology to the corpus luteum of lactation found during the quiescent phase of lactation in macropodids exhibiting embryonic diapause[2,3].

Twenty females without pouch young or dependent young-at-foot were taken into captivity in October 1964, and three of these were kept isolated from males. These gave birth, 3–4 months later, on January 29, February 1 and February 2, 1965. Sixteen of the remaining females also gave birth in January and February 1965 (that is, at the start of the breeding season), and although males had access to them the occurrence of mating before birth was not confirmed from the daily vaginal smear. There was no difficulty in recognizing the occurrence of post-partum mating, or of mating resulting in pregnancy in other females, from the vaginal smear.

A further sample of wild females was shot in February 1965, and from this sample, and from observations on captive females, the occurrence of post-partum fertilization and subsequent embryonic diapause was confirmed. Removal of pouch young during the breeding season was followed by development of the dormant blastocyst as happens in the quokka during the breeding season[3] and in the red kangaroo[4]. Blastocysts taken during the breeding season, from animals with pouch young, were exactly like those from females taken in the October 1964 sample. In both breeding season and anoestrous animals the blastocysts were from 0·24 mm to 0·33 mm in diameter and had between eighty and ninety cells, none of which was undergoing division in any blastocyst. Blastocysts carried by anoestrous females resumed development

following the same hormone injection procedures as resulted in the precocious development of blastocysts carried by females with pouch young during the breeding season. These experiments will be reported in full later, but are mentioned here as evidence for the viability of the dormant blastocysts of anoestrous females.

These results are interpreted as follows. Females breeding for the first time became fertilized during the early part of the breeding season. Post-partum fertilization, which leads to the production of a dormant blastocyst, occurs and loss or experimental removal of the young during the breeding season is followed by completion of development of the blastocyst, birth and another post-partum fertilization. Most females enter anoestrus carrying a pouch young, and loss of the young thereafter or its emergence from the pouch is not followed by resumed development of the blastocyst. However, the blastocyst is retained, through anoestrus, in a viable state and resumes development at the onset of the next breeding season. Thus embryonic diapause is initially lactation-controlled but the blastocyst remains quiescent, presumably because of the absence of pituitary stimuli, during the anoestrous period. The retention of a viable but totally quiescent embryo consisting of only eighty to ninety cells for a period of up to 11 months—that is, for one year minus the length of the gestation period—is regarded as established for the tammar. It may also occur in other seasonally breeding macropodids. The 11-month-long diapause is the longest known to occur in any mammalian embryo.

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Bispecific Chimerid Sponges from the Antarctic

IT is not unusual to find two different sponge species growing together intimately. One sponge often uses another as substrate, either encrusting, growing up from it, or surrounding it laterally. Topsent[1] and Kirkpatrick[2] have each reported a case in which one sponge is completely surrounded by another, with only its osculae projecting to the exterior. There is, however, always a definite delineation between the two unless the substrate portion of the host is dead.

The merging of two different sponge species to form one sponge is virtually unheard of. Evans[3] once reported peculiar spiculiferous bodies within a fresh-water sponge, and thought that perhaps they were parasitic sponges. However, he did not go into great detail and his illustration is unconvincing. The only other such case is de Laubenfels's *in vitro* induced "bispecific conglomerates"[4–6], the best case of which was still alive at 21 days when a storm washed away the live-car in which it was held[6]. These re-aggregates showed considerable sorting out of component cells, by species. Had they lived, they might well have produced a truly bispecific individual sponge.

During the course of investigations into the biota of the Antarctic seas, the Department of Biological Sciences and Allan Hancock Foundation of the University of Southern California have mounted, since 1961, a continuous sampling programme aboard the U.S.N.S. *Eltanin* under the sponsorship of the U.S. Antarctic Research Program of the National Science Foundation. On the sixth cruise a bispecific sponge was collected in the Drake Passage (62° 40·7′–62° 39′ S., 57° 50·7′–57° 45·5′ W., 662–1, 119 m).

The specimen, 428–2–43 (A.H.F. 1965–2), appeared on superficial examination to be a single sponge (Fig. 1). On the basis of its gross appearance, this sponge was placed as an extra specimen in a series later identified as *Oxymycale acerata* (Kirkpatrick, 1907). Collections from this station also included many individuals of a second species, a new *Sigmadocia*[7]. The *Sigmadocia* (Fig. 2*b*) is characterized by much thinner, dendritically branched, largely parallel fibre tracts, rather than the massive, very coarsely reticulate, fibre tracts of the *Oxymycale* (Fig. 2*a*). Variation in gross appearance had led to a few specimens being misplaced in series during the initial cursory examination and sorting. However, microscopic examination quickly indicated the proper placement.

A closer look at the specimen 428–2–43 gave the impression that it was one of the initially misplaced specimens and actually belonged in the *Sigmadocia* series, but stained sections and spicule slides from one area still indicated *Oxymycale*. New sections and spicule mounts from the side opposite were *Sigmadocia*. All slides were made in quadruplicate.

Spiculation of the two species involved is as follows: *Sigmadocia*, oxeas, microxeas and sigmas (Fig. 3*b*). *Oxymycale*, oxeas about half as long again, microxeas of about the same size, and two sizes of palmate anisochelas (Fig. 3*a*). Based on lumped dermis, fibre and "flesh" samples from other representatives of both species (428–2–3, *Sigmadocia*, s.s. 100, and 428–2–1, *Oxymycale*, s.s. 150) and a 0·1 per cent level of significance ($t = 15{\cdot}33$), it is concluded that the microxeas of the *Sigmadocia* are longer, although there is considerable overlap. Lumped microxea samples, size 150, from the first spicule slide of each of the representatives in the bispecific specimen also indicated that those of the *Sigmadocia* portion are longer at the 0·1 per cent level ($t = 55{\cdot}18$).

The area between those taken for the initial sections yielded two blocks designated as *A* (*Oxymycale*) inner and *B* (*Sigmadocia*) inner. At least one section from the *A* inner block demonstrates introgression of the *Sigmadocia* into the *Oxymycale* (Fig. 4). The converse is demonstrated by spicule slide No. 3 from the "flesh" of the outer region of the *Sigmadocia* side. This slide shows 40–60 per cent contamination by *Oxymycale* megascleres as well as 197 small and five large anisochelas (Fig. 3*c*). Cross-contamina-

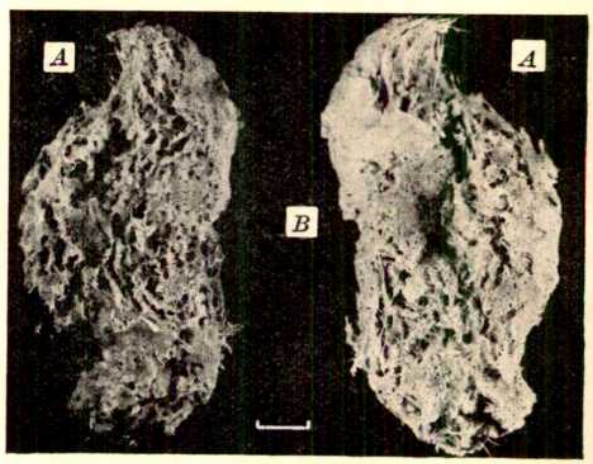


Fig. 1. Specimen 428–2–43, *A* (*Oxymycale* side), *B* (*Sigmadocia* side). Scale, 1·2 cm

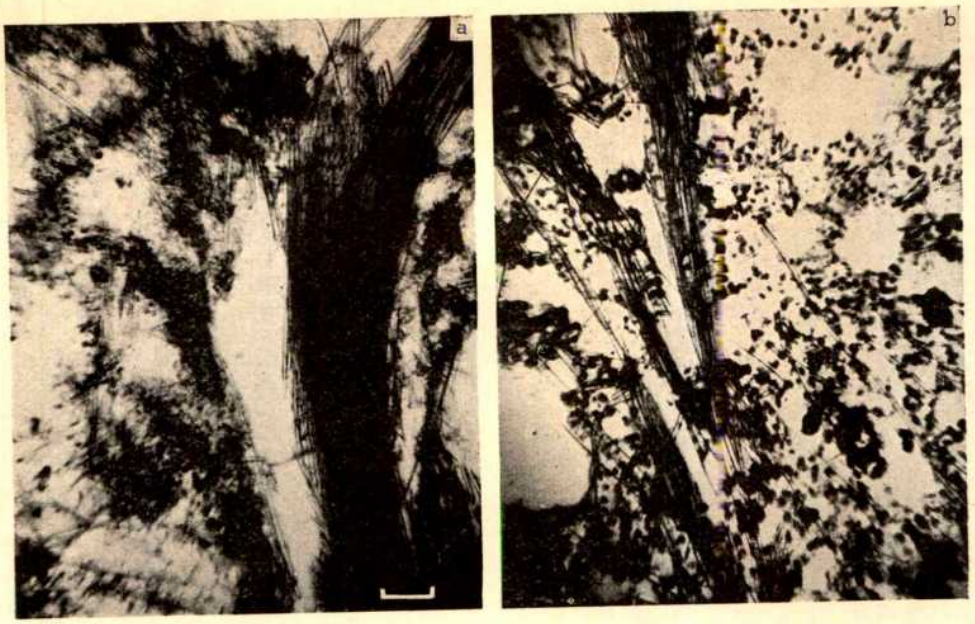


Fig. 2. *a*, Section from *A* outer side showing fibre tract. *b*, Section from *B* outer side showing fibre tracts. Scale, 214μ

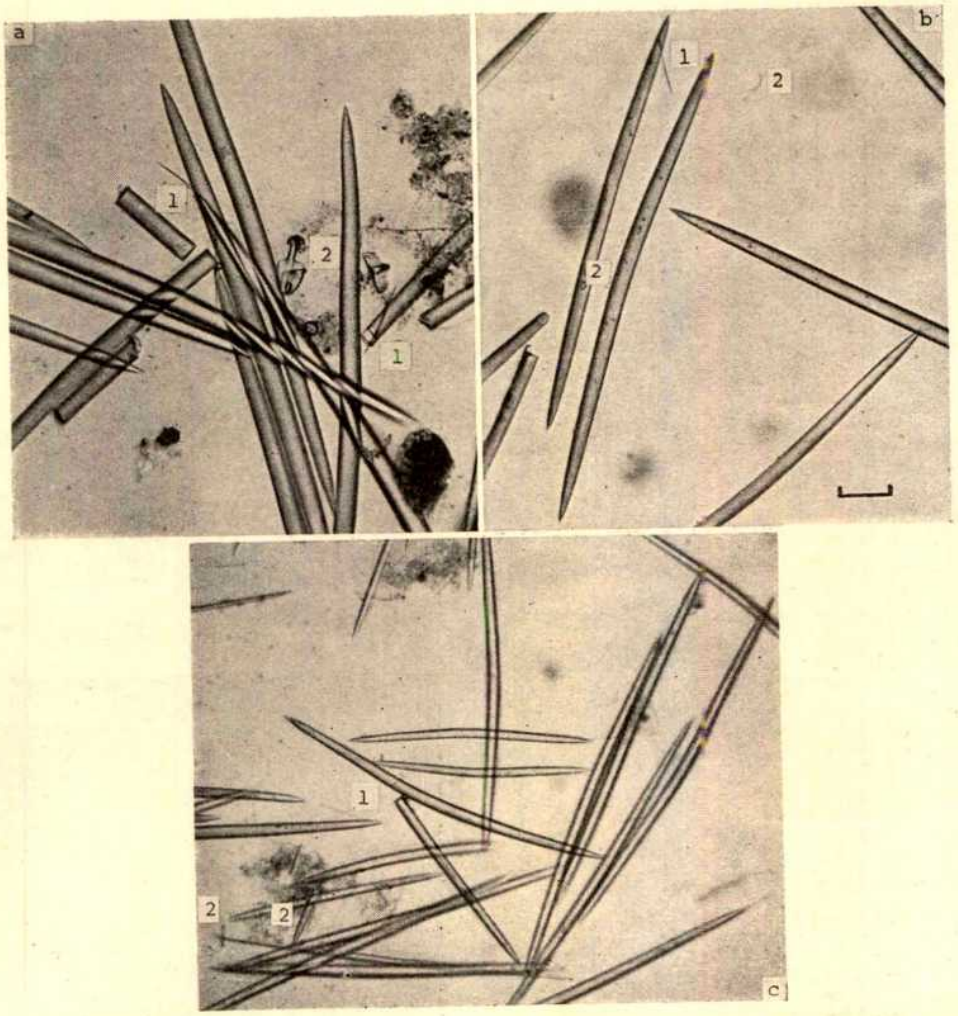


Fig. 3. *a*, Spicules of the *A* outer (*Oxymycale*) side showing oxeote megascleres, (1) microxeas and (2) chelas. *b*, Spicules of the *B* outer (*Sigmadocia*) side showing smaller oxeote megascleres, (1) microxeas and (2) sigmas. Scale, 56·6μ

c, Portion of spicule slide *B*3-Flesh, showing oxeote megascleres from both species, (1) microxeas and (2) chelas; the sigmas are present but do not show up well. Scale, 114μ

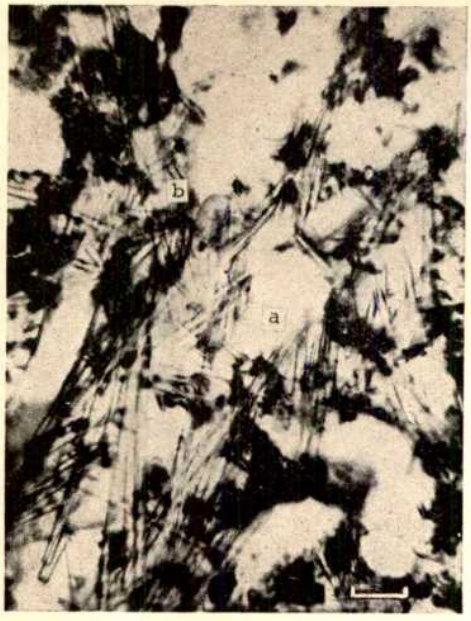


Fig. 4. Part of section from *A* inner (*Oxymycale*) block, showing (*a*) *Oxymycale* megasclere and (*b*) two *Sigmadocia* megascleres. Scale, 114μ

tion of sigmas and anisochelas in the other "flesh" mounts was minor and about the same as that found in specimens 428–2–1 and –3. The volume of material used in such preparations was about 2–4 mm³.

Further investigation yielded another such specimen, 428–2–283 (A.H.F. 1965–13), from the same station which, though much larger, is not nearly so impressive as the first. This is because the *Oxymycale* portion comprises the bulk of the specimen, the *Sigmadocia* part being about 10 per cent of the whole, and one can seemingly distinguish where one stops and the other begins. Cross-introgression, however, was repeatedly demonstrated in both directions in sections from the intermediate zone.

These specimens may be considered as the first naturally occurring "bispecific conglomerates". Two orders and families are represented, Desmacidonidae of Haplosclerina and Adociidae of Poecilosclerina.

Earlier ideas of a nearly infinite antigenic universe, and Spiegel's[8] demonstration that intercell adhesion in sponges is an antigen–antibody phenomenon, would virtually preclude the existence of such a bispecific specimen. More recent concepts of a definitely limited and even rather small antigenic universe coupled with eclipsed antigens and molecular mimicry[9,10] substantiate the possibility and even probability of such specimens in so lowly organized groups as sponges and some of the colonial protozoa.

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A Relict Priapulid from Northern Labrador

DURING the summer of 1960 a relict population of the priapulid *Halicryptus spinulosus* v. Siebold was discovered in Tessiarsuk, northern Labrador. Tessiarsuk, at 56° 30′ N., 61° 57′ W., is a small coastal meromictic lake, 544 hectares in area, which receives periodic volumes of sea water during high tides. At depth (54 m) the maximum salinity is 21·5 ‰ while the surface salinity increases from zero in the spring to about 16 ‰ in late autumn. As the lake is permanently stratified and the bottom waters anoxic, the benthos is mainly confined to the shallower littoral zone. *H. spinulosus* was found only in a small corner of the littoral about 6 hectares in area where the depth was 4–6 m, the salinity 10–15 ‰ and the bottom sediments consisted of silt and fine sand. A total of twenty-six specimens was obtained, the largest being 16 mm in length or approximately half the maximum length of 31 mm recorded by Fischer[1]. Other members of the community were the bivalves *Mytilus edulis* and *Macoma baltica* and the polychaete *Castalia aphroditoides*.

This is the first North American record of *H. spinulosus*. In a recent paper on the zoogeography of the Priapulida, Murina and Starobogatov[2] list the known areas of occurrence as western and eastern Greenland, Spitzbergen, southern Iceland, Trondjheim Fiord, Finmark, Øresund, Kategaat, Baltic Sea, Barents Sea, Kara Sea, Matochkin Shar in Novaya Zemlya and Taymirsky Zaliv in the Taymar Strait. Théel[3] first accounted for the peculiarly broken distribution of *H. spinulosus* by suggesting that the centrum of its range was the Baltic Sea, from which it had radiated westward to occupy a variety of habitats. Wesenberg-Lund[4] and Segerstråle[5], however, put forward the more convincing theory that the species is primarily Arctic and migrated into the Baltic during post-glacial

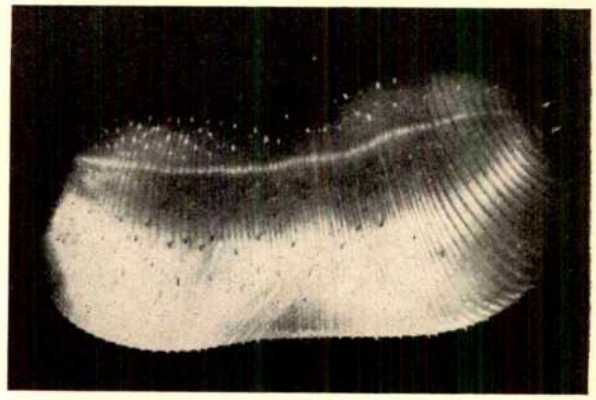


Fig. 1. *H. spinulosus*. Lateral view showing external annulations, spines and ventral nerve cord

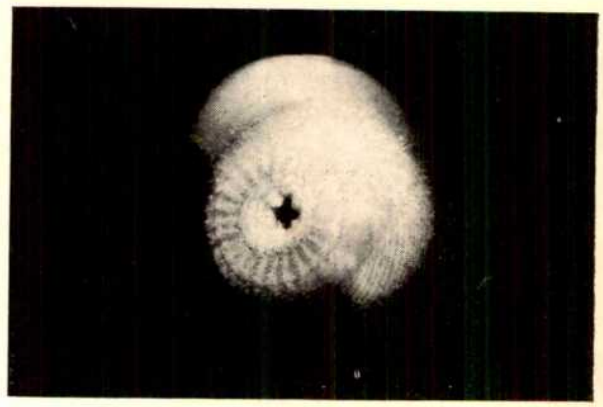


Fig. 2. *H. spinulosus*. Oral end

times when the salinity there was somewhat higher. When the salinity afterwards decreased, *H. spinulosus* was able to adapt to the modified conditions. A similar process presumably occurred in the case of Tessiarsuk, which probably contained water of a much greater salinity before the land rebounded in more recent times partially to isolate it from the sea. Since the closest recorded population is more than 500 miles distant it is probable that the range of *H. spinulosus* has shrunk considerably during the last few millennia.

Other faunistic parallels exist between Tessiarsuk and the Baltic Sea despite the great disparity in size. Besides *Macoma baltica* and *Mytilus edulis*, Tessiarsuk also contains populations of the amphipod *Pontoporeia femorata* and the medusa *Halitholus cirratus*, all common forms in the Baltic.

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A Decline in the Occurrence of *Diphyllobothrium* Plerocercoids in the Trout *Salmo trutta* L. of Llyn Padarn, Caernarvonshire

A SURVEY of the parasites of the trout, char, stickleback, minnow and eel in Llyn Padarn was begun in April 1963. The trout *Salmo trutta* L. and the char *Salvelinus alpinus perisii* (Günther) were heavily infected by the plerocercoids of *Diphyllobothrium ditremum* (Creplin, 1825) and *Diphyllobothrium dendriticum* (Nitzsch, 1824) which were found encapsuled in the viscera and body wall. The parasite survey was continued in 1964 when a marked fall in the occurrence of *Diphyllobothrium* plerocercoids in the trout was noted. Observations made in 1965 showed a further fall in the numbers of the plerocercoids in the trout. The numbers of plerocercoids present in the char remained more or less constant throughout the period of investigation (see Table 1).

In 1963 and 1964 a random selection of the trout population was sampled. In 1965 only trout longer than 15 cm were examined because smaller trout were found rarely to be infected by plerocercoids of *Diphyllobothrium*. All char examined were longer than 16 cm. All capsules were opened and the living plerocercoids were relaxed in cold water and preserved for further examination. Full details of the biology of the plerocercoids will be published later.

Table 1 shows that the mean number of *D. ditremum* and *D. dendriticum* per trout fell during the period 1963–65, while the ratio of occurrence of the two species remained constant. The numbers of plerocercoids in the char did not decline.

The fall in occurrence of plerocercoids in trout indicated that a change has taken place in the numbers, habits, or reactions to the parasite of one or more of the host animals involved in the life-cycle of the two species of tapeworm. No decrease in the numbers of the definitive hosts (gulls in the case of *D. dendriticum* and cormorant in the case of *D. ditremum*) has been observed during the investigation, nor is there any indication that the planktonic fauna of the lake has radically changed. These considerations, together with the fairly constant level of infection in the char and a recorded increase, from 1963 to 1965, of the number of capsules containing dead plerocercoids in trout, strongly suggest that the trout have developed an increasing immunity to the *Diphyllobothrium*.

A review of epidemics caused by plerocercoids of *Diphyllobothrium* in trout in the British Isles was presented by Fraser[1], but it is unfortunate that the fate of these outbreaks is not known. The date of the first occurrence of plerocercoids in the trout of Llyn Padarn is not certain, but the Gwynedd River Authority officials have only noticed the worms since 1959. The infection may have been building up at this time, and have reached a peak about 1961–62. Our observations have been carried out during a decline in the level of infection in the trout. We are continuing the observations in order to see the future pattern of the infection in the trout which may follow one of three possible trends: the infection of *Diphyllobothrium* may eventually die out, or may remain at a low level, similar to that found in the trout of Llyn Tegid (Bala Lake), or there may be cyclical changes between high and low levels of infection. In Llyn Padarn the presence of the infected char will affect the occurrence of the plerocercoids in the trout, by acting as a reservoir for the infection of the definitive hosts and the continuation of the life-cycle. As there has been no marked decline in the numbers of plerocercoids in the char, it is difficult to envisage the infection dying out completely in the trout.

We thank Mr. H. Evans, Fisheries Superintendent of the Gwynedd River Authority, and his staff for their help.

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MICROBIOLOGY

Observations on the Gametogenesis of *Plasmodium vinckei*

MANY authors in the past have observed the disappearance of gametocytes of certain blood protozoa as a result of their continuous and prolonged mechanical transmission. This phenomenon was especially investigated by Vincke[1], Rodhain[2], Jadin, Yoeli and Pierreux[3], and by Michiels[4] in strains of *Plasmodium berghei* maintained in the laboratory for years by 'syringe passages'. The rodent malaria parasite *Plasmodium vinckei*[5], isolated in 1952, behaves similarly.

It has recently been observed by Vincke, Scheepers-Biva and Bafort[6] that *P. berghei* (*Vinckeia* from Garnham[7]) which has lost its ability to produce gametocytes could

Table 1. INFECTION OF TROUT AND CHAR OF LLYN PADARN BY *Diphyllobothrium ditremum* AND *D. dendriticum*

	Trout			Char		
	1963	1964	1965	1963	1964	1965
Number of fish	13	17	48	5	23	30
Mean length of fish	22 cm	23·7 cm	22·2 cm	21 0 cm	19 2 cm	20 6 cm
Length standard deviation	±8 2 cm	±8·1 cm	±2 9 cm	±5·8 cm	±2 0 cm	±1·8 cm
Percentage of fish with *D. ditremum*	77	76	65	100	100	93
Percentage of fish with *D. dendriticum*	46	65	31	100	96	100
Total number of *D. ditremum* plerocercoids	1,100	651	148	331	559	1,094
Total number of *D. dendriticum* plerocercoids	191	198	28	23	180	360
Mean number of *D. ditremum* plerocercoids per fish	84·6	38·3	3 1	66 2	24 3	36 5
Mean number of *D dendriticum* plerocercoids per fish	14·7	11·6	0·6	4·6	7·8	12·0
Ratio of mean number of *D. dendriticum* to *D. ditremum* plerocercoids	1 . 5·8	1 : 3·3	1 : 5·2	1 : 14 4	1 . 3 1	1 : 3

recover this power if kept in a state of 'quiescence'. This 'quiescence' may be obtained by transmitting the infection to adult albino rats in the metacritical phase, where it survives in a mild, latent stage, and also by keeping the parasite at low temperatures at −75° C.

We wondered if we should observe the same phenomenon in *P. vinckei*. This parasite has been maintained since its isolation by blood transfers and has long lost its ability to produce gametocytes. We received the strain on September 14, 1962, from Algeria by the courtesy of Prof. Fabiani. No precise information is available on the number of mechanical transmissions undergone by the strain before its arrival in our laboratories. Our estimation is that the strain had passed at least 400 times when we received it, assuming that one blood transfer was carried out every 10 days from the time of its discovery by Vincke in January 1952 to November 1962.

We continued to maintain the strain by weekly transfers in mice and rats. On May 20, 1965, the strain, about 13 years old, has been passed 68 times in 934 days in our laboratory: 59 times in mice, four times in albino rats, and five transfers in tree rats, *Thamnomys surdaster*. Two transfers in deep freeze at −75° C were also carried out during this period.

Tables 1 and 2 summarize the details of the successive blood transfers in the two lines of animals which originated from the *P. vinckei*-infected animals received in September 1962.

It must be emphasized that the blood of all *P. vinckei*-infected animals was regularly checked for the appearance of gametocytes and for exflagellation, but none was observed. However, after keeping *P. vinckei*-infected blood from rat 362 at low temperature (−75° C) and afterwards passing it twice through albino rats, few gametocytes and sparse exflagellation were observed in the blood of mouse 734. More numerous exflagellations occurred in the blood of mouse 901 after the strain was maintained for a second period in deep freeze.

Table 1. SUCCESSIVE TRANSMISSIONS AFTER RECEIPT OF THE STRAIN ON NOVEMBER 14, 1962: MOUSE 901

Date	No. of passage	Animal	Observations
11/14/62–11/20/62	1–2	Young mice	Weekly transfers
11/27/62–1/4/63	3–7	*Thamnomys*	Weekly transfers
1/2/63–9/20/63	8–41	Young mice	Weekly transfers
9/27/63	42	Albino rat 362	Blood in deep freeze (−75° C) tenth day after inoculation. Time of stay at low temperature 67 days (10/8/63–12/12/63)
12/12/63–2/20/64	43–53	Young mice	Weekly transfers
2/27/64	54	Albino rat	Weekly transfers
3/13/64–5/8/64	55–63	Young mice	Weekly transfers
5/15/64	64	Rat reservoir	Rat clinically cured on the thirtieth day after inoculation and sub-inoculated on the 107th day
8/31/64	65	Rat reservoir	Rat clinically cured on the twenty-first day after inoculation and sub-inoculated on the 50th day
10/20/64	66	Young mouse	
10/30/64	67	Young mouse 734	Few exflagellations of microgametocytes observed in the blood on 11/5/64, the sixth day after inoculation. The blood was kept at −75° C from 11/6/64–5/13/65, 190 days
5/13/65	68	Young mouse 901	Exflagellation observed on 5/20/65, the seventh day after inoculation

Table 2. SUCCESSIVE TRANSMISSIONS AFTER RECHIPT OF THE STRAIN ON NOVEMBER 14, 1962: MOUSE 931

Date	No. of passage	Animal	Observations
11/14/62–11/20/62	1–2	Young mice	Weekly transmissions
11/27/62–1/4/63	3–7	*Thamnomys*	Weekly transmissions
1/2/63–9/20/63	8–41	Young mice	Weekly transmissions
9/27/63	42	Albino rat 362	Blood in deep freeze on tenth day after inoculation. Time of stay at low temperature, 414 days (10/8/63–11/26/64)
11/26/64	43	Young mouse	
12/3/64–12/11/64	44–45	Albino rat	Weekly transmissions
12/18/64–5/29/65	46–68	Young mice	Weekly transmissions
5/28/65	69	Young mouse	Few exflagellations observed in the blood on 6/3/6 5, 6 days after inoculation

We also observed exflagellation in the transfers originating from mouse 931 (Table 2). This animal was also infected with *P. vinckei* blood originating from rat 362, but the infected blood was kept for 414 days at −75° C and afterwards passed in successive transfers to mice. Exflagellation of microgametocytes in small numbers was observed at the sixty-ninth blood transfer. In the five observations in which exflagellation was hitherto observed, all were carried out on the sixth and seventh days after inoculation.

It may therefore be concluded that in *P. vinckei*, as in *P. berghei*, a period of 'quiescence' at low temperature (−75° C) favours the later reappearance of viable gametocytes. This phenomenon must, however, be confirmed by further observations as it conflicts with investigations made in human malaria in which gametogony is present in acute cases of malaria. However, more importance and more attention are now given to the possibility of transmission of infections by asymptomatic cases.

We thank Prof. Yoeli, who has very kindly corrected our translation.

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SOIL SCIENCE

Soils and Micro-organisms from Mount Erebus, Antarctica

WHILE conducting pedological and biological studies in the ice-free areas of East Antarctica, one of us (F. C. U.) and Dr. H. Janetschek had the opportunity in February 1962 to climb Mount Erebus. Considered the only active volcano known in Antarctica, Mount Erebus (3,721 m) consists of trachyte, kenyte, basalt and other volcanic rocks[1]. Extensive ice and snow cover limits the soil occurrence to the lower snow-free slopes, to exposed rock projections, a few areas near the hot fumaroles, and in the vicinity of the active crater.

Soil samples were secured at various locations along the mountain. This communication describes samples collected at two sites.

Sample E_3 was obtained from the vast field of active and inactive fumaroles. The area where the sample was collected is kept free from snow and ice by the hot fumarolic vapours. The dark-grey-brown soil is sandy, moist and warm to the touch in contrast to the air temperature of −26° C. A partial chemical analysis of the sample shows an acid reaction of *p*H 4·2, considerable quantities of chlorides, native sulphur and free iron oxides. Total nitrogen is 0·03 per cent, whereas total carbon is 0·1 per cent.

Sample E_4 was obtained from the edge of the active crater in an area covered by patches of sulphur. The very dry yellowish-brown soil is sandy with large loose feldspar crystals. The soil is very acid (*p*H 2·7); chlorides and native sulphur are abundant, but carbon and nitrogen are not detected. More information on the composition of these two samples has already been provided by one of us[2].

Neither site E_3 nor E_4 supports any form of megascopic plants. Bacteria, actinomycetes and fungi were not found in the material obtained from site E_4, not even the acid-tolerant sulphur bacterium *Thiobacillus thiooxidans*. Lack of micro-organisms is ascribed to the high acidity and dryness of the material. Various bacteria and fungi but no actinomycetes were detected in sample E_3 collected next to an active fumarole. Approximately 9×10^6 bacteria were found by plating on albumin agar and 6,500 fungi by plating on peptone–dextrose novobiocin agar. Among the fungi were species of *Penicillium*, *Aspergillus* and *Neurospora*, and a yeast and also a fungus that failed to produce spores and is considered to be a member of the Dematiaceae. The bacteria and fungi were as abundant in E_3 as in many fertile cultivated soils of temperate regions.

The soils of Mount Erebus are acid, contrary to the ubiquitous alkaline or neutral conditions of the soils of Southern Victoria Land. The oxidation of the native sulphur into sulphuric acid may be responsible for the low *p*H.

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HORTICULTURE

Chemical Control of Vegetative Growth and Fruitfulness of Tomatoes

IN 1957, Eaton[1] described the induction of selective male sterility in flowers formed on various cotton varieties which had been sprayed with sodium 2,3-dichloro-2-methylpropionate solutions and projected the possibility of mass-producing F_1 hybrid cotton seed from rigorously inbred parents by the use of these treatments. Similar gametocidal responses to foliar sprays of this chemical have since been demonstrated by Moore[2] in eleven tomato varieties. Additional gametocidal and growth regulatory properties have been described for tomatoes, cotton and sugar beets[3]. Further experimentation on all three crops, using foliar sprays of this chemical, invariably induced an excessive amount of female sterility in the process of achieving the desired sterility in male flower parts[4]. As a result, the mass production of hybrid seed in these crops using this chemical has been impractical.

The projected termination of the California Bracero programme in 1965 has stimulated interest in developing mechanical harvesting devices for use with various crops destined for processing in that area. All experimental models developed so far for tomatoes involve the destruction of the entire plant in a single picking harvest. The presence of either prematurely ripe fruit or small developing green fruit is undesirable at harvest. Renewed interest in the gametocidal properties of this chemical has been directed to its use as a possible aid in mechanical harvesting procedures. Normally, the perfect tomato flowers are self-fertilized before anthesis, but the stigmas remain receptive to hand pollination for a variable period after anthesis (depending on variety) when selective male sterility has been induced by chemical treatments. In the absence of hand pollination, natural crossing by other agents, or parthenocarpic fruit development, sterile tomato flowers continue to abscise without setting fruit. Thus, gametocidal treatments could eliminate the presence of early overripe fruit and facilitate the mechanical harvesting operation.

Three extensive field experiments were conducted at Tracy, Stockton and Saticoy, California, during the 1964 growing season. The locations selected represented a reasonably diverse range of geographic and cultural conditions normally encountered in California tomato production. Six treatments involving a factorial combination of two gametocidal dosage-levels (0·15 and 0·25 per cent water solutions) × three spray schedules (one versus two versus three sequential sprays) were used at each location in which four to six replications were used in a randomized block design. Attempts were made to adjust fertilization practices, water management and pesticide applications to the optimum benefit of each treatment, but the random arrangement of plots precluded complete adjustment in all instances. Each plot of any single treatment at all locations was harvested by hand to simulate a mechanical operation when the onset of overripe fruit was obvious. Accurate plant response data necessary for the appraisal of treatment responses listed in Table 1 were collected at each experimental installation.

All gametocidal treatments resulted in a "blanching" of the foliage, slight leaf epinasty, and obviously retarded growth rates at all locations. The severity of foliar injury increased with increasing numbers of sprays and, to a lesser extent, with spray concentration. The injury symptoms persisted for a minimum of 3 weeks at one location, a maximum of 7 weeks at another, but eventually vanished at all locations.

All treatments at each location induced obvious sterility in floral parts with resultant abscission of flowers and delay in the onset of fruiting. As treatment effects increased markedly with the increasing number of sprays, and were relatively uninfluenced by spray concentration, the average treatment responses for three sequential sprays over the range of two spray concentrations and three locations are compiled in Table 1.

Table 1. AVERAGE EFFECTS OF TWO CONCENTRATIONS OF THREE SPRAYS AT THREE LOCATIONS

Criteria	Untreated	Treated	*L.S.D.* ($P = 0.01$)
A. Delay in onset of fruiting (days)	0	24	
B. Vine weight (oz)	21·5	37·8	10·29
C. No. of fruit set per plant	45·3	58·8	10 15
D. Yield (tons/acre)	23·5	36 5	9 67
E. Yield (tons/acre day)	0·184	0·247	0 0735
F. Percentage usable fruit* (count basis)	48 4 (44·1)	63 7 (53·1)	(8·01)†
G. Percentage usable fruit* (weight basis)	60 7 (51·4)	74 7 (60 0)	(8·98)†
H. Percentage green fruit (weight basis)	35·3 (36 1)	18·6 (25 6)	(8·89)†
I. Fruit size (oz.)	2·60	2 25	0·436

* For processing.
† Percentage of data transformed (arc sin) for tests of significance.

Three sequential gametocidal sprays obviously induced floral abscission and delayed the onset of fruiting. These responses resulted in a longer period for continued vegetative growth and larger individual vines, which eventually set a greater number of fruit per vine at a later date. Increased yields resulted in all treatments using either tons per acre or tons per unit of production time as criteria. Fruit matured in a "peaked" condition favourable for either mechanical or hand harvesting procedures. The occurrence of underripe green fruit in a single-picking harvesting operation was also reduced by the treatments. Fruit size was somewhat adversely affected by the treatments, but this response may have resulted in part from our inability to adjust completely cultural practices to the optimum advantage of each treatment at each location. All the treatment responses in growth and fruitfulness reported here are statistically reliable at substantial levels of confidence.

In view of the common relationship between vegetative growth rates and fruitfulness in flowering plants generally, it would seem reasonable to expect corresponding responses to gametocidal treatments in other crops. The gameto-

cidal attributes of this chemical have already been established for cotton and various legumes.

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GENERAL

Kinematic Programming for Rain

The following statement and resolution of an elementary optimization problem may be of interest to those who, like me, have never been confident about the optimal speed and posture to adopt when caught without protection in a rain shower.

Consider a bounded rigid body B of arbitrary shape in a Euclidean space of k dimensions ($k > 1$). B is required to move one of its points, a reference point O, say, from a starting point S to a finishing point F. The space is dotted with hostile points generically denoted by the symbol R, which are all moving with the same velocity $\mathbf{v}$. It is B's object to move O from S to F in such a way as to minimize his contact with R.

An *itinerary* between S and F is specified by the triad $I = [P_0, \omega(t), \omega(t)]$, where P_0 is the initial posture of B at S, $\mathbf{u}(t)$ is the velocity of O at time t and $\omega(t)$ is the angular velocity vector. Let $\mathfrak{I}$ denote the class of available I's. Assume that, if a portion of the surface of B sweeps out a volume δV of the space, the mean contact with R is proportional to δV if δV, regarded as part of the moving space (R-space say) in which R is stationary, has not been previously swept by B. Also that, once a volume in R-space has been swept, that volume remains free of R thereafter. The problem is therefore to minimize:

$$V = \int_{(S)}^{(F)} \frac{\mathrm{d}V}{\mathrm{d}t}\,\mathrm{d}t \qquad (1)$$

where $\mathrm{d}V/\mathrm{d}t$ is the rate of sweeping by the whole surface of B of previously unswept volume. Now:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \int \varphi\,(\mathbf{r},t)\,[\mathbf{u}(t) - \mathbf{v} + \omega(t) \wedge \mathbf{r}].\ \mathrm{d}\mathbf{S} \qquad (2)$$

where $\mathrm{d}\mathbf{S}$ is the normal vector surface element of B at the point $\mathbf{r}$ relative to O and $\varphi(\mathbf{r},t) = 1$ if the rest of the integrand is not negative and $\mathbf{r}$ is adjacent to previously unswept volume: $\varphi(\mathbf{r},t) = 0$ otherwise. Equations (1) and (2) combine to give V for any itinerary.

Fortunately, the expected is the case. As long as $\mathfrak{I}$ includes "top-speed, unrestricted posture, straight-line" itineraries along SF, then choice of I to minimize V can be made using elementary considerations. Let:

$$u_m = \max_{I \in \mathfrak{I}} \max_{t}\ u(t)$$

and let $V_t(\mathbf{p})$ be the volume in k–1 dimensions of the projection of B at time t parallel to $\mathbf{p}$ on to the k–1 space orthogonal to $\mathbf{p}$. Let $\min V_t(\mathfrak{I}) = M$ (say) $= V_t(\mathbf{p}_t)$ (say). Let $\mathbf{l}$ be the unit vector along $\overrightarrow{SF}$.

Case A. If $\mathbf{v} \cdot \mathbf{l} \leqslant 0$, then an optimal itinerary in $\mathfrak{I}$ is $I_m = [P'_0, u_m\,\mathbf{l}, \mathbf{o}]$, where P'_0 has $\mathbf{p}_0$ parallel to $\mathbf{v} - u_m\,\mathbf{l}$. To see this, Fig. 1 in R-space should be examined.

S_0, F_0 are the positions of S and F, respectively, at $t = 0$. F_T is the position of F at $t = T$ if O arrives at F_T along I_m. $F_{T'}$ is the position of F at $t = T'$ if O arrives at $F_{T'}$ using the arbitrary(dashed) itinerary I_A. Clearly I_m minimizes the time of the itinerary, whence $T \leqslant T'$ and $S_0F_T \leqslant S_0F_{T'}$ as shown. Thus, using I_A, B must

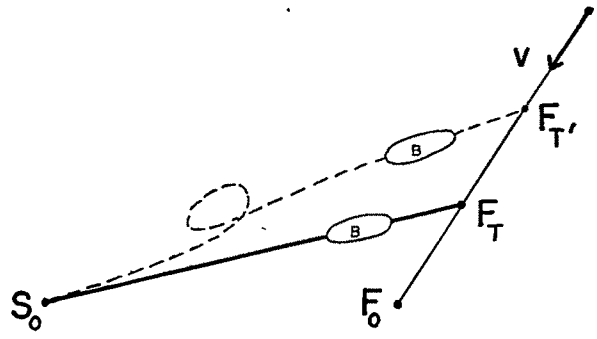


Fig. 1. Straight line and arbitrary itineraries in R-space

sweep out a volume of R-space which cannot be less than the volume for I_m. [The degenerate case of $\mathbf{v}$ parallel to $\mathbf{l}$ does not affect this finding.]

Case B. If $\mathbf{v} \cdot \mathbf{l} > 0$, then an optimal itinerary is: (i) I_m if $u_m \leqslant v^2/\mathbf{v} \cdot \mathbf{l}$; (ii) $I_0 = [P''_0, (v^2/\mathbf{v} \cdot \mathbf{l})\,\mathbf{l}, \mathbf{o}]$, where P''_0 has $\mathbf{p}_0$ parallel to $\mathbf{v} - (v^2/\mathbf{v} \cdot \mathbf{l})\,\mathbf{l}$, if $u_m > v^2/\mathbf{v} \cdot \mathbf{l}$. Case B (i) is proved in the same way as case A, because, for $u_m \leqslant v^2/\mathbf{v} \cdot \mathbf{l}$, an essentially similar figure is obtained. For case B (ii), S_0F_T is perpendicular to F_0F_T for I_0, which also establishes the result.

In cases A and B (i), the optimal itinerary is seen to be unique. Any deviation from it will increase V. However, in case B (ii) there is non-uniqueness. This is readily seen for the extreme case when $u_m = \infty$, when the solid itinerary indicated in original space in Fig. 2 has the same V as I_0.

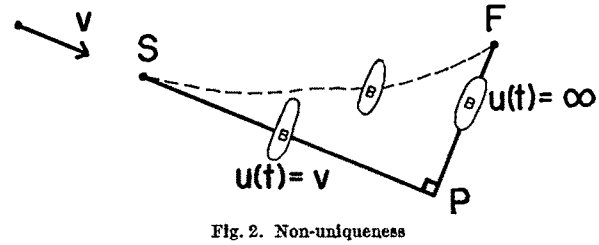


Fig. 2. Non-uniqueness

For $v^2/\mathbf{v} \cdot \mathbf{l} < u_m < \infty$, there are itineraries with O-paths interior to the triangle SPF of Fig. 2, as dotted, their characteristic being increasing distances from SP and a resolved velocity parallel to SP equal to $\mathbf{v}$.

The practical recommendations arising from these considerations are that, when rain is coming from the general direction of your destination, you should get your head down and go as fast as you can. When the rain is coming from behind, either lean backwards or walk backwards while leaning 'forwards', in neither case exceeding a certain optimal speed.

When the choice of posture is unrestricted, the only property of B affecting min V is the minimum projection M. When posture is restricted so that other properties of B affect min V, the following fact may be useful. For a given itinerary, V cannot be increased and, especially when posture is restricted, may well be decreased by filling in any concavities in B's surface with portions cut off anywhere from B.

The possibility of reducing min V by cutting B into portions and sending these portions separately to their assembly points is always available in certain applications (but not in that originally envisaged). Consider B shaped like a letter L. Break it at the angle. The flexibility thereby introduced can be used to reduce min V. However, care is needed because if B were cut into portions similar to B, it is easy to see from scalar considerations that min V would increase.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT to assist with computation and numerical analysis—Dr. C. D. Walshaw, Clarendon Laboratory, Department of Atmospheric Physics, Parks Road, Oxford (July 30).

TEMPORARY ASSISTANT LECTURER or LECTURER IN CHEMICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (July 30).

VETERINARY OFFICERS, Grade II (aged at least 23, Member of the Royal College of Veterinary Surgeons, with one year's appropriate postgraduate experience) with the Ministry of Agriculture, Fisheries and Food—Civil Service Commission, 23 Savile Row, London, W.1, quoting Ref. 130/66/2 (August 1).

TEACHING FELLOW (with a good honours degree with specialization in palaeontology) IN GEOLOGY (Palaeontology) at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 5).

SENIOR LECTURER (with an honours degree in electrical engineering or science, preferably a higher degree, and research or design experience in electronics or communications) IN ELECTRICAL ENGINEERING (August 26); and a LECTURER or SENIOR LECTURER (with a science degree specializing in geophysics or a related discipline and some research experience or recent experience in exploration geophysics) IN GEOPHYSICS (August 5) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1.

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER (chemist or biochemist, with a degree in science, H.N.C. in chemistry, or equivalent, and preferably some knowledge of chromatographic and radioactive isotope techniques) for work on mineral nutrition particularly of potassium in vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 6).

ASSISTANT EXPERIMENTAL (or Experimental) OFFICER (with a degree, or equivalent, in science or horticulture) FOR PLANT BREEDING WORK—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (August 6).

LECTURER or ASSISTANT LECTURER (graduate in medicine, veterinary science, dentistry or biology) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/136 (August 6).

RESEARCH FELLOW (with research experience in microbiology or biochemistry) IN THE DEPARTMENT OF CHEMISTRY to participate in a study of the mode of biosynthesis of antibiotics—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (August 11).

LECTURER or ASSISTANT LECTURER (with an honours degree in physiology or prepared to attend an honours course, and preferably with a veterinary qualification) IN THE DEPARTMENT OF VETERINARY PHYSIOLOGY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (August 12).

SENIOR LECTURER and a LECTURER IN PHYSICS at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 12).

SENIOR LECTURER and LECTURERS IN CIVIL ENGINEERING; LECTURERS IN ELECTRICAL ENGINEERING; and a READER, SENIOR LECTURER and LECTURERS IN MECHANICAL ENGINEERING at Ahmadu Bello University, Northern Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 12)

SENIOR LECTURER/LECTURER IN MATHEMATICS at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (August 12).

TEMPORARY ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (with at least two "A" levels including physics, or H.N.C. in applied physics, or equivalent. A degree in electrical engineering or equivalent is desirable) at the R.N. Medical School, Alverstoke, Hants, to act as assistant to the Health Physicist in charge of the Whole Body Counter—Ministry of Defence (Navy Department) CE3(N) 368, Old War Office Building, Whitehall, London, S.W.1 (August 12).

LECTURER (science graduate preferably with experience in microbiological chemistry, microbial genetics or an applied field of microbiology) IN BACTERIOLOGY at Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (August 13).

ASSISTANT LECTURER IN PURE AND/OR APPLIED MATHEMATICS; and a LECTURER or ASSISTANT LECTURER IN PHYSICS at the University of the West Indies, Barbados—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 15).

LECTURER or ASSISTANT LECTURER (with interests in taxonomy, morphology or plant breeding) TO TEACH THE BOTANY OF ECONOMIC PLANTS; and an ASSISTANT LECTURER to assist the Professor in teaching and research in crop physiology, crop ecology or the botany of tropical crops for two years—The Registrar (Room 39, O.R.B.), The University, Reading (August 15).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (with a first- or second-class honours degree, Dip. Tech., or equivalent or higher qualification in a natural science or technology, but, exceptionally, consideration will be given to a candidate whose main qualification is in a social science, preferably with experience in experimental psychology or with additional qualifications in natural science) in the Office for Scientific and Technical Information, London, to help promote the education and training of scientific and technical information staff at all levels and for all types of information work—Miss M. E. Gummer, Department of Education and Science, Curzon Street, London, W.1 (August 15).

LECTURER/SENIOR LECTURER IN BIOCHEMISTRY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 19).

LECTURER/SENIOR LECTURER IN MICROBIOLOGY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 19).

LECTURER/SENIOR LECTURER IN VETERINARY VIROLOGY IN THE DEPARTMENT OF VETERINARY PATHOLOGY AND BACTERIOLOGY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 19).

SENIOR LECTURERS or LECTURERS (2) (with a good honours degree and industrial or research experience in one of the following fields: circuit engineering, control, communications, microwave engineering, or power systems) IN ELECTRICAL ENGINEERING at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 19).

EXPERIMENTAL OFFICER and a TECHNICIAN (preferably with experience in mass spectrometry and/or electronics) to assist in the running of a unit for isotope geology being set up at the Reactor Centre—The Director, Scottish Research Reactor Centre, East Kilbride, Glasgow (August 20).

SENIOR LECTURER or LECTURER IN PHYSIOLOGY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 22).

SENIOR LECTURER or LECTURER (preferably with a special interest in organic chemistry) IN CHEMISTRY at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 22).

RESEARCH ASSISTANT for X-ray diffraction work—Dr. J. Zussman, Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford (August 25).

SENIOR LECTURER (with an honours degree in electrical engineering or science and preferably a higher degree, research or design experience in electronics or communications, and ability to lecture to postgraduate students and participate effectively in research projects) in ELECTRICAL ENGINEERING at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, August 26).

RESEARCH FELLOW (plant biochemist or microbiologist) to study the biochemical self-sufficiency of chloroplasts—The Registrar, University of York, Heslington, York (August 29).

CHAIR OF ANCIENT PHILOSOPHY—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife, Scotland (August 31).

LECTURER IN BIOCHEMISTRY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

LECTURER IN THE DEPARTMENT OF MICROBIOLOGY, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, August 31).

LECTURER or ASSISTANT LECTURER (physicist, physical chemist or others with an appropriate background) IN THE DEPARTMENT OF CERAMICS, Houldsworth School of Applied Science—The Registrar, The University, Leeds, 2 (August 31).

SENIOR LECTURER/LECTULER (preferably with interests in the field of inorganic chemistry) IN CHEMISTRY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

SENIOR LECTURER or LECTURER IN THE DEPARTMENT OF PHYSIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cardiff (September 5).

LECTURER or RESEARCH FELLOW (with some experience in the area of operational research and systems analysis, and particularly interested in the mathematical techniques of optimization) IN THE DEPARTMENT OF SYSTEMS ENGINEERING—The Secretary, University of Lancaster, Bailrigg House, Lancaster (September 16).

CHAIR OF PURE MATHEMATICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, Norfolk, NOR 88C (October 15).

ASSISTANT EXPERIMENTAL OFFICER (preferably with a degree in engineering or geology, with some instrumentation and/or soil mechanics research experience) IN THE ENGINEERING GEOLOGY GROUP OF THE GEOLOGY DEPARTMENT to be concerned with the development and experimentation of advanced soil laboratory equipment, and possibly carry out field work on the engineering geology of fissuring and consolidation of fine grained sediments—Mr. E. J. Hill, Departmental Superintendent, Geology Department, Imperial College, London, S.W.7.

ASSISTANT LIBRARIAN (reference and information services) (Chartered Librarian or graduate who has passed Part 2 of the Library Association examinations)—The Staff Officer, University of Aston in Birmingham, Gosta Green, Birmingham 4, quoting Ref 0/181/N.

CELL CULTURE TECHNICIAN (preferably with experience of bacteriological techniques) for work in the Radiobiology Laboratory—Dr. R. J. Berry, Radiobiology Laboratory, Radiotherapy Department, Churchill Hospital, Oxford.

JUNIOR TECHNICAL OFFICER (with a pass degree, H.N.C. or equivalent in physics or mathematics, and preferably with mathematical or electronic experience) to assist in physical research in the application of radioisotopes to medicine including use of an analogue computer—The Director, Medical Research Council Cyclotron Unit, Hammersmith Hospital, Du Cane Road, London, W.12.

LECTURER (with a degree in metallurgy, science, or engineering, together with research or industrial experience) IN PROCESS ENGINEERING IN THE METALLURGY DEPARTMENT—Prof. A. V Bradshaw, Department of Metallurgy, Imperial College of Science and Technology, Prince Consort Road, London, S.W 7.

LECTURER (with a first- or second-class honours degree or equivalent or higher qualification in mechanical engineering and practical experience associated with that of a Chartered Engineer) IN THE DEPARTMENT OF MECHANICAL ENGINEERING to teach applied thermodynamics to students reading for London External Degree in engineering—The Registrar, Royal Military College of Science, Shrivenham, Swindon, Wiltshire.

MICROANALYST to take charge of the Macroanalytical Laboratory—The Laboratory Superintendent, Chemical Laboratory, University of Sussex, Falmer, Brighton, Sussex.

OIL SEED CHEMIST (with a Ph.D. and experience in lipid research) for duties which will include directing an oil seed laboratory and conducting research on the quality of commercial oil seed crops—Dr. G. N. Irvine, Director, Grain Research Laboratory, Board of Grain Commissioners for Canada, Winnipeg, Canada, quoting Ref. 66-10C-GO.

PROFESSOR OF APPLIED BIOLOGY—The Registrar and Secretary, Welsh College of Advanced Technology, Cathays Park, Cardiff.

RESEARCH ASSISTANT (with a chemical or biochemical background) IN THE DEPARTMENT OF PAEDIATRICS, to work on aspects of carbohydrate metabolism in the newborn infant—Dr. H. R. Gamsu, King's College Hospital Medical School, Denmark Hill, London, S.E.5.

RESEARCH ASSISTANTS IN CHEMISTRY AND PHYSICS (with a first- or second-class (upper or lower) honours degree) to undertake postgraduate research leading to M.Phil. or Ph.D degrees of the University of London—Head of the appropriate department, Sir John Cass College, Jewry Street, London, E.C.3.

RESEARCH BIOLOGIST (with an honours degree in biology) to assist in investigations of aquatic invertebrates—Personnel Officer, Trent University, Peterborough, Ontario, Canada

SCIENTISTS (2) (with experience in either of the following fields: genetics and/or biochemistry, tumour biology and/or immunology, and preferably some experience in general virology) for research on tumour viruses and related topics—The Honorary Director, Prof. M. G. P. Stoker, M.R.C. Experimental Virus Research Unit, Institute of Virology, The University, Glasgow.

TECHNICIAN/JUNIOR TECHNICAL OFFICER (preferably with experience in microbiology or tissue culture techniques) at Pollards Wood Research Station, Chalfont St. Giles, to assist in molecular biological research—The Secretary, Institute of Cancer Research, 34 Sumner Place, London, S.W.7, quoting Ref. 301/B/259.

ZOOLOGISTS (M.Sc. or Ph.D., with research experience and preferably some knowledge of histology and histochemistry) to participate in experimental multi-disciplinary programmes of basic research in the field of food additive toxicology—The Director, British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey.

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STONEHENGE

At this time of the year, Stonehenge is crammed with tourists. The Ministry of Public Building and Works keeps the grass well cut, and will provide visitors with a guide-book for a modest fee. Refreshments are to be had in the car park across the trunk road which passes nearby, and on a sunny day those who have come to inspect the best preserved of the ancient monuments in Britain can also amuse themselves by watching the manoeuvres of the soldiers who practise incongruously with rocket weapons only a few miles away. Then, at sunrise on Midsummer Day, the Ministry of Public Building and Works will undertake to see that the monument is cleared of all who have no excuse for being there—usually the small company of eccentrics which re-enacts druidical rituals at Stonehenge once a year, and the Press photographers who come to take their pictures and that of the rising sun (when visible). The fact that this year the druids overslept and were half an hour late should not be held against them, for druids, in the twentieth century at least, are also human.

But, in spite of all the tomfoolery which it must from time to time accommodate, Stonehenge retains its trick of quickening the imagination and disturbing it. The technology of the monument, and the endless speculations about the ways in which the stones may once have been assembled, is more tangible and thus less awesome than the single-minded purposiveness of the whole enterprise. Why was it thought sensible to spend what must have been a large part of the energy of the community that built Stonehenge on this gigantic structure? To such a primitive people, this was an undertaking to dwarf the cathedral building of the Middle Ages in costliness. Surely a people preoccupied with survival would not choose to waste energy in such a lavish way merely out of superstition. But, if it is hard to conceive a superstition powerful enough, what sober purpose can there have been in building it?

Questions like these have informed speculation about Stonehenge for several decades. At the end of the nineteenth century Sir Norman Lockyer, the then editor of this journal, began to put flesh on the bones of the old assertion that Stonehenge must once have been a kind of astronomical observatory and nautical almanac office. Since then, there has been a steady stream of speculation about the way in which Stonehenge might have been used for astronomical observation and prediction. With the coming of electronic computers, it was possible to make detailed comparisons of the positions of the various stones at Stonehenge and the occurrences of conspicuous events in the movement of the Sun and Moon. Thus, three years ago, Professor G. Hawkins (*Nature*, **200**, 306; 1963) was able to claim a detailed correlation of the positions of several of the stones at the monument with the rising and setting of the Sun and Moon throughout the year and the occurrence of some eclipses. That work suggested that the men who built Stonehenge and afterwards operated the supposed observatory were able to glean a previously unsuspected wealth of detail from their observations. More recently, these conclusions have been questioned (*Nature*, **210**, 1302; 1966) on the grounds that Hawkins's alignments are not significant.

The most recent steps in the interpretation of Stonehenge as an astronomical observatory are to be found on pages 454 and 456 of this issue. Professor Fred Hoyle and Mr. C. A. Newham are both concerned to show how the post holes grouped to the north of the main circles (not accommodated in earlier interpretations) are somehow concerned in the more refined use of the circles of stones for measurement and prediction. Mr. Newham would have one group of stones function as a kind of vernier gauge. Professor Hoyle claims that it would be possible to use the array of stones for the prediction of all eclipses (which is a substantial improvement on Professor Hawkins) and also for making measurements of the regression of the line of lunar nodes in such a way as to ensure that predictions of eclipses are not vitiated by experimental errors. If Stonehenge was really designed as an astronomical instrument, the principles on which this was done accord well with the procedures by which modern experimenters pursue their craft. The scheme which Professor Hoyle describes is breathtaking not merely by its ingenuity—that of Professor Hoyle as well as of the supposed builders of Stonehenge—but by its practicality.

The cleverness which Professor Hoyle now attributes to the designers of Stonehenge is probably the most difficult part of his theory to accept. People will ask whether it is credible that people who had not then invented enduring houses in which to live, and who had not even invented the Royal Society, could possibly have been clever enough to build an optical instrument of such intricacy as Stonehenge may be. But this is not, by itself, conclusive. If archaeologists knew a great deal more than they do about life in Britain 3,500 years ago, they might be able to show that Professor Hoyle's suggestion is implausible. The difficulty, of course, is that archaeology is only able to describe a kind of lower limit to the sophistication of a society. The fact that there is no evidence of numeracy among the people who built Stonehenge is not conclusive proof that they could not predict eclipses. In the circumstances, it is only charitable that they should be given the benefit of the doubt, and that everybody concerned with their affairs should contribute to the upkeep of the monument. And, if the Ministry of

Public Building and Works should keep the grass in order, is it not appropriate that distinguished astronomers should try to puzzle out what the placing of the stones may mean ?

UNIVERSITIES WITHOUT STUDENTS

THE Collège de France is a splendid institution, and everybody will be delighted if Prof. Leprince-Ringuet's initiative (see this issue, p. 448) should eventually win for it a larger site in the centre of Paris. When institutions like this, which embody a substantial part of a nation's intellectual capital, are hampered in their work by lack of funds or facilities, the waste is every bit as real as when crops are lost through neglect or when the product of a factory is rendered useless by bad design or bad marketing. In other words, having decided that institutions like the Collège de France should exist, there is no sense in trying to run them on a shoestring.

The question remains of what place institutions like the Collège de France should assume in the modern academic world. Most often, the problems to which they were originally the solutions have long since disappeared. The Collège de France owes its existence to the wish of an ancient monarch to break down the intellectual tyranny of a medieval university. The calculation was that a group of able scholars free to pursue their interests without the restraint of conventional doctrines could not fail to be a liberalizing influence on the research and the teaching of a dogmatic university. In Paris in the sixteenth century, this expectation was most probably correct. In other words, what seems like an ivory tower can also be an intellectual Trojan horse.

At Oxford, the founding of All Souls College was similarly inspired and may, at the time, have been similarly influential. The modern equivalents of these institutions, among which the Institute of Advanced Study at Princeton is conspicuous, have more ethereal reasons for existence. They may, for example, cater for the needs of academics who hate to teach, or they may be based on the argument by which Prof. Leprince-Ringuet justifies in part the continued existence of the Collège de France at the centre of Paris—the view that the interaction between distinguished academics from widely different disciplines is likely to yield results which are qualitatively distinctive. The difficulty is that, in the modern world, these arguments are less and less convincing as justifications for what are, in essence, universities without students.

The truth is that the luxury of untrammelled intellectual enquiry is now as easy to find in a modern university as in an ivory tower on the old pattern. If a person is so engaged in his research that he does not want to confront a class of students once a week at nine o'clock in the morning, it needs very little ingenuity to escape such a commitment. Indeed, with growing size, many university departments are hard pressed to provide suitable captive audiences for all those who want to be engaged in formal teaching. In the same way, it is relatively simple for those who are so inclined to escape the chores of administration frequently thought inseparable from university life. Then the opportunities for interaction between disciplines are if anything more real and richer in a modern university than in an ivory tower. In other words, modern universities can offer scholars all the advantages of an ivory tower and also the benefits of size, and the stimulation which good students habitually provide. There are signs that the best ivory towers recognize for themselves the advantages of a vigorous interplay between research and teaching; the Rockefeller Institute in New York (which was not so much an ivory tower as a specialized research institute) has in this spirit chosen successfully to turn itself into the Rockefeller University. It is only fair to acknowledge that the contribution of the Collège de France to teaching at the University of Paris is substantial and distinguished, but it is also proper to suggest that a time when there is a chance to move to a new site may also be a suitable occasion for choosing to move some way along the road to being a university in the more usual sense. The example of All Souls is a sad proof of what happens when the integrity of an ivory tower is too successfully defended.

AT THE DEEP END

FIRST it was atomic energy; then it was aerospace. Now Washington's favourite science is oceanography. There is a danger that every project that smells of salt water is going to be so deluged with federal money that a considerable amount of research will have to be devoted to devising ways to spend it. Perhaps the explanation is to be found in this season's phenomenal heat; even in the best of summers, people stuck in Washington are driven to wild daydreams of the sea.

Whatever the cause, in the past few weeks, the result is startling. Congress has passed and the President has signed an Oceanographic Law creating, among other things, a National Council of Marine Resources and Engineering to promote "inner space" to the eminence of "outer space". Almost at the same time, a National Oceanography Association has formed itself. A new governmental body, the Environmental Science Services Administration, has unveiled a special report on the vast richness of the American continental shelf, neatly timed to impress a conference held in Washington by the Marine Technology Society. And, after all this, President Johnson last week released a report prepared by his Science Advisory Committee outlining what a national oceanographic programme should be and recommending that federal spending in this direction (now $310 million a year) be doubled.

So specific and practical is the report that it makes the new legislation, with the President's signature hardly dry, look waffly. For the new law sets up not

only the Council of Marine Resources at Cabinet level but calls for a special Commission to spend a year and a half doing what the report has done already—to decide what the United States wants from the oceans and what kind of federal agency should go after it. After that, the law would require, the Commission and the Council should both expire and a new body—what Washington wags are calling "the wet NASA"—could then emerge.

But the science advisers' report not only spells out what kind of research would be most useful—expanding the yield of food from the sea—but insists that any new agency to be formed should not be devoted exclusively to oceanography. Some new form of co-ordination is clearly needed: there are now 26 federal agencies and bureaux involved in one way or another with the salt water. These include a new Institute of Oceanography established last winter. The two biggest spenders are the Navy and the National Science Foundation, the second of which has not been swamped with applicants for the sizable sums that it has had for more than a decade to spend on oceanography. (Its budget for marine research and education for the current fiscal year is $138 million.) These would not be included in the new agency, described in the report, to co-ordinate all other efforts and to embrace all the other federal activities in the environmental sciences, land and air as well as sea.

The report recognizes that oceanography has a built-in deterrent in that scientists and students find irksome the need to go to sea, and suggests that it would be easier to do fieldwork if the research ships were clustered into fleets and made available to all universities and laboratories in a large region—the Great Lakes, perhaps, or the Gulf Coast. It also suggested that marine study centres be established, so that lawyers, economists, engineers and scientists could meet to discuss the special problems that exploitation of the oceans presents. In all these efforts, the seashore is to be given as much attention as the continental shelf (on which American oil industries are already spending about $2,000 million a year) and the deep seas. Half the American population lives near the water's edge. The shoreline needs to be restored and preserved for their recreation; in turn, they must learn to stop fouling the water. (Lake Erie is so polluted as to be in danger of turning into a swamp.)

It is hard to see how the new Oceanographic Commission will be able to improve on the Science Advisory Committee's labours. But, as long as the "wet NASA" does not emerge to lead a jingoistic race to the bottom of the ocean, the duplication may do no harm. One sensible requirement of the new law is that the President must present to Congress every year an outline of the national oceanographic plan. Perhaps by next year he may be able to report that Project Mohole has been set going again and that there are some large-scale projects of practical value to set alongside the performance of *Sea-Lab*, which has shown that men can live uncomfortably at the bottom of the sea for several weeks, and the development of clever craft which can find hydrogen bombs at the bottom of the Mediterranean.

NEWS AND VIEWS

Who Gives Most?

THE financial crisis in Britain last week kept at home most of the delegation that would otherwise have attended the meeting of the Development Assistance Committee (DAC) of the O.E.C.D. in Washington. This is entirely understandable. The calculation was not the saving of a dozen or so air fares across the Atlantic, but the need that the Minister of Overseas Development, Mr. Anthony Greenwood, should be in London to defend his department's budget against those of his colleagues bent on finding substantial economies in British public expenditure. Mr. Greenwood's counterparts from other O.E.C.D. countries will have been entirely sympathetic, for their agenda in Washington emphasized the need enormously to increase the volume of foreign aid. The fact that DAC was meeting in Washington rather than in Paris was itself a part of an attempt to increase the value of foreign aid by the advanced nations.

The league table of aid giving which informed the discussions in Washington was entirely flattering to the European members of the O.E.C.D. According to the calculations of the DAC officials, France spends a greater proportion of her national income on aid to developing countries than any other member of the O.E.C.D. Then, in order, come Belgium, the Netherlands and Britain, each of which spends more than 1 per cent of its national income on aid. By this test, the United States is surprisingly low on the list, and one of the motives of American officials in bringing the DAC to Washington was the hope that the United States Congress would be shamed by this oddly unflattering comparison into spending more on aid. Whatever the result may be, the DAC will be confirmed in its role as the keeper of objective international comparisons on aid giving; it is not, after all, so very long since the Committee pointed out that the official figures of the annual amount of British aid to developing nations should be adjusted by the amount returned as interest and capital repayments, thus converting roughly £200 million into £150 million, and also paving the way for the British Government's decision that development loans could be free of interest.

Another reason why some Americans were keen that DAC should meet in Washington was the hope that the Committee would bless the view that North American food stocks, if necessary bought with other nations' cash, will have to play an important part in foreign aid in the decades ahead. In the event, the meeting reaffirmed the need somehow to increase the capacity of developing nations to grow more food. But, one way or another, aid in the form of calories will be essential for decades to come. The O.E.C.D. brief for the Washington meeting included economic and demographic projections for the rest of this century for the advanced and the developing nations. In the developing nations (excluding China) the population is expected to multiply threefold by the end of the century, and the demand for food between four and six times. Food production, even on the most optimistic assumptions, will not grow as quickly as the population. But, in the advanced nations, food production could increase substantially, and much more quickly than the population. In other words, events seem to strengthen the

paradox first given point by Sir Arthur Lewis that, when it comes to foreign aid, the industrialized nations have most to offer in the form of agricultural produce.

Room in Paris

THE Collège de France is openly staking a claim on the site in Paris that will be vacated, possibly in five or six years, when the École Polytechnique moves out to Palaiseau in the south-west suburbs. The Collège has been in Paris since its foundation in 1530 by Francis I as part of an attempt to break the stranglehold of the schoolmen at the Sorbonne by creating a community of scholars independent of the politics of the University of Paris. The Collège de France, like many other academic institutions which are similarly placed in the hearts of capital cities, now finds the pressures of modern life—and particularly the non-academic demand for space—an increasing source of frustration. But, for one reason or another, moving out of town is unthinkable. It is no wonder that the professors of the Collège de France should be so excited by the prospect of a vacant lot in the gift of the French Government and only a few hundred metres from their present building that they have allowed themselves to express their yearnings in public.

Professor Louis Leprince-Ringuet put the claim of the Collège in an article in *Le Monde* on July 20. In the nature of things, the argument goes, the fifty or so professors who make up its membership must divide their time between research and teaching. But because their interests span the whole field of scholarship, they need facilities of all kinds. The humanists need access to the Louvre and the Bibliothèque Nationale, while the scientists—roughly twenty of them—must have their own laboratories and research assistants. Professor Leprince-Ringuet is eloquent about the great advantages which come from a system which requires distinguished men of diverse interests to work closely together in the same premises, and cites as an example the way in which Joliot-Curie and Robert Courrier worked on the study of thyroxine, using radio-iodine as a tracer. He argues quite openly that the Collège de France is "probably the only institution of higher education whose presence in Paris is almost essential", and goes on to say that, even though the Collège has no ambition to own large cyclotrons or nuclear reactors, it would gain enormously if the scientists could be gathered on to the same site rather than be scattered as at present up and down the Rue des Écoles and even farther away. Scholars everywhere will be interested to see how successfully it will be possible to sustain this claim on real estate in the centre of a capital city.

Doctors in Distress

MEDICAL manpower and utilization have been areas of debate and concern in Britain for a long time, but debate has often taken the place of action. In a pamphlet *Medical Manpower* the Office of Health Economics points out that more than 40 per cent of the hospital doctors in 1964 were born outside the country, while during the 1950's more than 5,000 doctors, the equivalent of one quarter of graduates of British medical schools, emigrated from Britain and did not return.

Coupled with these quantitative statistics, the study discusses the qualitative problems facing the hospitals; specialization has become increasingly necessary because of the complexity of modern surgical techniques and the need for medical and non-medical specialists in areas such as radiotherapy and heart surgery. Another problem facing the hospitals is the recruitment of junior staff; the incentive for staying at a hospital has usually been to reach the final goal of a consultant post. With far more registrars than consultants, more and more young doctors have gone directly into general practice so that, between 1961 and 1964, 1,000 out of the 1,100 increase in the numbers of junior hospital staff were born outside the British Isles. The Office of Health Economics blames this situation on three factors: a large error in forecasting the future need for doctors, much greater emigration from Britain than was expected or has been admitted, and a failure to take account of the rapid technological progress and changing pattern of medical care over the past twenty years when modifying medical education.

In the early 1950's there was a widespread belief in Britain that too many doctors were being trained. The Willink Committee was set up in 1955 to investigate this situation and, based on a population growth rate of 4·5 per cent—which in fact turned out to be 7·0 per cent—and assumptions about the growth of hospital staff which have proved to be half the actual growth, the committee held that student intake should be reduced by 10 per cent. This decision was reversed in 1961, but the effects are still felt in the present situation. Migration remains a major problem, yet the Council of the British Medical Association in 1958 reported that net emigration among doctors and dentists "can be considered of no special significance". Finally, while the specialized training and skills necessary for hospital staff have become less and less related to the community care demanded of the general practitioner, medical education and training have done little to recognize the variation in training that these distinct paths call for.

The study concludes that, while small advances are being made—closer links are growing between hospitals, general practitioners and local authorities—and automation and qualified auxiliaries are avenues to be explored, for the present the most pressing need is to increase the efficiency of the manpower now available.

Three Years of Data

THE National Bureau of Standards in the United States has just celebrated the third anniversary of the National Standard Reference Data System, which is the formal name for a programme intended to build up critical compilations of scientific data. Sensibly enough, the work is being co-ordinated with that of standards institutions outside the United States, most effectively by a committee of ICSU with a staff in Washington. Inevitably, however, the contribution of the United States is a very large part of the whole, and not simply because, in June 1963, the then director of the U.S. Office of Science and Technology said that the "intent is to provide an articulated system of activities under such co-ordination and direction as to ensure a compilation of data meeting quality standards and also to maintain continuous policy guidance of the system at the level of the Executive Office". The

experience of the past three years at the National Bureau of Standards is therefore a useful pointer to the problems of operating a thoroughly ambitious data system, and to some potential solutions as well.

There is no question that the plans of the National Standard Reference Data System are ambitious—and events may yet show that they have outstripped the budget. The physical sciences have been partitioned into seven fields, and separate programmes are under way in each of them. Only in nuclear physics do the existing arrangements approximate to what is thought necessary, so that the National Bureau of Standards can confine itself to the evaluation of data on elementary particles and cosmic rays. In other fields, several new projects have been started, sometimes within the Bureau of Standards but also frequently by letting contracts to independent organizations—universities, government laboratories and sometimes commercial organizations. Not surprisingly, activity is most conspicuous in thermodynamics and atomic and molecular spectra. Although the general field of "mechanical properties" is included in the brief for the reference data system, it will be hard to begin to make a start in this jungle, partly because of the difficulty of deciding what kind of information would be of value to potential users, partly because of the sheer bulk of it, and partly because of the way in which information about the mechanical properties of materials tends to depend on the method of measurement.

There is also the problem which has traditionally beset the compilers of critical tables—the frequently invidious task of deciding which published data to trust. The National Standard Reference Data System acknowledges that, in the last resort, value judgments of this kind cannot be left to machines. This is why it is good to see how it has been possible to encourage academic interest in the evaluation of data by the judicious letting of contracts. Elsewhere, scientists are still more eager to give their blessing to plans for collecting data than to undertake to carry out some of the hard work involved. But compilation is only half the story. There is also to be a huge effort on dissemination. The first of what will be a torrent of volumes of critical data have already appeared. At some stage, possibly within three or five years, a computer system will permit faster access to the files, and there will be a fast and comprehensive system by means of which individual laboratories will have access not merely to the computer files in Washington but to those in associated data centres as well.

Science Teaching Centre

THE Chelsea College of Science and Technology in London has announced a plan to create a centre intended to make a continuing contribution towards research in teaching methods in science. In a statement issued last week the principal of the College, Dr. M. R. Gavin, says that the College has in mind the possibility that there may be a hiatus in the development of new science curricula for British schools when the current plans of the Nuffield Foundation Science Teaching Project are worked out at the end of this decade. The College hopes to be able to be ready, before then, to play a full part in the development of methods of teaching science, and intends to cut its teeth in a number of fields of research and development ranging from the development of new curricula to the design of equipment and apparatus. The statement makes the point that such a centre would complement work in teacher training in a particularly valuable way, and that it would also serve as a means of providing teachers in the Greater London area with opportunities for in-service training. The first academic appointments will be made for the academic year immediately ahead. The College is evidently hoping that its plans for creating a centre for teaching science at Chelsea will match well with the secondment of the head of the Physics Department at Chelsea, Professor K. W. Keohane, to the Nuffield Foundation Science Teaching Project from July this year.

Chemical Research, Melbourne

DR. A. L. G. REES, chief of the Division of Chemical Physics, C.S.I.R.O. Chemical Research Laboratories, Melbourne, Australia, writes:

"A new laboratory for the Division of Chemical Physics has now been opened at Melbourne by Senator J. G. Gordon, minister in charge of C.S.I.R.O. The building has been named the David Rivett Laboratory after the late Sir David Rivett, for nearly 20 years chief executive officer and later chairman of the C.S.I.R.O. Sir David, a physical chemist, actively promoted the establishment of research in chemical physics in 1944 and encouraged its rapid development.

"The first steps taken in 1944 involved the establishment of specialized groups concerned with physical techniques and their application to chemical problems. Current sections include spectroscopy, electron and X-ray diffraction, electron microscopy, solid state and theoretical chemistry, and instruments and engineering services. The Division pioneered electron microscopy in Australia; its first work was largely on problems oriented towards biology. Early work on the fine histology of wool stimulated a more general interest in fibrous proteins. The Division entered the related field of electron diffraction in 1945 and its first task was the development of electron diffraction apparatus.

"Research in mass spectroscopy has been conducted in the Division since 1947. The major theme has been the elucidation, by experimental and theoretical methods, of the behaviour of molecules under electron and photon bombardment. In solid state chemistry, studies have been oriented towards establishing the significance of crystal defects in chemical behaviour. The occupation of new premises has allowed the expansion of research in this area, the most important of which concerns the low-temperature thermodynamic studies of non-stoichiometric solids.

"From the beginning, considerable attention has been paid to technique and instrumentation in chemical physics research, with the result that new techniques and new instruments have appeared as by-products. A number of these have encouraged the rapid development of the manufacture of scientific instruments in Australia. The most successful example to date is the manufacture of spectroscopic instruments. The total value of such equipment now produced each year in Australia exceeds one million Australian dollars."

Centres for Engineering

ENGINEERING education and training in Britain will have to be overhauled if it is to attract its fair share

of scientific and technological manpower. This is the conclusion of a working group on Engineering Training and the Requirements of Industry set up last year by Sir Willis Jackson of the Ministry of Technology's Committee on Manpower Resources for Science and Technology. In its report, the group calls for close co-operation between educationalists and industry to establish a programme of "matching sections" of formal education and training. By exposing engineering students to the intellectual and practical problems of manufacturing industries, they hope to maintain the interest and enthusiasm of those students who might otherwise go into research.

To co-ordinate these "matching sections", the group calls for centres of product technology, established by collaboration between an educational institution and one or more industrial units, with teaching at the former and the industrial plants used as full-scale demonstration laboratories. It envisages a training programme of approximately one or two years that would begin when a graduate enters a firm. At this time he would be exposed to possible jobs and specializations, begin to learn the principles of manufacturing engineering, and take part in a group project to expose him to the practical problems he will face. On choosing his first job and gaining some experience, he would enter a course at the centre in either product technology, manufacturing technology, or special techniques. He would then return to his firm for more supervised training.

Swings and Roundabouts

THE annual report of the British Post Office contains in microcosm the contemporary history of the balance between the different means of communication between people. On one hand, there is the traditional postal service which was introduced in Britain in 1840, with the Penny Post. On the other hand, there is the telephone system. The first of these services has had, in recent years, a tendency to run at a loss. As good luck and increased charges would have it, 1965–66 was not too bad a year. The postal services actually made a small profit—something like £0·9 million out of a total turnover of £318 million. But nobody is very hopeful about the years ahead. Already a further round of price increases has been announced.

By contrast, the telephone service is booming. In the course of a year, charges to the British public actually declined, chiefly because of the introduction of automatic dialling, yet the Post Office made a surplus of roughly 10 per cent on its telephone business. Demand from the public is at the same time growing quickly. Even local calls increased by 8 per cent in the course of a year. Trunk calls went up by 14 per cent and overseas calls by no less than 21 per cent, partly, no doubt, because of the arrival of satellite communications across the Atlantic and facilities for dialling direct to the mainland of Europe. All this, of course, is entirely to be expected. Telephones will keep on winning over posts. One practical question is whether those in charge of the Post Office should anticipate the future by weighting the odds even more heavily against the posts than the realities of handling pieces of paper entail. The second question, not quite so practical, is what will happen decades hence to all those letters written not for people now alive but for posterity.

Parliament in Britain

REPLYING on July 12 to an adjournment debate on the training of teachers, Mr. G. Roberts, Minister of State, Department of Education and Science, said that, since the announcement last year of the intention to establish departments of education in suitably located technical colleges, the local education authorities in Inner London, Birmingham, Manchester, Nottingham and Sunderland had been consulted. All had expressed a desire to participate and were preparing detailed proposals. It was hoped that the new departments of education would take their first students in the academic year 1967–68. The departments might be expected to attract to teacher training some technical college students who would not otherwise have thought of taking up teaching, and Mr. Roberts emphasized that the departments would provide courses comparable with the best afforded by the traditional college of education and the university departments of education. Initially, it was intended that the new departments should cater essentially for students taking a 3-year certificate course leading to qualified teacher status, but no doubt in due course the departments would introduce also 4-year courses leading both to degree and to professional teaching qualifications. Departments would start with about 100 students and rise to 250 to 300. For some years their main emphasis would be on training teachers for the primary schools.

REPLYING for the Government in a debate on July 8 on computers in universities, Mr. G. Roberts emphasized that the programme of the Flowers Group for new university computers, which the Government had approved, would provide for an integrated system. There would be regional centres in London, Manchester and Edinburgh. Of the 28 universities fitting into the Flowers programme, nine had delivery dates for their machines varying from July 1966 to the end of the year. Pending the establishment of the Computer Board, the membership and functions of which would be announced shortly, the University Grants Committee had made an interim arrangement for considering the most urgent cases and planning orders. Approval had already been given, at a cost of £4·8 million in the period 1966–69, for upgrading the *KDF* 9 computers at the seven universities recommended by the Flowers Group and for new installations for 21 other universities.

IN a written reply to a question in the House of Commons on July 20 regarding liaison with other countries on the courses of hurricanes, the Under-Secretary of State for Defence for the Royal Air Force, Mr. M. Rees, stated that the Meteorological Office kept in touch with other countries on hurricane research. There was liaison with the Hong Kong Meteorological Office and also with the United States National Hurricane Research Laboratory and other American weather agencies. In the near future, cloud photographs would be received at Muharraq, Gan and Singapore direct from U.S. weather satellites and would help to provide warning of hurricanes in the Indian Ocean and Far East areas. The Meteorological Office also participated in a recent North Atlantic Treaty Organization conference on cloud dynamics at which the problem of hurricanes was discussed.

University News : Bradford Institute of Technology

DR. G. D. H. LEACH, at present senior lecturer in pharmacy in the Institute, has been appointed professor of pharmacology.

Keele

DR. I. T. MILLAR has been appointed professor of organic chemistry. Dr. H. H. Greenwood has been appointed director of the University Computing Centre.

Leeds

DR. P. N. CAMPBELL has been appointed professor of biochemistry and head of the Department as from October 1, 1967.

Liverpool

DR. W. PETERS has been appointed to the Walter Myers chair of parasitology on the resignation of Prof. W. E. Kershaw.

Manchester

DR. R. B. NICHOLSON has been appointed to the newly-established chair of physical metallurgy. J. R. Moore has been appointed to the chair of oral surgery.

Southampton

THE following have been appointed to chairs: Dr. B. L. Clarkson (vibration studies); B. W. Cunliffe (archaeology); Dr. G. A. Kerkut (physiology and biochemistry). The personal title of Westland professor has been conferred on Dr. J. P. Jones, Westland reader in the Department of Aeronautics and Astronautics.

Appointments

DR. VIKRAM A. SARABHAI, a cosmic ray physicist, has been appointed chairman of the Indian Atomic Energy Commission in succession to Dr. H. J. Bhabha, who was killed in an air crash on Mont Blanc earlier this year. Dr. Sarabhai, who is 47, has been responsible in recent years for organizing Indian participation in international organizations for space research. He was a student at Bangalore and at Cambridge in the years immediately before the Second World War and took a Ph.D. at Cambridge in 1947.

THE National Science Foundation has appointed Dr. Robert Fleischer deputy head of the Office of International Science Activities. Dr. Fleischer joined the foundation in 1962, and has been programme director for solar-terrestrial research and co-ordinator for the participation of the United States in the International Year of the Quiet Sun. Between 1946 and 1962 Dr. Fleischer was professor of astronomy at Rensselaer Polytechnic Institute, where he worked on solar radio astronomy, interstellar dust and binary stars.

DR. E. A. ALEXANDER has been appointed scientific counsellor at the British Embassy in Moscow, where he will replace Mr. T. Garrett at the end of September. The post of scientific attaché was established in April, 1959; the title has recently been changed, but the purpose remains to advise the ambassador on scientific matters, to report on Soviet civil science and technology and to help visiting scientists and scientific and technological missions. Dr. Alexander, who is an Oxford physical chemist, has been with the Admiralty since 1941.

Announcements

MR. J. E. DENYER has been elected president of the Institution of Mining and Metallurgy for the session 1967–68.

MR. H. N. PEMBERTON has been elected chairman of Council of the Council of Engineering Institutions, in succession to Sir Robert Wynne-Edwards. He will take office in January 1967.

THE Royal Institution of Naval Architects has been elected a constituent member of the Council of Engineering Institutions.

THE National Bureau of Standards has established an Office of Engineering Standards Liaison and Analysis to serve as a focal point for NBS assistance to engineering standards-making bodies throughout the United States. Dr. G. S. Gordon, formerly chief of the Textile and Apparel Technology Center of the NBS Institute for Applied Technology, has been appointed chief.

THE 1966 international Industrial Diamond Conference will be held in Oxford during September 19–22. Further information can be obtained from the Organizing Secretary of the Conference, 2 Charterhouse Street, London, E.C.1.

A SYMPOSIUM on "Pyrolysis—Gas Chromatography" will be held at the Ecole Polytechnique, Paris, during September 15–16. Further information can be obtained from Dr. G. Guiochon, Ecole Polytechnique, 17 rue Descartes, Paris.

THE fourth symposium on "Chromatography and Electrophoresis", organized by the Belgian Society of Pharmaceutical Sciences, will be held in Brussels during September 12–13. Further information can be obtained from the Secretary of the Society, 11 rue Archimède, Brussels 4.

A SYMPOSIUM on "Nucleic Acids and Nucleotides in Higher Plants", arranged by the Phytochemical Group, will be held at Royal Holloway College during September 20–21. Further information can be obtained from A. H. Williams, Research Station, Long Ashton, Bristol.

A CONFERENCE on "Rare-earths", organized by the Institute of Physics and the Physical Society, will be held in the University of Durham during September 5–7. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE second international Biophysics Congress, organized by the International Organization for Pure and Applied Biophysics, will be held in Vienna during September 5–9. Further information can be obtained from Mrs. E. Weidenhaus, Wiener Medizinische Akademie, Alserstrasse 4, Vienna IX.

THE first international congress of the International Radiation Protection Association will be held in Rome during September 5–10. Further information can be obtained from the secretary-general of the congress, Dr. C. Polvani, International Radiation Protection Association, Casella Postale 2359, Rome.

A SYMPOSIUM on "Advanced Industrial Measurement and Control" will be held at the Welsh College of Advanced Technology during September 19–23. Further information can be obtained from the Organizer of Short Courses, Welsh College of Advanced Technology, Cathays Park, Cardiff.

AN international technical meeting on "The Biological Basis of Freshwater Fish Production", organized by the International Committee for the PF Section of the International Biological Programme, will be held in the University of Reading during September 1–6. Further information can be obtained from Mr. E. D. Le Cren, The River Laboratory, East Stoke, Wareham, Dorset.

ERRATUM. A note about the retirement of Prof. Eisenschitz from Queen Mary College on June 25 (210, 1314) gave the impression that Prof. Eisenschitz was retiring at the end of the current academic year. In fact, of course, Prof. J. G. Valatin has occupied the chair since September 1965.

CORRIGENDUM. In the article entitled "Memory and Spontaneous Alternation in the Rat" by A. W. Still (*Nature*, 210, 400; 1966), on page 401 in column 2, 'less' should read 'more' on line 8, 'more' should read 'less' on line 10 and 'less' should read 'more' on line 46.

OCEANOGRAPHY IN THE UNITED STATES

A RECOMMENDATION that expenditure in the United States on oceanography should be doubled is the principal conclusion of a report of a panel appointed by the President's Science Advisory Committee and now released to the public by the President with the title *Effective Use of the Sea* (U.S. Government Printing Office, $0·60). The report is likely to be influential because of the current interest in oceanography in the United States. The chairman of the panel, which was appointed in May 1965 at a time when the United States Congress was eagerly concerned to see that oceanographic research should not languish, was Dr. Gordon J. F. Macdonald of the University of California, Los Angeles. The other members of the panel were Dr. Douglas L. Brooks (Travelers Research Centre Inc.), Dr. Robert Charpie (Union Carbide Corporation), Dr. Robert Fleagle (University of Washington), Dr. Finn J. Larsen (Honeywell, Inc.), Dr. William D. McElroy (Johns Hopkins University), Dr. John Meyer (Harvard University), Dr. Walter H. Munk (University of California, La Jolla), Dr. Jack P. Ruina (Institute for Defence Analyses), Dr. Henry Stommel (Woods Hole Oceanographic Institution), and Dr. Gerald B. Whitham (California Institute of Technology).

Running through the report of the panel is the view that oceanography should be useful, and the suggestion that the U.S. Government should spend more money on oceanographic research is supported by the view that more research will bring tangible rewards. Thus it is argued that strategy requires the U.S. Navy to increase its efforts to learn more about submergence in the deep sea, partly so as the more effectively to navigate with submarines. The loss of the *Thresher* in 1963 and the loss of a hydrogen bomb off the Spanish coast earlier this year are cited as two of the reasons why the Navy should be able to recover objects from deep water.

The benefits from better use of the sea as a source of food are held to justify the considerable increase in the efforts spent on recovering edible protein from the sea. The panel would also like to see rounded studies of marine populations of organisms in estuaries and on the Continental Shelf so as to preserve the accessible parts of the ocean for fuller use by a growing population.

In pure oceanographic research the panel would like to see a shift of emphasis from the expensive survey of the oceans to an attempt to solve the specific problems related to physical oceanography and marine biology. It wants more higher education in oceanography although it questions the wisdom of proposals for granting Ph.D.s in oceanography; rather, it takes the view that oceanography is not a discipline in its own right but a field of studies to which specialists of all kinds must contribute. The panel also argues that the deployment of oceanographic research vessels needs to be planned more effectively, particularly because of the difficulty of persuading academics to go to sea. The panel therefore suggests that ships should be made available to institutions and universities on a regional basis, and grouped into fleets of reasonable size.

The argument on exploiting the oceans as a source of food is unusually specific. Comparing estimates of population with present resources of food, the panel concludes that improvements in conventional agriculture will not be sufficient to meet the future demand for protein. It points out that the fish caught in 1964 contained $17·1 \times 10^9$ lb. of protein, which would have been enough to provide 10 g of protein a day for 2×10^9 people. This, the calculation goes, would have been enough to get rid of chronic protein deficiency in the equatorial regions of the world.

The recent improvement in catches of fish from the sea is held to justify some of the panel's optimism. In the past ten years, the production of fish in the world is estimated to have increased from $60·5 \times 10^9$ lb. to 114×10^9 lb. The development of new fisheries is thought chiefly to have been responsible for the increase of production, and the fact that in 1964 nearly 20 per cent of the total production of fish was accounted for by anchovies taken off the coast of Peru is given as a sign of what new fisheries can produce.

Attempts deliberately to cultivate the sea are necessarily less well understood. The Japanese harvest is shown to have increased between 1951 and 1963 from 193×10^9 lb. to 857×10^9 lb.; even so, this is less than one-sixtieth of the total production of fish in Japan.

The panel in Washington considers that the technique of aquaculture, as it is called, needs much more development before it can be estimated what benefits are to be had. It points out that animals which feed directly from phytoplankton are likely to be among the most efficient captive sea animals. This suggests a future world in which oysters and other delectable shellfish are a part of the staple diet.

The panel plainly thinks it is easier to damage the natural ocean environment than to make it more favourable for growing things. It points to the dangers of pollution and tells the chilling story of how mussel culture in the Black Sea was brought virtually to an end by the introduction of predatory Japanese snails in 1949. To protect the oceans of the United States from disasters like this, the panel seeks to establish "marine wildernesses" in parallel with the wildernesses on dry land which have been set aside by the provisions of the Wilderness Act of 1964. It would also seek to improve food production and opportunities for recreation by cleaning some parts of the ocean and seek to win a better understanding of marine ecology so as to anticipate the effects, evil and otherwise, of interference with the natural sea.

Under the heading of what it calls "Undersea Technology", the panel argues that there is a need for better navigation, both where the Navy is concerned and as a means to exploiting mineral resources. Ideally it would

like to be able to navigate to within 30 ft. in those parts of the ocean within sight of land, and to within 150 ft. elsewhere. For drilling, even greater accuracy is necessary, and the report talks of oil companies which have been able to locate bore holes to within 2 ft. It also wishes to see more devices for producing pictures of the ocean bottom, not merely by means of better television techniques, but also by acoustic imaging. It has a number of schemes for improving survey techniques, and suggests that a surface ship should be fitted out with improved equipment, that a submarine equipped with sensing devices could do useful work, and that there is a case for developing towed submersible platforms for making more accurate and more convenient measurements of temperature near the surface of the ocean. Devices for preventing erosion, power plant that can be operated under the sea, and buoys for collecting information about weather and other physical variables need development. Then there is the programme known as "Man in the Sea" which the panel commends to the United States, and whose object is to enable people to function adequately at depths up to 1,000 ft. It seems to be acknowledged that divers will not be able to operate effectively below this depth.

In pure research, the panel argues that exploration of the oceans must become more systematic. It is concerned to have better understood the relationship between the circulations of the oceans and the atmosphere, which in turn is an essential step in formulating a theory of climate. Linked with this is the problem of sedimentation, for this is also a process governed by the same conditions within the oceans as those involved in climate.

The panel also speaks highly of new devices for following currents such as the "swallow" floats as it describes the instruments for this purpose developed by Dr. J. C. Swallow. Better data processing and telemetry have much to offer, as does the connexion between geophysics and oceanography.

The panel would like to see oceanographers turn their attention to a number of specific problems which are partly scientific and partly practical and which include prediction. For one thing, it would like to see better ways of predicting tides and exceptional oceanographic effects such as tsunamis. Predicting turbulence in the Gulf Stream, and meanders of this and other currents, is thought to be necessary and practicable. At the back of its mind, the panel let slip, is the possibility of modifying deliberately the character of the oceans—melting Arctic ice and so on.

It is argued that the work of the oceanographers which bears on geophysics ought also to be encouraged. The Benthic Boundary, at which water gives way first to ooze and then to solid mud, needs to be studied so as to understand long-range sound transmission as well as to make it easier to find things which fall to the ocean bottom. The panel gives its blessing to schemes for drilling holes into the crust of the earth as it is exposed on the sea bed, which will no doubt be welcomed by the organizers of the MOHOLE project. There is also a general commendation of studies involving floating buoys to study the circulation of the ocean, and programmes based on fixed platforms, moving buoys and aircraft to study such things as the upward mixing of layers of the sea near the surface, and the interaction between the physical and chemical environments and the biological life. The panel points out that plankton are not merely the means by which all sea populations obtain energy, but that a number of sea organisms—sea cucumbers, starfish and so on—are potentially important as sources of pharmacological chemicals.

How much will this cost? The panel is at pains to argue that it is not at this point feasible to assess the potential value of oceanographic research and development in strictly economic terms. In this sense the panel disagrees with the argument of the Committee of the National Academy of Sciences in the United States which argued, in 1964, that the case for a vigorous programme of oceanography could be strictly justified by the money it would earn. Instead the panel seeks to proceed by the prime objectives which are thought to be attainable in the not-too-distant future and then to add up the sums of money currently being devoted to these.

Outside the military field, it is thought that during the current financial year in the United States, government spending on oceanography will amount to $120 million. Of this amount, $14·5 million is being spent on better prediction services. Fisheries research in the United States seems to be using up $45 million in the year, while navigation accounts for $38 million. This sum does not, of course, include sums spent by universities and other institutions on oceanographic research. The panel argues for a steady growth of these expenditures in the next five years, with the suggestion that there should be a comparatively quick growth in expenditure on prediction and control, on marine biological resources, and on the "near-ocean environment".

How is all this to be organized? The suggestion is that there should be a major reorganization of civilian oceanography with the object of placing within a single agency all the government activities related to the description of, the prediction of, and the attempt to modify the environment—ocean, atmosphere or solid earth. The panel recommends this step because it believes there is a unity in the environmental sciences, that the development of the ocean for industrial purposes requires an ability to predict the environment, and that the effective execution of a national programme requires that one single agency should be in charge of it. The panel would form its new agency out of the existing Environmental Science Services Administration (which came into existence only at the beginning of this year), the Geological Survey, parts of the U.S. Coast Guard and the oceanographic interests in the Departments of Fisheries and of Mines.

Such a step would not, the panel argues, entail interference with the National Science Foundation in its present role of supporting academic oceanography. Similarly, the present activities of agencies such as the Office of Naval Research and the Smithsonian Institution would continue without interruption.

The operation of ships for oceanographic research is reckoned to be a special problem. Not merely are there a number of oceanographic vessels in commission, but there is a prospect of drilling platforms such as that intended for MOHOLE soon to come into commission. At present, the demand for ship time, and the complexity of modern exploration, means that schedules have to be prepared two years in advance. This, the panel argues, is extremely difficult when the cost of operation has to be met by a number of grants and contracts with individual researchers. For one thing, this means retrospective haggling about who pays what. It also means that the cost of operating an unpopular ship may seem to be more than that of operating a popular one. This in turn implies that small laboratories tend to find it hard to make proper use of their comparatively small funds. Thus, the argument goes, there is a need that ships should be operated centrally, and that the distribution of time among interested laboratories should be decided by groups of users with joint responsibility and privileges.

Among the other facilities for oceanographic research which the panel would like to create is a strengthening of the existing National Oceanographic Data Center which, the panel says, "needs upgrading very badly". The panel would also like to establish a number of special laboratories, including one in the Arctic for the study of living things at sub-freezing temperatures; a laboratory in the tropics for the study of marine environment and population; and a laboratory in the temperate zone to study the organisms involved in the food chains of food fishes.

STONEHENGE—AN ECLIPSE PREDICTOR

By PROF. FRED HOYLE
University of Cambridge

THE suggestion that Stonehenge may have been constructed with a serious astronomical purpose has recently received support from Hawkins, who has shown[1] that many alignments of astronomical significance exist between different positions in the structure. Some workers have questioned whether, in an arrangement possessing so many positions, these alignments can be taken to be statistically significant. I have recently reworked all the alignments found by Hawkins. My opinion is that the arrangement is not random. As Hawkins points out, some positions are especially relevant in relation to the geometrical regularities of Stonehenge, and it is these particular positions which show the main alignments. Furthermore, I find these alignments are just the ones that could have served far-reaching astronomical purposes, as I shall show in this article. Thirdly, on more detailed investigation, the apparently small errors, of the order of $\pm 1°$, in the alignments turn out not to be errors at all.

In a second article[2] Hawkins goes on to investigate earlier proposals that Stonehenge may have operated as an eclipse predictor. The period of regression of the lunar nodes, 18·61 years, is of especial importance in the analysis of eclipses. Hawkins notes that a marker stone moved around the circle of fifty-six Aubrey holes at a rate of three holes per year completes a revolution of the circle in 18·67 years. This is close enough to 18·61 years to suggest a connexion between the period of regression of the nodes and the number of Aubrey holes. In this also I agree with Hawkins. I differ from him, however, in the manner in which he supposes the eclipse predictor to have worked. Explicitly, the following objections to his suggestions seem relevant:

(1) The assumption that the Aubrey holes served merely to count cycles of 56 years seems to me to be weak. There is no need to set out fifty-six holes at regular intervals on the circumference of a circle of such a great radius in order to count cycles of fifty-six.

(2) It is difficult to see how it would have been possible to calibrate the counting system proposed by Hawkins. He himself used tables of known eclipses in order to find it. The builders of Stonehenge were not equipped with such *post hoc* tables.

(3) The predictor gives only a small fraction of all eclipses. It is difficult to see what merit would have accrued to the builders from successful predictions at intervals as far apart as 10 years. What of all the eclipses the system failed to predict?

My suggestion is that the Aubrey circle represents the ecliptic. The situation shown in Fig. 1 corresponds to a moment when the Moon is full. The first point of Aries γ has been arbitrarily placed at hole 14. S is the position of the Sun, the angle $\bigcirc$ is the solar longitude, M is the projection of the Moon on to the ecliptic, N is the ascending node of the lunar orbit, N' the descending node, and the centre C is the position of the observer. As time passes, the points S, M, N and N' move in the senses shown in Fig. 1. S makes one circuit a year. M moves more quickly, with one circuit in a lunar month. One rotation of the line of lunar nodes NN' is accomplished in 18·61 years. In Fig. 1, S and M are at the opposite ends of a diameter because the diagram represents the state of affairs at full Moon.

If the Moon is at N, there is a solar eclipse if the Sun is within roughly $\pm 15°$ of N, and a lunar eclipse if the Sun is within $\pm 10°$ of N'. Similarly, if the Moon is at N', there will be a solar eclipse if the Sun is within $\pm 15°$ of coincidence with the Moon, and a lunar eclipse if it is within roughly $\pm 10°$ of the opposite end of the line of lunar nodes. Evidently if we represent S, M, N and N' by markers, and if we know how to move the markers so as to represent the actual motions of the Sun and Moon with adequate accuracy, we can predict almost every eclipse, although roughly half of them will not be visible from the position of the observer. This is a great improvement on the widely scattered eclipses predictable by Hawkins's system. Eclipses can occur as many as seven times in a single year, although this would be an exceptional year.

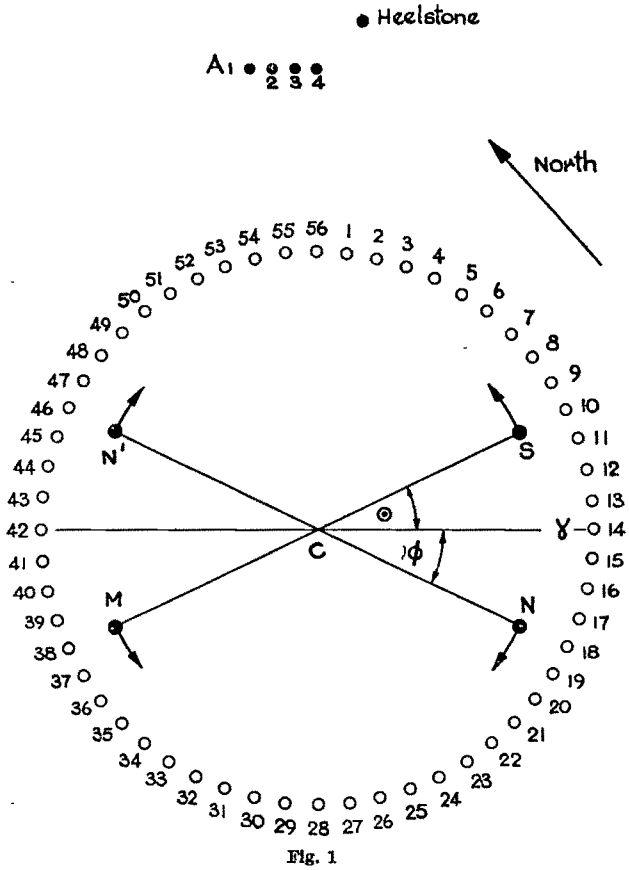


Fig. 1

The prescriptions for moving the markers are as follows: (1) Move S anticlockwise two holes every 13 days. (2) Move M anticlockwise two holes each day. (3) Move N and N' clockwise three holes each year.

We can reasonably assume that the builders of Stonehenge knew the approximate number of days in the year, the number of days in the month, and the period of regression of the nodes. The latter follows by observing the azimuth at which the Moon rises above the horizon.

If in each lunar month we measure the least value of the azimuth (taken east of north), we find that the "least monthly values" change slowly, because the angle $\varphi = NC\gamma$ changes. The behaviour of the "least monthly values" is shown in Fig. 2 for the range $-60° \leqslant \varphi \leqslant 60°$. (The azimuthal values in Fig. 2 were worked out without including a refraction or a parallax correction. These small effects are irrelevant to the present discussion.) The least monthly values oscillate with the period of φ, 18·61 years. By observing the azimuthal cycle, the period of φ can be determined with high accuracy by observing many cycles. At Stonehenge sighting alignments exist that would have suited such observations. With the periods of S, M and N known with reasonable accuracy the prescriptions follow immediately as approximate working rules.

Suppose an initially correct configuration for M, N and S is known. The prescriptions enable us to predict ahead what the positions of M, N and S are going to be, and thus to foresee coming events—but only for a while, because inaccuracies in our prescriptions will cause the markers to differ more and more from the true positions of the real Moon, Sun, and ascending node. The lunar marker will be the first to deviate seriously—the prescription gives an orbital period of 28 days instead of 27·32 days. But we can make a correcting adjustment to the M marker twice every month, simply by aligning M opposite S at the time of full Moon, and by placing it coincident with S at new Moon. The prescription for S gives an orbital period of 364 days, which is near enough to the actual period because it is possible to correct the position of S four times every year, by suitable observations made with the midsummer, midwinter, and equinoctial sighting lines that are set up with such remarkable accuracy at Stonehenge.

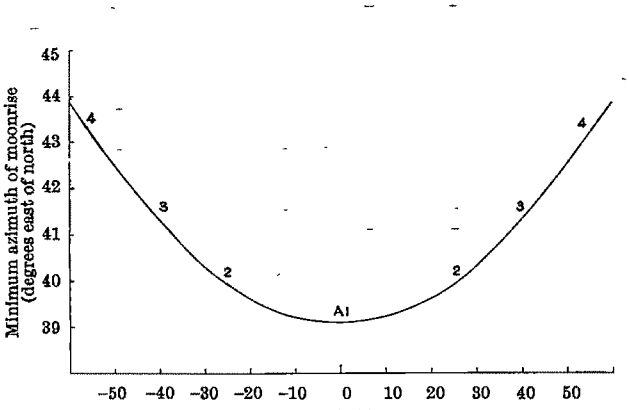


Fig. 2

Stonehenge is also constructed to determine the moment when $\varphi = 0$, that is, when N should be set at γ. The line C to $A1$ of Fig. 1 is the azimuthal direction for the minimum point of Fig. 2. By placing N at γ when the Moon rises farthest to the north, the N marker can be calibrated once every 18·61 years. The prescription implies only a small error over one revolution of N. If N started correctly, it would be out of its true position by only 1° or so at the end of the first cycle. The tolerance for eclipse prediction is about 5°, so that if we were to adjust N every cycle, the predictor would continue to work indefinitely without appreciable inaccuracy. The same method also serves to place N at the beginning.

But now we encounter an apparent difficulty. The minimum of Fig. 2 is very shallow and cannot really be determined in the way I have just described. Angular errors cannot have been less than $\pm 0·25°$, and even this error, occurring at the minimum of Fig. 2, is sufficient to produce an error of as much as $\pm 15°$ in φ.

The correct procedure is to determine the moment of the minimum by averaging the two sides of the symmetrical curve, by taking a mean between points 2, for example. The inaccuracy is then reduced to not more than a degree or two—well within the permitted tolerance.

What is needed is to set up sighting directions a little to the east of the most northerly direction. The plan of Stonehenge shows a line of post holes, $A1$, 2, 3 and 4, placed regularly and with apparent purpose in exactly the appropriate places.

The same point applies to solsticial measurements of the Sun. In summer the sighting line should be slightly increased in azimuth, in winter it should be slightly decreased.

Hawkins[1] gives two tables in which he includes columns headed "Error Alt.". These altitude errors were calculated on the assumption that the builders of Stonehenge intended to sight exactly the azimuthal extremes. The test of the present ideas is whether the calculated "errors" have the appropriate sign—on the argument given here "errors" should be present and they should have the same sign as the declination. In ten out of twelve values which Hawkins gives in his Table 1 this is so. The direction from C to the Heelstone is one of the two outstanding cases. Here the "error" is zero, suggesting that this special direction was kept exactly at the direction of midsummer sunrise, perhaps for aesthetic or ritualistic reasons. The other discrepant case is 91 → 94. Here my own calculation gives only a very small discrepancy, suggesting that this direction was also kept at the appropriate azimuthal extreme.

Negative values of the altitude error correspond to cases where it would be necessary to observe below the horizontal plane, if the objects in question were sighted at their extreme azimuths. This is impossible at Stonehenge because the land slopes gently upward in all directions. Such sighting lines could not have been used at the extremes, a circumstance which also supports this point of view.

It is of interest to look for other ways of calibrating the N marker. A method, which at first sight looks promising, can be found using a special situation in which full Moon happens to occur exactly at an equinox. There is evidence that this method was tried at Stonehenge, but the necessary sighting lines are clearly peripheral to the main structure. Further investigation shows the method to be unworkable, however, because unavoidable errors in judging the exact moment of full Moon produce large errors in the positioning of N. The method is essentially unworkable because the inclination of the lunar orbit is small. Even so, the method may well have caused a furore in its day, as the emphasis it gives to a full Moon at the equinox could have been responsible for the dating of Easter.

An eclipse calibrator can be worked accurately almost by complete numerology, if the observer is aware of a curious near-commensurability. Because S and N move in opposite directions the Sun moves through N more frequently than once a year, in 346·6 days. Nineteen such revolutions is equal to 6,585·8 days, whereas 223 lunations is equal to 6,585·3 days. Thus after 223 lunations the N marker must bear almost exactly the same relation to S that it did before. If the correct relation of N to S is known at any one moment N can be reset every 223 lunations; that is, every 18 years 11 days. The near-commensurability is so good that this system would give satisfactory predictions for more than 500 years. It requires, of course, S to be set in the same way as before. The advantage is that in the case of N it obviates any need for the observational work described above. But without observations the correct initial situation cannot be determined unless the problem is inverted. By using observed eclipses the calibrator could be set up by trial and error. This is probably the method of the Saros used in the Near East. There is no evidence that it was

used at Stonehenge. The whole structure of Stonehenge seems to have been dedicated to meticulous observation. The method of Stonehenge would have worked equally well even if the Saros had not existed.

Several interesting cultural points present themselves. Suppose this system was invented by a society with cultural beliefs associated with the Sun and Moon. If the Sun and Moon are given godlike qualities, what shall we say of N? Observation shows that whenever M and S are closely associated with N, eclipses occur. Our gods are temporarily eliminated. Evidently, then, N must be a still more powerful god. But N is unseen. Could this be the origin of the concept of an invisible, all-powerful god, the God of Isaiah? Could it have been the discovery of the significance of N that destroyed sun-worship as a religion? Could M, N and S be the origin of the doctrine of the Trinity, the "three-in-one, the one-in-three"? It would indeed be ironic if it turned out that the roots of much of our present-day culture were determined by the lunar node.

[1] Hawkins, G. S., *Nature*, **200**, 306 (1963).
[2] Hawkins, G. S., *Nature*, **202**, 1258 (1964).

STONEHENGE—A NEOLITHIC "OBSERVATORY"

By C. A. NEWHAM
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PROF. G. S. HAWKINS[1] has shown how Stonehenge could be regarded as a "computer" to predict the time when eclipses of the Sun and Moon were due, though not always visible at Stonehenge. He ingeniously relates the fifty-six Aubrey holes with a 56-year eclipse cycle. The principle is valid, but there is no evidence to support the idea that Stonehenge was intended to be a "computer" other than that the majority of main features embodied in the 'monument' unquestionably have some astronomical connexion. There are, however, a number of "post holes"; however, no satisfactory explanation has so far been put forward to explain their purpose.

An analysis of the position and number of post holes prompts a suggestion as to their purpose, and may well provide the clue to the method by which the builders of Stonehenge acquired elementary knowledge of Moon cycles, possibly including the approximate 56-year eclipse cycle.

Stonehenge post holes. Of the many post holes found in and around Stonehenge, there is one group of about forty holes situated in the "causeway" near the north-east entrance (Fig. 1). The holes seem to radiate from the centre of the Aubrey circle, and lie within a 10-degree arc north of the heelstone or solstice line. They are roughly arranged into six ranks crossing the line of the causeway.

A fairly reliable record of the behaviour of the Moon could be obtained by planting a temporary marker (wooden stake) to align on the point where the winter full-moon appeared above the horizon each year. The indications are such that this was done over a large number of years covering several 18·61-year cycles. Such a period would be sufficient to ascertain a 19-year phase or metonic cycle and possibly the approximate 56-year eclipse cycle as suggested by Hawkins (that is, $3 \times 18{\cdot}61 = 55{\cdot}83$). It should be appreciated, however, that they would have considerable difficulty in defining this eclipse cycle by their crude methods.

Starting from the premise that the post holes were used for the purpose suggested, the azimuth bearing of each post hole was first ascertained in rank order, numbered 1 to 6 counting in a north-easterly direction.

The number of holes attributed to each rank and their azimuth bearings taken from the centre of the Aubrey circle are given in Table 1.

Preliminary calculations reveal that the causeway post holes lie within the arc or sector of the most northerly limits of winter full-moon rise as seen from the centre of the Aubrey circle (C.A.C.); also, the number of holes in any one rank did not exceed the number of risings that could appear north of the heelstone or solstice line in any one cycle.

If individual bearings of moonrise in a sequence of cycles were found to agree with the "hole alignments", any doubt as to their significance would be eliminated; furthermore, dating of the post holes would be feasible. Unfortunately, the necessary data concerning the Moon are not available; to obtain such information would severely tax the ability of the most expert celestial mechanician.

Table 1

Rank No.	1	2	3	4	5	6
	–	–	(40·4)	(40·7)	–	(40 3)
	41·9	41·9	–	–	41·8	–
Azimuth	43·1	43·0	43·0	42·9	42·9	42·7
bearings	–	44·1	44·0	43·8	44·0	–
degrees	–	45·3	45·3	45·5	45·6	–
E. of N.	–	45·6	45·6	–	–	–
	46·3	46·4	46·5	46·3	46·4	46·4
	–	– *47·4	–	–	–	46·7
	–	47·8	47·5	47·2	47·6	47 5
	–	49·3	49·4	49·0	48·9	49·1
	–	49·8	–	–	49·8	–
	–	–	50·4	50·2	50·8	50 3

The figures in parentheses refer to holes beyond the line of full orb but are reached by the line of first gleam of moonrise.

* This hole is situated between ranks No. 2 and No. 3 and cannot be allocated to either rank.

However, computed moonrise bearings covering the period of 2000–1000 B.C. were first used in an attempt to determine whether any similarity existed between general grouping of the post holes and a sequence of moonrisings. The data used were kindly supplied by Prof. G. S. Hawkins. His calculations are based on first-order terms and refer to the full-moon nearest the winter solstice and also require that the instant of moonrise should coincide with the time when the Moon reaches its appropriate maximum declination. This particular feature seldom applies (see Fig. 2).

The pattern of risings that would apply to the early Stonehenge period is more truly represented in groups '*B*' and '*C*'. Even so, their arrangements could be subject to slight variations as many individual moons have possible alternative rising positions. This applies in those cases when the Moon reaches "full" during the daytime, for example, on December 12, 1875, the Moon was full at 0745 h (shortly before setting). In similar circumstances, would they regard the critical Moon as being that which rose on the afternoon of December 12, or that of the following day? If midnight was their datum, the former Moon would apply. On the other hand, it would be the latter if the critical time was on its rising. A similar condition arises in respect of those Moons which are full

14 or 15 days before the solstice and would be followed by the next Moon 14 or 15 days after the solstice.

There is no question that people of early civilizations had to contend with the same difficulty which was partly obviated by choosing the critical Moon as the one which was full after a specified time, such as the day of the winter solstice or equinox. In the second millennium B.C. the Delians regarded the critical Moon as the first one that was full after the winter solstice, and the possibility that such was the case with the Stonehenge people cannot be overlooked. However, the remarkably close relationship of the moonrise sequences and hole patterns must surely be more than a coincidence. If this suggested method of observation had been carried out by the Stonehenge people, they would have had little difficulty in recognizing the 19-year phase or metonic cycle.

It seems fair to assume that these and other post holes served a purpose in obtaining preliminary information of the behaviour of the Sun and Moon. Once satisfactory alignments had been established, markers of a more permanent kind would be installed. If the foregoing assumptions were correct, then the long period of time that elapsed between the inception of the bank, etc., and the building of the first major stone structures would be accounted for.

The position and spacing of the four large post holes near the heelstone indicate a relationship with the causeway holes, especially so in conjunction with stone '*D*' and the heelstone. The size of the holes indicates that the posts were much larger than the causeway posts and, presumably, more permanent. If stone '*B*' were also included, then the seven markers which could be observed would act as a crude 'vernier'. When correlated with the setting Sun seen in the reverse direction, it would provide a means of defining the time when an eclipse of the Moon was probable.

Whether or not Stonehenge people discovered the 56-year cycle is a matter for conjecture, but their ability to predict pending eclipses after a crude fashion was certainly possible.

It can be shown that a person assisted by nothing more than the crudest equipment in the form of a peg and line and a few stakes could acquire the necessary information. The observer, however, would have to have the necessary tenacity of purpose to undertake systematic observations over a long period of time and a simple method of recording numbers by means of tokens, for example, notches on a stick or pebbles in a bag.

Doubtless, several generations of observers were involved, and if we are to believe that the writings of Diodorus referred to Stonehenge, then the position of supervisor of the "Spherical* Temple" was held by a member of the same family from one generation to another.

The strong lunar influence with which Stonehenge must now be associated necessitates revision of hitherto

* Early Greek term for "Astronomical". Heath: *History of Greek Mathematics.*

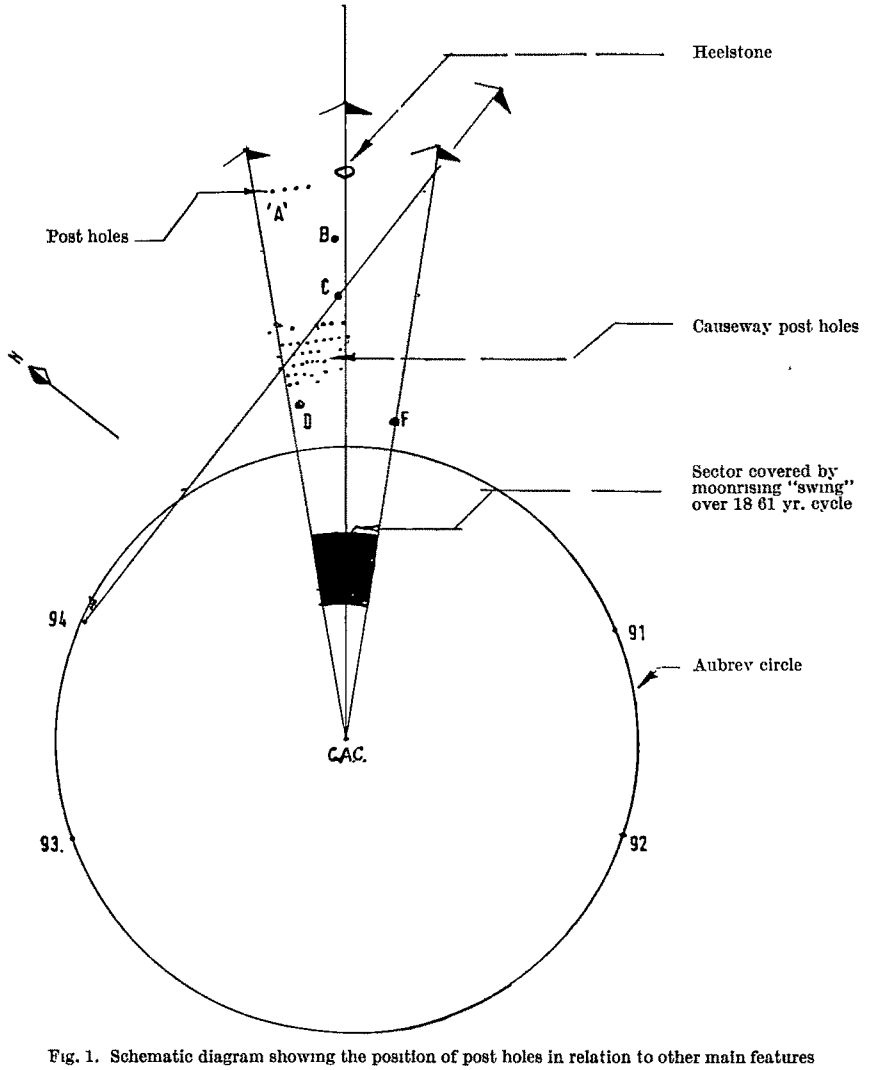


Fig. 1. Schematic diagram showing the position of post holes in relation to other main features

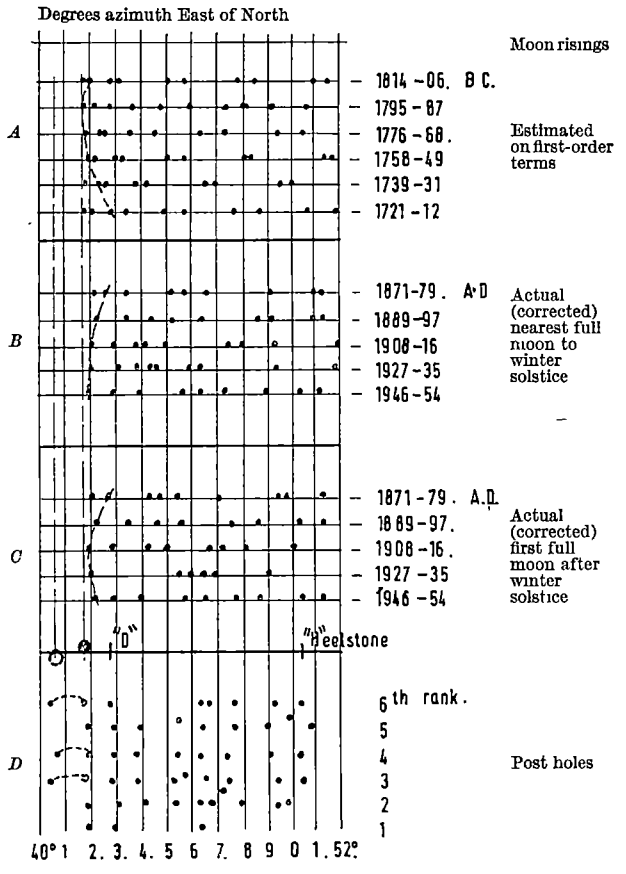


Fig. 2. Comparable azimuth bearings of winter full-moon risings. (Full-orb tangent to horizon) and post hole alignments

Group '*A*': Six cycles of moonrisings based on first-order terms as supplied by Hawkins for period 1814–1712 B.C.

Group '*B*': Similar to '*A*' but the "Moons" correspond to the known rising position covering the period A.D. 1871–1954 after correction to allow for the change in the angle to the ecliptic of the Earth–Moon system over the past 3,800 years

Group '*C*': Basically similar to '*B*', but here it applies to the first full-moon after the winter solstice

Group '*D*': Depicts the post holes arranged according to their azimuth bearings

The data connected by broken lines are all separated by a period of 6,940 days or exactly 19 years within a few hours, and differ from other similar Moons in that their rising positions are practically the same. The broken lines in group '*D*' merely indicate the position the three holes would occupy if they were aligned on full orb. The two larger circles immediately above represent the most northerly position of first gleam and full orb of moonrise. The positions of stone '*D*' and the heelstone are indicated on the same line

accepted explanations of some salient features. Until new evidence is found pointing to the contrary, it is more logical to conclude:

(*a*) That the small stone (No. 11) in the sarsen circle was intentional, and that the circle represented the 29·5 days of the lunar month.

(*b*) The double circle or spiral[2] of the '*Y*' and '*Z*' holes represented the 59 days of two lunar months. The strong possibility that there were fifty-nine blue stones inside the sarsen circle would provide a more suitable means of representing the same thing.

(*c*) The 19-year phase or metonic cycle was represented by the nineteen blue stones inside the trilithon "horse shoe".

All things considered, including other similar post holes, there seems little doubt that Stonehenge, in its early stages, was a kind of "observatory". It provided a suitable site wherein systematic observations of 'Soluna' (Sun and Moon) phenomena were carried out by these neolithic peoples.

[1] Hawkins, G. S., *Nature*, **200**, 306 (1963); **202**, 1258 (1964).
[2] Sale, J. L., *The Secrets of Stonehenge* (private publication, 1965).

BOOK REVIEWS

NEW CONCEPTS FOR OLD

Science, Folklore and Philosophy

By Harry Girvetz, George Geiger, Harold Hantz and Bertram Morris. Pp. xi + 547. (London and New York: Harper and Row, Publishers, 1966.) 72*s*.

THE authors of this volume seem to have run up against a common difficulty for teachers of philosophy, that of justifying to their pupils the seemingly abstract and recondite studies included in the philosophy curricula. Why study methodology, why do conceptual analysis? And in particular why read Aristotle on substance, or Locke on qualities, or Austin on performative utterances? In this book there seems to me to be exactly the right answer. It is that the growth of science has thrown up, from time to time, new ranges of phenomena which have required new concepts and systems of concepts for their understanding. The system with which the discoverers were equipped (called by the authors the "folklore") broke down in the face of the new situation and a new system was painfully forged to accommodate it. They see philosophy as the activity of forging (by critical analysis) new conceptual schemes. Their answer then to their pupils and to all who cannot see the point of philosophical studies is that philosophical issues have had to be resolved in each of the great periods of conceptual change initiated by scientific advance (or *vice versa* of course) and that the resolutions that were made by the Greeks, by the British empiricists and Descartes, by the post-Darwinians, and which are being made these days in the philosophy of mind and of behaviour, have created the system of concepts which we now use. In short, to understand our present science we operate with a system of concepts and methods of reasoning forged in the past and carelessly acquired in the present. We should understand the reasons why we have adopted in our culture the intellectual machinery that we have.

The project is quite admirable in my view and the plan of the book excellent. It is distressing then to have to say that the detailed execution of the plan leaves a great deal to be desired. There are a great many historical errors, some minor, for example that "Tycho's view [of the structure of the solar system] was too clumsy to be entertained", when that very view was not only entertained by some of the first mathematicians of Europe, such as Ursus, but was the most widely taught in the first half of the seventeenth century. There are also major errors. The most remarkable of these is the contention that science and religion parted in about 1600, their divorce being pronounced by Francis Bacon. The authors can have had very little conception of the attitude to Nature of the circle of Boyle, Locke and Newton. Indeed there is a general failure to get the historical settings quite right. Nevertheless the book has great value in showing, even in a rather unsatisfactory way, how the great philosophical problems were generated, and the place of their resolutions in our conceptual scheme.

R. HARRÉ

AN INSPIRED EXPERIMENTER

Collected Papers of P. L. Kapitza

Edited and with an introduction by D. ter Haar. Vol. 1: 1916–1934. Pp. xvi + 503 + 31 plates. 140*s*. net. Vol. 2: 1938–1964. Pp. viii + 505–993. 140*s*. net. (London and New York: Pergamon Press, Ltd., 1964–65.)

THE collection of papers under review makes fascinating reading: I have found browsing through them an enjoy-

able and educational experience. It is interesting to compare Kapitza's collected papers with those of Landau, recently issued by the same publisher, for the two men can be regarded as occupying comparable positions with respect to experimental and theoretical physics in Russia. The Landau collection occupies only one volume and contains a surprisingly large number of papers that one would read nowadays for their scientific content. While some of the Kapitza papers one still reads for their scientific content—most notably the series on superfluidity of helium—the interest of the majority is in the light they throw on the development of physics and on the character of a remarkable physicist. One can find resemblances both to his teacher, Rutherford, and to Brunel. In his love for and skill in experimental physics, and in his feeling for the important simple features of his results he resembles Rutherford. But he is also to a considerable extent an engineer, and shows something of the inventiveness, the feeling for the grandiose, and the forcefulness of Brunel. One feels that Kapitza's wide-ranging abilities and interest in practical applications would have been more at home in the Royal Society of the seventeenth century than the twentieth.

The two volumes under review neatly separate Kapitza's Cambridge period, up to 1934 (together with a few early papers from Leningrad), from his Moscow period. Kapitza came to Cambridge in 1921 and secured acceptance by Rutherford by his now famous lesson in orders of magnitude repeated in Dr. ter Haar's introduction. His work on α-particle tracks soon led to an interest in producing high magnetic fields, and his skill in orders of magnitude is very apparent in his comparison of different forms of energy storage for the production of impulsive fields, making these papers an object lesson in the design of apparatus. Kapitza's impulsive 300 kG was to stand as a record for many years, and he rapidly exploited fields of the 100 kG order in numerous measurements of magnetoresistance and magnetostriction. But Kapitza not only produced and used high fields, he also succeeded in getting the Royal Society Mond Laboratory built for his work, and built the way he wanted it—a very Brunel-like achievement. It was his decision to equip this laboratory with low temperature facilities that led to his ingenious design for an expansion engine liquefier for helium, avoiding the use of liquid hydrogen, which has proved of immense importance for low-temperature technology. Not only have large liquefiers in the U.S.S.R. descended from it, but also the Collins liquefier which has so transformed low-temperature physics since the Second World War and the giant 100 l./h liquefiers at Texan oil wells.

On Kapitza's return to Russia he created a similar laboratory (even to the marble switchboards) at the Institute for Physical Problems in Moscow, and almost at once began his pioneering work on the superfluidity of helium. Of these papers, one can select two as showing quite exceptional ingenuity and insight. In the first, before Landau's theory, he showed that heat flow from a bulb of helium is accompanied by a jet capable of moving a vane, even though the whole apparatus is full of liquid. It is interesting that he was led logically to this experiment by the idea that negligible viscosity (which he had already observed) would lead to extremely easy convective transfer of heat, and he thus came near to deducing the two-fluid model from experiment. The second experiment, after Landau's theory, was the first quantitative evidence for the two-fluid model; in a simple but ingenious experiment Kapitza measured the thermomechanical effects associated with superflow through a narrow slit. These two classic papers are required reading for anyone in the field, and it is good to have them readily available in English.

After the Second World War, Kapitza turned his attention to high-power microwave engineering. I myself have no knowledge of this field, but I was impressed by the technique Kapitza developed for calculating the essential features of the motion of electrons in electric and magnetic fields (also applied by him to the problem of a pendulum with a rapidly oscillating support, which led to a typical Kapitza demonstration experiment), and by the ingenious experimentation which accompanied these calculations. These papers (and also a few others) were specially translated for the present volumes; it is to this, no doubt, that we owe the addition to the vocabulary of microwave plumbing of the "sewerage" of microwave power. This latest work of Kapitza's is in a sense a return to his earliest. It involves the same type of original electrical engineering, and shows the same delight in taking things to extremes, as his work on high magnetic fields.

The brief introduction by Dr. ter Haar gives a useful summary and classification of the papers, but little more. A larger introduction giving much more biographical information would have considerably enhanced the interest and value of the volumes. As it is, many questions, such as the enigma of Kapitza's wartime eclipse, remain unanswered, as well as a number of fascinating sidelines—for example, the genesis of the paper on the nature of ball lightning.

At £7 per volume the books are expensive, even for collected papers, and few but libraries will afford them. Every science library should buy them, and they should be widely read. The books are well printed and open comfortably, but at the price the publishers should have managed a better cloth binding. H. E. HALL

THE IONOSPHERE *IN EXTENSO*

Physics of the Earth's Upper Atmosphere

Edited by C. O. Hines, I. Paghis, T. R. Hartz and J. A. Fejer. Pp. xiv+434. (Englewood Cliffs, N.J., and London: Prentice-Hall, Inc., 1965.) 105*s*.

To the scientist the "upper atmosphere" means broadly that part of the Earth's envelope in which physical processes are profoundly affected by the presence of charged particles, and are therefore intimately associated with the fields of electromagnetic waves and the Earth's magnetism.

Effective study of the upper atmosphere began some 40 years ago with the discovery of the ionosphere by radio sounding, and much of the early work was concerned with problems of radio communication. More recently, through the extension of observational techniques by means of rockets and satellites, the study has developed into a major discipline which embraces an astonishing variety of interlinked phenomena. There are, of course, reasons in addition to scientific interest for the scale of the present research effort, but it is to the purely scientific aspects of the subject that this book is devoted.

A wide range of topics is covered. Details of theory, and of the results and methods of observation, are generally omitted. There are plenty of diagrams, but, as a phrase from the dust jacket puts it, emphasis is on descriptive physics The account is none the worse for that. Indeed, it is hard to imagine a different approach serving better either the student just entering the field, whom the editors are particularly anxious to help, or the specialist in some part of the discipline looking for an authoritative guide to the wider implications of his work.

The various sections are written by experts in each topic. Nevertheless, the whole is remarkably well knit, and the level of exposition uniformly high; of the battle to reconcile inevitable differences over the selection and presentation of material, lightly touched on in the preface, no scar remains.

Roughly, the first half of the book is concerned with so-called "normal" or "undisturbed" conditions, and the second half with "disturbed" conditions. In this context a disturbance in the upper atmosphere means an effect

caused by a disturbance on the Sun, so that the later chapters contain a good deal about the part played by solar emission of particles or radiation.

After an introduction, there is a chapter on the solar optical radiation that affects the ionosphere below 120 km, and then, successively, chapters on the *D* and *E* regions of the ionosphere, on the *F* region, and on the outer ionosphere. The latter includes a review of the motion of charged particles in a magnetic field, prefacing a discussion of the Van Allen belts, those regions where energetic charged particles are trapped in the geomagnetic field. Next, windlike motions are considered, first of the neutral atmosphere, and then of ionization; the main topics here are tidal oscillations and smaller scale gravity waves (the only purely gas dynamic phenomena treated in the book), and the "dynamo" theory of the diurnal variations of the geomagnetic field. Discussion of the undisturbed atmosphere ends with a chapter jointly on ionospheric noise, that is, audio-frequency radio emissions which produce the delightful medley of sound known as "dawn chorus", and on geomagnetic micropulsations.

Disturbances on the Sun are divided into those emitting ultra-violet and X-radiation, and those emitting charged particles. The former give rise to "sudden ionospheric disturbances", and are dealt with in a single chapter. The latter are thought to be the origin of numerous effects, in particular, polar cap absorption, magnetic storms, and aurorae; and the discussion here occupies a further six chapters. Of these the final one, on storm theory, is an enlightening account of the different suggestions that have been put forward.

The two appendixes give some fifty pages on aspects of electromagnetic wave propagation. They might, perhaps, have been linked a little more closely to the main text. For example, they contain no reference to Faraday rotation, quoted earlier as giving a measure of electron content.

The authors rightly emphasize the incomplete state of knowledge in many of the topics under discussion, and throughout strike a fair balance between rival theories, not a few of which have been contributed by one or other of their company. Their book is to be highly recommended to a wide class of readers. P. C. CLEMMOW

SILICATE ANALYSIS AT THE BENCH

Methods of Silicate Analysis

By H. Bennett and W. G. Hawley. Second edition. Pp. xv+334. (Published for the British Ceramic Research Association.) (London and New York: Academic Press, 1965.) 63*s*.

FOR many decades there was little change in the methods of silicate analysis. Standard works such as Hillebrand and Lundell's *Applied Inorganic Chemistry*, itself based on an earlier text, served a generation of analysts. With the development of instrumental methods and the introduction of new reagents a move was made to introduce simplified methods of silicate analysis. The older, generally applicable, systematic schemes of gravimetric analysis, often lengthy and involved, were replaced by rapid direct determinations using spectro-photo-colorimetric and complexometric methods. Although this process was often accompanied by considerable loss of precision, the fresh impetus given to the subject induced many workers in the field to review methods generally with the result that many of these were simplified, often with a gain in accuracy.

Although this branch of analysis is now progressive, the once neat pattern of analysis has disappeared, so that these new simplified, but often restricted, methods of analysis exist side by side with the "classical" general methods of analysis. The task for the compiler is therefore not easy and for some time there has been a need for a critical survey of the variety of methods scattered in reference books and original literature. Bennett and Hawley, in a new and much improved edition of their work, *Methods of Silicate Analysis*, have gone a long way towards effectively summarizing the present state of silicate analysis, especially in the context of ceramic materials. The title is perhaps slightly misleading, for although materials which are not silicates but are of ceramic interest find a place in this work, certain silicates do not. The work is, in fact, directed primarily at the industrial ceramic analyst rather than the silicate analyst, although it will prove useful to the latter.

The book falls into three sections. Part I is introductory and is followed by a discussion on the basic principles behind the theory and practice of silicate analysis (Part II). The main section (Part III) deals with the analysis of materials; full practical details are given, but general comment and discussion are placed separately in Part II. This makes for easy reading of the practical text. The authors have not hesitated to duplicate material to avoid the necessity for cross-references which would be tiresome and out of place in a work clearly designed to be used at the bench. Chapters in this section of the work are classified on the basis of the material analysed and the general analytical approach adopted. The authors attempt to provide different schemes for different analytical requirements; these depend on the apparatus available, the accuracy required, and the material being analysed.

Materials discussed are those which have some bearing on the ceramic industry such as siliceous and aluminous materials, carbonates, refractories, frits, glazes, etc. Methods described include the generally applicable classical schemes of analysis, accurate direct determinations, and rapid gravimetric, volumetric and spectrophotometric procedures. There are chapters devoted to the analysis of alkalis, the determination of fluorine and to methods connected with the solubility and resistance of materials toward aqueous and acid attack.

The text is thoroughly reliable, for it is abundantly clear that a considerable amount of personal practical experience has gone into this book, and its use should steer an analyst clear of those hidden pitfalls of practical silicate analysis. Although it emphasizes ceramic analysis, the book should prove of value to all silicate analysts provided they are aware of the limitations of certain specialized methods. It is thoroughly to be recommended.
A. D. WILSON

CATIONS AND CATALYSIS

Friedel–Crafts and Related Reactions

Edited by George A. Olah. Vol. 1: General Aspects. Pp. xxxiv+1031. 220*s*. Vol. 2: Alkylation and Related Reactions. Part 1: Pp. xxix+1–658. Part 2: Pp. xxv+659–1362. 378*s*. the two parts. Vol. 3: Acylation and Related Reactions. Part 1: Pp. xxvi+1–910. Part 2: Pp. xxii+911–1606. 450*s*. the two parts. Vol. 4: Miscellaneous Reactions; Cumulative Indexes. Pp. xvii+1191. 340*s*. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1963–65.)

ONE of the ways which is in use at present to render down to digestible proportions the vast mass of chemical information is the publication of multi-author books on a wide theme. Each specialist then selects and presents his own considerations of what he considers to be significant. This procedure, if it is to be successful, requires a firm and wise editor who must be able to obtain co-operation from his authors and who must possess the vision which will ensure that the theme is well served.

Dr. George Olah has presided over the production of a magnificent work on, essentially, catalysed cationic reactions. *Friedel–Crafts and Related Reactions* is the combined effort of fifty contributors from nine different

countries. Half of these, including the editor himself, hold industrial positions; some have international reputations while others were unknown to me. As the net has been very widely cast in terms of the topics to be dealt with, the writers are by no means restricted to organic chemists.

The book, and it is to be considered as a whole, is immense. There is a total of 5,189 pages, of which 870 in Volume 4 are given over to the subject and author indexes. It has been published in parts from 1963 to 1965 and it is a tribute to Olah's editorship that such a large work should contain references as late as 1963. No claim for complete comprehensiveness is made and many references to previous reviews are given.

One might expect that, with such a wide diversity of contributors, the style of the book would be uneven. Surprisingly, this is not generally so. With few exceptions, the very variegated subject matter is dealt with in a remarkably cohesive manner. Further, a book of this size might be expected to present its own retrieval problems. These have been eliminated by carefully cutting each chapter into sections and providing very detailed plans of contents. These enable the reader to pick out a specific topic very readily and encourage the luxury of browsing. The subject index is also very detailed and provides references not only to conceptual material in the text but also to the thousands of individual compounds which appear in tables throughout the book.

Readability and overlap are two further problems in a book of this type. The first has largely been overcome by separation of the 'catalogue' material into tables apart from the text, so as to leave behind discussion of general principle and topics of specific controversy. Those chapters which conform to this pattern are best suited to the aims of the book; the others are, quite frankly, dull. Some overlapping is bound to occur in a multi-author treatment of a broad theme. There is a good deal in this book particularly with respect to discussion of mechanism, but at least the reader is spared from chasing cross-references to discussions in another part of the book. The amount of detail provided for the various topics is inevitably variable. The chapter in Volume 2 on alkylation with haloalkanes, which one might regard as the fundamental reaction, is disposed of in sixty pages. Perhaps this is all it deserves.

Volume 1 deals with general aspects. A very interesting historical introduction includes photographs of the laboratory notebooks of the originators who produced the three original papers in the summer of 1877. This is followed by Olah's own review of the entire field in which the scope of the book is defined. Olah himself contributes to eleven of the fifty-four chapters, and these are all extremely readable, critical and informative. The other chapters deal with inorganic catalyst systems, investigations of reaction mechanisms by spectroscopic and isotopic methods, and considerations of stereochemistry, selectivity and thermodynamics. The last provides a very timely reminder of fundamental principles for the less numerical reader. This volume is the only one which can well stand apart from the set. It presents the basis for the remainder and provides an excellent introduction to them.

Volume 2, in two parts, deals with alkylation. The coverage is very wide, and in addition to the well-known reactions with olefines, halides and carbonyl compounds, embraces dehydrogenative coupling and isomerization of hydrocarbons, together with consideration of the Prins reaction, hydrogen exchange and cationic polymerization. The treatment is now, of course, much broader than in Volume 1. The recitation-of-lists approach is more common and in some chapters the inclusion of practical details in the text is overdone. Pitfalls of structure numbering have not always been avoided: in Chapter 22, the Roman numerical horror CCCLXXXVIII appears. A chapter on alkylation of saturated hydrocarbons is heavy going because of its large amount of technical detail and sometimes unusual English. The reviews of hydrocarbon isomerization, alkylation with alcohols and ethers, and of hydrogen exchange are particularly interesting. The last of these could, with advantage, have been placed in Volume 1.

Volume 3, also in two parts, covers acylation and related reactions. 'Related reactions' again covers a wide range including sulphonation, halogenation and nitration. Within the theme of the book it is right that they should do so. The more conventional Friedel–Crafts acylations are dealt with in two enormous chapters by Gore and by Peto which occupy most of the first part. Gore's chapter alone contains two hundred pages of tables and two thousand references. As in the rest of the book, careful attention is paid to the pathways and mechanisms of the reactions involved. Stimulating pointers are given to the areas where further effort is required against the background of a vast amount of observation already made. Olah again displays his gift for interesting reviewing in the chapters on formylation and nitration, both of which include accounts of his own important work. A chapter on the curious perchloryl-ation reaction gives much practical information in this new field.

Volume 4 deals with eight miscellaneous topics. Olah again contributes—this time on metathetic cation-producing salts—and gives tabular surveys of this rather specialized field. The chapter dealing with ethers includes a valuable review of epoxide polymerization, and the strange reactions between carbohydrates and aromatics are also allotted a chapter. Reactions with organometallic compounds (Russell) comprise one of the topics farthest from the general theme of the work. Olah rounds off in a chapter dealing with technical applications (mandatory for academics) and future outlook. That the outlook is bright is due in no little measure to his own contributions. This volume also contains the very large cumulative author and subject indexes.

On the whole, the printing of formulae and text is good and errors trivial. One might perhaps have expected a stronger binding on books that will be much used for reference and which are as expensive as these. Contemporary book reviews frequently finish with a complaint that the price is far too high. This work costs, in all, £69 8*s*. This may be thought excessive. On the contrary, because of its wide breadth of coverage, its extremely detailed indexes and contents pages and, above all, because of the almost uniformly high quality of its contents, this book provides a direct entry to this highly topical field. The saving of time devoted to inefficient searching of the literature alone makes this book worth its cost. After all, only about one hundred hours of searching time need to be saved to pay for it.

The book is dedicated to the memory of Charles Friedel and James Mason Crafts and to the investigators who have followed them. It is a fitting tribute.

C. J. M. STIRLING

SEMICONDUCTING MATERIALS

Materials Used in Semiconducting Devices

Edited by C. A. Hogarth. Pp. vii + 243. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, Inc., 1965.) 105*s*.

THIS book consists of a series of articles, each by an author with experience of the particular material, reviewing the properties of several semiconductor materials which find extensive application in commercial devices. A notable omission is that of gallium arsenide; the editor states that the rapid technological development of this material would make any article out of date before it was published. In view of the time taken to publish this book, this view is perhaps reasonable. The article on selenium has no references to work later than 1962,

those on germanium, silicon and bismuth tellurides only one reference each later than 1962. The article on the lead compounds is brought up to date by an appendix; the articles on the antimonides are otherwise the only ones which are up to date as written. It would appear that the main part of the book has undergone a gestation period rather longer than that for an elephant.

Each article is roughly similar in layout; an introduction is followed by a section on methods of preparation, purification, crystal growth and doping. General properties, which include crystal structure, density, and thermal and elastic properties, are next discussed. The band structure is described and the resulting transport and optical properties are listed. The properties of alloys may then be given, and finally some comments on applications of the materials to devices. Mechanical properties, which are presumably of interest to device engineers, are completely ignored except for a very brief mention in the article by Putley, which is also the only one to give consideration to the making of contacts. Nowhere is there any serious attempt to explain the theory of semiconducting materials.

It is generally considered unfair to criticize a book for not being what its author never intended it to be. In this case, however, it is extremely difficult to determine the purpose of this book. Its lack of theory renders it useless as a text-book for students. If it is intended as a guide to the device engineer in selecting suitable materials for his devices, then a summary of the properties of all the materials in tabular form would be an invaluable aid, particularly for comparison purposes. To be really helpful to him, the sections on applications could also undergo a considerable expansion. There is such a lack of detail with regard to techniques that only someone who is already experienced with semiconductor materials would find it of use, perhaps when switching his attention to, to him, a new material. The collection of so much information into one volume is to be welcomed, but a division by properties and applications rather than by materials would be more appropriate.

The book is pleasingly produced and is very well indexed.

DAVID DEW-HUGHES

MECHANISMS OF MIND

The Nature of Psychology

By Kenneth J. W. Craik. Edited by Stephen L. Sherwood. Pp. xx+184. (London: Cambridge University Press, 1966.) 30*s*. net: 6 dollars.

KENNETH CRAIK, had he lived, would by now have been recognized as one of the most original and versatile psychologists of his generation. This selection from his unpublished writings, introduced by a brief biography from Sir Frederic Bartlett, should be welcome to all who knew him and highly instructive to those who did not. Born in 1914, a classical scholar at the Edinburgh Academy, and a student of philosophy under Prof. Kemp Smith, Craik was eventually attracted to psychology by the lectures of the late Prof. Drever. After graduating in that subject, he moved to the Cambridge laboratory to embark on a series of experimental researches on the problems of sensation. Besides attending the recognized classes on psychology, we are told, he went off to the Cambridge Technical School to take a course in engineering, and then under Lord Adrian's guidance steeped himself in the anatomy and physiology of the brain.

In 1940 he obtained the degree of Ph.D., and a year later a Fellowship at St. John's College with a thesis on "The Experimental Study of Visual Adaptation". The bibliography of his published work runs to eighty numbers —an output of almost eight papers every year. During the Second World War, so Prof. Bartlett relates, "he became a key man in the scientific service of his country". He was chairman of the Target Tracking Panel of the Ministry of Supply, and an active member of the Panel on Servo-mechanisms, of the Military Personnel Research Committee, and of the Vision Committee for the Flying Personnel Research. For security reasons, it has unfortunately been impossible to publish any of his official reports. In 1945, on the eve of V.E. Day, while cycling along King's Parade, he was knocked off his bicycle and killed by a passing car. In describing what happened the editor and his collaborators observe that they "have come upon about ten recorded presentiments of the circumstances of his lethal accident".

Of his numerous manuscripts, the earliest that survives is apparently an essay on "Immortality", written when he was only 17. Prof. Bartlett quotes a couple of short extracts, but it seems a pity it was not included in the present volume. The selection begins with "the epilogue from the Ph.D. thesis", and then proceeds to a couple of draft chapters and several isolated notes for a book on "The Mechanism of Human Action". The next two sections include reports of "experimental work" (for example, observations on a prolonged monocular scotoma, the effect of boldly fixating the noonday Sun for two minutes) and suggestions for the "quantification of sensation". Of the four papers that make up the third section—on "Aspects of Psychology"—the most noteworthy is a lecture on "Psychology for Medical Students"; this brings out with a shrewd impartiality both the weaknesses and the strong points of all the different schools, and stresses the need for an eclectic text-book of psychology specifically for the student of medicine. What he has in mind, he tells us, is a book which would omit all that is unnecessary for the practising physician and concentrate solely on topics of direct utility.

Here, as elsewhere, Craik fully recognizes the value of introspective techniques; but in almost all these papers his aim is avowedly to think first of the human mind as functioning through the mediation of brain-mechanisms. And throughout these selected excerpts, which, it must be remembered, were written more than 20 years ago, and in the ingenious "brain-models" which he describes, he reveals a remarkable anticipation of the later contributions made by cybernetics and information theory. The "standpoint of the electronic engineer" is equally prominent in his only book (on *The Nature of Explanation*) and in the last of his papers, the two published posthumously on the "Theory of the Human Operator in Control Systems".

The collection ends with a section headed "Philosophy", consisting of a dozen short papers dealing with the purely mechanistic approach. Of these perhaps the most illuminating is his reply to the criticisms urged by W. H. Barnes in reviewing his book in this journal ("Is the Mind a Calculating Machine?" *Nature*, 153, 603; 1944). Here he reverts once again to the subject of his youthful essay, and explains that his intention was "to pursue the physiological and mechanistic approach" as the most fruitful and profitable line available at the moment, and to "convey its relevance to the body–mind problem, to the explanation of thought and behaviour, and to the immortality of the soul". This fascinating set of papers, thirty-five in all, reveals the many-sided interests of Craik himself and the brilliant promise of his early work—a promise which unfortunately was never to be fulfilled.

CYRIL BURT

Dictionary of Nuclear Physics and Nuclear Chemistry

German – English/American; English/American – German. Second revised and enlarged edition. Edited by H. Rau. Pp. 351. (London: Sir Isaac Pitman and Sons, Ltd., 1964.) 55*s*.

THIS dictionary, though considerably enlarged from its first edition (*Dictionary of Nuclear Physics*, published

by Brandstetter, 1957), still gives the impression of a miscellaneous collection of words and phrases *aus der Praxis für die Praxis* (to quote the German preface; no corresponding phrase appears in the English preface). The terms in each half number about eight thousand, according to the preface, occupying some 130 fairly small pages. In addition to a reasonable selection of terms to be expected from the title, many expressions are included which have no direct relevance to nuclear science. For example, on page 335, *thread microscope, threaded sleeve, three-finger rule, throw-over switch, thymene* appear. On the same page is the extraordinary entry *time for scattering of an electron by distant encounters with ions*. Surely these entries, evidently *aus der Praxis*, are of little use *für die Praxis*. Again, '*Modell* (1 : 1)*/mock-up*' is all too obviously a reversal of the entry under *mock-up*, and indeed this seems to have been the method by which the two parts of the dictionary have been built up, every entry having its counterpart in the opposite direction.

Many terms are annotated or explained, mainly in German, in the same way in both parts. Genders are given. Obsolete or uncommon terms abound: *celtium, Chariot project* (but *Plowshare* is not given), *Contracid* (but not *Zircaloy*), *cooky* (but not *cobalt bomb*), *cubo-cubocube* (but not *power function*); *cathode ray tube* is rendered as 'Kathodenstrahlröhre' but not as the equally common 'Braunsche Röhre'. There are several misprints and one apparent misapprehension, *dyadic product* being rendered as 'dyadisches Erzeugnis'.

One good feature is the list of some fifteen hundred annotated symbols and abbreviations, including many acrostics and chemical formulae, though again one wonders at the inclusion of 'ACS' (American Chemical Society) and of 'ACTION' (American Council to Improve Our Neighborhoods), the latter with its postal address. The book ends with three pages of conversion factors.

J. B. SYKES

Agricultural Insects of Ghana

Compiled by John Forsyth. Pp. 163. (Accra: Ghana University Press; London: Oxford University Press, 1966.) 35*s*. net.

THIS is not a book for those desiring a narrative account of the insect pests of crops in Ghana, since the information is entirely in the form of lists of names, but, apart from its local use, it will be of great value to those dealing with the distribution of plant pests and their natural enemies in Africa or the pest fauna of particular crops, since it provides a compilation of authentic records of the kind that these require. It is much to be hoped that Mr. Forsyth's example will be followed in other countries.

The work is divided into five main parts, dealing with field crops and plants, stored products, parasites of plant-feeding insects, predators of plant-feeding insects, and insects associated with or attacked by fungi. Each part consists of two complementary lists, so that, for example, in the first and main part there is a list of the various plants showing the insects that attack them, and then a list of the insects arranged in their orders and families, showing the plants attacked by them, the plant part concerned and some details of local distribution. The lists are based on records dating back to 1909, and there is a useful bibliography of sources containing references to the appropriate abstracts in the *Review of Applied Entomology*, where further details can be found.

A good deal of care has been taken to see that the scientific names used are those accepted by modern systematists, and there are commendably few misprints, but a few minor inaccuracies have inevitably crept in, notably in the part on stored products. This part, however, contains something that the others do not, namely, an indication of which pests are the commonest.

R. F. AVERY

MEETINGS

CONDENSATION NUCLEI

SINCE the discovery of condensation nuclei by Coulier (1875) and by Aitken (1881) they have been of great interest to physicists and meteorologists. In recent years, with increasing awareness of atmospheric pollution, both chemical and radioactive, atmospheric chemists and health physicists have been drawn into the field and the resulting interdisciplinary exchange has proved to be very fruitful. This was exemplified at the International Conference on Condensation Nuclei held during May 9–13 at Albany, N.Y., and State College, Pa., which was the sixth of a series started by the late Prof. L. W. Pollak in 1955 at the Dublin Institute for Advanced Studies. More than eighty scientists from twelve countries attended and fifty papers were presented. Abstracts were available before the meeting and, with the exception of the invited review paper, presentations were limited to ten minutes to enable authors to stress the main points of their papers and an hour and a half was allowed for discussion of each group of five or six papers. This arrangement proved very successful and throughout the conference discussion was lively and sustained.

At its Albany session the conference had the honour of being addressed by the Governor of the State of New York, Mr. Nelson D. Rockefeller, who gave an impressive account of the measures being taken in the State to determine the causes and counteract the effects of environmental pollution.

In a review of recent work on small ions and Aitken nuclei (particles smaller than 0·4μ diameter), W. J. Megaw (Harwell) quarrelled with the view that such particles are of little importance in the atmosphere because they contribute little to the mass of atmospheric aerosol and therefore cannot act as centres of condensation at atmospheric super-saturations. The small ions were responsible for the important electrical properties and the Aitken nuclei, because of their size, tended to remain airborne for long periods. It had been shown that most of the natural radioactive decay products were carried on the Aitken nuclei and there was some evidence that they could act as carriers of radioactive effluent. Megaw described recent British and French work on small ions and concluded that, even after 50 years, little was known of the nature and constitution of the so-called "cluster ions". He stressed that care was needed in the use of radioactive tracers of high specific activity in aerosol work because of the production of additional particles by radiolytic reaction with impurities in the air.

In a preliminary report, H. Weickmann (Boulder) suggested that measurements of the concentration of Aitken nuclei in the neighbourhood of clouds could contribute to a better understanding of convective circulation in and around cloud fields, and P. Squires (Boulder) gave measurements of the concentration of both Aitken and cloud nuclei in the neighbourhood of cities. These showed that human activity results in a noticeable production of cloud nuclei as well as of Aitken nuclei. He concluded, however, that the total production of cloud nuclei by this means is small compared with the natural production by continental surfaces. J. Podzimek (Prague) discussed the relative importance of diffusiophoresis and thermophoresis, Stefan flow and Brownian motion on the binding of condensation and ice nuclei on the surface of cloud elements; he held that for slightly supersaturated air, the effect of the first three of these processes was negligible. G. Schumann (Heidelberg) derived expressions for the attachment of radioactive atoms to particles taking account of charge and size distribution of the particles and confirmed them by experiments on the atmospheric aerosol.

J. P. Lodge (Boulder) gave a valuable account of techniques for the identification of individual sub-micron particles. Chemical methods exist only for chloride and sulphate particles, but he has found that a number of species, including sulphuric acid in various stages of neutralization by gaseous ammonia and phosphate, can be identified by their characteristic appearance in the electron microscope.

V. J. Schaefer (State University of New York) described the detection of surface active molecules on airborne particles by the use of indicator oils. Pollen grains, ocean spray nuclei and many particles in industrial smog had been found to exhibit surface active properties by this technique.

Papers on electrical properties of particles were given by J. Bricard (Paris), G. Madelaine (C.E.A. Fontenay aux Roses) and by R. Siksna (Uppsala). Bricard had found that only 4 per cent of the radioactive small ions from the decay of radon and thoron have the expected mobility of 2·15 $cm^2V^{-1}sec^{-1}$; the remainder have a mobility spectrum with peaks at 0·51 and 0·81 $cm^2V^{-1}sec^{-1}$. He attributed this to the rapid formation of cluster ions, but in the discussion it was suggested that cluster ions as normally understood could scarcely have so different a mobility and that the effect was more probably due to the formation of Aitken nuclei by radiolytic reactions.

In papers on the development and use of instruments, T. A. Rich (G. E. Coy, Schenectady) described an interesting combination of diffusion and electrical techniques to investigate the size of aerosols and R. E. Davis (U.K.A.E.A., Winfrith) the use of the Nolan Pollak counter in the testing of high efficiency filter systems. G. F. Skala (G. E. Coy, Schenectady) advocated the use of ion chambers to investigate the production of particles from heated substances in conditions of temperature and pressure which would preclude condensation methods. H. Israel and N. Nix (Aachen) re-opened the question of the role of Aitken nuclei in atmospheric condensation by quoting measurements of the temperature drop recorded on expansion in the Nolan–Pollak counter. While their results are in conflict with other measurements, they suggest that the counter detects nuclei activated at a supersaturation of 2 per cent instead of the 280 per cent calculated if an adiabatic expansion is assumed.

It is hoped that the next conference of the series will be held in Prague and Vienna in 1969. W. J. MEGAW

OBITUARIES

George de Hevesy

PROF. DE HEVESY, Hungarian born, who lived latterly in Copenhagen and Stockholm, died on July 5. He was the pioneer in the use of isotopes as revealers of the paths taken by elements in chemical and biological processes—discoveries which have been of immense consequence. Born in Budapest in 1885, he took his doctorate in Freiburg before coming to England in 1911. Later he worked in Vienna before gaining a chair at Freiburg in 1926. Some years later he moved to a professorship in Copenhagen and finally settled before retirement in a research institute at Stockholm. He was the holder of many awards and honorary degrees. These included the Nobel Prize for chemistry in 1944 and the Copley Medal and foreign membership of the Royal Society. He found time to write notable books on his main subject as well as to publish a large number of papers. With Coster he discovered hafnium.

Von Hevesy, as he then was, found himself, a chemist, among the physicists who clustered around Rutherford in Manchester a few years before the Great War. His first success there was his showing that the whole of a minute amount of a radio-element deposited itself from a solution on nickel, immersed in it, even when the radio-element was actually chemically more reactive than nickel. His first failure—not at all his fault—was his complete inability to separate radium-*D* from its isotope lead. But with Von Hevesy, this failure led to success, for it showed him that minute concentrations of radio-elements must be successful tracers of their common isotopic elements in chemical and biological processes. Later he found that the tracer-isotope need not be radioactive; so long as its oddness could be characterized, an inactive isotope might do—for example, deuterium. Later still he extended possibilities by making a suitable inactive isotope reveal itself in a mixture by its being made artificially radioactive by bombardment by neutrons; the measurement of its characteristic radiation determined the amount of the element in the mixture quantitatively. His discoveries in all these fields have since had a magnificently fruitful following.

Hevesy was a European of the best sort, genial, kind-hearted, a good friend. He did not look strong, but he was a mighty worker dedicated to research. He died in Freiburg. A. S. RUSSELL

Hans Pettersson

PROF. HANS PETTERSSON, who died in Goteborg on January 25 at the age of 77, was particularly noted for his leadership of the Swedish Deep Sea Expedition in the *Albatross*, and for his many contributions to the study of the deep sea floor and its sediments. His father, Otto Pettersson, with other Scandinavian scientists, particularly Nansen, Ekman and Helland-Hansen, did much to establish the serious study of physical oceanography at the turn of the century. Hans studied physics in Uppsala under Knut Angstrom and in London under Sir William Ramsay. At first he joined his father in the study of currents and internal waves in Swedish coastal waters, but he soon developed his interest in optical and radiochemical studies. Between 1922 and 1936 he worked frequently at the Institut für Radiumforschung in Vienna, where he interested a number of specialists in marine problems. He found that although radium is concentrated in deep sea sediments, its concentration in sea water is much less than would be expected from the much greater concentration of uranium. He believed the deficit to be due to co-precipitation of thorium and ionium with the hydrated oxides of iron and manganese. He used the variation of radium content with depth in the sediments as a basis for geochronological estimates. He did much to further the development of deep sea coring devices.

His tremendous enthusiasm for the subject and the success of his work and that of his colleagues persuaded some of his successful compatriots to provide funds necessary for the founding of the Oceanografiska Institutet in 1939 and, in 1947–48, for the round-the-world expedition in the *Albatross*. The expedition was very successful, and its observations and sampling did much to stimulate the growth of interest in marine geochemistry there has been in the past 20 years. Although mainly concerned with the sea floor, studies of the transparency of the water and scattering of light at different depths were also important features of the work.

Pettersson was interested in the contribution of cosmic dust to the ocean sediments, and his latest communication to *Nature* dealt with this subject. He was elected a Foreign Member of the Royal Society in 1956, and retired in the same year from the post as director of the Oceanografiska Institutet. G. E. R. DEACON

Eva G. R. Taylor

PROF. EVA GERMAINE RIMINGTON TAYLOR, who died on July 5, 1966, at the age of 87, was bedridden for the later years of her life and almost totally blind for the last year, but she nevertheless retained her acute critical faculties to the last, and only a few weeks before her

death had dictated her doubts on the authenticity of the Vinland map.

She was educated at the North London Collegiate School for Girls and, after taking a degree in chemistry in the University of London, spent several years as a science mistress at Burton-on-Trent. Seeking to improve her teaching qualifications, she went up to Oxford to take the Diploma in Education. There she came under the influence of A. J. Herbertson and was swept into his activities in developing the 'new geography', both in the Oxford department and through his text-books in the schools. She stayed on as his assistant, working on the preparation of the famous Oxford wall maps and, on a vacation course, met J. F. Unstead. The series of text-books she produced with him had a great influence in the schools.

It was, however, her appointment as lecturer at Birkbeck College under Unstead in 1921 which marked the turning-point in her career. She turned her attention to historical geography and published *Tudor Geography* and *Late Tudor and Early Stuart Geography*. They established firmly her academic standing so that, when Unstead retired in 1930, she succeeded him as professor of geography and so remained until she retired in 1944. In those years she became not only the acknowledged expert in the maps and literature of the Tudor and Stuart periods, but also in the instruments of navigation and the work of contemporary explorers. A series of papers in the *Mariner's Mirror* and the *Journal* of the Institute of Navigation culminated in her *The Haven-finding Art*.

But Prof. Taylor was far from being immersed in the past. She threw herself with enthusiasm into physical planning and became a member of Lord Reith's Consultative Panel in 1941, with the inauguration of the Ministry of Town and Country Planning. She is still remembered by planners as the originator of the 'sieve method'—by eliminating or sieving out areas unsuitable for development for one reason or another there remains a positive field within which to work.

A woman of strong personality and trenchant language verging on abuse, there were occasions when it was easier to be on the other side, for she never minded opponents who hit back. But her positive opinions were based on wide knowledge, and the bubble reputations she delighted to prick usually deserved this treatment. She retained her maiden name throughout her life and, though proud of her two sons, she kept her domestic life entirely separate from her academic. Shortly after her retirement her work was recognized by the award of the Victoria Medal of the Royal Geographical Society, and her students, subscribing in large numbers, endowed a named lecture at the Society in her honour. L. DUDLEY STAMP

Fritz Zernike

PROF. FRITZ ZERNIKE, who died on March 10 in Groningen, Holland, was born in Amsterdam on July 16, 1888. His father was headmaster of an elementary school and his mother had been a schoolteacher. Their six children formed a lively and intellectually stimulating family. At nineteen, as a student of chemistry at Amsterdam, Zernike was awarded a gold medal by the University of Groningen for an essay on a probabilistic treatment of a certain parlour game. In 1912 he received another prize for work on critical opalescence, and in 1914 his collaboration with L. S. Ornstein resulted in the Ornstein–Zernike formula, which plays a central part in the theoretical and experimental investigation of the critical point. In 1915 he obtained his doctor's degree with a thesis, *L'opalescence critique, théorie et expériments*, in which the formula for extinction by scattering near the critical point was derived by means of the grand canonical ensemble. The experiments he reported agreed with the theoretical result and made possible a determination of Avogadro's constant to within a few per cent.

In the meantime, Zernike had become an assistant to J. C. Kapteyn, the astronomer at Groningen, but soon afterwards he was appointed a reader in theoretical physics at the same university, and then, in 1920, a full professor. He continued the investigation of the pair correlation function $g(r)$ occurring in the Ornstein–Zernike formula, and in collaboration with J. A. Prins applied it to X-ray diffraction in liquids. His interest in statistical physics also led to a study of the influence of Brownian motion on the measurement of small currents and, characteristically, he put his theoretical understanding to practical use by constructing an improved galvanometer.

Zernike's unique combination of mathematical insight and technical ability proved very fruitful when he directed his attention to optics. Rather than obscuring a problem by laborious calculations, he would reduce it to its simplest mathematical elements which would then serve as stepping-stones for his physical intuition and inventiveness. For example, he showed that the difference between the rival theories of Abbé and Rayleigh concerning the microscopic image formation is nothing but a difference in order of integration. The discovery of phase contrast microscopy in 1932 was virtually a natural consequence of this method; in fact, when Zernike explained the principle it was hard to understand why it had not been discovered earlier. Perhaps that makes understandable why, when he offered his invention to Zeiss, the leading optical industry in those days, the haughty reply was: "Wenn es interessant wäre, so hätten wir es schon längst selbst erfunden". Yet some development work was carried out at Zeiss, and the first motion picture of cell meiosis was made. Phase microscopy did not become widely known, however, until Arthur T. Bryce, of the U.S. Army, arrived in Jena in 1945 and immediately realized its importance.

Meanwhile, Zernike turned his attention to another problem in microscopy—What is the influence of small aberrations on the diffraction pattern in the image plane? As an appropriate mathematical tool he introduced "circle polynomials" and, in collaboration with B. R. A. Nijboer, the work was successfully completed in 1942. In the same years Zernike developed the concept of "degree of coherence", constructed a useful poor man's ultracentrifuge using the motor of a vacuum cleaner, published a quantitative treatment of the Senftleben effect with C. van Lier, gave an approximate treatment of the phase transition in binary alloys, and worked on the technical problem of how to manufacture the phase rings needed for his microscope.

Relativity theory and quantum mechanics were of less interest to Zernike, presumably because they lacked the intimate connexion with technical applications which made classical physics so fascinating to him. He was not easily persuaded to publish his results and ideas, but when he wrote an article it was carefully planned and lucidly written. His public addresses were full of brilliant ideas and he had a masterful way of utilizing a simple analogy to throw new light on an old problem. His contacts with other scientists were rather limited, partly because he was so much absorbed in his own research, and partly because he felt ill at ease with people who could not appreciate that the short, often witty, and sometimes caustic way in which he expressed his opinion was just another aspect of his search for unadulterated truth. Unfortunately, this gentleness made him rather defenceless in the turmoil of university politics. His friends, however, will not forget how his great knowledge, widespread interest, and penetrating and refreshingly unprejudiced views made his conversation an intellectual enjoyment.

Official recognition came late, first in the form of the Rumford Medal in 1952, and in the next year the Nobel Prize for his invention of the phase contrast microscope. Zernike was a Foreign Member of the Royal Society of London. N. G. VAN KAMPEN

IS GRAVITATIONAL COLLAPSE REALLY IRREVERSIBLE?

By DR. WERNER ISRAEL
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Quasi-stellar Sources

IF the cosmological interpretation of their large red-shifts is accepted, the quasi-stellar sources lie near the outskirts of the visible universe and close to its origin in time—"sparks of the primeval fireball". A lower limit to the masses which must be involved (10^8 solar masses) can be obtained from the enormous energies stored in the relativistic electrons which gyrate in their magnetic fields[1], whereas the observed optical and radio variability[2,3] places an upper limit of about a light-week (10^{16} cm) on the size of the central emitting nucleus. A non-rotating ball of 10^8 solar masses becomes gravitationally unstable if its radius becomes smaller than $4{\cdot}7 \times 10^{17}$ cm[4]. It seems difficult to escape the conclusion that the typical quasi-stellar source is in a state of gravitational collapse approaching free fall.

The predicament to which this leads is well known. According to the picture of gravitational collapse which has become traditional[5], the collapse proceeds irreversibly to zero volume. An external observer sees the body asymptotically shrinking to its Schwarzschild radius. The observed time of collapse is thus infinite; but progressive dimming due to the Doppler and gravitational red-shifts, and to the falling back of light rays sent out obliquely from the surface, will render the object invisible to the external observer in a time comparable with the Newtonian free-fall time of 25 years[6]. This picture is obviously incompatible with the life-times of 10^6 years inferred from the linear dimensions of the sources ($\sim 10^6$ light-years), and the clear evidence (from the extended life-times and the variability) of repeated injections of relativistic particles.

It has been known for some years that the continued activity in the supernova remnant of 1054, the Crab nebula, confronts us with a similar difficulty[7].

In the face of this apparent impasse, it is very curious and perhaps suggestive that a naive application of Newton's theory will account in a perfectly natural way for all the major observed features of quasi-stellar sources. Imagine the Newtonian collapse of a ball of 10^8 solar masses. If support from gas and radiation pressure fails, the collapse continues until, at zero radius, there is a violent "self-collision" and rebound, with the ejection of high-speed streams of ionized gas and particles. For a radius at maximum of 10^{17} cm, the resulting pulsations have a period of about 20 years. (Observations of the quasi-stellar source *3C* 273 over the past 70 years suggest cycles of about 10 years for major fluctuations[2].) We can elaborate this model a little by imagining the ball deformed into an irregular lumpy mass. Each lump, while sharing in the oscillation of the mass as a whole, executes its own quicker pulsation. This reproduces the "light flashes" and irregular variations observed in many quasi-stellar sources.

At this point I should make it clear that I am not arguing for the reinstatement of Newton's theory. However, this model, which is based essentially on the reversibility of gravitational collapse, fits the observed facts so naturally that one cannot help asking whether, on this issue, Einstein's theory is—not wrong—but misinterpreted. Is it really true that general relativity predicts the irreversibility of gravitational collapse? The remainder of this article is devoted to a critical examination of this question.

Radial Motion in the Schwarzschild Field

The history of a particle on the outer boundary of a collapsing ball is a radial time-like curve of the exterior Schwarzschild field. Thus, many of the essential features of gravitational collapse are brought into focus by studying the radial motion of particles in the Schwarzschild vacuum manifold.

In terms of Schwarzschild's familiar r,t co-ordinates, the paths of incoming and outgoing radial light-pulses have the equations u = a constant and v = a constant respectively, where:

$$4m u^{-1}\,du = (1-2m/r)^{-1}\,dr + dt$$
$$4m v^{-1}\,dv = (1-2m/r)^{-1}\,dr - dt$$

These null curves are shown in Fig. 1, together with the future light cones which they define.

The time-like path ABC of a radially moving test particle can now be traced out qualitatively if we remember that it must always be directed into the future light-cones. The particle reaches $r=2m$ at a perfectly definite event C, attained in a finite proper time. But, because of failure of Schwarzschild's t co-ordinate at $r=2m$, the event C cannot be mapped on to the finite part of the r,t plane. We can remedy this deficiency by straightening the null lines, that is, adopting u, v as new co-ordinates. The new form of Schwarzschild's line-element is[8]:

$$ds^2 = \frac{32m^3\,du\,dv}{re^{r/2m}} + r^2\,d\Omega^2$$

with $r(uv)$ defined by (1)

$$\left(\frac{r}{2m}-1\right)e^{r/2m} = uv$$

and this is regular down to $r=0$.

Possible Interpretations of the Extended Manifold

In the new map (Fig. 2), we can follow the career ($ABCD$) of the falling particle down to $r=0$.

To proceed further, we have first to decide how to interpret the peculiar duplication which occurs in the uv

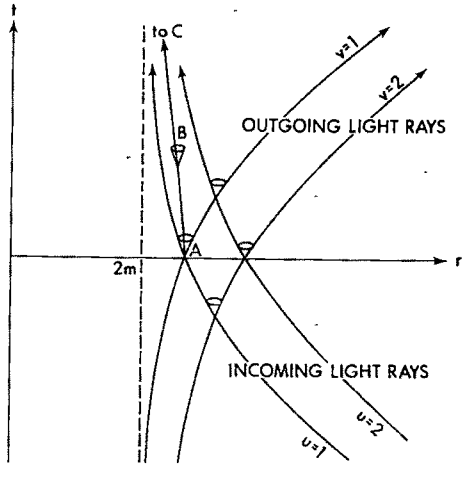


Fig. 1

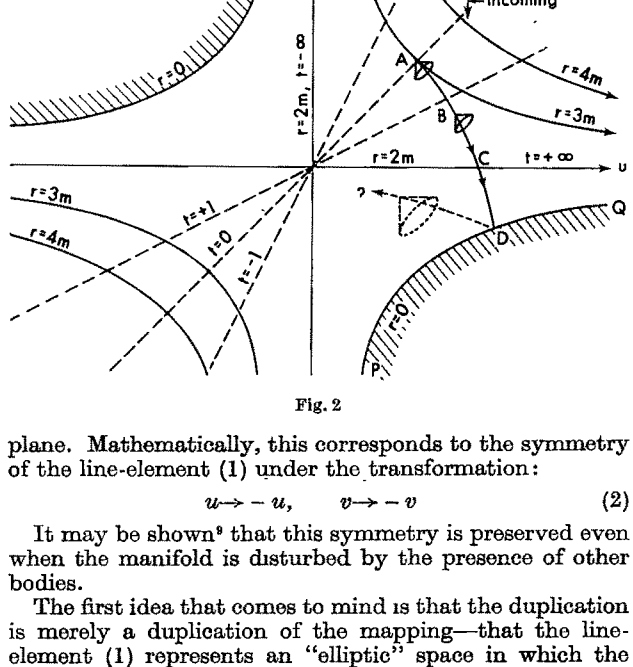


Fig. 2

plane. Mathematically, this corresponds to the symmetry of the line-element (1) under the transformation:

$$u \to -u, \qquad v \to -v \tag{2}$$

It may be shown[9] that this symmetry is preserved even when the manifold is disturbed by the presence of other bodies.

The first idea that comes to mind is that the duplication is merely a duplication of the mapping—that the line-element (1) represents an "elliptic" space in which the events (u, v) and $(-u, -v)$ are physically identical.

Actually, until very recently[10,11] this idea was not followed up. The major discouraging factor seems to have been this: Let us assume that future light cones in quadrant I of the uv plane have a south-easterly direction, as indicated in Fig. 2. If we accept the identification just proposed, then the future light cones assigned to quadrant III must point north-west. Because this assignment cannot be extended continuously into II and IV, we would be forced to admit a breakdown of the global distinction between past and future in the domain with $r < 2m$. This raises the spectre of possible causal violations.

Consequently, it has been traditional to escape these worries by the (implicit) introduction of Postulate X: Past and future can be globally distinguished throughout the extended Schwarzschild manifold.

Postulate X and Irreversibility

Postulate X permits us to mark future light cones into the four quadrants of the uv plane, all pointing (say) south-east.

On this basis, let us return to consider the fate of the particle which has reached $r = 0$ at event D. Can it rebound from the centre or pass through the centre into the radially opposite space ? Fig. 2 shows that every time-like path which emerges from the branch PDQ of $r = 0$ is directed into the past defined globally in quadrant IV, and this is forbidden. We have no alternative to the conclusion that a particle which has fallen to $r = 0$ is irreversibly swallowed up by the singularity. Correspondingly, a spherical body (boundary $ABCD$) which has collapsed under its own weight to zero radius can never re-expand: its subsequent history is the space-like singular curve DQ.

It is clear from this argument that the irreversibility of gravitational collapse is a consequence not of general relativity but of the *ad hoc* Postulate X.

Irreversibility is not required by the elliptic interpretation. As future and past are not globally distinguished in quadrant IV, it is possible—and, indeed, most natural—to assume a "bounce" at $r = 0$. (For an account of how this gives rise to an externally observed pulsation with period in agreement with Newtonian predictions, see refs. 11 and 12.)

Difficulties with Postulate X

Because the traditional picture of gravitational collapse hinges critically on the validity of Postulate X, it is important to test the soundness of this postulate. Examination reveals a number of disturbing features. I list a few of them.

(i) The imposition of a global past-future arrow on to the uv plane breaks the symmetry, since the arrow is not invariant under (2). To this extent, Postulate X is alien to the geometry and runs counter to the clear indications of general relativity.

(ii) It is unprecedented and very strange that a postulate based on the essentially statistical coarse-grained concept of a past-future distinction should need to be inserted into the foundations of the more primitive subject of dynamics.

(iii) Every slice t = constant divides the extended manifold into two sheets with $r > 2m$, joined by an Einstein–Rosen bridge. These sheets are in all respects geometrically identical, even in the presence of external disturbances. But—if Postulate X is accepted—only one is the ordinary space of our experience; the other is a physically distinct space in which each of us has an identical twin. This seems uncanny. Even more uncanny is the fact that, while no signal can pass between us, my twin appears to be in some kind of instant telepathic contact with me: when I crook my little finger he simultaneously (at the same "time" t) crooks his.

(iv) The global past-future arrow defined by Postulate X cannot agree with the direction of increasing $t = 2m \ln(u/v)$ in both quadrants I and III. This has the following curious consequence. Suppose that a sequence of slices $t = 1, 2, 3, \ldots$ shows me ageing. My twin, identical with me on each slice, will go through the same sequence of physical transformations. But, according to Postulate X, he would have to describe himself as getting younger! This drains most of the meaning from the words "past" and "future" as used in Postulate X.

(v) According to the traditional picture based on Postulate X, the final history of a collapsed star is a (singular) space-like curve. Relativistic principles would lead us to expect the history of any material body to be time-like.

Individually, the foregoing objections are perhaps not fatal, but together they add up to a formidable indictment.

The elliptic interpretation is free of these objections. Moreover, detailed investigation[11] shows that the causality bogey does not materialize under actual physical conditions. There is thus good reason to expect that the most natural interpretation of the Schwarzschild manifold is, in addition, self-consistent and concordant with the astronomical evidence.

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APPEARANCE OF RELATIVISTICALLY EXPANDING RADIO SOURCES

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IN this article it is suggested that the central parts of some radio sources may expand with relativistic velocities. Relativistic effects could then have a decisive influence on the observable properties of these sources. In particular, the flux from a radio source expanding with relativistic velocity can change sufficiently rapidly to account for the variations observed in some sources, even if they are at cosmological distances. A related relativistic effect would imply that the ages of some types of radio sources may be significantly less than the usual estimates.

Variable Radio Sources

Intensity variations[1-3] have been observed in several radio sources associated with quasi-stellar objects, the red-shifts of which indicate that they are probably at cosmological distances. Well-known arguments concerning self-absorption[4,5] enable us to place a lower limit on the dimensions of a source which is at a known distance (if the radio flux is synchrotron radiation), and it has proved difficult to understand how a source at a cosmological distance can vary with the observed rapidity unless its size is less than this limit. This problem arises if the assumption is made that the 'time-scale' of the variations cannot be much less than the time light would take to cross the emitting region. If[6,7] (*a*) the variations are periodic, or (*b*) they result from changes in the brightness of a region of fixed size, this assumption is certainly necessary, but I shall describe a type of model where special relativistic effects arise which permit it to be relaxed.

In the model proposed, the source is assumed to be spherical and the radio variations are principally due to changes in its apparent diameter rather than in its surface brightness (in contrast to (*b*)). We shall show that, if the boundary of the source expands with velocity $\sim c$, the apparent rate of increase in its angular size and luminosity can be extremely high. Before discussing the physics of the model, and arguing the reasonableness of relativistic velocities, we shall illustrate its essential geometrical properties.

Consider a sphere, centre S, the surface of which expands with radial velocity v from zero initial radius. An observer O at a large distance R from S (and at rest relative to S) will observe an increase in the apparent size of the sphere.

If $v \ll c$ the observed angular diameter will be $2\frac{v}{R}t$, where t is the time measured from the moment when the expansion is seen to begin. However if $v \sim c$ this result needs modification. The locus of points from which radiation reaches the observer at time t is not a sphere, but a spheroid with S as focus, axis along SO, eccentricity v/c and semi-latus rectum vt, as shown in Fig. 1. Owing to aberration, the parts with velocity perpendicular to the observer are invisible if the sphere is opaque, and the apparent limb, in fact, has a velocity making an angle $\cos^{-1} v/c$ ($\sin^{-1} = 1/\gamma$, where $\gamma = (1-v^2/c^2)^{-\frac{1}{2}}$) with SO. Furthermore the apparent component of velocity of the limb at right angles to the line of sight is γv (which can be $\gg c$ if γ is large) and the observed angular diameter of the sphere at time t is $2\gamma vt/R$. If, for example, $\gamma = 5$, the apparent diameter of the source will increase by almost 10 light years each year. Because the observed intensity of a source, for a given surface brightness, is proportional to the apparent size, it is already clear that an expanding source could exhibit a rate of increase of flux density high enough to explain the observations.

This geometrical fact is obviously relevant to a variety of physical models, of which only one will be described here. Suppose that the source consists of a massive object (probably identifiable with a quasi-stellar object) which accelerates particles to relativistic energies. Suppose also that the surrounding magnetic field is weak, in the sense that $H^2/8\pi$ is small compared with the kinetic energy density of the particles. A burst of relativistic particles ejected from the massive object will not be confined by the field, but will expand outwards, 'dragging' the field with it (that is, there will not be an electric field in a reference frame sharing the mean outward motion of the particles, though there will generally be in other frames). This explosion will have a relativistic velocity provided that the thermal gas density is small. If we assume for simplicity that the outward radial velocity is constant, we have an expanding shell resembling the sphere already described.

The electron density and the field strength will decrease as the shell expands, and the spectrum of the synchrotron radiation will alter. The total observed flux at time t is obtained by integrating the contributions from all parts of the shell, but this calculation is complicated by the fact that the emission from different parts was emitted at different stages in the expansion. Also the Doppler blue-shift varies from γ on the limb to $\gamma(1+v/c)$ at the

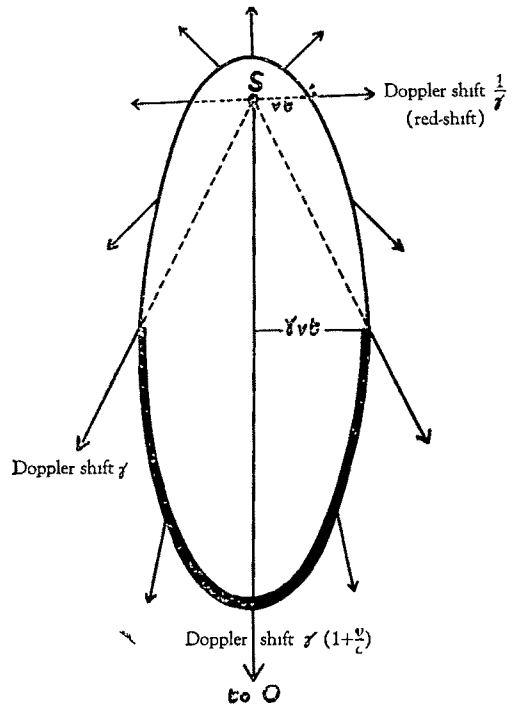


Fig. 1. If a distant observer O sees a spherical shell start to expand from S with velocity v, the locus of the points from which radiation reaches him at time t later is a spheroid. If the shell is opaque, the only visible parts are those the velocity of which makes an angle $< \cos^{-1} v/c$ with SO. These parts all have a Doppler blue-shift $> \gamma$, and the limb has an apparent transverse velocity γv

centre. The total flux from the shell obviously cannot be determined exactly without specifying the detailed behaviour of the model during the expansion. However, the problem is simpler if we restrict attention to low frequencies, for the following reason. The spectral index of the radiation from each part of the source will be 2·5 at frequencies at which synchrotron self-absorption occurs ($< \nu_c$, say). The shape of the spectrum at higher frequencies depends on the energy spectrum of the electrons. ν_c will decrease as the expansion proceeds, but at sufficiently low frequencies of observation (or for small t) all the contributions to the flux will have a spectrum of slope 2·5, and the spectrum of the whole shell will have this slope. The observed flux density will then exceed that from a static source with the same apparent size and magnetic field $\bar{H}$ (where $\bar{H}$ is a mean value of the field strength in the shell when the radiation is emitted) by a factor $\sim\gamma^{\frac{1}{2}}$.

Such a model might apply to 3*C* 273, the variability of which is well established at several frequencies. The hardest observations to explain[2] (which consequently constitute the most crucial test of any model) are the increase of ~ 6 flux units (F.U.) over a 3-year period, in the flux density at 1,400 Mc/s (the lowest frequency at which variations have been detected). Maltby and Moffet[2] have shown that this can be understood on the basis of a non-exploding model only if H has the improbably small value of $< 10^{-4}$ gauss. If, however, a source at the distance of 3*C* 273 were to start to explode with a velocity corresponding to $\gamma = 5$, and if H (measured in a frame sharing the mean particle motion) $\sim 10^{-2}$ gauss, the flux density would have risen to ~ 15 F.U. in 3 years, which is greater than is required to explain the data. The parts of the shell which would contribute most of this flux have a Doppler blue-shift > 5, and therefore the radiation received at 1,400 Mc/s would all have been emitted at frequencies below 300 Mc/s. Furthermore, after 3 years, these parts would be ~ 100 light years from S, so we would require ν_c (a decreasing function of distance from S) to be $\gtrsim 300$ Mc/s out to this distance, which is not an unreasonable requirement. The non-variable flux from 3*C* 273 at frequencies below 1,000 Mc/s must originate in a larger region surrounding the variable component, and its spectrum would mask the steep slope of the low-frequency spectrum of the exploding source. The spectrum of the shell at higher frequencies (or at later stages in its expansion when ν_c has decreased) is more difficult to calculate, because some of the radiation would come from transparent regions. We would expect the highly blue-shifted part of the source with velocity almost towards the observer to be the first to become transparent at a given frequency (though this cannot be definitely stated without detailed calculation of a precise model). It is this part which contributes most of the flux in the early stages of the expansion, so we might expect to observe a decrease in the flux density when it becomes transparent. This decrease could be almost as rapid as the original increase.

It should be emphasized that no attempt has been made to incorporate the optical frequency radiation in the foregoing model for a quasi-stellar radio source. The thermal gas which radiates the lines in the visible spectrum from which the Hubble red-shift is determined presumably has a velocity $\ll c$ relative to the massive object, in contrast to the fast-moving clouds of relativistic electrons in the model. We have, incidentally, neglected a correction factor involving the cosmological red-shift in this model.

The foregoing has demonstrated that the radio variations of quasi-stellar sources do not provide strong evidence for the so-called 'local' theory[6,8] (according to which quasi-stellar radio sources are debris from an explosion in our own or a nearby galaxy, and their red-shifts are a Doppler effect due to high peculiar velocities), since we can account for the available observations on the assumption that the sources are at cosmological distances. Nor is it necessary to invoke 'coherent' radiation mechanisms, which might permit a higher surface brightness temperature to be reached before the onset of self-absorption. If future observations should prove to be inconsistent with this model, a wider range of behaviour could be accommodated by abandoning our assumption of spherical symmetry (for which there is no physical reason, and which was adopted here merely for simplicity), and considering asymmetrical exploding sources with particular orientations.

Other Implications of Relativistic Bulk Motions

The most important feature of the model described in the previous section has been the simple fact that an object moving relativistically in suitable directions may appear to a distant observer to have a transverse velocity much greater than c. The remainder of this article is concerned with other possible implications of this fact for theories of radio sources.

Most strong extragalactic radio sources consist of two or more components separated by distances of up to 10^6 light years[9]. We may assume that their existence is due to some disturbance (of unknown nature) in the associated visible object. If the radiating regions had themselves moved bodily to their observed positions from the parent galaxy, their speed must be relativistic unless their age is $\gg 10^6$ years. Alternative theories[10,11] postulate shock waves or streams of relativistic particles ejected from the galaxy, radio sources being formed as a result of their interaction with intergalactic material or an intergalactic magnetic field. Present observations cannot prove or refute any of these hypotheses, but if more sensitive future observations fail to confirm the presence of a significant amount of intergalactic material (the existence of which is still an open question), theories of the latter type will become less plausible than those according to which both particles and magnetic fields are ejected from galaxies. These 'plasmoids' may well have velocities $\sim c$. No process capable of accelerating them is yet known, but clouds of relativistic particles could certainly be accelerated to relativistic velocities more easily than super-massive stars, as is postulated in the 'local' theory of quasi-stellar radio sources.

If these relativistic velocities exist, the possibility of apparent transverse velocities $\gg c$ implies that the minimum observed lifetime of a source can be less than its observed distance from the associated visible object divided by c. (This conclusion also holds if the radio source does not itself move, but is 'triggered' by some influence which propagates out from the visible object with relativistic velocity.) The total energy contained in radio galaxies is, however, generally sufficient to maintain their observed rate of energy output for a time $\gg 10^6$ years, so that there is no reason to suspect that their lifetimes are in fact much shorter. Quasi-stellar sources, on the other hand, radiate enormous energy at optical and infra-red as well as radio frequencies, and the problem of understanding this great power output from a small region is aggravated if their lifetime is long. Furthermore, they are more likely than other astronomical objects to be the sites of explosions violent enough to accelerate clouds of relativistic particles to high velocities. The conjectures which we shall put forward will therefore be particularly relevant to 'double' quasi-stellar radio sources such as 3*C* 273 and 3*C* 47 (refs. 12 and 13).

If a 'plasmoid' consisting of relativistic electrons and magnetic fields is ejected with a velocity $v(\sim c)$ its apparent transverse velocity relative to the quasi-stellar radio source can be as great as $\sim\gamma v$ (that is, $\gg c$) if its velocity makes an angle $\sim\cos^{-1} v/c$ with the line of sight. During t years the 'plasmoid' would appear to have moved a transverse distance $\sim\gamma\, vt$ light years, and its actual distance from its point of origin would be $\sim\gamma^2\, vt$ light years. A proper time $\sim\gamma t$ would have elapsed in the 'plasmoid' since the explosion. (If $\gamma \gg 1$ this is approximately equal

to the time it would take to reach its observed position if it had moved with a velocity $\sim c$ perpendicular to the line of sight.) Of course it is very unlikely that an object ejected at random would have its velocity oriented at $\cos^{-1} v/c$ to the direction of the observer, especially if $\gamma \gg 1$. But the associated quasi-stellar radio sources may well have ejected a large number of such clouds, which would be too faint to be detected unless they were moving almost towards us. If $\sim 2\gamma^2$ clouds were thrown out with the same velocity, for example, we would expect only $\sim\gamma$ of them to be blue-shifted, and only ~ 1 to have a Doppler blue-shift $>\gamma$; but it is the cloud which does have a large blue-shift, and is therefore bright enough to be detected, which is likely to have the greatest apparent velocity ($\gg c$) perpendicular to the line of sight. The radiation from each cloud would be emitted predominantly in a narrow beam of angle $\cos^{-1} v/c$ pointing in its direction of motion, and we would receive more radiation from the one cloud with large Doppler blue-shift than from all the others combined. These others would appear closer to the quasi-stellar radio source, though we would expect them to be too faint to be detectable individually. The energy in all the clouds (including their kinetic energy) would be no greater than the energy required by other models.

The most attractive feature of the possibility suggested here is that it enables us to adopt a lower estimate for the duration of the superluminous phase of quasi-stellar radio source. Unfortunately the model would not be tenable if the intergalactic density were as high as $\sim 10^{-5}$ atoms/c.c., since the clouds would then be braked too rapidly by the ambient gas.

The foregoing discussion has shown that bulk velocities $\sim c$ may occur in radio sources, and that relativistic effects may drastically alter estimates of the size of a source from its rate of variation, or conversely of its age from its apparent size. In this way we can resolve some of the difficulties which have been encountered in attempts to understand variable radio sources if they are at cosmological distances. It is also possible that the lifetimes of some radio sources and quasi-stellar objects may be substantially shorter than is generally believed.

I thank Dr. D. W. Sciama for advice and encouragement.

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LUMINESCENCE CAUSED BY PROTON IMPACT WITH SPECIAL REFERENCE TO THE LUNAR SURFACE

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RECENTLY, Derham and Geake reported[1,2] some results of laboratory experiments on the luminescence of meteorites by proton impact in the keV-region. The figures supplied by them, however, are mainly relative. Because we are able to perform absolute measurements on excitation by proton and electron beams, we decided to carry out a similar investigation. The apparatus was calibrated by means of a standard tungsten ribbon lamp (a description of the apparatus and the calibration procedure has been given elsewhere[3,4]). Some of our results are given in Fig. 1. The graphs show the luminescence of the meteorites Holbrook, Arispe and Odessa, the lava Fuertaventura and molecular hydrogen, under proton bombardment with an energy of 30 keV. The choice of this energy results from a suggestion made by Kopal[5], who supposed that a correlation exists between solar flares and lunar luminescence. The observed time lag was between 8 and 48 h, whereas an energy of 30 keV corresponds to a time lag of about 26 h.

For the reason that our measurements gave very low excitation values, we also measured the luminescence of willemite. The results are plotted as curves 1 and 3 in Fig. 2.

The maximum number of photons obtained in this way was about 10^7 per μamp and per unit of wave-length.

If it is assumed that the normal flux of protons in the solar wind is $1{\cdot}6 \times 10^{-8}$ cm^2 s^{-1}, that the bandwidth in the optical range is 500 Å, and that there is an area on the lunar surface contributing to the luminescence of 100 km^2, a result of 5×10^{16} photons per sec is obtained. An observer on Earth with a 50-in. telescope at a distance of 385,000 km would receive on the average from this flux 1 photon per 1,000 sec.

* Formerly: F.O.M. Laboratory for Mass Separation.

As we used one of the strongest luminescent materials, we believe it very improbable that even during solar flares, when the intensity of the solar wind increases by a factor of 100, the observed excitation can be caused by proton impact in the keV-region. Even the fact that the lunar night-time is 10^4 times fainter than the normal brightness cannot be explained in this way.

For the luminescence of molecular hydrogen we find about the same values in curve 1 (Fig. 1), assuming a pressure of 10^{-2} torr (this corresponds to a density of about 3×10^{14} cm^{-3}). This seems rather high when the lack of atmosphere and the relatively low escape velocity are considered. Because our laboratory values differ considerably from those of Derham and Geake, we tried to find a reason for this discrepancy.

We believe the following two points to be worth mentioning: (1) In most cases the bombarded target consists of insulating material, so during proton bombardment it is charged up to a potential determined by the conductance of the material, the current density and the proton energy. This means that under laboratory conditions a potential drop develops across the bombarded target. Protons hitting the target surface create secondary electrons, and these electrons are accelerated to the most positive part of the target. As the efficiency of electrons in the keV-region is about 10^5 times higher than that of protons, the luminescence caused by these secondary electrons is several decades greater than the luminescence caused by protons.

This effect becomes clear from a consideration of Fig. 2, where curve 5 corresponds to the light-output by electron-bombardment, and curve 4 by proton-bombardment with an insulating target—which means that a great number of high-energy secondary electrons is formed—whereas

curve 1 corresponds to proton-bombardment on a grounded target with aluminium coating, and curves 2 and 3 with a thin insulated layer deposited on a grounded copper plate, at a proton energy of 10 and 30 keV, respectively.

(2) The background pressure in the apparatus may be a second cause for the high luminescence effects. During their passage through the gas, the protons tend to ionize the gas molecules, thus creating a number of electrons. These electrons are then accelerated to the most positive part of the target, and this results in a relatively important contribution to the luminescence. The result of the increase of background pressure in our apparatus from 10^{-5} torr to 10^{-4} torr is indicated in Fig. 3, where a thin layer of willemite was deposited on a grounded copper plate. It can be seen that by increasing the background pressure by a factor of 10 the luminescence increases by the same magnitude.

We conclude that the luminous emissions observed from some parts of the lunar surface are not caused by impact of protons from the solar wind. The high luminous yields of protons reported by other investigators are most probably due to secondary instrumental effects. We see no evidence to support the existence of a correlation between the protons from solar flares and lunar luminescence—unless some unknown storage mechanism is present.

The creation of photons on the lunar surface seems to be an established fact, and for this reason other mechanisms must be sought to explain the presence of energy and its conversion into light. We think it probable that the effect is caused by electro-magnetic radiation of short wavelength (ultra-violet, X-rays). Evidence for this supposition could be provided by an extended lunar satellite programme, in which absolute measurements of radiation intensities as a function of wave-length could be performed, and a correlation with luminescence established.

This work is part of the research programme of the Foundation for Fundamental Research on Matter (F.O.M.)

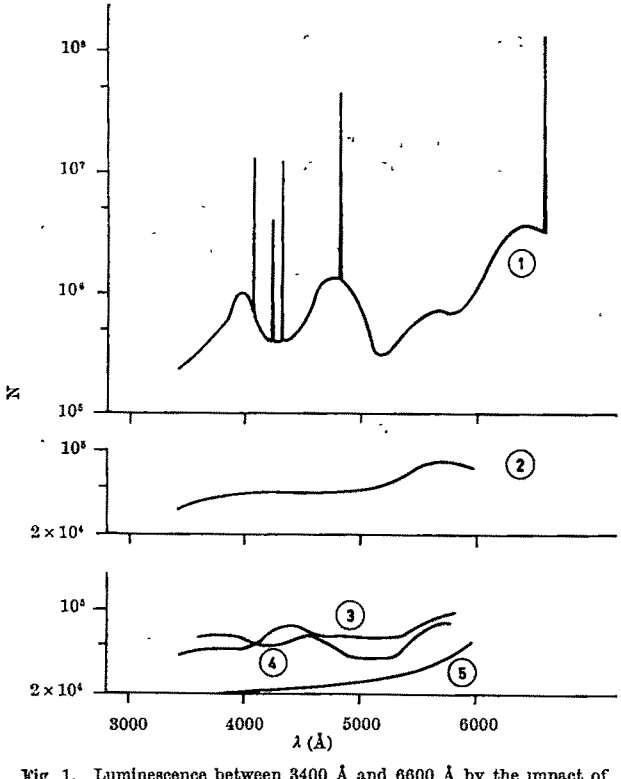


Fig. 1. Luminescence between 3400 Å and 6600 Å by the impact of 30-keV protons. The intensity I is given in number of photons per μamp beam current and per unit of wave-length (Å). Curve 1 is for molecular hydrogen (pressure 10^{-3} torr) at an observed beamlength of 25 mm; curve 2 is for the lava Fuertaventura; curve 3 for Arispe; curve 4 for Holbrook, curve 5 for Odessa

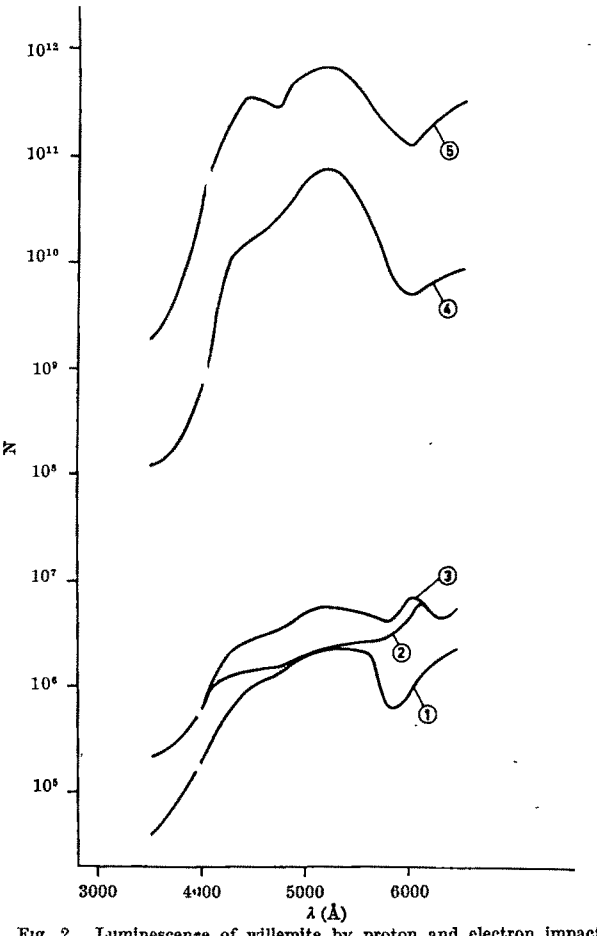


Fig. 2. Luminescence of willemite by proton and electron impact. (1) Proton impact 30 keV on aluminized target, (2) proton impact 10 keV, thin layer on copper target, (3) proton impact 30 keV, thin layer on copper target, (4) proton impact 30 keV on insulated target; (5) electron impact 10 keV, aluminized target

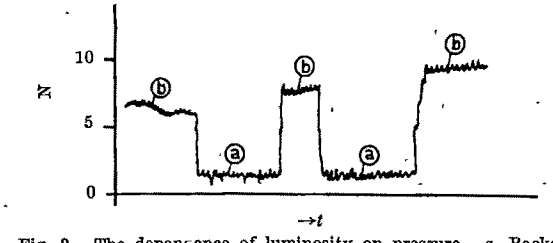


Fig. 3 The dependence of luminosity on pressure. *a*, Background pressure 10^{-5} torr; *b*, background pressure 10^{-4} torr. The other conditions were kept constant

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WE should like to comment on some of the points raised by Schutten and van Dijk.

The efficiency figure of about 1 per cent which we have given[1] was for the most efficient class of meteorite we found—the enstatite achondrites. We are surprised that Schutten and van Dijk have, for comparison, taken the meteorites Arispe, Odessa and Holbrook. The first two are listed as irons, and as it seems inconceivable that almost pure nickel-iron should luminesce at all, even the weak emission they observed was probably due to surface

contamination. It is indeed difficult to make any target clean enough not to luminesce at this sort of level, especially if a large beam current is used. We find that Holbrook, a hypersthene chondrite, has an efficiency (when fresh) of about 1/25 of that of the enstatite achondrites[2], and we can only suppose that the even lower output they found was obtained from a heavily damaged sample. Indeed, the spectrum they give for it resembles ours for a damaged sample rather than a fresh one. The difficulty with this kind of measurement is that unless the proton beam current density is kept very low, the sample damages rapidly, resulting in a greatly reduced efficiency and a changed spectrum—and to obtain a spectrum at all with a sufficiently low beam current a very powerful spectrograph is needed. We find that a proton beam current density of about 1/4 μamp/cm^2 is about the most that can safely be used. Schutten and van Dijk do not give any information about their sample preparation, or state their beam current density, which is actually more important than the particle energy. Also the spectral range of their measurements does not extend far enough into the red to include the region where we have found the efficiency to be highest for the most efficient meteorites.

The second point which surprises us is the reported difference of 10^5 between the efficiencies for electron and proton excitation. Such other published evidence as is available suggests that the efficiencies are about the same. There is a shortage of this sort of data, but it is available for some scintillator materials; for example, Allison and Casson[3] conclude that for sodium iodide, protons and electrons have about the same efficiency, and even for α particles it is not much lower. Organic scintillators are known to be less efficient for heavy particles than for electrons, but even so, the figures collected by Brooks[4] from various workers only show factors for anthracene ranging from 2 to 5 depending on the energy. Silicates are likely to show much smaller differences[5], and we feel justified in taking the efficiencies as about the same for protons and electrons. We have therefore used the electron excitation figures given by Bril and Klasens[6] for the material of our comparison phosphor, as applying, within the accuracy of our measurements, to proton excitation. One effect which could produce an apparent reduction in efficiency for protons is that protons damage the target much faster than do electrons of the same energy. If a large beam current were used, the proton efficiency could become very low in a matter of seconds, although we would hesitate to ascribe the whole of the factor of 10^5 to this.

As regards the effect of secondary electrons, the process by which protons excite luminescence emission at all presumably involves the production of secondary electrons within the material as its first stage—it is unlikely that protons interact directly with the material[5]. However, the liberation of secondary electrons from the surface in large quantities, and their attraction back again by a positive surface charge, seems a very unlikely mechanism for enhancing luminescence by a large factor. All our measurements have been made with a thin dust target on an earthed metal plate, and it seems unlikely that the surface could charge up to the high potential needed to attract the electrons back with appreciable energy. Also the effect, if appreciable, should cause the apparent efficiency to vary with the surface potential, which should in turn increase with target resistance and therefore thickness. We have tried the effect of varying the thickness of the dust layer, and find the efficiency to be about the same for a thick pile of dust as for a single grain smeared out on the plate, only falling off slightly as the target becomes too thin for it to cover the beam area uniformly. Only when we put the dust on a glass plate do we notice charge effects—the beam becomes unstable and disperses convulsively, giving a wildly fluctuating luminescence output—but the peaks are still roughly at the previous steady level.

We have also checked the effect of the pressure in our apparatus. If, during normal operation, we increase the pressure by isolating the pumps, a small increase in the luminescence output indeed occurs, but this arises from a build-up of the hydrogen pressure in the ion chamber which, up to a point, increases the proton output and therefore the beam current. If, on the other hand, we increase the pressure by leaking air into the system, the luminescence output initially decreases, followed by a series of more complicated changes which we can explain, but which are in any event too small to be important. We conclude that, for a given beam current, pressure changes over the range likely to occur in our apparatus do not produce significant effects.

Unfortunately, Schutten and van Dijk do not give enough information for us to attempt an explanation of their results, except to say that they seem consistent with the use of a rather high beam current density.

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ABSORPTION OF HIGH-ENERGY GAMMA-RAYS WITHIN QUASARS AND OTHER RADIO SOURCES

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THE conspicuous absence of positive results[1] in the many attempts in recent years to detect high-energy gamma-rays from selected objects in the universe has led many to question the transparency of space to such radiations. It has gradually become apparent that high-energy γ-rays, if produced, may suffer absorption *en route*, especially by pair production, in photon–photon collisions[2] with low-energy quanta present in intergalactic and interstellar space. Collisions with the photons of intergalactic starlight[3] and radio-frequency fields[4] were first considered, and it is now realized[5,6] that the recently discovered, and supposedly universal, microwave thermal radiation[7,8] may be sufficiently strong to produce significant attenuation of γ-rays even within the Galaxy. There is now also indirect evidence[9,10] for this universal microwave radiation, from interstellar CN absorption spectra from stars in our own Galaxy.

The cases of γ-ray absorption considered so far have all been concerned with the attenuation in the relatively weak photon fields between the sources and the Earth. The purpose of this article is to point out that the same absorption process may be significant within the sources

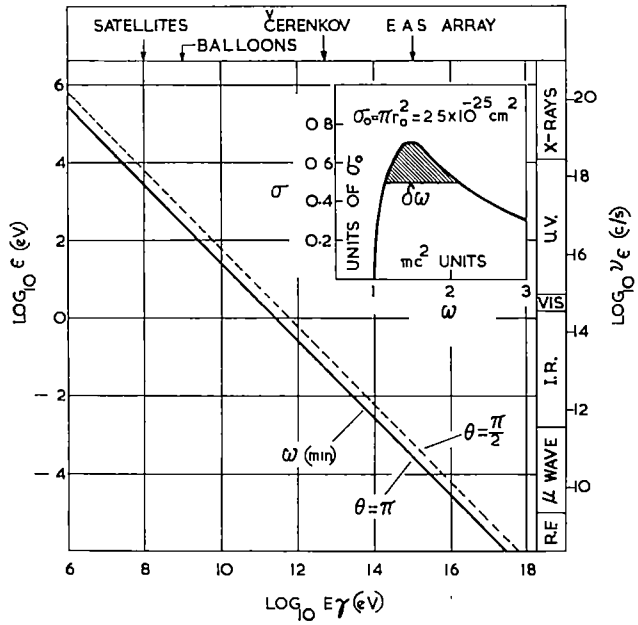


Fig. 1. A plot of the threshold condition for pair production by photon–photon collisions, between high-energy γ-rays and local radiations of low quantum energy ε. Inset: the form of the cross-section σ close to the threshold. $\omega = (E_\gamma \varepsilon)^{1/2}/m$

themselves. This idea is prompted by the realization that the potential sources of high-energy radiations are in general also intense sources of radiation of low quantum energy. In such cases the high photon density of the local fields of the lower-energy quanta may more than compensate for the relatively short interaction path lengths, as the γ-rays move through and away from the sources.

The absorption of a γ-ray of energy E_γ by pair production[2], on collision with a photon of energy ε, has a threshold at $E_\gamma \varepsilon\,(1-\cos\theta) = 2\,m^2$, where m is the rest-energy of the electron, and θ the angle between their directions of motion. This threshold is lowest for head-on collisions, $\theta = \pi$, when $E_\gamma = (m^2/\varepsilon)$. To the accuracy required here, we consider only head-on collisions, for which the threshold condition is shown in Fig. 1. The cross-section σ for the process (see insert) rises from zero at the threshold to a maximum just above it, where it attains an absolute value $\simeq 0{\cdot}7\,\pi\,r_0^2$, where r_0 is the classical radius of the electron. If $\omega = (E_\gamma.\varepsilon)^{1/2}/m$, σ is significant (that is, $> 0{\cdot}5\,\pi\,r_0^2$) over a band-width $\delta\omega \simeq \omega$, or $\delta\varepsilon \simeq 2\varepsilon$, for a given E_γ; a value of $\sigma = 10^{-25}$ cm^2 will be used throughout this article. If u_r is the energy density of the soft quanta, the numbers-density of these quanta $n = (u_r/\varepsilon)$, and the mean free path against photon–photon absorption is $L = (1/\sigma n)$.

For our purpose here we make the important assumption that the volume occupied by the lower-energy quanta is the same or larger than that from which the γ-rays may originate, and that the flux of the soft quanta is distributed approximately isotropically, so that we will ignore the variation of threshold and absorption cross-section with θ. In this model it is therefore implicitly assumed that in general the photon (and particle) energies decrease monotonically with radial distance from the core. If the source has a core and halo, as for example in the model of Rees and Sciama[11] for the quasar *CTA* 102, we would assume that the γ-rays originate in the stellar object at the centre, and not appreciably in the halo. It should be emphasized that if the energy distribution is reversed, so that some higher-energy particle and radiation fields occur in the outer halo regions, for example through the inverse Compton process, the conclusions deduced in this paper will be invalid, or will at least have to be modified (N. A. Porter, private communication).

Assuming the volume occupied by the low-energy photons to have an effective radius R, then, if the source is at a distance d, and s is the flux of these quanta at the Earth, per unit bandwidth, $u_r = (4\,s/c).(d/R)^2.\delta\nu$ erg. cm^{-3}, in a bandwidth $\delta\nu$. Taking an effective bandwidth $\delta\nu = 2\nu$ (since $\delta\varepsilon \simeq 2\varepsilon$), where $\nu = (\varepsilon/h)$, the numbers-density of photons over this effective band, is:

$$n_\varepsilon = (8\,s/c).(d/R)^2.h^{-1} \text{ quanta cm}^{-3}$$

which, we note, is independent of ε. s is assumed to be approximately constant over $\delta\nu$.

Existing techniques in γ-ray astronomy cover broadly three energy regions, with thresholds $\sim 10^8$–10^9 eV in satellite[12] and balloon-borne experiments[13], $\sim 5\times 10^{12}$ eV by the Čerenkov technique[14], and $\sim 10^{15}$ eV by large air shower arrays[15]. Let us now consider possible source absorption in these three energy regions, and for three markedly different types of object, namely quasars, galactic supernova remnants, and 'peculiar' galaxies. The main interest attaches to the 5×10^{12} eV and the 10^8–10^9 eV bands, since the microwave absorption will preclude the observation of all but local galactic objects at $E_\gamma \sim 10^{15}$–10^{17} eV. First take the case of 5×10^{12} eV γ-rays:

Quasars warrant special attention in view of their present-day interest and high energy content. 3*C*273 has been selected, as it is one for which both flux limits have already been obtained[16] and for which there is at the same time adequate information on its energy content at lower frequencies[17,18]. It is appreciated that in some respects this choice is not ideal, in view of the complex structure[19] of this particular quasar.

Of the many models proposed we consider three; the first two are simplified theoretical models, and the third is based on interferometric measurements[19] on structure, at $\lambda \simeq 21$ cm.

Model 1. Consider first the recent model of Hoyle, Burbidge and Sargent[20], in which it is assumed the object is extragalactic but local, with $R \simeq 10^{17}$ cm (determined by its light variations) and $d \simeq 10$ megaparsec (Mpc). At $E_\gamma = 5\times 10^{12}$ eV, from Fig. 1, $\varepsilon = 0{\cdot}052$ eV ($\nu = 1{\cdot}26\times 10^{13}$ c/s, $\lambda \simeq 24\mu$). Extrapolating from the infra-red measurements[18] at $\lambda = 10\mu$, we find $s \simeq 2\times 10^{-22}$ erg. cm^{-2}sec^{-1}(c/s)$^{-1}$, $n_\varepsilon = 7{\cdot}7\times 10^{11}$ photons cm^{-3} and thus $L \simeq 1{\cdot}3\times 10^{13}$ cm, that is, only $\simeq 1{\cdot}3\times 10^{-4}\,R$.

Model 2. Assume instead that the source has the same linear size, but that it lies at its cosmological distance of 470 Mpc (for Hubble's constant, $H = 100$ km sec^{-1} Mpc^{-1}). n_ε is then $1{\cdot}7\times 10^{15}$ photons cm^{-3} and $L \simeq 6{\cdot}1\times 10^9$ cm, only $\sim 6\times 10^{-8}\,R$, again at $E_\lambda = 5\times 10^{12}$ eV.

Model 3. Now take the linear dimensions of 3*C*273, as measured at $\lambda = 21$ cm (ref. 19). This source has two components of which one, component B, coincides with the optical source, and this in turn has two parts. The other and larger component, A, does not emit in the optical region. Because at $E_\gamma = 5\times 10^{12}$ eV the effective value of ε falls in the infra-red spectral region which is nearer to the optical than to the decimetre band, we will assume the observed infra-red[18] arises from one or both parts of component B.

The radio measurements set a limit of 0·1 sec arc for the size of either of the two parts of component B. This, in turn, implies $R \leqslant 2{\cdot}5$ pc or $\leqslant 114$ pc on the 'local' and cosmological models respectively, again with $H = 100$ km sec^{-1} Mpc^{-1}. Because this component B has not been resolved optically, we will assume the infra-red radiation divided equally between the two parts, in which event we deduce $n_\varepsilon \geqslant 6{\cdot}5\times 10^7$ quanta cm^{-3} and thus $L \leqslant 1{\cdot}5\times 10^{17}$ cm, that is, $\leqslant 0{\cdot}021\,R$ on the local model, or $\leqslant 4{\cdot}5\times 10^{-4}\,R$ on the cosmological model. From Low's extrapolated spectrum of 3*C*273, the infra-red yield of component A is at least three orders of magnitude lower than that from component B, so that any γ-rays ($E_\gamma \sim 10^{12}$ eV) generated in A would doubtless survive the local infra-red fields in this component.

We conclude from these arguments that if 3C273 is typical of quasars, and if the real object resembles any of these three models, it is unlikely that γ-rays in the 10^{12}–10^{13} eV energy region will be observed from quasars —at least if such γ-rays are emitted in the region of the core. This is consistent with the observations[16], with the exception of 3C147 for which there remains a suggestion of a positive effect.

Consider now the absorption of lower-energy γ-rays within this quasar. At $E_\gamma \simeq 10^8$ eV (typical of satellite experiments), $\varepsilon \simeq 2{\cdot}5$ keV which occurs close to the 3 keV X-ray band, for which galactic and intergalactic space are transparent. This band has already been explored in rocket experiments, for example, by the American Science and Engineering, Inc.–Massachusetts Institute of Technology[25], and U.S. Naval Research Laboratory[26] groups. Although no X-rays have as yet been observed from quasars, a flux limit has been quoted[1], $s_x < (1/20)$ of that from the strong source *SCOX*–1, for which $s_x = 1{\cdot}6 \times 10^{-7}$ erg. cm^{-2} sec^{-1} over a band $\delta\varepsilon = 1$–10 keV. Taking $\bar{\varepsilon} = 3$ keV and $\delta\varepsilon = 2\bar{\varepsilon}$, we find s_x (quasars) $< 3{\cdot}7 \times 10^{-27}$ erg. cm^{-2} sec^{-1} (c/s)$^{-1}$. For the local model, model 1 ($R = 10^{17}$ cm, $d = 10$ Mpc), one obtains from this figure $n_\varepsilon \leqslant 1{\cdot}4 \times 10^7$ X-ray photons cm^{-3}, or $L \geqslant 7{\cdot}2 \times 10^{17}$ cm, or $\geqslant 7R$, that is negligible attenuation. On the other hand, with this same limiting flux, on the extreme model 2, one finds $L \geqslant 3{\cdot}2 \times 10^{14}$ cm or $\geqslant 0{\cdot}003\,R$, in which case the absorption is very heavy. On model 3, that is $(R/d) < 0{\cdot}05''$, we have $L < 535R$ and $< 11R$, for the local and cosmological models, respectively.

There is no observational evidence so far for γ-rays from 3C273, or from any other quasar either in balloon-borne or satellite experiments, and the recent search[13] at $E_\gamma > 10^9$ eV with an angular resolution $\sim 2°$ unfortunately did not embrace the co-ordinates of this source.

Crab nebula. This object has always been, and remains, an object of special interest in γ-ray and X-ray astronomy. We shall assume a distance $d = 1{\cdot}1$ kpc, and that it has a diameter of $\sim 5'$ at optical and radio frequencies. Its spectrum is known over a wide range of frequencies[21], and has been discussed in detail by Gould[22]. At $\varepsilon \simeq 0{\cdot}052$ eV, appropriate to $E_\gamma = 5 \times 10^{12}$ eV, we find $s = 4{\cdot}8 \times 10^{-22}$ erg. cm^{-2} sec^{-1} (c/s)$^{-1}$, which leads to $n_\varepsilon \simeq 37$ photons cm^{-3}, and thus $L \simeq 2{\cdot}7 \times 10^{23}$ cm or $\sim 10^5\,R$, implying negligible absorption within the source. Photon–photon absorption cannot therefore be responsible for the apparent absence of γ-rays from this object[16,23] at this energy. Moreover, it will not frustrate the search for γ-rays at the lower energy $\sim 10^{11}$ eV which it is hoped to attain with a giant mirror system proposed[24] by the Smithsonian Astrophysical Observatory.

For 10^{15} eV γ-rays which could be observed with the BASJE array[15], and which would survive the universal microwave field, the corresponding figures are: $\varepsilon \simeq 2{\cdot}6 \times 10^{-4}$ eV ($\lambda \simeq 4{\cdot}8$ mm), $s = 2{\cdot}85 \times 10^{-21}$ erg. cm^{-2} sec^{-1} (c/s)$^{-1}$, $n_\varepsilon \simeq 220$ photons cm^{-3} and $L \simeq 4{\cdot}5 \times 10^{22}$ cm, or $1{\cdot}8 \times 10^4\,R$; again the attenuation is negligible.

It is readily shown that the attenuation of 10^8–10^9 eV γ-rays within the Crab nebula, owing to the observed X-rays from this object, is quite negligible, even allowing for the fact that these X-rays are concentrated within a relatively smaller volume, of angular diameter $\sim 1'$ as deduced from the occultation experiment[27]. While many of the known X-ray sources are exceedingly strong on the basis of total energy, they are relatively impotent for this absorption process, as the large bandwidths lead to correspondingly small values of s.

Galaxy M87. This object has attracted considerable attention as it is believed that much of the optical as well as the radio-frequency radiation arises from synchrotron radiation[28]. The optical emission, moreover, is concentrated in a thin jet the volume of which is small compared with that of the main Galaxy. We will assume that γ-rays may be generated in the region of the jet, the dimensions of which, following Burbidge[28], are those of a cylinder of length 20″ and diameter 2″. With a revised distance $d = 11{\cdot}4$ Mpc ($H = 100$ km sec^{-1} Mpc^{-1}), this jet has dimensions 1,100 pc × 110 pc, so that $R \simeq 55$ pc. The total optical emission from this jet is $6{\cdot}8 \times 10^{41}$ ergs. sec^{-1} (over 4000–5500 Å), so that for $\nu_{opt} = 6{\cdot}5 \times 10^{14}$ (c/s), the source strength S_{opt} is $\simeq 1{\cdot}0 \times 10^{27}$ erg. sec^{-1} (c/s)$^{-1}$.

For $E_\gamma = 5 \times 10^{12}$ eV, $\varepsilon \simeq 0{\cdot}052$ eV and $\nu_\varepsilon \simeq 1{\cdot}3 \times 10^{13}$ (c/s). The spectrum of the radiation from M87 can be written as a power law $P(\nu) = k\nu^{-\alpha}$, for which, in the optical band, $\alpha = 0{\cdot}65$ (ref. 28). The infra-red source strength S_{IR} is then $(\nu_{opt}/\nu_\varepsilon)^{0{\cdot}65} S_{opt} \simeq 1{\cdot}3 \times 10^{28}$ erg. sec^{-1} (c/s)$^{-1}$ and, at the Earth, $s = 8{\cdot}7 \times 10^{-25}$ erg. cm^{-2} sec^{-1} (c/s)$^{-1}$. For $\delta\nu = 2\nu_\varepsilon$, we deduce $n_\varepsilon \simeq 1{\cdot}51 \times 10^3$ quanta cm^{-3} and hence $L \simeq 6{\cdot}6 \times 10^{21}$ cm, or $\simeq 39R$ if R is here the radius of the jet. Absorption within the jet is thus insignificant, but not by such a large margin as in the case of the Crab. Burbidge has predicted the γ-ray fluxes which might be expected from M87. While these figures are clearly model dependent and cover a broad range, they fall in the general region of sensitivity of existing equipment. No radiation has been observed at $E_\gamma \simeq 5 \times 10^{12}$ eV although a limiting flux has been obtained[23].

While X-rays have now been observed from M87 (ref. 29), the first such source known definitely to be extra-galactic, their effect on 10^8–10^9 eV γ-rays is again quite negligible, for the same reasons already discussed for the Crab nebula.

Finally, consider the survival of high-energy γ-rays as they traverse the solar radiation on their last stages to the top of the Earth's atmosphere. The highest radiation flux to which these γ-rays are exposed in the solar system occurs at $\varepsilon \simeq 2{\cdot}5$ eV (corresponding to $E_\gamma \simeq 1{\cdot}0 \times 10^{11}$ eV). At the Earth's orbit, the solar flux is $\simeq 1{\cdot}4 \times 10^6$ erg. cm^{-2} sec^{-1} over the optical band, so that $n_\varepsilon \sim 1{\cdot}2 \times 10^7$ photons cm^{-3} (for $\delta\varepsilon = 2\varepsilon$), and thus $L \simeq 8 \times 10^{17}$ cm or $\sim 6 \times 10^4$ a.u., which represents negligible absorption. It is worth noting, however, that if γ-rays are generated in the early phases of a supernova, during which the light intensity rises to $\sim 10^9$ times that from the Sun, it is possible that this same absorption mechanism may prevent or reduce their escape from the source; in such a case, however, the energy thresholds and the photon–photon collision frequencies will be modified owing to the small values of θ.

To summarize, it would appear that γ-rays in the energy region 10^{11}–10^{13} eV may not be able to escape from the core regions of quasar sources, though they may be free to do so from the more extended halo regions. It is concluded, however, that the recently discovered X-ray emissions from various 'point' sources, strong though they are, are unlikely to frustrate the escape of lower-energy γ-rays in the energy region 10^8–10^9 eV. The survival of γ-rays of $\sim 10^{12}$ eV energy over interstellar and intergalactic distances depends critically on the level of infra-red background radiations in the energy region $\sim 0{\cdot}26$ eV, that is, for $\lambda \simeq 4{\cdot}8\mu$; it is therefore most important for the future of γ-ray astronomy that the extra-terrestial flux of infra-red radiations should be established as soon as satellite-borne equipment of adequate sensitivity can be flown.

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DATING PLEISTOCENE EVENTS IN SOUTH-WEST WALES

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Recent investigation has revealed a broadly identical stratigraphy of coastal Pleistocene deposits between Cardigan Bay and Gower (Fig. 1). The key to this stratigraphy occurs in Gower where a cave earth yielding a mammalian fauna together with a ubiquitous palaeosol enables the west Wales stratigraphy to be dated independently of recent radiocarbon dates[1] which are not as unequivocal as could be hoped and which, in the light of investigations reported here, seem misleadingly young. It seems likely that the shells from which Dr. John obtained his dates have been contaminated by ground water percolation through the gravels in which they lay.

The last glaciation of south-west Wales, with the possible exception of a small area in north Pembrokeshire and south Cardiganshire on which a Weichselian Irish Sea glacier moving down St. George's channel may have impinged[2–4], would now appear to be Saale (Riss) in age and not Weichselian (*c.* 28,000 B.P.) as suggested by Dr. John[1].

In 1932 Prof. George described a four-fold stratigraphy at Minchin Hole cave, Gower: (i) *Patella* beach; (ii) a cave breccia 8 ft. (2·44 m); (iii) marine sand, the *Neritoides* beach—1–3 ft. (0·30–0·91 m); (iv) blown sand merging laterally with limestone head. Since that date the section has receded and a recent investigation revealed:

(6) Upper head 1 22 m	(Weichsel)
(5) Cemented sand 2·31 m	(Eem)
(4) Loose sand (the *Neritoides* sand) 0 46 m	(Eem)
(3) Stratified cave earth 114 m	(Eem)
(2) Lower head 2 m	(Saale)
(1) *Patella* beach 0 51 m	(Hoxnian)

The *Patella* beach (1) is ubiquitous in Gower[5] and rests on shore platforms between 4·88 m (16 ft.) and 15·24 m (50 ft.) *O.D.*[6], the episode it represents having been one of multiple sea-level within that height range. From its included erratics it has been inferred that it is interglacial in age[7]. The lower head (2) described by George as a cave breccia "very similar to normal head" and believed by Hinton[8] to have accumulated in a 'temperate' period by virtue of its fauna, is a periglacial deposit produced by thermoclastic processes as is shown by the angular and sub-angular sharp-edged nature of its constituents. It is not unlikely that the fauna contained was derived from a previous warm period to which the *Patella* beach may be referred. The mammalian fauna yielded by the cave earth, which included *D. hemitoechus*, is similar to that recovered from the Upper Flood Plain (Ilford) terrace of the lower Thames and is regarded by Dr. A. J. Sutcliffe as Last Interglacial (Eem) in age[9]. For Minchin Hole this provides a horizon which can be dated and to which the other members of the stratigraphy can be related. The lower head (2) is suggested to be Saale (Riss) in age, the upper head (6) to be Weichselian, and the *Patella* beach deposit (1) to be Hoxnian.

Resting on the cave earth is the 'gritty marine sand' (4) of George which contained species of *Neritoides*. This was believed to indicate a higher and younger sea-level than that recorded by the *Patella* beach. Dr. M. R. Dobson kindly undertook an analysis of the sand, and on the basis of available parameters for distinguishing beach and dune sands[10], it would seem to be wind blown. It is just possible, however, that the sand may have been reworked by the sea after wind deposition.

Elsewhere, as for example between Shirecoombe and Minchin Hole, where George recognized the *Neritoides* beach, it can be shown that it is merely a facies of the lower head, beach pebbles, shells and sands having been incorporated within the limestone head. In short, the evidence from Minchin Hole and elsewhere suggests that the concept of a *Neritoides* beach indicating a specific sea-level must be rejected. The sea-level contemporaneous with the accumulation of the Minchin Hole cave earth and sands is adduced in the following.

Resting on *Patella* beach and lower head at many sites in Gower is a *terra fusca* palaeosol. George described it as a "fluvioglacial deposit"[11], but Wirtz first recognized its interglacial character[2]. Later, Ball described it at Worms Head and from its fabric concluded that it had formed under conditions similar to those of the present day but with warmer summers[12]. At many sites the lower head has been weathered and under Pwll-du head the *Patella* beach has been completely weathered. On the basis of the position of the palaeosol (that is, resting on both *Patella* beach and lower head and covered by later upper head) it is correlated with the cave earth and sands of Minchin Hole which are also considered to indicate a warm period, namely the Last Interglacial (Eem). It is unlikely that the palaeosol is any younger, for it is overlain by the thickest Pleistocene deposits in Gower, the upper head, which must be referred to the Weichselian, and from what is known of the non-glacial phases of the Last Glaciation it is unlikely that summer temperatures would have been sufficiently warm for its development[13]. At Mewslade the palaeosol rests on a shore platform at 4·88 m (16 ft.) *O.D.*, suggesting that the platform pre-dated the Last Interglacial. This, however, may not be conclusive because here, as elsewhere, the palaeosol has been subject to solifluction and may not be *in situ*.

Overlying the palaeosol occur the thickest Pleistocene deposits of the area, the upper head. Without exception in Gower they accumulated by solifluction and the 'glacial' horizons discussed by George and Wirtz consist of solifluted glacial deposits. Nowhere does till occur *in situ* in the coastal sections. This is shown by the fabric of the deposit, the long axes of stones showing a remarkable preferred orientation parallel to and changing in sympathy with local slope changes; the included head masses of local bedrock material; the cryoturbated silt horizons; the well-developed bedding; the lateral merging of glacial material with local bedrock head; and finally the intimate relationship between local topographic depressions and accumulations of solifluted till material. At several sites, for example at Horton, the surface of the upper head is

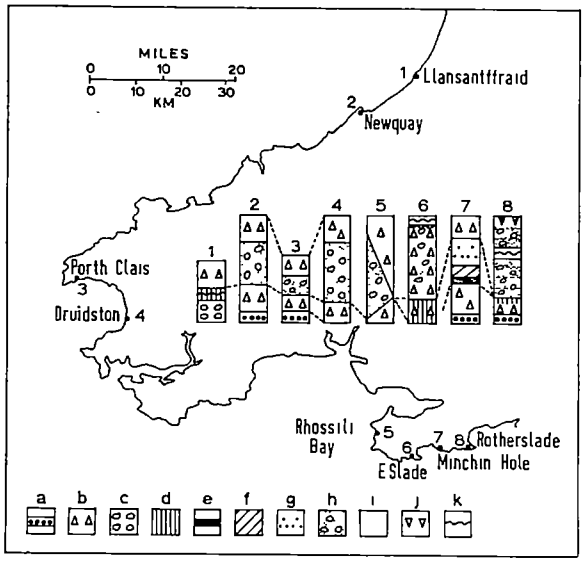


Fig. 1. Sections not to scale. Key: *a*, *Patella* beach; *b*, head; *c*, till; *d*, weathering horizon or interglacial palaeosol; *e*, cave earth; *f*, Minchin Hole sand; *g*, Minchin Hole cemented sand; *h*, solifluсted till (part of the upper head); *i*, silt horizon; *j*, frost wedge casts; *k*, involutions

frost heaved, and frost wedge casts occur at Rotherslade. Capping most of the coastal sections is the upper loam of George[11], a periglacial deposit which sometimes reveals a three-fold stratigraphy, the two lower members having been frost heaved. The upper loam is probably Late Glacial in age.

The Heatherslade beach rests on the 'modern' shore platform and, because it contains larger and a higher percentage of erratics than does the *Patella* beach, George inferred that a glaciation occurred between the formation of the two beaches. It seems likely that this glaciation was a later manifestation of the cold period which commenced with the accumulation of the lower head. The deposits of this glaciation were solifluсted during a later cold period which post-dated the formation of the palaeosol. The last glaciation of Gower must, therefore, be referred to the Saale (Riss/Gipping) Glaciation, and the changing facies and frost structures within the upper head indicate changing environmental conditions during the Weichselian. These are being investigated.

The sea-level of the Last Interglacial is probably indicated by the height of the undercut notch at the inshore margin of the Heatherslade beach shore platform and lies at 1·52 m (5 ft.) *O.D.* It is unlikely that the shore platform is any younger for radiocarbon dates suggest that, during the Weichselian, world sea-level was below the present[14], and as the platform passes beneath the solifluction terrace at Eastern Slade it cannot be Flandrian. This suggestion is consistent with a belief that the Minchin Hole sands were not flung up any great distance.

The Gower stratigraphy is repeated in west Wales where, at Porth Clais, Leach recognized a four-fold stratigraphy resting on a shore platform: raised beach, head, boulder clay (solifluсted), head[15] (Fig. 1). At Druidston Haven, John described a calcareous till, which I believe has been solifluсted, resting between two heads[16], while at Newquay, a calcareous solifluсted till occurs between two deposits referred to by Mitchell as local boulder clays[17], but considered to be head deposits by Watson[18]. The lower head rests on what might be raised beach gravels[17]. Finally, at Llansantffraid a cryoturbated interglacial palaeosol developed on weathered till is overlain by periglacial torrent gravel[7].

With the possible exception of a small area in north Pembrokeshire and south Cardiganshire[2–4], Weichselian ice did not reach the shores of south-west Wales. The basic stratigraphy is identical over wide areas and in particular within and outside Prof. Charlesworth's end moraine line[19] which has been subject to doubt recently as an ice margin by investigations of fluvioglacial features[3,4] and periglacial phenomena[20]. In Carmarthenshire the limit of local Weichselian ice may be marked by well-defined valley moraines and related outwash[6,17].

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FORMATION OF QUARTZ SAND

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Quartz sand is the universal continental detrital material and, as Kuenen[1] has noted, its abundance has led to its relative neglect. It is generally accepted that quartz sand is derived from quartz crystals in granitic rocks; this is a view of respectable antiquity, for example, it was stated by Sorby[2] and by Dawkins[3]. A careful modern statement has been made by Kuenen[4]: "It is assumed that the bulk of non-volcanic and non-organic sands is derived from weathered coarse crystalline rocks, mainly granites and gneisses".

There does not seem to be any acceptable alternative source and, in fact, Nemecz[5] has shown experimentally the similarity between quartz crystals in granite and sand grains found nearby.

There are two aspects to the study of the formation of sand grains; the first concerns the formation of the quartz crystals in the granite and leads to a field rich in controversy, the second concerns the release of the quartz crystals from the granite matrix by weathering.

The chemical composition of most granites is such that they can conveniently be represented on the silica–nepheline–kalsilite diagram as shown in Fig. 1. This is based on the dry diagram determined by Schairer and Bowen[6] and revised by Schairer[7]. The quaternary silica–albite–orthoclase–water is perhaps more significant and useful where the formation of the granite is being considered. Sorby[8], from his investigation of the liquid inclusions in quartz, came to the conclusion that the

magma of granite had solidified in the presence of water under great pressure but at low heat. This still appears to be a reasonable assumption. Turner and Verhoogen[9] state that the mineralogy and coarse holocrystalline fabric of granites suggests origin by slow crystallization of siliceous magma. This will be taken as the starting point in the process of sand formation.

It appears that the quartz crystals in most granites are part of a coarse eutectic of quartz and feldspar. In most granites (or at least those formed by crystallization from magma) large feldspar primary crystals are formed and then the quartz–feldspar eutectic. The other minerals (mica, apatite, etc.) at the beginning of the Rosenbusch succession have been ignored. It has been suggested that the graphic granite structure is a eutectic structure[10]; if it is, its existence would lead one to expect the existence of granites with primary crystals of silica. Very large crystals have been reported in pegmatite structures and some of these have been extensively investigated, in particular by Jahns[11], but they do not appear to form part of a primary silica plus eutectic system. The eutectic crystals must be the major source of quartz sand and the granites containing them all have compositions very close to the silica–feldspar boundary in Fig. 1.

Fig. 2 is based on a figure by Tuttle and Bowen[12] and shows the system silica–albite–orthoclase at various pressures of water vapour. The silica–feldspar boundary does not move much with increased pressure, but high quartz replaces tridymite as the silica form at the liquidus. Tuttle and Bowen also show a section through the 1,000 kg/cm² isobaric prism from $Ab_{50}Or_{50}$ to the silica apex in the silica–albite–orthoclase diagram, and this shows the temperature of the eutectic valley to be of the order of 710° C. The eutectic temperature in the dry system is of the order of 1,000° C, but in actual rock systems the lower temperature probably applies. The high pressures and the presence of water suppress the formation of tridymite and cristobalite and the silica crystallizes as high quartz with an axial ratio of the order of 1·092 and hexagonal symmetry.

At the inversion temperature the high quartz crystals undergo a displacive transformation[13] into low quartz. This transformation is characterized by the change in bond angle at the oxygen atoms. If, at the transformation temperature, the bonds all change simultaneously the low quartz crystal suffers a uniform stress. If, however, the change in the bond angle is a progressive phenomenon, there will be a time when part of the crystal is high quartz and part is low quartz. If there is any sort of temperature gradient in the high quartz crystal the low quartz will

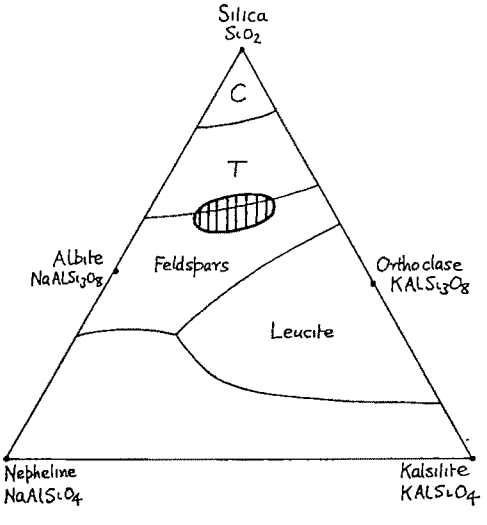


Fig. 1. The ternary system nepheline–kalsilite–silica. The shaded area represents compositions similar to those found in granites. (*C*, cristobalite, *T*, tridymite)

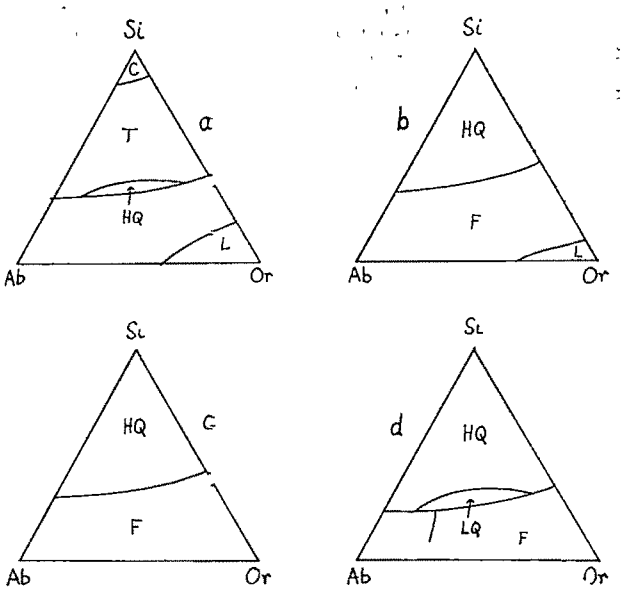


Fig. 2. Stability fields at the liquidus in the system albite–orthoclase–silica–water for various pressures of water vapour; *a*, 200 kg/cm²; *b*, 2,000 kg/cm², *c*, 3,000 kg/cm²; *d*, 4,000 kg/cm²

start to form at the lower end. The existence of an influential temperature gradient does not seem likely. It is observed that in most granites the quartz is among the last part of the system to solidify and could therefore be under some constraint; there could be compressive crystallization stresses. For the maximum relief of well-distributed compressive stresses a uniform contraction is indicated, starting at a point at which the stresses are concentrated. This will be somewhere in the interior of the crystal and from this point the low quartz structure will develop. The high–low interface will spread until it encompasses the entire crystal. There are several types of interface which may occur between separate structures in solid systems. Fine[14] has defined three kinds of interface between two crystalline phases (α and β): (1) Incoherent interface; there is no similarity in the arrangements of atoms in the α and β planes facing each other across the interface. (2) Coherent interface; here there is a perfect match in the arrangements, angles and spacings of atoms in the α and β planes facing each other across the interface. (3) Semicoherent interface; this is the case in between 1 and 2. The high–low interface is probably type 2. Nabarro[15,16] has considered the system in which the interface between α and β is coherent and all the strain caused by the phase change is taken up by the matrix. The new phase β is hard, the matrix α is soft. Nabarro, using the elasticity theory assuming elastic isotropy, computed the strain energy for expanding different-shaped holes hydrostatically. The holes were taken to be ellipsoids of revolution with a the long axis and b the short axis of the original ellipse. The axis of revolution is about b. The result of Nabarro's calculation is:

$$E \text{ (per unit vol.)} = K(\Delta V)^{1/3} f(b/a)$$

where K is the compressibility, ΔV is the difference in volume between the two phases and E is the energy.

E is least for a disk and maximum for a sphere. A low interfacial energy tends to favour a sphere; a large change in volume favours a disk. The equation appears to be applicable for an expansion or a contraction caused by the phase change. In the case of the high–low transformation the system is contracting. The volume change is relatively small, and being a simple displacive transformation the interfacial energy is probably low. It seems reasonable to expect low quartz to be slightly harder than high quartz; it has the same bonding system but a

more compact structure; high pressures certainly favour low quartz more than high quartz as the pressure–temperature diagram for silica shows. Both forms are, however, markedly anisotropic and this would influence the shape of the interface. An ellipsoid with three unequal axes would appear to be the most likely interface shape. This would influence the stress distribution in the crystal and the shape of the eventual sand grains.

If the alternative and apparently more likely assumption is accepted and a more or less simultaneous change of all relevant bond angles is postulated, a different approach must be made to the problem. It is observed that during the displacive high–low transformation the *c*/*a* ratio changes considerably. Fig. 3 shows the change in *c*/*a* ratio with temperature; the data are from Frondel[17]. As quartz cools from 800° C to about 50° C the *c*/*a* ratio changes from 1·0916 to 1·1000. If it is assumed that *c* stays constant, the volume change due to the change in *c*/*a* ratio can be calculated. The volume change is calculated with reference to a hexagonal prism and found to be 1·5 per cent. The value quoted[17] for contraction occurring at the high–low transformation is 0·86 per cent. This contraction can be accounted for by an increase in the *c*/*a* ratio from 1·0916 to 1·0950. This could virtually be achieved with an isothermal transformation at the high–low inversion point. It appears that the observed contraction at the high–low transformation could be accounted for by contraction about the *c*-axis caused by the change in bond angle at the oxygen atoms. The stresses caused by this type of contraction would act in a tensile manner perpendicular to the *c*-axis and would tend to cause cracks parallel to the *c*-axis and lead to elongated sand grains.

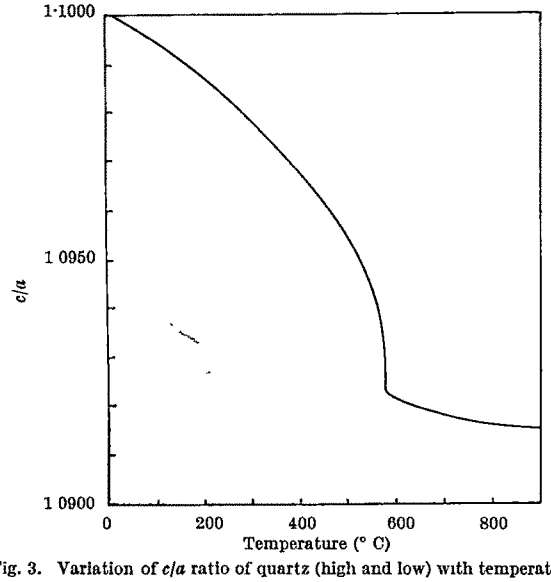


Fig. 3. Variation of *c*/*a* ratio of quartz (high and low) with temperature

Wayland[18] and Rowland[19] have observed that the quartz grains in sandstones tend to be elongated, and Wayland has noted a correlation between elongation and *c*-axis direction. Their observations and the suggestion that quartz particles fracture parallel to the *c*-axis led Ingerson and Ramisch[20] to carry out a series of abrasion tests on quartz. They also performed a statistical survey of the angles between *c*-axes and long axes of the grains of freshly crushed quartz and determined the relation of elongation to optic axis for much weathered but undisturbed quartzose rocks. They inferred that (1) quartz has no pronounced tendency to fracture parallel to the *c*-axis, (2) elongation parallel to the *c*-axis is not likely to be developed by differential abrasion, and (3) unbroken and unworn quartz grains show elongations parallel to the *c*-axis and the rhombohedron faces. They conclude that the elongation of quartz sand grains is due to the original shape rather than to fracture and differential abrasion during transport.

It may be that forces applied externally do not tend to fracture crystals parallel to the *c*-axis but that internal forces generated while the quartz crystal is part of a granitic system do cause this type of cracking. As a result, quartz grains would tend to be elongated in the *c*-axis direction.

There appear to be three factors which could influence the shape of quartz sand grains: (1) the shape of the original crystal in the igneous rock; (2) the possible distortion of this crystal by metamorphic action; and (3) internal stresses due to the high–low transformation and external stresses due to weathering. Abrasion during transportation does not materially affect the shape of the sand grain. Bokman[21] analysed the shapes of quartz crystals in source rocks and found that the mode elongation of quartz in granite was of the order of 1·3. The elongation of crystals in schists was much more marked. The elongation is expressed as an elongation quotient; this is the long axis/short axis ratio.

The metamorphic action which creates schistose rocks will account for the higher proportion of elongated quartz crystals and will therefore exert an influence on the shape of the eventual sand grain. The quartz crystals in granite are not shaped by such external forces but have more chance to form random shapes. A crystal growing in a magma system has a three-dimensional existence, and a measure of its shape can be gained by three measurements of length taken at right angles and referred to three orthogonal axes *a*, *b* and *c*. An ideal case can be considered where a limited number of lengths is possible. If each of *a*, *b* and *c* can have any integral value from 1–10 units the relative abundance of the different shapes can be calculated; the total number of solid figures possible is $(10)^3$; ten choices for each of three measurements. These can be classified into shapes using the Zingg system. There are ten configurations which fulfil the condition $a = b = c$; these fall into Zingg class 2. There are 720 configurations which fulfil the condition $a \neq b \neq c$; these fall into class 3. The remaining 270 configurations have tetragonal shapes and fall into classes 1 and 4. Thus, in this random model, Zingg class 3 shapes predominate; with an increasing number of available values for *a*, *b* and *c* the fraction of class 3 shapes increases.

If observations are made on crystals which have grown in a magma system or sedimentary particles formed by some random process one would expect the majority of them to possess the elongated shapes of class 3. This is what Bokman observed in granite. The random conditions required are more likely to be encountered in the formation of particles than in the formation of crystals, where the atomic structure and bonding of the crystal have considerable influence on the external form.

The size and shape of sand grains are largely determined at the moment the eutectic quartz solidifies in a cooling granite magma. The crack system encouraged by the stresses generated by the high–low transformation ensures a correlation between elongation and *c*-axis direction.

Schubert[22], among others, has stated that "sand is produced by continuous abrasion and action of sedimentary processes". There seems to be some confusion over the role of abrasion in the formation of sediments. Rogers, Kreuger and Krog[23] set out to show that abrasional methods of production of sand and silt are qualitatively different. If abrasion is caused by the mechanical action of one sediment unit on another it is difficult to see how it can contribute effectively to sand formation.

It is observed that there is a scarcity of certain particle sizes in sediments. Tanner[24] quotes 0·03–0·12 mm and 1·0–8·0 mm as the particular values. These effectively divide sediments into different types: gravel, sand and silt. The relevant units may be called blocks, grains and flakes. The experiments of Rogers *et al.* were designed to explain the scarcity of 0·03–0·12 particles by proving

that there are two distinct modes of abrasion, one giving sand (grains) and the other giving silt (flakes). So far as quartz sand is concerned, it seems more reasonable to suggest that abrasion plays no effective part in its formation. Its size is determined by the size of the eutectic quartz crystals in granites; its shape is determined largely by chance; its separation from the granite matrix and its final shaping are due to the combination of internal and external forces. The separation is materially aided by the tensile stresses in the quartz crystal caused by the contraction at the high–low transformation. If these stresses are concentrated inside the quartz crystal they will ensure that the grain splits cleanly away from the matrix when the crack caused by the weathering agency arrives. The glacier provides a powerful and efficient rock splitting action[25] and it seems likely that most of the quartz sand found in Britain and N. Europe is of glacial origin[26]. The problem of abrasion has been aptly summarized by Guilcher[27]: "There is no progressive change from one grade to another by gradual attrition: in particular, sand appears to be derived only to a small extent, if at all, from the reduction of shingle". Blocks and grains and flakes are formed by completely different mechanisms and it would be very surprising if a continuous distribution of size were observed.

The origin of sand cannot, with any confidence, be traced further back in time than the granite stage. From the sedimentological point of view this is a satisfactory starting point. A safe maxim is that the formation of a sediment should be traced back as far as is needed to establish the factors which influenced the properties of the detrital particles; this article is an attempt to do this, in outline, for quartz sand.

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ARRANGEMENT OF ATOMS IN THE FIRST MONOLAYER OF NICKEL ON TUNGSTEN

By DR. J. P. JONES

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AN investigation of the properties of adsorbed layers of copper on tungsten by field emission microscopy led to the suggestion that copper atoms in the first monolayer conformed exactly to the tungsten structure by taking up positions offered by the array of potential wells present at the tungsten surface. The observed maximum work function was attributed to the formation of a complete monolayer of copper–tungsten dipoles[1]. Some support for these suggestions has now been obtained by ion microscopy applied to nickel layers adsorbed on tungsten; for, like copper, nickel produces a maximum work function which can be attributed to a complete dipole layer (Fig. 1).

A bakable field ion microscope of conventional design has been used to obtain helium and neon ion images of nickel condensed on tungsten. A source of nickel was formed by enclosing a 3 mm length of Johnson-Matthey 'Specpure' nickel wire 0·5 mm diameter in a closely fitting helix of tungsten wire 0·25 mm diameter. The helix, supported on a tungsten-through-glass press seal, was situated 1·5 cm from the tungsten point. The tip, supported on a tungsten loop, was heated by direct current and temperatures were estimated by measuring the resistance of the central portion of the loop. The tip shank consisted of a length of tungsten wire 2 mm × 3 × 10^{-4} mm which constituted a thermal barrier, so that during evaporation of nickel at $T \sim 1{,}200°$ K radiant heating is calculated to have raised the temperature of the tip by 40° K at room temperature and somewhat less at 78° K.

After preliminary bake-out to attain a pressure $< 5 \times 10^{-9}$ torr, the tip was maintained at 1,600° K for 2 h in 10^{-2} torr pressure of purified hydrogen. Following bake-out at 400° C, helium was admitted, and the tip heated to 1,500° K for 2 min before final cleaning by field evaporation at 78° K, to produce the familiar evaporation end-form of tungsten. The helium was then pumped out and the surface examined by field emission to obtain Fowler–Nordheim data at pressures below 2×10^{-9} torr. With no field applied, nickel was then condensed on the tip maintained at a pre-selected temperature, and after

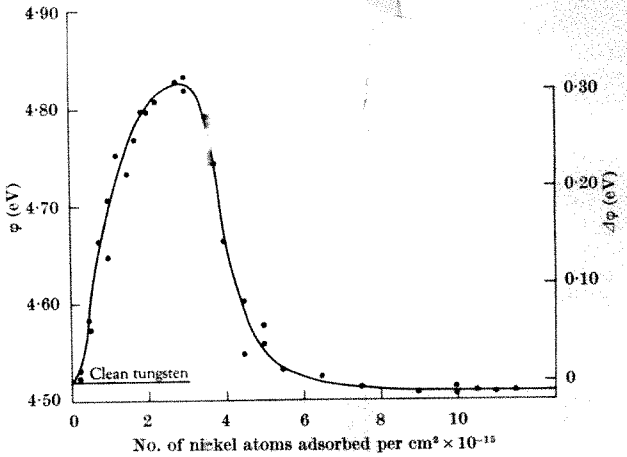


Fig. 1. Change in work function φ with amount of nickel condensed on to a clean tungsten point and spread uniformly over the surface

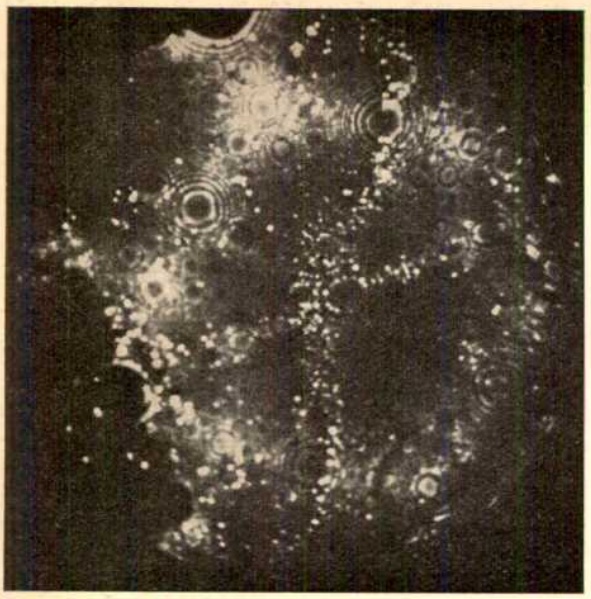


Fig. 2. An ordered nickel layer formed by condensation of vapour on to a tungsten tip cooled to 78° K. Second and higher layers of nickel are disordered and have been removed by field evaporation. Due to radiant heat from the nickel source the tip temperature during evaporation is calculated to have risen to ~118° K. (×1·2×10⁶)

examination of the deposit by field emission the tip was cooled to 78° K, neon admitted to $P \sim 2{\cdot}0 \times 10^{-3}$ torr, and the deposit examined by field ion microscopy.

Nickel condensed on to the tip cooled to 78° K showed no detectable order in the third and higher layers, and these fields evaporated at less than 9·5 kV to reveal a second layer having some degree of order. The second layer field evaporated at 10·5 kV, revealing a further layer of nickel which had a high degree of order and resembled closely the tungsten substrate except at the leading edge of the deposit (Fig. 2), where the adatom population decreased. With the tip held at room temperature, nickel atoms, which condensed to form the first monolayer, diffused forward to form a perfectly ordered layer over much of the visible surface. Second and higher layers exhibited little order and field evaporation revealed the final layer. Field evaporation of this final layer occurred first from {111} (Fig. 3*b*) at a field strength of about 3·1 V/Å and progressed inwards towards the central 110 plane as the field was increased (Fig. 3*c*). Gold condensed on tungsten has been observed to form a similar well-ordered layer[2]. The surface remaining after complete evaporation contained atoms that were clearly not part of the original surface but which nevertheless withstood fields up to that required to evaporate tungsten itself. They are thought to be tungsten atoms, but their origin is uncertain.

In order to obtain Fowler–Nordheim data, nickel was condensed on to the tip at 430° K. This temperature allowed complete coverage of the emitting areas by nickel and was sufficiently low to ensure stability of the tungsten surface during the experiment[3]. Considerable order was observed in the second and third layers, and the first layer was readily isolated by field evaporation of those overlying it. The first layer exhibited slightly less perfection than first layers formed at lower temperatures. Fowler–Nordheim data on clean tungsten obtained before and after deposition of nickel, together with data obtained for the isolated monolayer, showed that the nickel layer

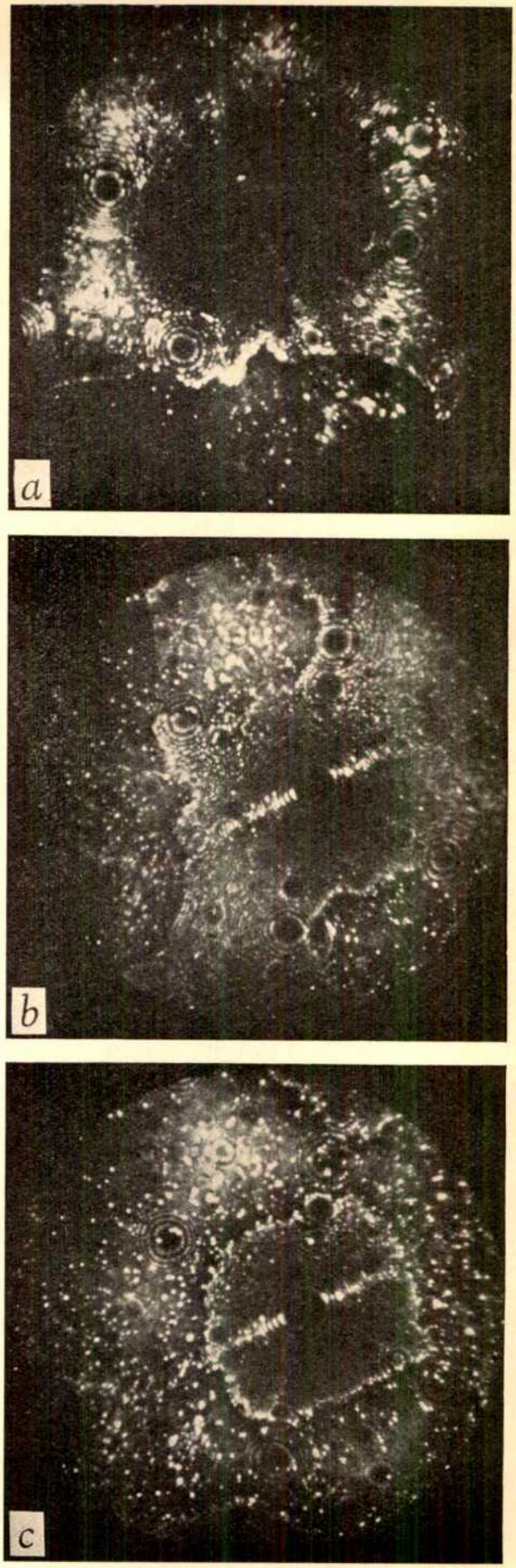


Fig. 3. *a*, The first monolayer of nickel, obtained by condensation on to a tip at 300° K, retains its ordered appearance up to the leading edge of the layer, which has spread by diffusion beyond the directly accessible target area. *b*, Field evaporation of the first monolayer at 78° K has removed nickel from {111} and their environs at a field strength of ~3·1 V/Å. *c*, With increasing voltage, field evaporation progresses by removal of atoms from the edges of the nickel layer which thus shrinks in towards the central 110 plane. (*a*, *b* and *c*, ×1·2×10⁶)

increased the work function by 0·27 ± 0·03 eV. This supports the belief that the maximum in Fig. 1 resulted from adsorption of one monolayer of nickel. It should be added that the good agreement between the work function change obtained on field evaporated tungsten and that observed on thermally cleaned tungsten (Fig. 1) is consonant with other observations of the similarity between the two forms of surface[4].

Current nucleation theory[5,6] is based on the belief that condensed material forms isolated nuclei which have the structure of bulk condensate. As applied to the condensation of gold[2] and nickel on tungsten the theory now requires some modification to take account of the formation of at least one, and possibly more, continuous monolayers of condensate on the surface, before nucleation takes place. The ordering achieved at 118° K would seem to imply that condensing nickel atoms make at least one atom jump before finally coming to rest on the tungsten surface[7].

I thank Dr. T. I. Barry for helpful discussion.

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PHOTO-OXIDATION OF ORGANIC MATTER IN SEA WATER BY ULTRA-VIOLET RADIATION, ANALYTICAL AND OTHER APPLICATIONS

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Following on the observations of Beattie *et al.*[1] that organic matter in fresh water could be oxidized to carbon dioxide on exposure of samples to radiation from a medium-pressure mercury vapour lamp, we found that exposure of sea water to such a light source caused a decrease in the extinction coefficient at 2000–2500 Å, characteristic of the presence of organic matter. This behaviour, indicative of the rapid oxidation of at least some of the organic material in sea water, led us further to examine the laboratory applications of high intensity ultra-violet radiation to analytical and other problems associated with the presence of dissolved organic matter in the sea. These preliminary findings may be of interest to workers investigating sea water chemistry as well as to others concerned with dilute aqueous solutions of organic matter.

In the work described here samples were irradiated by a 1,200-W mercury arc tube (Hanovia Engelhardt '189 *A*'), 30 cm in length mounted axially in a vertical aluminium cylinder 50 cm high and 20 cm in diameter. An air extractor fan at the lower end of the cylinder provided cooling. Dimming of the discharge through excessive cooling of the lamp was avoided by using a 4·5-cm diameter jacket of fused silica around the lamp. A small-bore silica tube delivered a stream of filtered air to the bottom of this jacket to carry off some of the heat produced by the lamp. Samples for irradiation were contained in stoppered fused silica tubes 35 cm long and 2·5 cm in diameter (containing about 110 ml.), twelve of which could be set parallel to the lamp and equidistant 7 cm from it. The temperature of samples stabilized at 60°–80° C at the tops of the tubes after prolonged irradiation.

The actinic energy incident on the samples was measured by oxalate actinometry[2] and was found to lie between 0·20 and 0·25 W/cm², some variation between tubes being found. The total actinic energy intercepted by twelve full tubes was therefore about 200 W out of some 400 W radiated in the range 2000–4400 Å (from the manufacturer's data). However, since the absorption of sea water is only appreciable below about 2500 Å, it can be calculated that only some 10 per cent of the incident actinic energy can be absorbed by the samples, at least one-half of this being at wave-lengths shorter than 2500 Å. That this was the more effective radiation was demonstrated by irradiation of samples in a 'Vycor' tube, which cuts off radiation of wave-lengths shorter than about 2400 Å. The oxidation reactions described here were then markedly slower.

* On leave from the Marine Biological Association, Citadel Hill, Plymouth.

Oxidation of Carbon in Organic Matter

The method of Menzel and Vaccaro[3] for the determination of dissolved organic carbon in sea water, rarely present in amounts outside the range 0·4–1·5 mg carbon/l., has been used by us to check the amounts of carbon left in sea water after irradiation with ultra-violet light.

In sea water the oxidation of organic matter followed first-order kinetics with respect to carbon, with a rate constant of about 0 05/min. Oxidation was effectively complete within 1 h with all samples of sea water that we have investigated.

To assess the percentage oxidation of various classes of organic compounds these were added to give concentrations of 1–2 mg of carbon/l. in artificial sea water Samples were irradiated and the residual organic carbon determined.

The following compounds were oxidized substantially to completion with a 2–3 h irradiation period: pyridine, 2,2'-bipyridine, adenine, ethyl alcohol, methyl alcohol, glucose, glucosamine, acetic and oxalic acid, formic acid, palmitic acid, dimethylamine, casein, glycerol, phenylalanine and "humic acid". Urea, on the other hand, was decomposed only to the extent of 50 per cent or less, even with the addition of hydrogen peroxide to the water as a source of excess oxygen The surprising behaviour of this compound was confirmed by the low oxidation rate of nitrogen. Palit and Dhar[4] reported incomplete oxidation of urea in experiments on the photodecomposition of organic compounds sensitized with zinc and ferric ions aerated and exposed to sunlight over long periods, but we would not have predicted our results and cannot explain them. Simple hydrolysis of urea does not occur to any extent at the hydrogen ion concentrations used here (*p*H 8).

The use of photosensitizers, such as Fe^{3+} and UO^{2+}_2 was not investigated. Heckler[5] has shown that carbon dioxide is the main product in the UO^{2+}_2-sensitized photodecomposition at 2537 Å of organic acids in solution, where carbon monoxide, hydrogen, methane and ethane are produced in lesser amounts.

The presence of appreciable quantities of end products other than carbon dioxide is contra-indicated in our work. Measurements of the decrease in *p*H after irradiation of known compounds in sea water gave values which corresponded with those calculated for the stoichiometric addition of carbon dioxide to the system. For example, the change in *p*H for sea water samples having a concentration of 1 mg of carbon/l. was of the order of 0·250 *p*H units.

If corrections are applied for the increase of hydrogen ion concentration due to the oxidation of dissolved organic nitrogen to NO_3^- and NO_2^-, then *p*H changes may be used as a rapid and fairly precise measure of dissolved organic carbon in sea water samples and such a method would be applicable to monitoring continuous flow systems.

Liberation of Inorganic Phosphate from Organic Phosphorus Compounds

Organically combined phosphorus in sea water (generally present between zero and about 30 μg/l.) has till now been estimated from the difference between two determinations of inorganic phosphate, the one on a sea-water sample after the destruction or hydrolysis of organic matter and the other on an untreated sample. Organic matter is generally destroyed by modifications of methods requiring wet oxidation with perchloric acid[6] or necessitating an autoclave treatment with sulphuric acid[7]. These methods are tedious and their precision is poor because of the difficulties of controlling contamination introduced by the reagents used to destroy organic matter. It has never been certain whether or not these procedures give interference from any inorganic polyphosphates that might be present in the sea, as these compounds would be depolymerized by the processes used to degrade organic matter and would then react with molybdate.

By contrast, exposure of sea water to ultra-violet irradiation liberates phosphorus as inorganic phosphate from organic matter rapidly and without the necessity of adding any reagents except one or two drops of 30 per cent hydrogen peroxide introduced to ensure the presence of an excess of oxygen. The method of Murphy and Riley[8] now enables *ortho*phosphate to be determined in sea water with a high precision (σ of 0·2 or less on 10 μg of phosphorus/l.) so that the differences of phosphate concentration found between irradiated and non-irradiated samples give a measure of the organically combined phosphorus present in filtered sea water with a precision far exceeding that of any analyses previously obtained. Even in deep ocean water one can detect with confidence as little as 0·5 μg organically combined phosphorus/l.

The method has been checked by us by adding phosphorus-containing organic matter to artificial sea water at concentrations of about 100 μg of phosphorus/l. and irradiating the solutions for known times before determining *ortho*phosphate in the water. Solutions of RNA were completely degraded to give the theoretical yield of inorganic phosphate after about 20 min of irradiation. For surety, 1–2 h exposure has been used with natural samples. After such a period we found complete mineralization to *ortho*phosphate of the phosphorus present in known additions of β-glycerophosphate, ribose-5-phosphate, RNA, choline phosphate and 2-aminoethanephosphonic acid (containing a carbon–phosphorus bond). In view of the destruction of these compounds, in particular the last two, it seemed pointless to test many other substances, but a trial was made with several linear inorganic polyphosphates with between three and seventy phosphorus atoms in the chain and also adenosine triphosphate with a polyphosphate group attached to an organic molecule. None of these compounds liberated any *ortho*phosphate, so that it can be assumed that ultra-violet radiation of the wave-lengths used does not bring about a significant degree of depolymerization and hydrolysis of inorganic phosphate polymers and that the phosphate liberated in sea water by ultra-violet irradiation must be present initially only in the form of organic esters. A detailed profile of organically combined phosphorus present in the sea off southern California is shown in Fig. 1.

Oxidation of Organic Nitrogen Compounds

Soluble nitrogenous matter and the ammonium ion in sea water were oxidized by dissolved oxygen, under the influence of the ultra-violet radiation used here, to give what we believe to be initially a quantitative yield of the nitrate and nitrite anions.

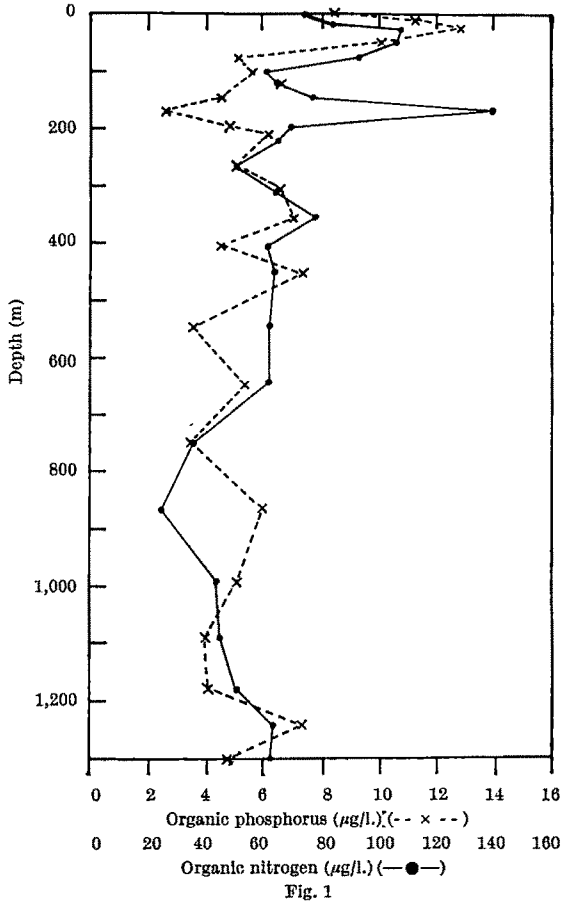


Fig. 1

It has been reported that ammonia can be photo-oxidized to nitrate and nitrite[9,10] and, therefore, because oxidative deamination occurs at an early stage in the photolysis of organic nitrogen compounds[11] nitrate and nitrite can be expected as end products of the irradiation experiments.

There are reports in the early literature[12,13] that sea water samples enriched with ammonium ion and exposed to sunlight yield nitrite, but a more recent investigation[14] did not confirm this.

The reduction of nitrate to nitrite is well documented for photolysis of solutions with or without ammonia or organic compounds present as reducing agents[15–18]. Such reactions have often been promoted by the presence of surfaces of tin, iron or titanium oxides[19,20] or by adding uranium or transition metal ions to the solution as photosensitizers. There is no information available in the literature on the products of exhaustive irradiation of small concentrations of organic matter in the presence of excess oxygen such as was undertaken in this work.

The method of Morris and Riley[21] for nitrate in sea water as modified by Strickland and Parsons[22] enables a rapid analysis to be made for this anion with high precision (σ of 3 or less on 200 μg of nitrate nitrogen/l.). The method consists essentially in passing sea water through a column of amalgamated cadmium filings and determining the resulting nitrite colorimetrically. Nitrate is reduced to nitrite with a yield in excess of 90 per cent, but any nitrite present initially in the sea water passes through the column unchanged.

The nitrogen in organic nitrogen compounds is first oxidized almost completely to nitrate which is then slowly reduced to nitrite on further irradiation. After 3 h about

30 per cent of the total nitrogen is present as nitrite. We have no explanation for this behaviour, which we have confirmed, but which clearly requires a major investigation for its elucidation.

We have made a preliminary investigation of the kinetics of the oxidation of organically combined trivalent nitrogen to give nitrate, using 2-2′-bipyridine, a convenient nitrogenous base in which the element is strongly bonded in a cyclic system. The rate of oxidation of amounts equivalent to about 200 μg organic nitrogen/l. in well-oxygenated water was found to be first order with a rate constant of about 0·03/min, which is appreciably slower than the carbon oxidation. The formation of nitrate was effectively complete in 2–3 h. Hydrogen ion concentration had no effect below *p*H 8, but above this value the reaction rate decreased, as if the ammonium or a similar ion, but not the corresponding hydroxide, were being attacked. (The ammonium ion, however, when added as such to sea water was oxidized considerably more rapidly than was organically combined nitrogen.)

Oxygen is required for nitrate formation and neither nitrite or nitrate is produced unless sufficient dissolved oxygen is present to oxidize all carbon compounds. Excess oxygen then becomes available for nitrate production by a reaction which is rate dependent on oxygen concentration raised to a fractional order of about 0·25. It is desirable, therefore, to have solutions saturated with oxygen and to this end we have added one or two drops of 30 per cent hydrogen peroxide of analytical reagent quality to each 100 ml. of sea water. This addition ensures the supersaturation of all samples with oxygen. Excess hydrogen peroxide appears to be rapidly decomposed and after 1 or 2 h of irradiation it cannot be detected.

Under these conditions solutions of ammonium chloride, 2-2′-bipyridine, casein, thiourea, adenine, guanidine, and even pyridine were quantitatively oxidized after 3 h of irradiation and, especially in view of the refractory nature of pyridine, we feel little doubt that most likely nitrogenous compounds dissolved in the sea would be transformed to give nitrate. It was interesting, however, to note that the behaviour of urea was again anomalous and this compound was only partially oxidized to give nitrate after an exposure time found adequate for the other substances. The resulting method is incomparably more rapid and precise than any existing method for determining organic nitrogen in sea water and enables detailed and reliable profiles like that shown in Fig. 1 to be obtained easily.

Other Uses

Several important applications of this technique for the destruction of organic matter in sea water can be envisaged, some of which have already been given a preliminary trial in our laboratories.

The ability to destroy all organic matter is of particular value in culture, nutrition and vitamin assay studies; water which is free from all organic growth factors and inhibitors can easily be delivered from a suitably constructed assembly at a rate of many litres an hour. The irradiated water contains no oxidants capable of liberating iodine from acidified iodide and appears to be usable for culture purposes with no further treatment or, at most, after only a brief treatment in an autoclave.

Most metallo-organic complexes should be readily decomposed by ultra-violet irradiation making for more definitive analyses of the total concentration of metal ion in sea water. By liberating metal ions in this manner from organic complexes they would be easily removed by subsequent complexing with standard organic reagents such as dithizone and separation of these new complexes by solvent extraction. Any excess of organic reagent could then be destroyed by irradiation in the presence of excess hydrogen peroxide, giving a sample completely free from organic compounds and from specific inorganic ions of nutritional significance.

We anticipate using ultra-violet irradiation in a continuous-flow system to combust the dissolved organic matter in several thousand litres of sea water in order to obtain carbon dioxide for radioactive dating. The problem of scavenging this organic material quantitatively from sea water in sufficient amounts for the purpose of dating has not, until now, been solved.

Finally, algal suspensions (marine diatoms) in the presence of peroxide can be destroyed and the carbon and nitrogen oxidized quantitatively during an overnight irradiation in the apparatus herein described. This technique has interesting possibilities for the rapid elemental analysis of cell suspensions without the necessity of preliminary filtration and washing which can lead to cell destruction and other difficulties.

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Use of Ultra-violet-irradiated Sea Water in the Preparation of Culture Media

SERIOUS difficulties are encountered in the culture of marine bacteria and algae, as well as in investigations of their physiology, on account of the toxic effects of natural sea-waters[1–5]. In order to overcome such effects the use of 'aged' sea-water in the preparation of media has been recommended[6]. However, such 'aged' water still contains unspecified amounts and types of organic matter and its behaviour is far from being reproducible for physiological investigations. In attempts to obtain reproducible organic-free media, artificial sea-waters have been used[3,7], but it has recently been demonstrated that artificial sea-water can also be inhibitory[8,9]. More recently, a charcoal-treatment of sea-water has been shown to be an effective method of preparing waters free from certain classes of metabolites[10]. However, this method has limited application as the complete removal of organic matter is not possible. Armstrong *et al.*[11] irradiated sea-water with a high-energy ultra-violet light source and found that its content of organic matter was reduced below detectable levels. It seemed reasonable to suppose that if this treatment produced no toxic by-products it would have application to investigations now in progress in these laboratories on vitamin B_{12} assays of natural waters and the kinetics of organic matter uptake by marine bacteria, as well as to the culture of marine bacteria and algae in general.

Sea-water was irradiated with ultra-violet light in the manner described by Armstrong *et al.*[11] for 2–4 h. This irradiation sterilizes the water and all subsequent handling of the water was, therefore, performed aseptically. Samples were not autoclaved unless specifically noted.

As a preliminary investigation into the possible toxicity of irradiated water, cultures of marine bacteria and algae were grown in media prepared with natural and irradiated natural sea-waters and observed for gross differences in growth behaviour. Shake-tube cultures of a marine pseudomonad were made in a medium containing glucose, ammonia and inorganic phosphate. The cultures were incubated at room temperature and their growth determined turbidimetrically. Growth curves of bacteria in the irradiated water showed no significant deviations from those observed in the natural sea-water cultures. Cultures of the diatom *Skeletonema costatum* and chrysophyte *Monochrysis lutheri* were grown in the same medium used for their routine maintenance in this laboratory. The medium was prepared with enriched natural sea-water, which had been shown to be suitable for their culture, and with an enriched irradiated sample of the same water. On one occasion there appeared to be a slightly lower initial growth rate in the medium made with irradiated sea-water, but this effect did not appear in subsequent experiments. Microscopic examination showed the algae cells to be normal in all respects in both experiments.

Sea-water samples are routinely assayed in our laboratories for the presence of vitamin B_{12} using the marine centric diatom *Cyclotella nana*. External standards of known B_{12} concentrations serve as the calibration values and are prepared in charcoal-treated[10] sea-water. Known amounts of crystalline vitamin B_{12} were added to media prepared with irradiated sea-water, irradiated sea-water which had been autoclaved (5 min at 15 lb.), and charcoal-treated sea-water. The growth response of *C. nana* in each of these waters was determined by measuring the incorporation of carbon-14 dioxide after a 2-h exposure to the isotope following a 47-h incubation. The growth response was shown to be a function of vitamin B_{12} concentration[10]. At all the concentrations tested there was little difference in the carbon-14 dioxide uptake rate in each of the media. In an earlier experiment, however, the uptake rate of carbon-14 dioxide in irradiated sea-water had been found to be about 80 per cent that in charcoal-treated sea-water. The slight inhibition found with this irradiated sea-water was overcome by a short autoclaving which may be a desirable precaution when using irradiated water for preparing standards for vitamin bioassaying.

An investigation into the kinetics of the uptake of organic matter by marine bacteria is in progress in this laboratory. The methods being used are similar to those described by Wright and Hobbie[12] where the velocity of substrate assimilation is measured over a range of substrate concentrations. The curves thus obtained and the mathematical constants describing them are extremely sensitive to the physiological state of the bacterial cells. Artificial sea-water has been used because of the possible presence of the metabolite under investigation in natural sea-water. We have compared the uptake velocity by a pure culture under rigidly standardized conditions in artificial and irradiated natural sea-waters. The two curves were identical at lower substrate concentrations. At higher concentrations, however, there was a slightly lower maximum velocity in media made with irradiated sea-water. It is probable that a short autoclaving treatment, as was performed with the B_{12} assay media, would have eliminated this effect.

While it is acknowledged that these results are preliminary in nature, they indicate that sea-water treated by the method of Armstrong *et al.*[11] may be used in the preparation of media for marine microbiological studies. On the basis of two different tests, both of which were extremely sensitive to inhibitory effects, it appears that the level of toxic by-products is very low or non-existent. Irradiation produces water of a quality equivalent to that obtained by charcoal treatment for vitamin B_{12} bioassay. There appears to be no reason why waters treated by this method could not be used, in preference to artificial and charcoal-treated sea-waters, in investigations of marine microbial physiology.

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EFFECTS OF ULTRA-VIOLET ALONE AND SIMULATED SOLAR ULTRA-VIOLET RADIATION ON THE LEAVES OF HIGHER PLANTS

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ONE of the factors often cited[1,1a] to support the idea that earth-type organisms could not survive the environment on Mars is the postulated occurrence of relatively high levels of ultra-violet radiation in the light which impinges on the surface of the planet. This, together with the recent *Mariner* photos of Mars, and the possible use of plants in bio-regenerative systems in space, adds new impetus to the question of the relative resistance of plants to ultra-violet radiation, as the ultra-violet light present in unfiltered solar radiation occurs at levels which are considered harmful to most living organisms. The deleterious effects of ultra-violet light on higher green

plants have been investigated for nearly three-quarters of a century[2,3].

Leaves and flowers are generally the most sensitive plant organs. Leaves respond to ultra-violet light by turning a bronze or dark colour and sometimes appearing shiny. If the radiation dose is sufficient, the leaf will die. Despite this, many plants will recover by sending out new shoots when they are returned to a normal environment. Ultra-violet radiation does not penetrate deeply; thus roots and, to a lesser extent, stems are protected by soil and by bark. Extended periods of high-intensity ultra-violet radiation will eventually kill most plants, but the exact cause of injury is not known. Present lines of investigation indicate that it may involve the formation of poisonous peroxides[4] and the deactivation of nucleic acids[5] and proteins.

Investigations[6] are being carried out at Colorado State University to determine the relative resistances of a wide variety of plants to ultra-violet alone and to a high-intensity simulated solar spectrum. Possible adaptive mechanisms are also being considered. Although several approaches are being taken, the present article is concerned with two leaf survival experiments involving a total of sixty-seven species with special emphasis on *Xanthium pennsylvanicum*, *Zea mays* and *Pinus nigra*.

The first experiment was concerned with a survey for resistance among widely diverse species of the plant kingdom carried out with a germicidal lamp (Sylvania 15 W, low-pressure mercury arc—90 per cent of its energy is at 2537 Å). The second experiment was an investigation of resistance to ultra-violet in the presence of a complete, simulated solar spectrum (high-pressure xenon arc). The total emitted radiation has been measured with a thermopiler adiometer (Colorado Research and Engineering Inc., Boulder, Colo.). So far as is known, these are the first such surveys of this type which have been made since the development of the germicidal lamp and the xenon arc.

In the first experiment there was an extremely wide variation in the resistance of the sixty-seven different species tested. Leaves of young seedlings of *Pharbitis nil* were killed with less than 1 cal/cm² of germicidal light, whereas leaf blades of week-old corn seedlings required over 33 cal/cm². The lethal exposure for young needles of ponderosa pine was about 800 cal/cm². It should be emphasized that these lethal exposures do not refer to that quantity of radiation necessary to kill all foliage on the plant but only the uppermost leaves or needles. For corn it refers only to the exposed portions of the leaf blade and not to the sheath. Leaves were examined for damage within several days after irradiation. A leaf was considered to have been killed if it showed significant signs of curling, of colour change to yellow or brown, and of drying.

In the second experiment, a 2,500-W high-pressure xenon arc lamp (Osram Co.) was used as the light source. The spectral distribution of emitted light (especially in the ultra-violet) was quite similar to that of unfiltered solar radiation above the Earth's atmosphere[7]. The radiation emitted from the lamp was projected on to the plants (*Xanthium*, *Zea* and *Pinus nigra*), placed in a wind tunnel. In this experiment no filters were used. The total radiation levels used were 0·86, 2·00 and 3·82 cal/cm²/min. These intensities occurred at various distances from the focus of the arc and are equivalent to those above the atmosphere of Mars, Earth and Venus, respectively[8]. Two pots of plants could be tested at each of the three radiation levels simultaneously. A total of eighteen cocklebur plants (eighteen pots), about eighty corn plants (nine pots), and six 11-in. pine seedlings (six containers) were tested.

The only purpose of the wind tunnel in this experiment was to keep the leaf temperatures below a lethal level. Air temperatures ranged between 21° and 23° C. Wind velocity averaged about 6 m.p.h. Leaf surface temperatures (Table 1) were well below the lethal point for the three species. The plants were usually irradiated for 13–16 h each consecutive day. No attempt was made to simulate precise photo-periods, gravity effects, or gas composition or pressure of the Martian or other environments.

Table 1. LEAF SURFACE TEMPERATURES (°C) IN SECOND EXPERIMENT AS MEASURED BY COPPER-CONSTANTAN THERMOCOUPLES

	Radiation-level (cal/cm²/min)	*Xanthium*	*Zea*	*Pinus*
Martian	0 86	24	22–23	22·5–25
Terrestrial	2·00	27–32	25–28	22·5–26
Venusian	3 82	24–30	24–28	23–27

It was sometimes difficult to determine the exact radiation exposure necessary for the death of an entire leaf. Thus, the values presented in Fig. 1 are approximate. *Xanthium* leaves were killed after only 1·8 h of Venusian-level radiation, whereas leaf-kill by Martian-level radiation required about 4·5 h. The shoots of week-old *Zea* plants were significantly more resistant. The destruction of the exposed portions of these corn leaf blades required 8 h of Venusian radiation and about 32 h of Martian radiation. *Pinus* seedlings were exceedingly resistant. The newly-sprouted Austrian Pine shoots did not show serious

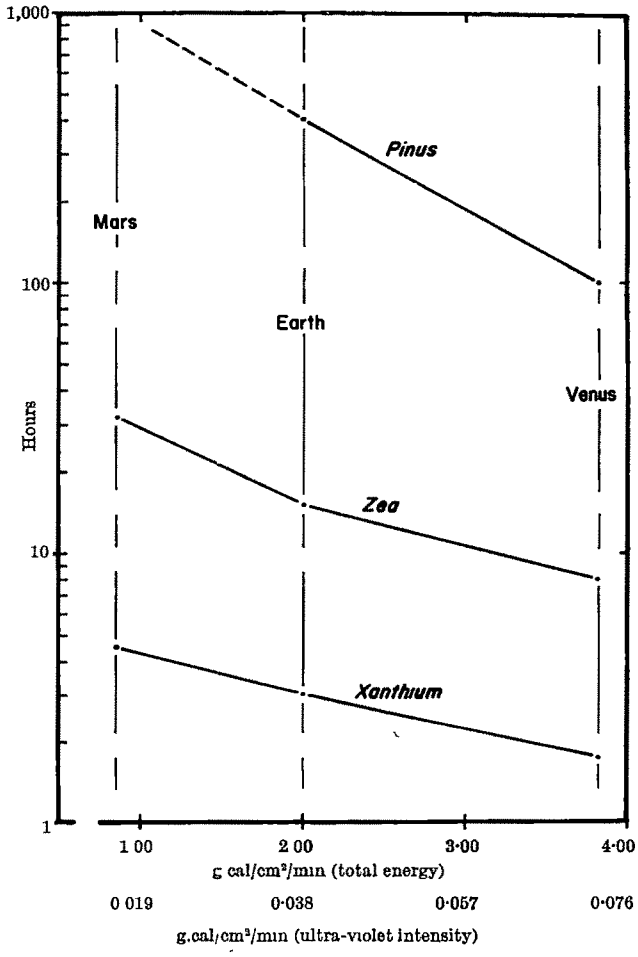


Fig. 1 Leaf survival time under different intensities of extra-terrestrial solar radiation in the second experiment. The values on the upper scale refer to the total simulated solar energy as measured; whereas those below refer only to the ultra-violet portion ($\lambda = 2000$–2900 Å) which was 1·9 per cent as determined by an analysis of the xenon spectral curve published by the General Electric Co. for 5-kW lamps (Large Lamp Dept, Nela Park, Cleveland, Ohio, 1962). The *Pinus* curve has been extrapolated to indicate its probable resistance to higher doses based on the fact that there was only slight damage after 635 h at the termination of the experiment

damage until after nearly 100 h of Venusian radiation. In this case there appeared to be evidence suggesting that a significant portion of the injury was not due to ultra-violet light but rather to water stress in the needles. Thus, the estimate is probably low. Nearly 400 h (30 days) were required to bring about serious damage to the needles of the plants at terrestrial radiation-levels, but at the Martian level there was an almost complete absence of damage in seedlings after 635 h (44 days) of irradiation.

In both experiments, nearly all tests were repeated except for the long investigation with *Pinus nigra*. For most species the results of the second treatment closely duplicated those of the first.

A fascinating aspect of the role of possible protective adaptations against ultra-violet damage in plants is the reversal of such damage by subsequent visible light. This is known to occur in a wide variety of organisms[9]. In the present investigation, photoreversal was found by the method of Tanada and Hendricks[10] in *Xanthium* leaves with both germicidal light (0·019 cal/cm of ultra-violet alone) and xenon light (8·4 cal/cm² total energy including ultra-violet, high-intensity visible and infra-red) followed by about 4 h of outdoor sunlight. The ultra-violet exposures already mentioned were near the maximum for reversal. When the exposures given were much larger than these values then the damage was not reversed. The significance of photoreversal in space is still largely unknown, but the effect could be an important one.

An interesting feature of the Martian atmosphere is the presence of a high violet layer (the 'blue haze') which is thought to be opaque to a considerable portion of the ultra-violet component of sunlight[11,12]. Occasionally this blue haze clears for a week or so, apparently allowing a considerable increase in the ultra-violet penetration through the atmosphere. A 10-day clearing in the blue haze (14·5-h days) would represent about 145 h of irradiation, or less than a fourth of the total time (635 h) used in this experiment. Furthermore, the total radiation on any particular Martian 'plant' would average considerably below 0·86 cal/cm²/min, mainly owing to the diurnal fluctuation, but also owing to latitudinal differences on the planet, some remaining atmospheric scattering, and mutual shading. Even certain earthly plants could, then, be expected to survive the ultra-violet bombardment during a blue clearing on Mars. Some of these (such as *Pinus nigra*, *Pinus ponderosa*, and *Agave* sp.) might even survive well if the blue haze were less opaque than is at present assumed[1a].

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DISCRIMINATING POWER OF RODENT DEOXYRIBONUCLEIC ACID ON INCUBATION IN AGAR

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THE discovery[1] that denatured deoxyribonucleic acid (DNA) from higher organisms can be trapped in an agar gel, and used to combine specifically with DNA fragments from similar or different sources, enabled the genetic approach, pioneered by Doty and his group[2] in bacteria, to be extended to mammals. Using this technique, Hoyer, McCarthy and Bolton[1] demonstrated a difference between radioactively labelled DNA fragments from mouse and man: human fragments bound better than mouse on to human or monkey DNA; mouse bound better than human on to mouse, rat or hamster DNA.

We have been concerned to explore further both the limitations and the possibilities of this technique[3,4]. There seems little doubt that the degree of binding between samples of DNA from different organisms at least in part reflects similarities in base sequence, which in turn reflect genetic relationships. The failure of more than about a third of the fragments to be bound to the trapped DNA, even when both are derived from the same source, is due to a true heterogeneity among the initial population of fragments[3]. For this reason, the proportion of fragments bound cannot be relied on to give a quantitative estimate of genetic relatedness. Further, different samples of DNA differ non-specifically in their binding capacity for fragments from any source[4]. Two species can therefore only be compared if labelled fragments are available from both, so that within-species and between-species binding can be tested on the same sample of DNA-agar. In the initial study of Hoyer *et al.*[1], labelled fragments were available from mouse and man only; thus the relationships between the various rodent species could not be examined.

Despite these drawbacks, the technique has made it possible for the first time to make qualitative distinctions between the DNA from different vertebrate species. But if common ground with traditional genetic methods is to be sought, the discriminating power of the DNA-agar technique will need to be very greatly increased, so as to bring more closely related groups within its scope. To this end, we have selected a single pair of fairly closely related species, laboratory mice (*Mus musculus* L.) and rats (*Rattus norvegicus* L.), and used it as a model on which to test a number of possible approaches to the problem of increasing the resolving power of the technique.

DNA is extracted[3] from liver or testis, from primary cell cultures of embryonic tissue, or from long-established cell strains; it is then denatured, trapped in agar,

sieved and washed[1]. To prepare DNA fragments, the cell cultures are labelled with radioactive phosphorus or tritium and, once extracted, the DNA is broken up with ultrasonic treatment and then denatured. DNA-agar is mixed with fragments in a water-jacketed column, kept usually at 60° C, and incubated overnight[3]. Unbound fragments are washed off at a high salt concentration; the temperature is then raised and the salt concentration lowered so as to elute the bound fragments. The result of an incubation is expressed as the percentage of the total radioactivity which is washed out at the higher temperature.

Discriminating between Mouse and Rat DNA

Initially we tried two approaches, which we termed "blocking" and "fractionation". If the DNA trapped in agar is exposed to an excess of unlabelled fragments from the other species, all shared binding sites should in theory be blocked. Labelled fragments added afterwards from the other species should be unable to find binding sites, and should therefore be rejected, while those from the same species should still be bound, though the number of available sites will, of course, be reduced. The discriminating power of the technique should thus be increased. In practice this proved to be so[4], but only to a rather small extent, owing to the difficulty of saturating more than a small proportion of the shared binding sites.

Table 1. Binding Ability and Discriminating Power of DNA Fragments during Three Successive Cycles of Incubation

Fraction selected from between-species incubation		Cycle 1	2	3
Bound	Within-species binding (per cent)	33	42	48
	Between-species binding (per cent)	23	35	47
	Discrimination (per cent)	30	17	2
Unbound	Within-species binding (per cent)	33	20	17
	Between-species binding (per cent)	23	8	5
	Discrimination (per cent)	30	60	71

For the second and third cycles, either the bound or the unbound fraction from the previous between-species incubation was taken. For each within- or between-species estimation in this and other tables, both mouse and rat fragments were used, and the binding percentages averaged. "Discrimination" is calculated as the percentage by which between-species binding falls short of within-species binding.

More effective discrimination was achieved when the DNA fragments were fractionated by means of successive between-species incubations. Those fragments which are bound by the DNA of the other species should preferentially contain shared sequences, and should therefore discriminate less well on a second incubation, while those which are rejected should include the species-specific regions which might be expected to discriminate maximally. Table 1 shows the results of three successive incubations, testing at each step for discriminating power, defined as the percentage by which between-species falls short of within-species binding. As predicted, the level of discrimination of the bound fragments declined at each successive incubation, and was virtually non-existent by the third cycle. By contrast, the rejected fraction increased in discriminating power at each successive cycle. Some asymmetry was evident, in that the rat fragments tended to discriminate less well than the mouse ones (see later).

More recently, we have found that the discriminating power of a DNA sample depends on the temperature at which the agar mixture is incubated. At 40° C, 70 per cent of fragments are bound in a within-species incubation, but the duplexes formed are very imperfect, judging by the shape of their melting curves[3]. As the temperature is raised, the proportion of fragments bound decreases, and the duplexes approach nearer to the native condition. The decrease in binding is proportionately greater in a between-species than in a within-species combination, from which it follows that the discrimination improves as

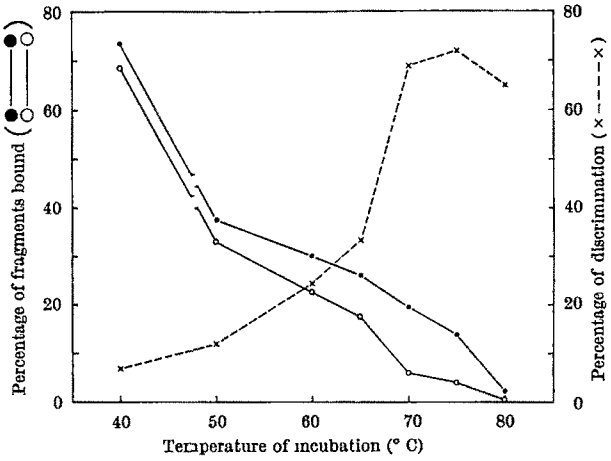


Fig. 1. The effect of temperature of incubation on the binding in DNA-agar of DNA fragments from the same (●——●) or another (○——○) species. Both mouse and rat fragments were tested at each temperature, and the results from the two species averaged to give the percentage of fragments bound. "Discrimination" (× - - - ×) is defined as the percentage by which between-species falls short of within-species binding

the temperature increases (Fig. 1). In our mouse–rat system, it is about 30 per cent on a first cycle at 60° C (Table 1), but by 70°–75° C (the highest temperatures at which the technique is satisfactory) it has risen to 70 per cent. At 70° C, fractionation by successive between-species incubations no longer improved discriminating power.

The poor discrimination at low temperatures does not mean that no specific binding has occurred, but is due rather to the masking effect of relatively non-specific binding. This can be shown by eluting the bound fragments at successively higher temperatures (Table 2). Whether the temperature of incubation is 40°, 50° or 60° C, discriminating ability is confined to those duplex regions stable at 60° C, and remains in the neighbourhood of 30 per cent.

Table 2. Binding Ability and Discriminating Power of DNA Fragments Stably Bound at Various Temperatures

Temperature of incubation (° C)	Temperature of elution (° C)	Percentage of fragments bound: Within-species	Between-species	Discrimination (per cent)
60	75	26·8	19·9	*26*
50	60	13·5	15·5	−15
	75	20·9	14·5	*31*
40	50	40·7	40·7	0
	60	13·7	14·7	−7
	75	16·7	11·7	*30*

Unbound fragments were washed off in high salt at the temperature of incubation; the temperature was then raised in steps, the eluted fragments washed off in high salt at each successive temperature below 75° C, and finally in low salt at 75° C.

The greater sensitivity to temperature of between-species, as compared with within-species binding which we have found, must in part reflect the fact that at higher temperatures, matching sequences will need to have longer complementary runs if they are to give stable binding. 'Species-common' and 'species-specific' sequences must be in continuity with one another, rather than segregated, and must constitute a gradation of increasingly imperfect matching between species. The greater thermal stability of the within-species duplexes might also indicate a higher cytosine-guanine (C-G) content. To investigate this possibility we tested the binding ability in DNA-agar of fragments separated on a hydroxyapatite column[5,6].

Binding Behaviour of Hydroxyapatite Fractions

When DNA fragments are eluted at increasing temperature and constant salt concentration from a hydroxyapatite column, the successive fractions show an increasing

C-G content[5]. Fig. 2 summarizes the results of a number of experiments in which the within- and between-species binding ability of successive thermal fractions of mouse and rat DNA fragments was tested. In between-species incubations, the results for mouse and rat fragments were very similar, and have been combined. There was no indication that within-species duplexes were richer in C-G than between-species ones; the greater thermal stability of the former is therefore presumably due entirely to the greater mean length of the complementary sequences involved in duplex formation.

Fractions with a high A-T content consistently bound less well on DNA-agar than did those from the intermediate or high C-G regions of the thermal chromatogram. In addition, the mouse fragments incubated with mouse DNA-agar showed a region of high binding towards the centre of the range, declining not only towards the high A-T but also towards the high C-G fractions (Fig. 2). This feature was not evident in the between-species or in the rat within-species incubations. It was characteristic of mouse DNA fragments incubated with homologous DNA-agar at 40° C and 70° C, as well as at 60° C (Fig. 3). A consequence of this was that the discriminating ability of mouse DNA fragments showed a strong and highly significant ($P < 0.001$) negative regression on the temperature of elution from hydroxyapatite (Fig. 4). High A-T fractions discriminated well, high C-G ones very poorly.

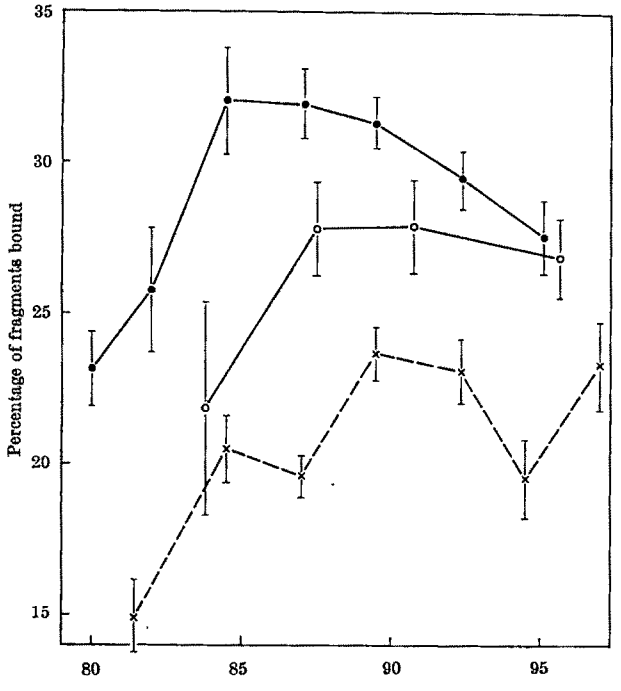


Fig. 2. Percentage binding, ± *S.E.*, of mouse and rat DNA fragments eluted at increasing temperatures from a hydroxyapatite column at constant phosphate concentration. The two between-species combinations gave very similar results, and have been combined. The total number of estimations carried out was 62, 23 and 56 for the mouse/mouse (●), rat/rat (○) and between-species (×) combinations respectively; in some cases estimates from different elution temperatures have been pooled so as to ensure that no points are based on one or two observations only. For calculating means and standard errors, the percentages were first subjected to angular transformation

The complementary fractionation procedure, whereby DNA fragments are eluted from a hydroxyapatite column at increasing phosphate concentration, at a constant temperature, fractionates DNA according to its secondary structure[5]. We find that, at 70° C, a small fraction of DNA, after denaturation, elutes in the native position (0·16–0·20 M) while the remainder elutes at lower salt concentrations (0·07–0·12 M) in the single-stranded position. We have termed these fractions "stable" and "labile", respectively[6].

Melting curve experiments on mouse DNA show that the stable fraction is partly duplex in nature, but that it is not identical with native DNA[6]. This is strikingly

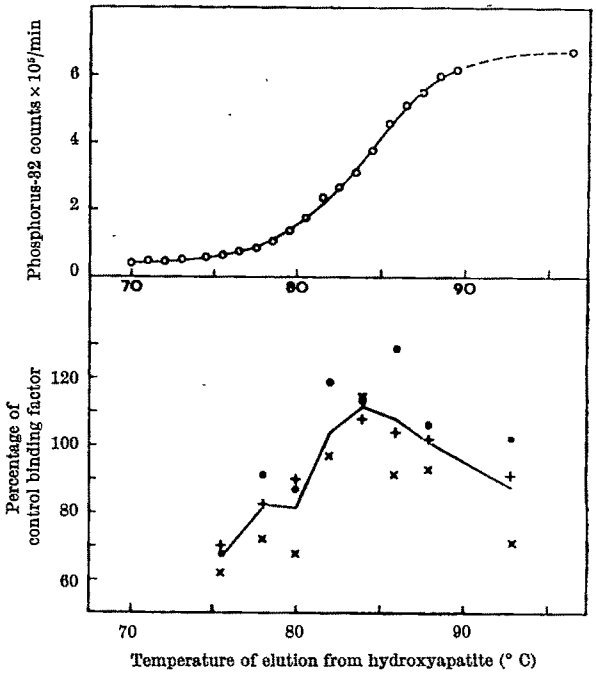


Fig. 3. The effect of temperature of elution from a hydroxyapatite column on the percentage of fragments of mouse DNA bound on mouse and rat DNA-agar during overnight incubation at 40° (+), 60° (●) and 70° C (×). For each incubation temperature the binding ability of each thermal fraction is expressed as a percentage of the binding ability of the unfractionated DNA fragments incubated in agar at the same temperature

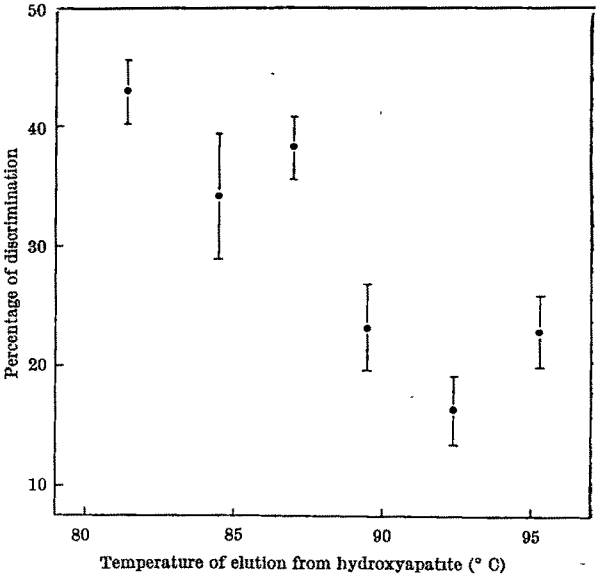


Fig. 4. The relation between the discriminating power, ± *S.E.*, of mouse DNA fragments, and their temperature of elution from a hydroxyapatite column. The points are based on a total of forty-one estimates, comprising all those experiments included in Fig. 2 where mouse DNA fragments were tested simultaneously on mouse and rat DNA-agar

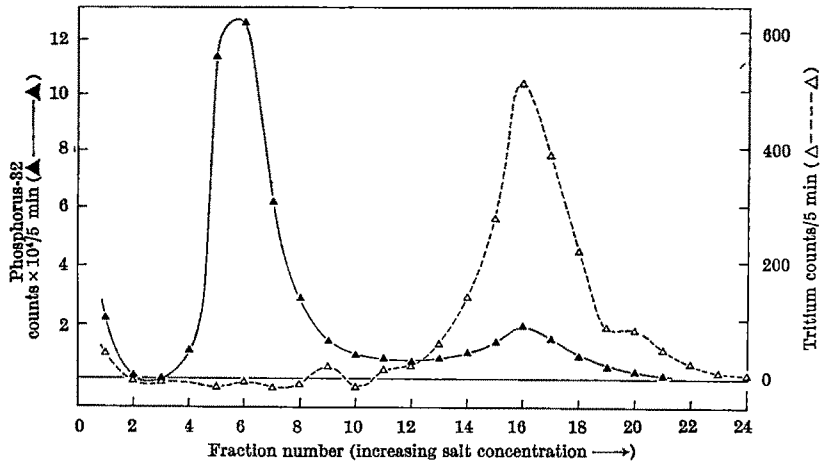


Fig 5. Hydroxyapatite fractionation at 60° C and increasing phosphate concentration (0·03–0·30 M phosphate), of native and denatured fragments of mouse DNA labelled with tritium (△) and phosphorus-32 (▲) respectively

confirmed by its binding ability on incubation with DNA-agar. Native and denatured mouse DNA, labelled with different isotopes, were fractionated simultaneously on a hydroxyapatite column (Fig. 5). The fractions corresponding to the native and single-stranded peaks were then incubated with homologous DNA-agar, and the results set out in Table 3 were obtained. The native DNA, as expected, showed very little binding; but the stable fraction contained in the same incubation mixture bound with remarkable efficiency. The labile fraction bound only about half as well as the stable fraction.

Table 3. BINDING ABILITY OF HYDROXYAPATITE FRACTIONS WHEN INCUBATED WITH HOMOLOGOUS (THAT IS, MOUSE) DNA IN AGAR GELS AT 60° C

Exp.	Hydroxyapatite fraction taken for incubation with DNA-agar	Denatured (phosphorus-32) Labile	Denatured (phosphorus-32) Stable	Native (tritium)
(1)	0·07–0·11 M	17·7	—	—
	0·14–0·17 M	—	42·8	3·7
	0·17–0·21 M	—	30·9	3·6
(2)	0·14–0·21 M	—	42·4	3·1

The fractions were taken from a hydroxyapatite column at 60° C, loaded with a mixture of native DNA labelled with tritium, and denatured DNA labelled with phosphorus-32. Figures represent the percentage of labelled DNA bound to the DNA-agar gel.

Table 4. EFFECT OF TEMPERATURE OF FRACTIONATION ON THE SIZE OF THE STABLE FRACTION OF MOUSE DNA, AND ITS BINDING ABILITY, WHEN INCUBATED WITH HOMOLOGOUS DNA IN AGAR GELS AT 60° C

Temperature of hydroxyapatite fractionation (° C)	Percentage of radioactivity in stable fraction (0·3 M)	No of DNA-agar incubations	Average binding (per cent)
50	22·8	3	51·9
60	15·0	2	50·8
70	12·2	3	51·2
Unfractionated	—	6	28·9

Table 5. EFFECT OF THE TEMPERATURE OF HYDROXYAPATITE FRACTIONATION ON THE DISCRIMINATING POWER OF MOUSE DNA FRAGMENTS

Temperature of fractionation on hydroxyapatite (° C)	Discrimination (per cent) shown by Labile	Discrimination (per cent) shown by Stable
40	42·0	58·6
50	32·5	65·2
60	28·5	77·9
70	23·4	80·7

A sample of DNA deliberately cross-linked by treatment with nitrous acid[6,7] proved to have a lower binding ability than control material; the fraction that eluted in the native position bound very poorly indeed (< 10 per cent), in contrast to the stable fraction of untreated DNA.

If the temperature at which the hydroxyapatite fractionation procedure is carried out is reduced to 50° C, more single-stranded material is included in the stable fraction[6], but this does not affect its binding ability (Table 4). In another experiment, stable and labile fractions of mouse DNA fragments from hydroxyapatite chromatograms run at 40°, 50°, 60° and 70° C were incubated with mouse and rat DNA-agar. The labile fractions bound relatively poorly, as expected, and showed less discrimination as the temperature of fractionation increased (Table 5). The stable fractions, on the other hand, all bound well in the within-species situation, but showed increasingly poor binding with the rat DNA as the temperature of fractionation increased. This led to a strikingly high level of discrimination on the part of the 70° C hydroxyapatite stable fraction.

When rat DNA from a primary embryonic cell culture is subjected to 70° C hydroxyapatite fractionation at increasing phosphate concentration, the fraction which elutes in the native position is smaller than in the case of mouse DNA (a mean of 3·9 per cent against 8·6 per cent from seven rat and seven mouse fractionations; $P < 0·001$). In marked contrast to the findings with mouse DNA from either *L*-strain or embryonic cells, the small stable fraction from rat DNA showed low binding—lower than did the labile fraction (Table 6). The discriminating power of the two rat fractions was similar. Evidently our fractionation procedure, which for mouse DNA results in segregating the "discriminant" fragments in the stable fraction (Table 6), does not exert any detectable differential effect on the discriminant fragments of rat DNA.

Table 6. DISCRIMINATING POWER OF 70° C HYDROXYAPATITE FRACTIONS OF MOUSE AND RAT DNA INCUBATED IN A DNA-AGAR GEL AT 60° C

Source of fragments: Species	Source of fragments: Cells	Hydroxyapatite fraction	Percentage of binding in agar containing: Mouse DNA	Percentage of binding in agar containing: Rat DNA	Discrimination (per cent)
Mouse	Embryonic	Stable	43·1	9·2	79
	L-cells	Stable	45·1	8·7	81
		Labile	26·1	20·0	23
Rat	Embryonic	Stable	14·0	21·6	35
		Labile	19·6	30·5	36

The two hydroxyapatite fractionation procedures can be combined by first eluting DNA from a hydroxyapatite column at increasing temperatures, and then subjecting these thermal fractions to a second fractionation at increasing phosphate concentration. When the stable and labile components of the various thermal fractions of mouse DNA were incubated with mouse and rat DNA-agar, we obtained the results summarized in Fig. 6. The labile components from all elution temperatures showed low binding and a relatively constant degree of discrimination; the stable components showed very low binding in between-species incubations, but a peak of within-species binding in the fraction eluting at 85° C. This suggests that the similar peak seen in Fig. 2 results from the stable component of the fraction. In other words, both fractionation procedures have been sequentially effective in segregating the discriminant fragments, leading to an eventual level of discrimination (Fig. 6) of more than 90 per cent.

It has already been stated that our preparations of mammalian DNA fragments are initially heterogeneous. Fragments, bound on homologous DNA-agar and later

eluted, show a higher binding capacity when re-incubated on a fresh sample of DNA-agar than did the initial population; conversely, fragments which failed to bind on a first incubation, bind poorly on subsequent incubations[3]. When bound and unbound fragments of mouse DNA from a 60° C DNA-agar incubation were subjected to hydroxyapatite fractionations, more of the bound than the unbound were included in the high phosphate fractions (27·2 per cent compared with 15·5 per cent). The heterogeneity revealed by incubation on DNA-agar is therefore not independent of that revealed by the hydroxyapatite procedure.

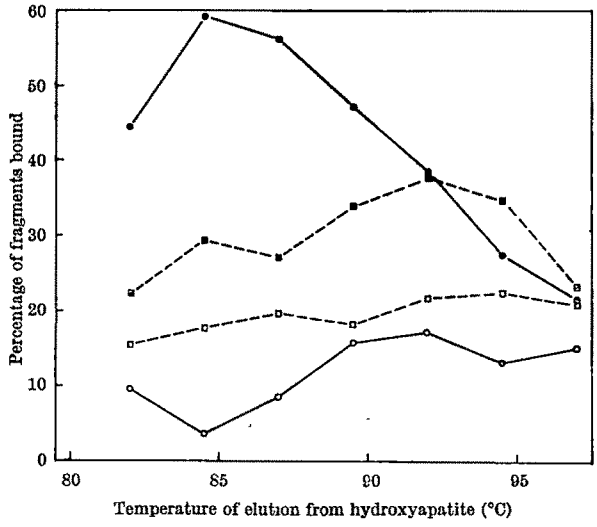


Fig. 6. The within- (● and ■) and between-species (○ and □) binding ability of stable (● and ○) and labile (■ and □) components of various thermal fractions of mouse DNA from a hydroxyapatite thermal chromatogram. Mouse DNA fragments were first eluted from a hydroxyapatite column at constant phosphate concentration (0·06 M phosphate) and increasing temperature; successive elution fractions were pooled to give a total of seven samples, which were then separately fractionated on a hydroxyapatite column at 70° C and an increasing phosphate concentration. The 0·07–0·12 M (labile) and 0·16–0·20 M (stable) fractions of each were recovered, and incubated overnight with mouse and rat DNA-agar at 60° C

Earlier evidence[3] suggested that failure to bind might be partly due to the presence of intra-strand secondary structure, stable at the temperature of incubation in agar, and associated with C-G rich regions of the genome. This interpretation is supported by the binding capacities of the various thermal chromatogram fractions illustrated in Fig. 2. If C-G regions were the main contributors to DNA duplex stability at 60° C in 0·3 M sodium chloride, then we would expect, unless there was some impediment, that the binding efficiency of thermal fractions should increase as the temperature of fractionation and thus their C-G content increased. But this was not so: above a point midway up the chromatogram, binding efficiency levelled off or even, in the mouse, decreased. Our finding of a DNA fraction with both a high degree of secondary structure and a high binding capacity does not necessarily invalidate this view, because here too it is the lower and middle temperature components of the stable material which bind best (Fig. 6).

Conclusions

It seems unlikely that our failure to isolate a fraction of rat DNA with high binding and discriminatory abilities reflects any fundamental distinction between the genetic material of rats and mice. In the experiment summarized in Table 6, mouse and rat DNA differed little, after fractionation, in the overall degree of binding and discrimination, when the relative size of the stable fraction is taken into account. What did clearly differ (Table 6) was the extent to which the discriminant fragments have been successfully segregated; this is presumably dependent on the precise fractionation technique used. On the other hand, we have on a number of other occasions observed that unfractionated rat DNA fragments discriminate somewhat less well than mouse fragments[4]. It is possible that some virus-like contaminant in the genetic material of the mouse contributes to the superior discrimination. The very rapid renaturation undergone by the stable fraction might, in particular, suggest a viral origin. This rapid renaturation occurs whether or not the stable material has been isolated from the rest of the DNA; in an analogous situation, the rate of renaturation of *T4* phage DNA is not affected by a 500-fold excess of mouse DNA (ref. 8).

On the other hand, when a stable fraction is isolated from high molecular weight DNA at 70° C, only about 25 per cent of it shows a sharp spectrophotometric melting transition; the remainder is single-chain DNA which must be linked, presumably by covalent bonds, to the stable component. No such linkage would be expected if the stable material were viral or cytoplasmic in origin. More recent experiments[9] have shown that the stable fraction discussed in this paper renatures at the same rate as, and is therefore presumably identical with, the satellite fraction isolated by Britten[10]. The rapid renaturation of both fractions is thought to be due to the repetition, many times over, of a sequence perhaps only 250 base-pairs in length. The existence of a large amount of nucleotide sequence repetition in the DNA of higher organisms, if confirmed, would have important evolutionary implications.

The binding ability of the stable fraction of mouse DNA is surprising, because the reformation of duplex structure might be expected to reduce the number of hydrogen-bonding sites available. The imperfections in duplex structure revealed by melting curve experiments must, however, expose a certain number of sites, and it is presumably these which are responsible for binding on DNA-agar. This implies that partially renatured DNA can be stabilized in DNA-agar by the specific binding of the unpaired bases in a relatively short segment.

However, we have also seen that the high binding ability is only manifested in within-species incubations, and then only by fragments eluting over a certain portion of the hydroxyapatite chromatogram. Fractions of mouse DNA eluted at higher temperatures bind worse with mouse but better with rat DNA than do those eluted at lower temperatures. For this reason, use of the stable component of the thermal fraction eluting at 85° C achieves a higher level of discrimination between mouse and rat DNA than any other method tried so far. Either the species-specific sequences must be rather rigidly segregated into this particular fraction, or the degree of complementarity required for stable binding by the exposed sites in the stable fraction of mouse DNA is so stringent that they are unlikely to be satisfied by more than a small minority of sequences present in DNA of the other species.

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INCREASED ANGIOTENSIN FORMATION IN RESPONSE TO CAROTID OCCLUSION IN THE DOG

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A FALL of renal arterial pressure raises the rate of secretion of renin[1] and its concentration in the blood[2,3]. A reduction of blood volume brought about by severe haemorrhage also raises the concentration of renin in blood[4,5], an effect thought to be mediated by the local effect of the fall in renal arterial pressure. Recent evidence suggests that this interpretation is not correct. Thus in dogs a reduction of renal arterial pressure by clamping the aorta fails to increase the concentration of circulating angiotensin, though an increase is brought about by an equivalent fall of systemic arterial pressure caused by haemorrhage[6]. We have found[7] that in dogs haemorrhage causes an increase in the concentration of circulating angiotensin even when there is no fall of arterial mean or pulse pressure. These results suggest that the secretion of renin is modified by nervous reflexes, and Vander[8] showed that stimulation of the renal nerves raises the concentration of renin in the blood. We have also shown that the rise of angiotensin concentration during haemorrhage is prevented by blocking the renal nerves with lignocaine[9]. The experiments described here provide direct evidence that the concentration of angiotensin in blood is affected by cardiovascular reflexes.

Dogs of both sexes, weighing 18–32 kg, were used. Anaesthesia was induced with halothane, maintained with chloralose (100 mg/kg intravenously) and supplemented with pentobarbitone (60–120 mg intravenously) as necessary. Cannulae were inserted into the trachea, a femoral artery and a femoral vein; an animal was mechanically ventilated and given heparin (1,000 U/kg intravenously). Mean arterial pressure was recorded by a mercury manometer, and in some experiments the undamped pressure was recorded photographically from a Statham pressure transducer (type *P23Db*) and amplifier. The bladder was catheterized and the output of urine recorded by drop counter. For each millilitre of urine excreted, an equal volume of 0·9 per cent saline w/v was automatically injected intravenously. Both common carotid arteries were dissected in the neck and mobilized for temporary occlusion with bulldog clamps.

The concentration of angiotensin in circulating blood was assayed continuously by the blood-bathed organ technique, developed for catecholamines by Vane[10,11] and modified for angiotensin by Regoli and Vane[12,13]. Femoral arterial blood was pumped at 15 ml./min to superfuse in series three assay tissues and was returned to the femoral vein by gravity. Contractions of the tissues were recorded on a kymograph with frontal writing pendulum levers[14] of 16 : 1 magnification and an initial load of 1–3 g. The assay tissues were chosen from: *rat stomach strip*[15], which distinguishes between angiotensin (which causes a small contraction) and catecholamines (which cause relaxation); *chick rectum*[16], which relaxes in response to adrenaline, is less sensitive to noradrenaline and is insensitive to angiotensin; *rat colon*[12], which is highly sensitive to angiotensin (responding by contraction) and feebly sensitive to catecholamines (responding by relaxation). The specificity of the assay, which is described in detail elsewhere[9,13], depends on the differential responses of the three tissues and on the demonstration that a response characteristic of a change of endogenous angiotensin concentration no longer occurs when the kidneys are excluded from the circulation. Angiotensin can be distinguished from histamine, acetylcholine, 5-hydroxytryptamine, bradykinin, vasopressin, oxytocin, catecholamines, adenosine and its derivatives, corticosteroids, and many other compounds.

The response of the tissues to changes of concentration of endogenous angiotensin was calibrated by infusions of synthetic angiotensin to match the response as follows: to determine the *rate of generation* of angiotensin in the dog, angiotensin (Val_3—Hypertensin, II Asp.β.amide, 'Hypertensin Ciba') was infused intravenously at 0·1–2·0 μg/min. To determine the concentration of angiotensin in blood, angiotensin was infused at 3–15 ng/min directly into the blood superfusing the tissues.

Occlusion of the carotid arteries always caused a rise of blood pressure. This varied from 10 to 120 mm mercury; occlusion for 5 min or less caused no detectable change of

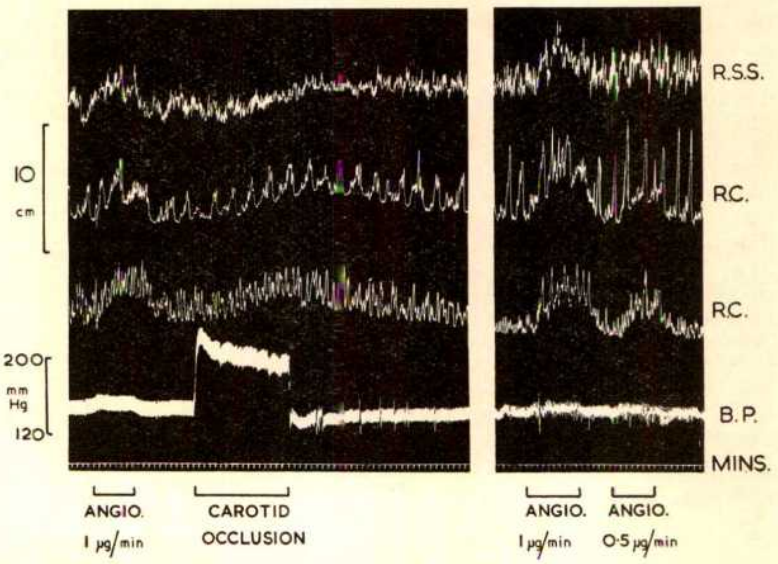


Fig. 1. Dog No. 73 (27 kg). The kymograph tracing shows the movements of a rat stomach strip (*R.S.S.*) and two rat colons (*R.C.*) superfused with carotid arterial blood from the dog. Blood pressure (*B.P.*) in mm mercury and time in min. An infusion of angiotensin (angio) at 1 μg/min intravenously shows the sensitivity of the system. During the period of carotid occlusion, the contractions of the blood-bathed organs indicated an increase in the generation rate of angiotensin of 0·5 μg/min

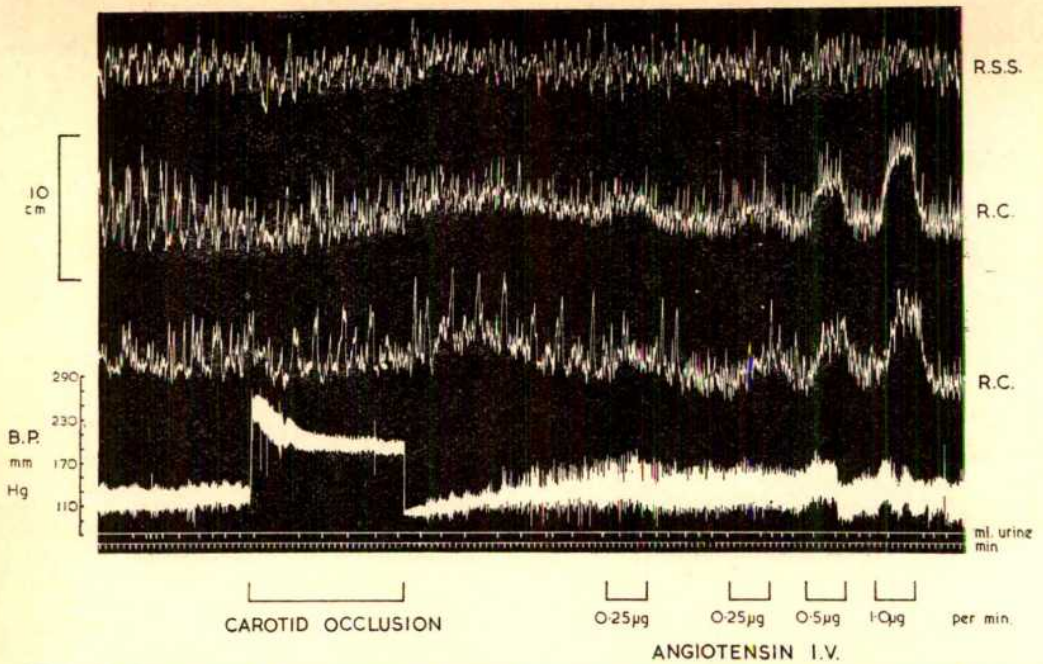


Fig. 2. Dog No. 75 (24 kg). Record as in Fig. 1, with the addition of urine output in ml. The carotid occlusion caused an increase in the angiotensin concentration which was maximal after the release of the occlusion. The peak increase corresponded to a generation rate of between 0·25 and 0·5 μg angiotensin/min

angiotensin concentration (14 dogs); longer periods of occlusion (7–26 min) caused a rise in the concentration of circulating angiotensin in ten out of seventeen animals. One of these experiments is illustrated in Fig. 1, which shows a slow rise of angiotensin concentration during occlusion, followed by a slow fall after its release. This time course is typical of the effect of injections of renin, which has a half-life in the circulation of 15–20 min[13]; angiotensin itself has a half-life of less than 3 min[9]. In two of these ten dogs, carotid occlusion was repeated after the vagus nerves had been cut in the neck; in both dogs the second occlusion caused a greater increase of blood pressure and of angiotensin concentration. In six of the ten dogs the angiotensin concentration reached a maximum after release of the occlusion (for example, Fig. 2). Of the seven dogs in which carotid occlusion was ineffective in causing a rise of angiotensin concentration, haemorrhage was effective in four and ineffective in three.

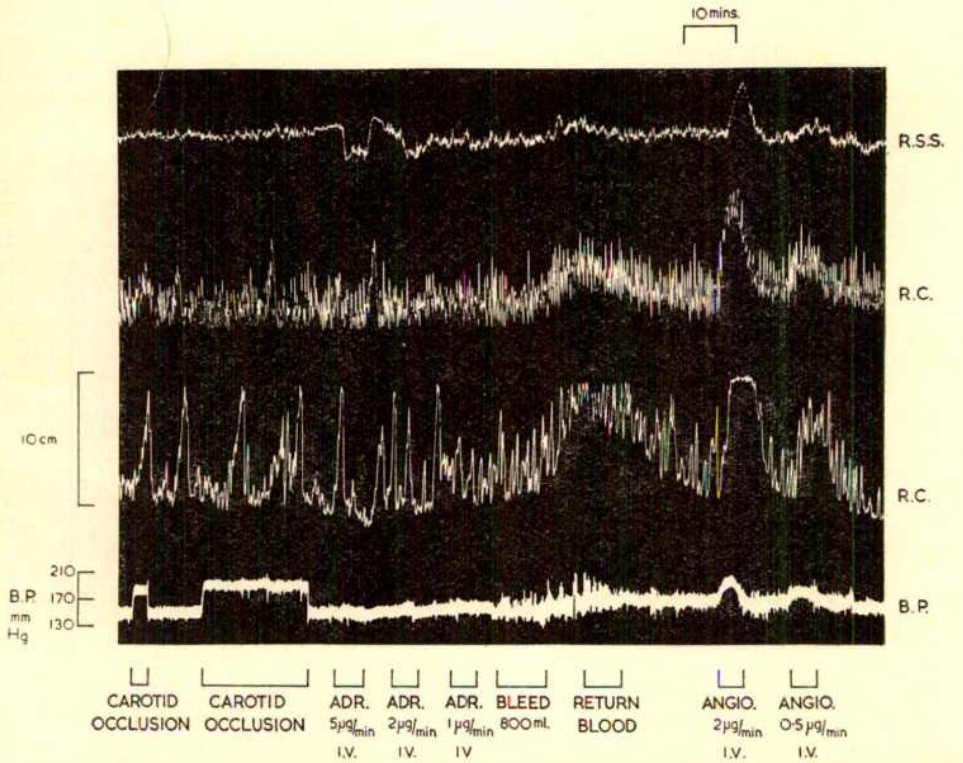


Fig. 3. Dog No. 64 (31 kg). Record as in Fig. 1. Carotid occlusion failed to change the blood angiotensin concentration, although subsequent haemorrhage was effective. Note the different responses of the tissues to intravenous infusions of adrenaline (*ADR*) and angiotensin

Table 1. SUMMARY OF RESULTS

The experiments have been arranged according to the rise in blood pressure induced by carotid occlusion (duration 7–36 min)

Dog No.	Weight (kg)	Duration of occlusion (min)	Mean rise of blood pressure due to carotid occlusion (mm mercury)	Increase of angiotensin generation rate due to carotid occlusion (μg/min)	Rise in blood pressure (mm mercury) produced by angiotensin infusion at the rate shown in the preceding column
68	26	15	110	1·0	8
68	27	15	100	1·0	14
37	26	10	90	0·1	5
73	27	18	80	0·5	6
74	30	24	80	0	0
75	24	26	74	0·25	2
67	26	16	72	0·2	6
65	28	18	72	0·1	2
72	9	7·5	70	0·2	4
70	23	12	70	0	0
61	26	7	60	0	0
36	23	10	52	0	0
41	16	8	50	0·1	2
69	22	18	50	0	0
66	21	11	38	0	0
64	31	18	36	0	0
38	20	8	10	0·1	2

To determine whether the observed increase of angiotensin concentration arose from an increase in its rate of generation, the rate of destruction of infused angiotensin was measured before and during carotid occlusion in two dogs. Angiotensin was infused intravenously at the same rate before and during carotid occlusion. The resulting increases in concentration were similar in the two circumstances, indicating that its rate of destruction was unaltered. Assuming that synthetic and endogenous angiotensin are inactivated at the same rate we can conclude that the rise of angiotensin concentration during carotid occlusion resulted from an increase in its rate of generation.

The results of the experiments are summarized in Table 1. The increased blood angiotensin concentration in response to carotid occlusion was equivalent to an increased generation rate of 0·1–1 μg/min: infusion of synthetic angiotensin at these rates had detectable effects on arterial pressure which were, however, quantitatively unimportant in contributing to the rise of blood pressure during carotid occlusion.

The results show that, in anaesthetized dogs, occlusion of the common carotid arteries can cause a rise of the concentration of angiotensin in blood. This rise was the result of an increase in the rate of generation of angiotensin, presumably owing to an increased rate of secretion of renin, and could account for the increase of aldosterone secretion rate which occurs during carotid occlusion or constriction[17,18].

The cardiovascular effects of carotid occlusion in the dog result entirely from a fall of pressure in the carotid sinus, which causes complex reflex circulatory adjustments resulting in a rise of arterial pressure[19]. The renal vasomotor nerves contribute to the general increase of peripheral resistance so that the effects on the kidney may include changes of volume; of afferent arteriolar pressure; of glomerular filtration rate and filtration pressure; and of renal blood flow. Any of these could be the effective stimulus to the release of renin, whether this is brought about by cardiovascular reflexes or by changes of renal arterial pressure caused, for example, by partial occlusion of the renal artery. It therefore does not seem necessary to postulate the existence of nerves the special function of which is to regulate the secretion of renin.

The role of carotid sinus reflexes in the control of renin secretion remains to be elucidated. Such reflexes cannot be the only ones mediating the changes of angiotensin concentration during haemorrhage. Thus, despite a smaller fall of sinus pressure, haemorrhage caused a greater rise of angiotensin concentration (for example, Fig. 3) than did carotid occlusion. Furthermore, none of the systemic arterial baroreceptors is likely to be important in causing the increase of angiotensin generation rate during slow haemorrhage, as this can occur without change in systemic arterial mean or pulse pressure[8].

The significance of the results described here lies in the demonstration that a cardiovascular reflex can bring about increased generation of angiotensin despite a rise of renal arterial pressure. Thus in the control of the renin-angiotensin system, reflexly induced changes in the kidney can override the direct effects of changes of renal arterial pressure.

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SPECTROPHOTOMETRIC DETECTION AND MEASUREMENT OF SENSITIZING ANTIBODIES

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METHODS for detecting tissue-sensitizing antibodies in the sera of chickens experimentally infected with *Salmonella gallinarum* have already been described. Buxton and Allan[1] recorded experiments demonstrating the presence of cytophilic antibodies on circulating chicken leucocytes, while techniques were described by Allan, Buxton and Thomlinson[2], using an established line of *ERK* cells to detect antigen–antibody reactions *in vitro*.

In all these techniques an eosin–*y* dye exclusion method, modified from that of Hanks and Wallace[3], was used to detect the resultant cytopathic effect[1] (CPE). This, like other dye-exclusion techniques, is open to certain criticisms, and these have been ably illustrated in experiments carried out by Black and Berenbaum[4]. The main criticism of dye-exclusion methods for estimating CPE is that such estimations are achieved by subjective means. A proportion of cells invariably shows intermediate morphological changes when dye exclusion is not conclusive, and interpretation becomes speculative. Consequently, an alternative technique for estimating CPE based on more exact methods of assay seemed desirable.

Ultra-violet spectrophotometry, because it precludes the necessity of including colour reactions, seemed likely to produce a direct and uncomplicated method of assay.

Table 1

0·5 ml. volumes incubated with cells; *A* for 30 min followed by *B* for 20 min	Tubes containing an equal volume of HeLa cells of known concentration					
	1	2	3	4	5	6
Chicken antiserum	A	A	—	—	—	—
Antigen in PBS	B	—	B	—	B	—
Chicken negative serum	—	—	A	A	—	—
PBS alone	—	B	—	B	A	A,B

Romeyn and Rigby[5] measured immune cytolysis of guinea-pig peritoneal exudate cells (chiefly mononuclears) and Ehrlich's ascites tumour cells, by monitoring cell-free supernatants for nucleic acid, at a wave-length of 260 mμ, following incubation of the cells with specific antisera. By this means an *in vitro* type 2 reaction[6] was produced and complement was titrated.

As the intention was to measure tissue sensitizing antibodies in sera, the possibility was considered of passively sensitizing a suspension of an established line of either *ERK* or HeLa cells *in vitro*, washing them, exposing them to a Westphal-type antigen and measuring the release of cellular ribonucleic acid into the supernatant suspending medium at a wave-length of 260 mμ. In other words, an attempt was to be made to induce passively *in vitro* a type 1 reaction[6].

As a preliminary step, HeLa cells were examined microscopically for cellular RNA by staining with methyl-green pyronin, using perchloric acid as a substitute for ribonuclease in the histochemical detection of RNA (refs. 7–10). These preliminary observations were encouraging[11] and led to the design of a test which it was thought might measure release of cellular RNA following controlled passively induced type 1 reactions[6] on *in vitro* cultured cells. In this way it was hoped to calculate a cytopathic index based on the absorbancies of supernatants from experimental and control HeLa cell suspensions, which would allow the titration of sensitizing or cytophilic antibody in sera under test.

The test was initiated by dispersing a number of aliquots of thrice-washed HeLa cells of known concentration, suspended in phosphate buffer-saline (PBS), into tubes. Some of these cells were incubated with 0·5 ml. of a 1 : 2 dilution of chicken serum under test, others with 0·5 ml. of a 1 : 2 dilution of known negative chicken serum, and others were incubated with 0·5-ml. PBS. After 30 min at 37° C the cells were washed as before in PBS, some were further incubated for 20 min at 37° C, resuspended in 1-ml. suspensions of antigen in PBS, and some in 1-ml. volumes of PBS alone (see Table 1).

After the second period of incubation (*B* in Table 1) all tubes were centrifuged at 236*g* for 2 min, and to the supernatants were added 1-ml. volumes of 2 N Analar perchloric acid. This precipitated any protein and freed nucleic acid and nucleotides by hydrolysis. The supernatants were incubated with perchloric acid for 30 min at 60° C and were then centrifuged at 1,720*g* for 30 min to separate the acid-soluble nucleic acid and nucleotides for monitoring at 260 mμ. Analysis of the results of several hundred absorbance readings consistently showed that minimal release of cellular RNA from HeLa cells occurred owing to manipulation of the cells during the experiment (tube 6, Table 1). Absorbance of supernatants from cells in tubes 2, 3, 4 and 5 (Table 1) were all significantly less than from cells in tube 1 which had been passively subjected to a type 1 reaction[6].

Table 2. CYTOPATHIC INDICES OF WHOLE SERUM AND 'SEPHADEX' FRACTIONS OF CHICKENS ARTIFICIALLY INJECTED WITH *E coli*

Day	Chicken	Cytopathic index (as percentage) Whole serum	'Sephadex' fraction 32–34	35–36	37–38	39–40	41–42	43–44	45–46	47–48	49–50	51–52	Serum titre HA	AG	H
0	1	26·4	13 3	31·2	18·2	33	21 2	37·2	33 6	35·6	NT	NT	16	512	20
	2	10·7	11·1	13·8	11·1	0	0	0	25·3	27·2	11·1	11·8	4	256	10
	3	0	14·7	0	26·5	14·8	14 2	16·9	14·4	17 6	0	NT	4	256	20
	4	15 0	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	4	256	20
	Average	13·0											4	320	18
1	5	4·5	22 2	17·8	17 4	14 8	17·4	12	17 8	12	14 8	0	32	512	40
	6	5·3											4	256	20
	7	10·4	17 5 / 25 5	13 / 13·5	7 0 / 7·0	10·5	NT	NT	NT	NT	NT		4	256	10
	8	0	NT	NT	NT	NT	NT	NT	NT	NT	NT		4	64	40
	9	0											4	64	20
	Average	4·4											6	230	26
			33–37		38–42		43–47	48–52		53–57		58–62			
2	10	13·8	15·6		0		0	0		0		0	4	256	10
	11	0	15·2		0		0	0		0		0	8	256	10
	12	4·4	11·5		0		0	0		0		0	8	64	20
	13	13·8	NT		NT		NT	NT		NT		NT	8	256	160
	14	11·3											4	256	40
	Average	8·7											6	192	48
3	15	15·7	23 1		0		0	0		0		11·8	16	1,024	10
	16	15·1	14·5		0		0	0		0		0	16	128	10
	17	0											8	128	10
	18	14·1					NT						64	1,024	40
	19	17·6											16	512	40
	Average	11 2											24	562	20
4	20	25·2	30 1		0		0	0		14 7		28 3	32	512	20
	21	16·3	16 6		18·1		22·2	9 9		14·1		10 8	64	2,048	20
	22	12·7	27·2		0		27·3	0		0		25·5	8	128	40
	23	17·9											32	2,048	10
	24	31·4					NT						64	2,048	40
	25	22·8											4	2,048	40
	Average	21·7											34	1,472	28
5	26	27·4	22 5		0		21·5	30 8		10 3		0	8	2,048	20
	27	30·7	32·6		23·1		18·9	27·4		18 9		26 4	256	2,048	20
	28	21·1	48·0		51·8			53 2		42 5		53·6	32	512	80
	29	22·8						NT					64	2,048	10
	30	7·1											16	512	40
	Average	21·05											75	1,434	34

H: H agglutinating antibody. HA: O haemagglutinating antibody. AG: O antiglobulin haemagglutinating antibody.

It was possible, in consequence, to calculate cytopathic indexes from data which were so controlled that the only variable was the sensitizing activity of the chicken antiserum. The formula used was:

$$\text{Cytopathic index (CI)} = \frac{E\ 260\ \text{tube}\ 1 - E\ 260\ \text{tube}\ 5}{E\ 260\ \text{tube}\ 1} \times 100$$

Tube 5 was deliberately chosen as the control, as it consistently gave slightly higher absorbancies than tubes 2, 3, 4 and 6 (see Table 1).

To determine the efficiency of this cytopathic test a series of experiments was carried out in which serum responses to oral infection of individual birds were followed. As a result, it was shown that sensitizing activity was present in these sera. The next step was to see if serum fractions possessed cytophilic properties and if it was possible to characterize sensitizing antibodies. Accordingly, thirty Light Sussex 5-week-old cockerels were obtained and divided into six groups. One group was killed immediately and maximum serum samples recovered from each bird. These are the samples of day 0. The remaining five groups immediately received 1×10^6 live *E. coli* 02 organisms into the posterior air sac. Serum samples were recovered by killing one group from the remaining five at daily intervals to give serum samples for days 1–5. These sera were used passively to induce type 1 antigen–antibody reactions[4] with *E. coli* 02 polysaccharide at a concentration of 60 μg/ml. on suspensions of HeLa cells.

Cytopathic indexes were calculated for these whole serum samples, and detailed results of these can be seen in Table 2. Fig. 1 illustrates the mean cytopathic index for each day of the experiment, and these show a typical secondary response to infection with live organisms. The presence of antibodies in the sera before experimental infection is of interest as *E. coli* 02 is a normal inhabitant of the alimentary tract of the chicken. The mean haemagglutination and anti-globulin haemagglutination titres (Table 2) follow similar patterns to the mean cytopathic index figure except that the minimal mean antiglobulin haemagglutination titre was reached one day later, that is, on day 2. It is of interest to note that Buxton and Allan[1] showed that antiglobulin haemagglutinins in sera from birds orally infected with *Salm. gallinarum* were capable of sensitizing leucocytes from normal chickens. When challenged with *Salm. gallinarum* polysaccharide, these leucocytes were damaged.

For 'Sephadex' fractionation 3 sera from each day were used. A 5-ml. volume of each serum was used, with the exception of birds 5 and 6, when 2·5 ml. of each of these sera were fractionated together. The fractions were bulked and reduced to a fixed volume by overnight dialysis at 4° C against polyethylene glycol ('Carbowax', Union Carbide Co.). By this method a tenfold concentration was achieved, and the ultra dialysate was used in place of whole serum to induce a type 1 reaction[3] on HeLa cells.

The concentration of the 'Sephadex' fractions followed three patterns: (*a*) Day 0 and day 1: consecutive pairs of fractions totalling 10 ml. were reduced to 1 ml. (*b*) An exception to this was bird 7 on day 1, when each individual fraction of 5 ml. was reduced to 0·5 ml. (*c*) Days 2, 3, 4 and 5: five consecutive fractions totalling 25 ml. were reduced to 2·5 ml.

Fig. 2 shows the profile of a typical fractionated serum taken from each day. The absence of peak I in day 0 is

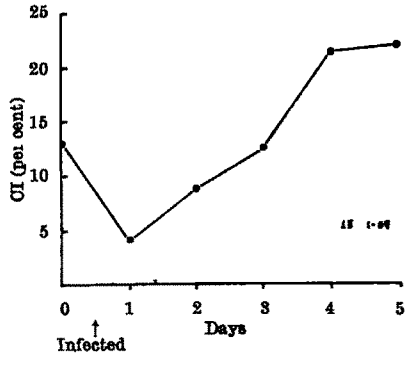


Fig 1

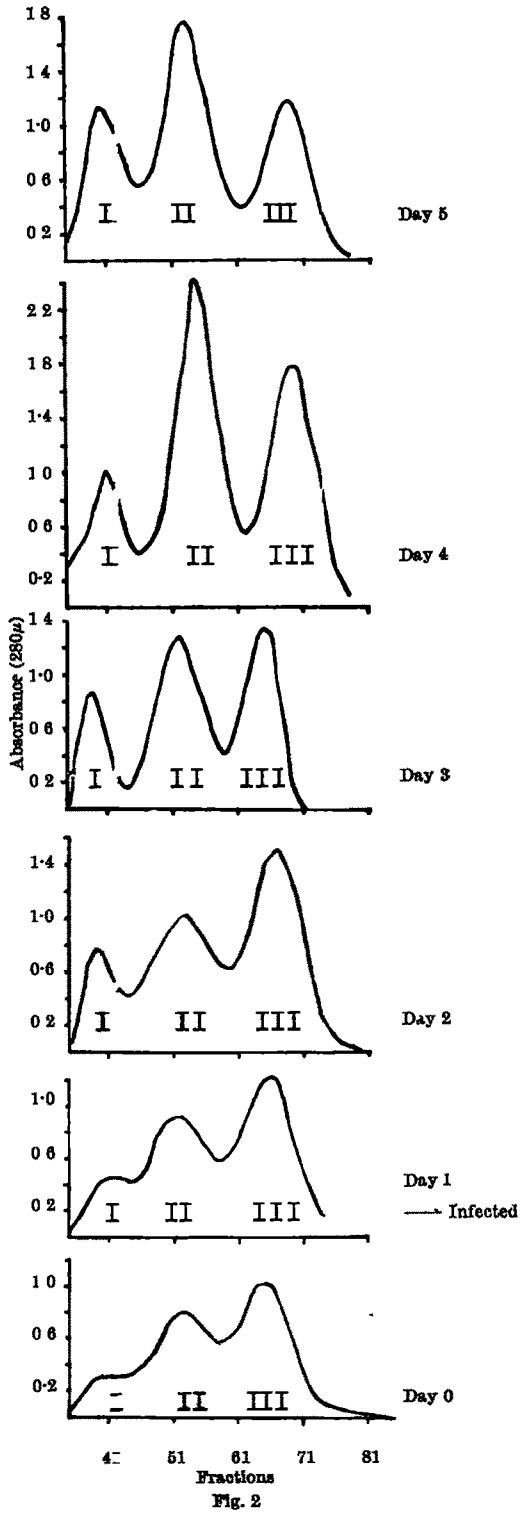


Fig. 2

indicative of the low IgM levels in normal 5-week-old chickens. The rapid increase in formation and height of peaks I and II as a result of infection is readily discernible. The consistent reliability of 'Sephadex G 200' as a separating medium meant that it was possible from day 2 onwards to bulk the fractions from each serum in the same manner (*vide supra*, and Table 2).

Table 2 sets out the cytopathic indexes of all the fractions tested. It will be seen that the sera from days 0 and 1 show sensitizing activity throughout the initial fractions which correspond to peaks I and II on the respective serum protein profiles (Fig. 2). In contrast, sera from days 2 and 3 show activity confined to the IgM peak only. Sensitizing activity gradually reappeared throughout the whole range of fractions during the last 2 days, with the cytopathic indexes becoming approximately equivalent.

When the whole sera were subjected to 2-mercaptoethanol treatment, it was found that all haemagglutinating activity was removed while the antiglobulin haemagglutinating titres were reduced to 1 : 8 or less.

From these experiments it appears that first IgM and then IgG antibodies may be sensitizing and concerned in cell damage. Turner, Jenkins and Rowley[12] showed that mice vaccinated with living *Salm. typhimurium* produced two types of immunoglobulin. The first phase of production was characterized by a 19*S* macroglobulin response which lasted for about 4 weeks, leaving a 7*S* IgG antibody which persisted for about 6 months. Rowley, Turner and Jenkins[13] also showed that passive transfer of immunity to mouse typhoid was possible by transfer of phagocytic cells from immune mice. This resulted from a cell-bound antibody that could be eluted from these cells and was shown to be 19*S* IgM.

The fact that both IgM and IgG immunoglobulins are capable of sensitizing heterologous HeLa cells may to some extent be explained by a report of Brambell[14]. This suggested that uptake of globulins by cells, whether passively as in type 1 reactions[6] or actively, depends on the presence of "recognition units" on the immunoglobulin molecules which match receptors on the pinocytotic vesicles of the tissue cells involved in the adsorption process[14].

From the experimental results obtained by the cytopathic test described in this article, it seems that this test is capable of detecting 7*S* IgG activity at an earlier period in the immune response than conventional *in vitro* methods.

The emergence of 7*S* IgG antibody as a more potent cell sensitizing agent may be related to a higher circulating serum level. However, recent reports indicate that homologous $7S\gamma_1$ is more effective than $7S\gamma_2$ in sensitizing skin in passive cutaneous anaphylaxis (PCA) tests in guinea-pig and mice[15,16].

Roe[17] stated that the uncertainty in interpreting the ultra-violet absorption spectra of macronuclear material when the conditions of examination are difficult to control has made many workers sceptical about possibilities of obtaining quantitative results from the microspectroscopy of biological cells. The knowledge that nucleic acid is present in high concentration, and may undergo local changes in *p*H or in ionic environment with changing cell metabolism, and at different stages of cell division, leads to the conclusion that quantitative information may be difficult to obtain[17]. By using an established cell line such as HeLa in the carefully controlled environment of the cytopathic test just described, it is possible to fulfil Roe's criteria and thus present quantitative results which, at least under the same experimental conditions, appear quite valid.

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EFFECT OF SULPHYDRYL REAGENTS ON A MECHANICAL PROPERTY OF SKIN

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INVESTIGATING the effect of various treatments on the tensile strength of the skin of the rat's tail, with the object of defining more precisely the mechanical linkages holding it together, we had occasion to try thioglycollate and this was found to produce a large reduction in tensile strength. We have since tried a number of other thiol reagents and found they all have this effect.

The experiments were carried out on the tail skin of rats 40–60 days old. This skin was cut into rings, and loaded stepwise to the point of rupture at a constant rate (breaking time 3–5 min in untreated skin[1,2]). The tensile strength of this material is almost entirely due to the dermis, the effect of the epidermis being relatively unimportant[1,2]. It has therefore, in general, been ignored. However, some of the results have been checked on epidermis-free skin.

Usually, rings of skin were treated with the reagent under investigation overnight or for longer periods in the refrigerator at about 4° C. Fig. 1 shows effects of treatment with thioglycollate, mercaptoethanol cysteamine, cysteine and reduced glutathione (in 0·1 M concentration, except for glutathione), at different hydrogen ion concentrations obtained by adjusting the *p*H with hydrochloric acid or sodium hydroxide. Lower concentrations (not shown in Fig. 1) of glutathione (2·5 mM) and cysteine (10 mM) have also produced similar effects. In Fig. 1 the effect of alteration of *p*H in buffered physiological saline alone is shown as a dotted line (from ref. 2). The effect of the experimental reagents is to move this curve in the direction of higher *p*H, and large reduction in tensile strength is produced by them in the range of *p*H from 6 to 7·5. It should be noted, however, that the curves represent effects at standard times and not necessarily final equilibrium conditions.

Compounds related to two of the above but containing oxygen instead of sulphur (ethylene glycol and ethanolamine) had no significant effect (at *p*H 7). The normal effect of *p*H on tensile strength appears to be relatively insensitive to changes in the composition of the buffer[2]. It is much the same in 0·1 M *tris* or acetate as in the

physiological saline buffered with phosphate, or bicarbonate, we have generally used. Other substances which in single tests have not had an effect like these thiols are ascorbate and oxalate. To this extent the effects of thiols appear to be specific.

The effects are not confined to tail skin. We have obtained them on ankle and wrist skin with cysteamine.

The method of adjusting the hydrogen ion concentration leads to small variations in ionic strength, but there is no reason to suppose these have more than second-order effects[3] except perhaps at the lowest *p*H value for mercaptoethanol which represents a simple solution.

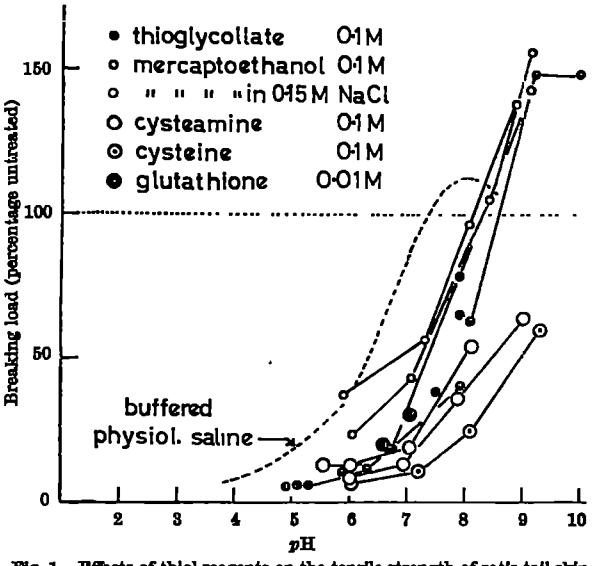


Fig. 1. Effects of thiol reagents on the tensile strength of rat's tail skin. Rings of skin were treated at 4° C, overnight, or for three days (glutathione) in 10 ml. of solution. Reagents were in simple aqueous solution or physiological saline buffered with phosphate (glutathione, in sodium chloride 0·13 M and 0·02 M phosphate; this was also boiled out and gassed with nitrogen). EDTA was added to some of the solutions to a final concentration of 1 mM. The *p*H values are those obtained by measurement after removal of skin sample for test. The lines join means of pairs of skin samples from individual tails. The dashed line shows the simple effect of *p*H in physiological saline (ref. 2). Reagents were obtained from commercial sources as follows: thioglycollic acid, Light; mercaptoethanol cysteamine, Light and B.D.H.; cysteine, B.D.H; reduced glutathione, B.D.H.

It has been suggested to us that the effects of these reagents might be produced by activation of proteolytic enzymes, but this seems unlikely in view of the following: (1) Previous treatment of the skin with acetone did not prevent the effect. (2) Treatment with cysteamine at room temperature for 0·5 h, followed by 10^{-2} M iodoacetamide for 2 h (which should activate and then inactivate an enzyme), did not prevent the effect. (3) The effect was at least partly (up to about 70 per cent) reversible by leaving in phosphate-buffered physiological saline in the refrigerator. After reversal, the effect, so far as we have investigated it, can be repeated (indicating that the reversal is a true one and not due to formation of new linkages).

Also it is difficult to explain the increase in tensile strength found at high *p*H values with some of the reagents on the basis of enzyme activation. However, one cannot draw definite conclusions from this increase at the moment in view of the similar effect of cyanide[3] in low concentration. There is no reason to suppose it was a contaminant of the reagents used, but an equally active material would only have to be present to the extent of one part in a thousand of the reagents to produce the effect.

The conditions of some of the experiments were similar to those used to encourage growth of anaerobic bacteria. However, the participation of such organisms can virtually be excluded from some of the effects, because, at room temperature or 37° C, those can be demonstrated in 30 min. In experiments overnight at 4° C the addition of sodium azide (10^{-3} M), an inhibitor of bacterial growth, made no significant difference.

There are a number of possible explanations of those effects of thiols. Removal of a metal ion is one. We have been able to find no effect of EDTA (up to 0·1 M); but acetyl acetone reduces the tensile strength of this skin[2], though not nearly as effectively as these thiols. Another possible effect of these could be the breaking of disulphide links, which would agree with the fact that we also have found sulphite to reduce the tensile strength of this skin.

Whatever the ultimate explanation of these effects, they do not appear to be capable of interpretation in terms of what is at present known of the structure of collagen. There is, for example, no evidence of the presence of disulphide links in its structure. We have tried cysteamine on reconstituted collagenous sausage skin (kindly provided by Ethicon, Inc.) and found no significant effect.

It is reasonable to conclude that these thiol reagents are acting on another material, between the collagen fibrils, and that this forms an essential link in the mechanical integrity of the dermis. Previously recorded effects of *p*H and other agents on the tensile strength of skin, which are equally inexplicable in terms of known properties of collagen, support the same conclusion[1,2].

We have previously suggested the possibility[1] that local changes in hydrogen ion concentration could be the basis of a mechanism by which cells could mould connective tissues and move through them. These effects of thiol reagents could be used for the same purpose, with less or no alteration of *p*H from normal. They also suggest that more attention might usefully be concentrated on the role of non-collagenous materials in mechanical properties of other soft tissues, for example in wounds.

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SUBSTRATE- AND INHIBITOR-INDUCED CHANGES IN THE OPTICAL ROTATORY DISPERSION OF ASPARTATE TRANSCARBAMYLASE

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One of the main homeostatic control processes in cells is the modulation of the activity of certain enzymes by small metabolites and catabolites. Allosteric enzymes[1] show large changes in catalytic activity in the presence of effectors (inhibitors or activators) that are often not stereochemically related to the substrate or product of the enzyme of interest. A model for the allosteric enzyme regulation mechanism, based largely on kinetic evidence,

has been proposed by Monod, Changeux and Wyman[2]. This model proposes that small molecule effectors alter enzyme catalytic activity by bringing about conformational changes in the enzyme structure. Independent evidence for or against the alteration of protein conformation by allosteric effectors is important for assessing the correctness of the point of view of this model. This article reports some results on optical rotatory dispersion which demonstrate changes in the conformation of the enzyme aspartate transcarbamylase caused by substrate and by an allosteric inhibitor.

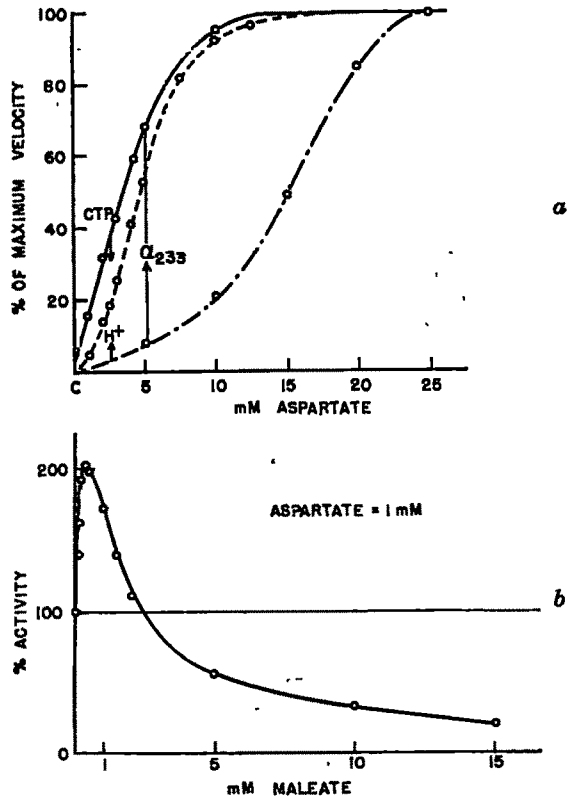


Fig. 1. *a*, The effect of CTP and *p*H on the reaction velocity substrate dependence of ATCase. 3·6 mM carbamyl phosphate and 40 mM buffer present in all samples. ———, *p*H 6·1; - - - -, *p*H 7·0 or *p*H 6·1 plus 2×10^{-4} M CTP; —·—·, *p*H 8·6. All the curves (except the *p*H 6·1 plus 2×10^{-4} M CTP curve) are taken from ref. 3. The arrows on the graph are explained in the text. *b*, The effect of the substrate analogue, maleate, on the reaction velocity of ATCase. 1 mM aspartate, 3·6 mM carbamyl phosphate, and 40 mM potassium phosphate; *p*H 7. Curve taken from ref. 3

Aspartate transcarbamylase (ATCase)[3] is one of the best characterized examples of an allosteric enzyme and is suitable for physical investigation because it can be highly purified. ATCase is the first enzyme in the pyrimidine biosynthetic pathway. Aspartic acid and carbamyl phosphate are coupled by ATCase to form carbamyl aspartate which is transformed by a series of enzymatic reactions to the pyrimidine nucleotides, uridine triphosphate (UTP) and cytidine triphosphate (CTP). As first shown by Yates and Pardee[4], cytidine derivatives exert a regulatory influence on the activity of the enzyme in whole cells and in cell extracts. Kinetic measurements on purified ATCase show that CTP binds reversibly at a site distinct from the catalytic site, and inhibits the enzyme by reducing its affinity for the substrate aspartate[5]. The level of the ultimate product, CTP, is controlled by feed-back inhibition of ATCase.

ATCase has a sigmoidal substrate saturation curve for aspartate at *p*H greater than 6·1, indicating a co-operative (homotropic) interaction[2] between catalytic sites, that is, ATCase increases its aspartate affinity with increasing aspartate concentration. Fig. 1*a* shows the effect of CTP and *p*H on the substrate saturation curve of ATCase. Co-operative interactions between catalytic sites and the modification of this interaction by CTP (heterotropic interaction[2]) are examples of indirect interactions between binding sites that are characteristic of allosteric enzymes. The co-operative substrate site interaction allows non-reactive substrate analogues that are simple competitive inhibitors at high concentrations to activate the enzyme at low concentrations. Fig. 1*b* shows the activation of ATCase by a substrate analogue at low concentrations, and the competitive inhibition at high concentrations. Several substrate analogues show this effect.

The kinetic effects, in summary, are as follows: CTP promotes a decrease in the affinity of ATCase for aspartate while substrates, substrate analogues, and protons increase enzyme affinity for aspartate. At a given aspartate concentration, the aspartate affinity of the enzyme determines the catalytic activity (reaction velocity) of ATCase. The activity changes at a fixed aspartate concentration are represented in Fig. 1*a* by the arrows showing the direction of the activity shift on addition of CTP and H^+.

Materials and methods. All chemicals used were obtained from commercial organizations. The sodium salt of poly-L-glutamic acid, supplied by Mann Research, Inc., had an average degree of polymerization of about 180, estimated by the intrinsic viscosity in 0·1 M sodium chloride. The poly-L-glutamic acid concentration was estimated from the dry weight, assuming one water of hydration per residue. ATCase was a gift from Dr. John Gerhart. The enzyme assay[5] and method of preparation[21] were previously described. All enzyme solutions contained 2 mM mercaptoethanol and 0·2 mM EDTA. Potassium phosphate was used for the *p*H 7·0 and *p*H 6·1 buffers, while *tris* hydrochloric acid was used for *p*H 8·6. The buffers were 40 mM for the kinetic measurements and 4 mM for the optical measurements at less than 240 mμ. The enzyme concentrations were determined from the absorption at 279 mμ, using $A^{1\,cm}_{1\,mg/ml.} = 0{\cdot}59$. The average residue weight ($\bar{M}$) of ATCase was estimated, from a preliminary amino-acid analysis[17], to be 120.

Optical rotatory dispersion measurements were made with a Cary Model 60 recording spectropolarimeter. The rotations were independent of slit width and the specific rotations were independent of the product of (concentration) × (path length) over a ten-fold range. Lamp position adjustment and slit width adjustment were made for minimum noise-level at the wave-length region of interest. The cells, made by Pyrocell, Inc., were selected for minimum birefringence and were cleaned inside and out by soaking in concentrated nitric acid–sulphuric acid (50/50 by volume) and by exposure for 1 min to methanol–3 M potassium hydroxide (50/50 by volume). When properly cleaned, the cells could be removed and replaced in the polarimeter with no observable change in the rotation. Cells of 0·1, 1·0 and 10 mm path length were used. All measurements were performed at 4·5° ± 1·0° C. The specific rotation measurements for different samples at the same solution conditions varied 1–2 per cent. The variation probably arises from volumetric errors in sample preparation. This variation of specific rotation from sample to sample was too large to permit a satisfactory determination of the absolute specific rotations of the enzyme in the presence and absence of effectors. However, we could determine the relative rotation in the presence and absence of effector by a titration method. For the titration experiments, 2·5 ml. of enzyme solution was pipetted into a 0·1 or 1·0 mm cell and the optical rotatory dispersion spectrum measured. Ten- to twenty-lambda portions of concentrated effector solutions, previously adjusted to the appropriate *p*H, were added with a micro-pipette. The enzyme solution was mixed by slow inversion

of the cell and the optical rotatory dispersion spectrum measured. Corrections for dilution and CTP rotation were applied. The magnitude of rotation at selected wave-lengths was most precisely determined by chart recording of the rotation at each fixed wave-length for extended periods (*c.* 10 min) with a 10- or 30-sec pen time constant. After running a spectrum, the peak and trough wave-length points were repeated and the rotations were unchanged within the noise level in all cases. The specific residue rotation $[R']$ was calculated according to the relation:

$$[R'] = \frac{3}{n^2 + 2} \frac{\alpha_\lambda}{l\,c} \frac{\bar{M}}{100}$$

where α_λ is the observed rotation in degrees, l is the path length in decimetres, c is the concentration in g/ml., $\bar{M}$ is the average residue weight, and n is the refractive index of the solution at wave-length of measurement. The refractive index of water at 231 mμ[6] and 20° C was used for all wave-lengths. The Lorentz factor, $3/(n^2 + 2)$, for water at 231 mμ is 0·7635.

Absorption measurements were made with a nitrogen-purged Cary model 15 spectrophotometer. Absorption spectra were taken relative to a buffer solution of identical concentration. The phosphate buffer used for the far ultra-violet absorption measurements had a maximum absorption of one at 180 mμ in a 0·1 mm cell.

Helix content of ATCase. Optical rotatory dispersion in the far ultra-violet region of peptide bond absorption is sensitive to protein secondary structure[7,8]. Fig. 2 shows the ultra-violet optical rotatory dispersion of a model polypeptide, poly-L-glutamic acid (PLGA), in the helical (*a*) and random coil (*b*) conformations. Similar curves, differing slightly in magnitude, have previously been published by other workers[8]. Fig. 2c shows the ultra-violet optical rotatory dispersion of ATCase; the spectrum is strikingly similar in shape to that of helical PLGA, but the magnitude/residue is much smaller. The optical rotatory dispersion curve of ATCase can best be fitted with about 47 per cent of the PLGA helix specific residue rotation (Fig. 2*a*) with no contribution of the non-helical residues. To a first approximation, we conclude that about 37 per cent of the residues of ATCase are in the helical conformation. Because there may be many short regions of helix in the molecule, the actual helix content may be somewhat higher than 37 per cent owing to an expected dependence on chain-length of the rotation/residue of a polypeptide helix[8]. The only appreciable deviation of the fit of a 37 per cent PLGA helix specific residue rotation to the ATCase optical rotatory dispersion occurs in the region between 220 mμ and 205 mμ. The small deviation of the fit has a rather complicated wave-length dependence, and is thought to result from an almost complete cancellation of opposing contributions of the Cotton effects of the various non-helical residues. In an investigation of the far ultra-violet optical activity of myoglobin, using circular dichroism, Holzwarth and Doty[10] have found a similar cancellation of the contributions of the non-helical residues. For myoglobin, the circular dichroism can best be fitted by 70 per cent model polypeptide helix dichroism with no contribution from the non-helical residues[11].

Several other methods are available for helix content estimation from data for optical rotatory dispersion[12,13]. The other treatments of this problem rely on the monotonic visible and near ultra-violet data, and we feel that the most reliable method should be the direct observation of the peptide helix Cotton effects as has been done here. The exact absolute value of helix content of ATCase is not of prime importance in our work and, furthermore, we did not want to use the large amounts of material required for precise visible measurements. None of the methods for estimating the helix content is really satisfactory for proteins with low helix content, as local charge and dipole fields[14] of regions of the protein wrapped around the helical segments are not present in the reference polypeptides. It should be mentioned that PLGA may not be the ideal reference material for an estimation of helix content, and these problems have been discussed by Yang and McCabe[15].

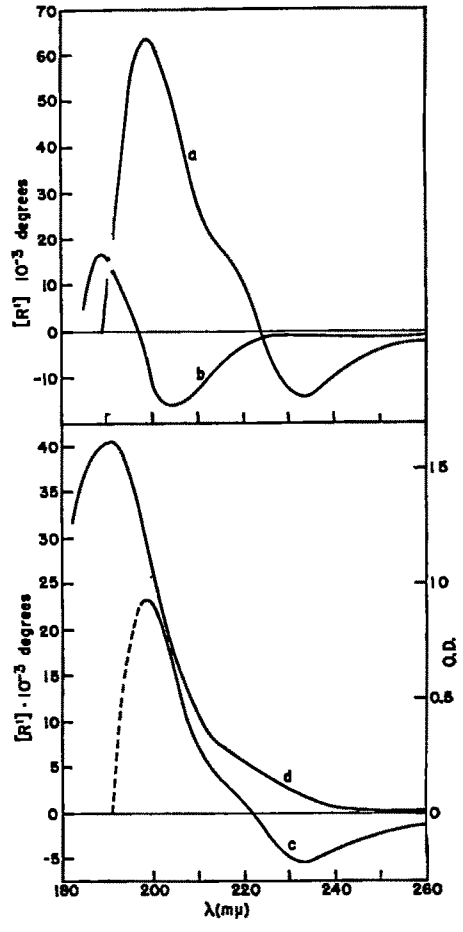


Fig. 2. Optical rotatory dispersion (ORD) and absorption. *a*, ORD of poly-L-glutamic acid, *p*H 4·8 (no added salt), helix form; *b*, ORD of poly-L-glutamic acid, *p*H 7, random coil form; *c*, ORD of ATCase, *p*H 6·1, the average residue weight is taken as 120 g/mole; the dotted portion of the curve is in a region of relatively high noise-level and is less reliable than the other data; *d*, absorption of ATCase, 2·08 mg/ml., *p*H 6·1 in a 0·1 mm cell measured versus buffer

The helix content of proteins can also be estimated from the hypochromic effect that the helical conformation exerts on the ultra-violet peptide bond absorption[16]. This method agrees well with the optical rotatory dispersion for simple polypeptides and some proteins. The use of this method for proteins is complicated by the contribution of aromatic amino-acid side-chains, as well as arginine, methionine, cysteine, amide, and carboxylate groups, to the absorption in the region of peptide bond absorption. The method is attractive because it requires small amounts of material and we felt that more cases should be tried to fully explore its possible usefulness. The side-chains contribution to the absorption of ATCase was estimated from an amino-acid analysis[17] and the amino-acid side-chain extinction coefficients given by Rosenheck and Doty[18]. The absorption spectrum of ATCase is given in Fig. 2*b*. The helix content of ATCase was estimated by this method to be 95 per cent at 190 mμ, 51 per cent at 197 mμ, and 48 per cent at 205 mμ. The 190 mμ point has the largest relative correction for side-chain absorption and is least trustworthy. The 197 mμ point has the

smallest relative correction, and Rosenheck and Doty[18] believe 197 mμ to be the most reliable.

The peptide hypochromism gives a value for the helix content of ATCase which is larger than that predicted by the optical rotatory dispersion. If the 190 mμ point is neglected, the peptide bond hypochromism indicates about 50 per cent helix. This is in acceptable agreement with the optical rotatory dispersion value of 37 per cent. We consider the hypochromism value as the less reliable, as the side-chain as well as the peptide bond absorption may be considerably perturbed by the environment inside the protein.

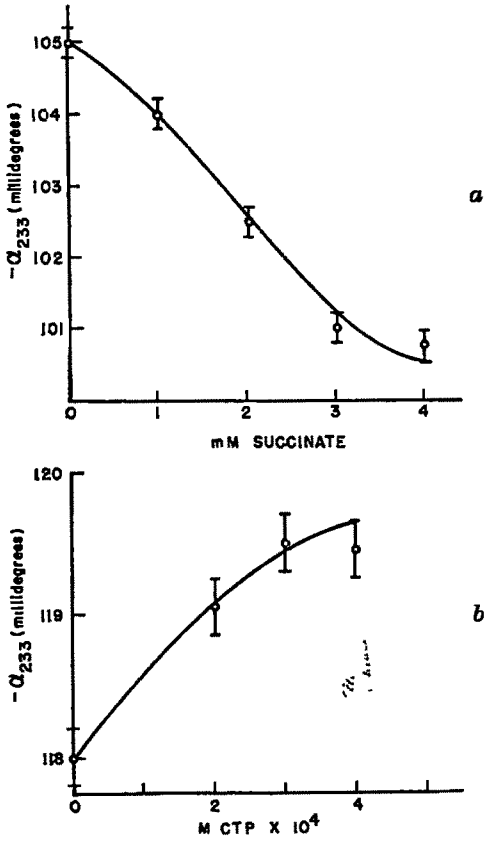


Fig. 3. The dependence of the rotation of ATCase, at the trough of the 233 mμ Cotton effect, on the concentration of CTP and substrate analogue succinate. *a*, Enzyme (1·75 mg/ml.) at *p*H 8·6, and equal portions of a mixture of 150 mM potassium succinate and 130 mM carbamyl phosphate (*p*H 8·6) added. *b*, Enzyme (2·08 mg/ml.) at *p*H 6·1, and equal portions of 20 mM CTP (*p*H 3·1) added

Substrate and inhibitor-induced changes in optical rotatory dispersion. The entire curve for optical rotatory dispersion of ATCase changes in magnitude when CTP, substrate analogues, or protons are added to the enzyme. The observed changes are small (about 5–6 per cent maximum) but can be measured over most of the wave-length range of interest. Table 1 shows the specific rotation/residue of ATCase at the trough of the 233 mμ Cotton effect in the presence of various substances that affect its kinetic behaviour. We use 233 mμ as a convenient wave-length for tabulating the optical rotatory dispersion change because the relative errors are lowest at this wave-length. These measurements were repeated several times and always showed the trends indicated in Table 1; however, the variations in the observed differences were large enough to make the magnitude of the change uncertain. We found that titration of the change in optical rotation shows the rotational changes clearly (see the section on materials and methods).

Fig. 3*a* shows the change in ATCase rotation observed on addition of small portions of a concentrated mixture of carbamyl phosphate and succinate, a non-reactive substrate analogue that is bound to the enzyme. The rotation decreases with increasing substrate concentration, while the catalytic activity increases. It is important to note here the role of carbamyl phosphate, in that carbamyl phosphate is required for the rotatory change, but causes no change by itself (within experimental error). Perhaps carbamyl phosphate is necessary for the binding of succinate. Fig. 3*b* shows the increase in rotation of ATCase as the CTP concentration increases. The catalytic activity decreases with increasing CTP concentration. The effect of added protons on the enzyme shows increased rotation (Table 1) with decreased activity (Fig. 1*a*). All measurements consistently show an inverse correlation between the magnitude of the rotation and the catalytic activity of ATCase.

Table 1. VALUES OF SPECIFIC RESIDUE ROTATION OF ATCASE AT 233 mμ IN VARIOUS SOLUTIONS FROM A TYPICAL EXPERIMENT

Sample	$[R']_{233\ m\mu}$		
*p*H = 6·1	5,240 ± 20		
*p*H = 6·1 + 10^{-4} M CTP	5,330 ± 20	Δ = 90	Δ = 350
*p*H = 8·5	5,590 ± 20		
*p*H = 8·5 + 5 mM succinate and 3 mM carb. PO_4	5,330 ± 20	Δ = 220	

The Δ values are the differences caused by solution alteration. All experiments show the same trends of effector-induced changes in the rotation. The errors shown in the table are the observed noise-level in the optical rotatory dispersion spectrum.

The changes in rotation clearly show that there is a change in the relationships of amide residues in the protein accompanying changes in the catalytic activity. Fig. 4 shows the maximum change in the optical rotatory dispersion of ATCase caused by the addition of substrate analogue. The shape of the difference curve for optical rotatory dispersion is very close to that of the polypeptide helix, indicating that, on the face of it, some residues of the protein have undergone a coil-to-helix transition. The noise-level is relatively high in the region of 200 mμ and below, and it is difficult to follow the details of the change; however, it is clear that the maximum change in

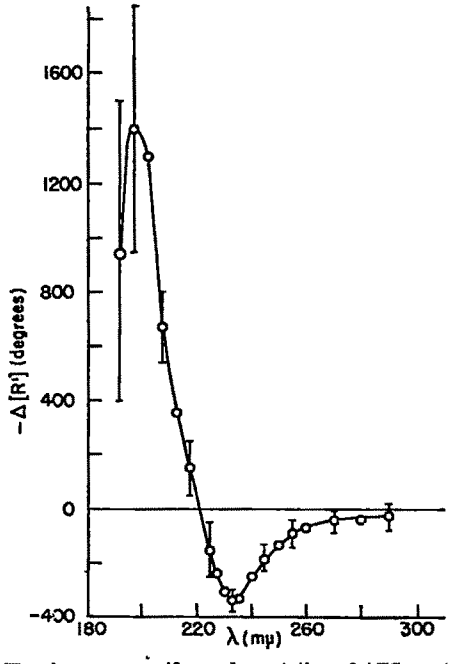


Fig. 4. The change in specific residue rotation of ATCase at *p*H 8·6 brought about by 4 mM potassium succinate and 4 mM carbamyl phosphate. The difference is presented as (enzyme + effector)–(free enzyme)

this region relative to the maximum change at 233 mμ is consistent with a change in helix content.

Care must be taken in assigning such small changes to any particular structural alteration, and alternative explanations must be carefully examined.

(1) Small molecules can acquire optical activity when bound to an optically active large molecule[18,19]. If the optical rotatory dispersion changes observed arose from induced optical activity in the bound effectors, specific rotations in excess of 10^6 degrees/mole of bound substrate analogue would be required to explain the observed change in rotation (even if as many as ten substrate analogues/mole of enzyme were bound). Specific rotations of this magnitude are much larger than can reasonably be expected. Furthermore, induced Cotton effects will be centred very close to the absorption maxima of the bound molecules. CTP has a weak optical rotatory dispersion spectrum with a Cotton effect centred at 270 mμ. An induced Cotton effect in CTP would show up in the 270 mμ region and could not give rise to a change in optical rotatory dispersion with the wave-length dependence observed. Substrate analogues, succinate and malate, have somewhat different absorption spectra and both produce a change in the optical rotatory dispersion spectrum of the same shape. Because the same changes in optical rotatory dispersion are caused by substances with different absorption spectra (CTP, succinate, malate) we can rule out Cotton effects induced in the bound molecules as the source of the changes.

(2) Effector-induced alterations in the non-helical regions of the protein structure could change the charge and dipole environment around the helix and give rise to an altered helix optical rotatory dispersion. Changes in the charge and dipole fields would be expected to have the largest effect on the helix optical rotatory dispersion in the electronically forbidden n/π^* region around 225 mμ[9,10,14] and to be less important in the electronically allowed $N-V$ peptide bond transition in the region of 190 mμ[10,14]. It is very unlikely that alterations in the static fields would produce the same percentage of change in the optical rotatory dispersion at 200 mμ and at 233 mμ as is observed. We can conclude with some confidence that the change in the optical rotatory dispersion does not result from alterations in the non-helical regions of the protein. (The non-helical regions may well change under the influence of the allosteric effectors, but this is not the source of the changes in optical rotatory dispersion we observe.)

(3) In principle, one other possible explanation for a change in optical rotatory dispersion with the wave-length dependence of the helix-coil type is a change in the local refractive index resulting from swelling or shrinking (change in internal hydration), thereby altering the local electric field of the light inside the protein and leading to an altered amplitude of optical rotatory dispersion. If we assume a Lorentz local field, a very large change in refractive increment for the protein (of the order of 6 per cent maximum) would be required to explain the observed effect. A change in refractive increment of this magnitude is extremely unlikely.

In summary, the addition of allosteric effectors causes a physical change in the protein structure. The interrelationships of the amide residues clearly change, and, although static field and hydration effects are not strictly excluded, the allosteric transition is most probably accompanied by a change in helix content of the enzyme.

ATCase appears to be composed of six sub-units, four regulatory and two catalytic, the interaction of which controls the catalytic activity[20,21]. The helix-coil transition that has been found could change the geometry of the sub-units and thereby affect the sub-unit interaction. It has been observed that substrate analogues induce a small change in the sedimentation coefficient of ATCase[17]. The magnitudes of the change in optical rotatory dispersion and sedimentation are both small. The molecular weight of ATCase[21] is about 3×10^5, which corresponds to about $2{\cdot}6 \times 10^3$ residues, about $8{\cdot}3 \times 10^2$ (37 per cent) of which are in a helical environment. The number of residues involved in the allosteric helix-coil transition can be estimated to be about 40–50 residues from the maximum change in rotation of about 5–6 per cent. This small alteration of the enzyme structure occurs during a change of substrate affinity of two orders of magnitude or more. It is not surprising, however, that small alterations of enzyme structure could lead to large changes in activity by affecting the shape, polar environment, or charge on the catalytic site. The allosteric enzyme acts like a biochemical amplifier[22] in that a small signal causes a large effect.

The estimate of the number of residues involved is a lower limit. The net observed effect could be the result of opposite transitions, one helix-to-coil and the other coil-to-helix; the difference in the number of residues would be seen by our experiments. It is also possible that a large number of residues modify their helix dimensions as compared with a smaller number undergoing a complete helix-to-coil transition. The former point of view is unfavourable because helix-coil transitions tend to be co-operative.

The model for allosteric proteins put forward by Monod *et al.*[2] supposes that there are two forms of the enzyme in equilibrium. The two forms are assumed to have different catalytic activities and that the binding of substrate or inhibitor shifts the equilibrium between the two forms. The model predicts that the physical effect of the inhibitor is equivalent but opposite to that of the activator. Another possibility, which runs contrary to the model under consideration, is that the inhibitor and activator cause the same kind of physical change but do so in different parts of the molecule. Our results show that the change occurring on addition of inhibitor is opposite (and approximately equal to) the effect of activator. In terms of Monod, Wyman and Changeux's model, the evidence from optical rotatory dispersion would imply that the catalytically more active form is the less helical form.

An investigation of the responses of the separated regulatory and catalytic sub-units[21] to the allosteric effectors would be desirable to separate their rotatory contributions. Correlation of the binding of effectors with the physical change produced by the effectors is central to the understanding of the mechanism of this enzyme.

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LETTERS TO THE EDITOR

ASTRONOMY

Expected Number of Blue-shifts and Red-shifts of Ejected Sources

IT has recently been suggested[1] that the quasi-stellar radio sources are objects that have been ejected by relatively nearby peculiar galaxies. If their large red-shifts are due to the Doppler effect, we shall show that one would, in fact, expect to see many more objects with spectral shifts towards the blue than those with shifts towards the red.

We make the following assumptions: the explosion centres are distributed randomly in space; the distribution of the ejected objects is isotropic; the explosion centres are at rest with respect to the observer (we neglect the small red-shifts of the parent galaxies); the geometry over the volume of space considered is Euclidean; the spectra of all the ejected objects are the same over the region of interest and are representable by a simple power law; and the proper luminous lifetime of the ejected objects is small compared with the time taken for light to travel from here to the ejection centre.

The Doppler shift $(1 + z) = \lambda_{obs}/\lambda_{emitted}$ is given by equation (1) (see, for example, ref. 2).

$$(1 + z) = \frac{1 + v/c \cos\theta}{(1 - v^2/c^2)^{\frac{1}{2}}} \tag{1}$$

where v is the ejection speed, and θ is the angle between the line of sight and the velocity vector. The probability that an object is ejected into a given element of solid angle is just $d\Omega/4\pi$, as the ejections are assumed isotropic. Thus, if $P_0(z)dz$ is the probability of an ejection in the interval $[z, z + dz]$, we have:

$$P_0(z)dz = \frac{d\Omega}{4\pi} = \tfrac{1}{2} \sin\theta \, d\theta \tag{2}$$

and from equation (1):

$$dz = \frac{-v/c}{(1 - v^2/c^2)^{\frac{1}{2}}} \sin\theta \, d\theta \tag{3}$$

so, letting $1 + z_m = [(1 + v/c)/(1 - v/c)]^{\frac{1}{2}}$ be the maximum observed red Doppler shift (note that the maximum blue Doppler shift is $(1 + z_m)^{-1}$), we obtain:

$$P_0(z)dz = \frac{1 + z_m}{z_m (2 + z_m)} \, dz \tag{4}$$

For a given proper lifetime, the observed lifetime is proportional to the Doppler shift (the Doppler shift is very nearly constant, because the last assumption ensures that the angular distance travelled by the object is small), and for this reason, the probability of observing an object with Doppler shift $(1 + z)$ is enhanced by a further factor $(1 + z)$. Thus if $P(z)$ is the distribution function taking this effect into account, we have:

$$P(z)dz = \frac{2(1 + z_m)^2}{(1 + z_m)^4 - 1} (1 + z) \, dz \tag{5}$$

Let the intrinsic luminosity of each source be $L_0(\nu_0)$. Then the flux crossing a shell of radius R_0 (in the frame of the emitter) in the frequency interval $(\nu_0, \nu_0 + d\nu_0)$ is:

$$F_0(\nu_0)d\nu_0 = \frac{L_0(\nu_0)d\nu_0}{4\pi {R_0}^2} \tag{6}$$

The flux seen by an observer with a Doppler shift $(1 + z)$ relative to the emitter is:

$$\begin{aligned} F(\nu)d\nu &= (1 + z)^{-2} F_0(\nu_0)d\nu_0 \\ &= (1 + z)^{-1} F_0[\nu(1 + z)] \, d\nu \end{aligned} \tag{7}$$

The factor $(1 + z)^{-2}$ can be understood either by considering a flux of photons, both the energy and rate of passage of which are reduced by $(1 + z)^{-1}$ (the area enclosing a given bundle of rays can be easily shown to be invariant; see, for example, ref. 3), or by directly transforming the Poynting vector. The distance R to the emitter measured by the observer can be easily shown to be:

$$R = \frac{R_0}{1 + z} \tag{8}$$

so we can write:

$$F(\nu) = \frac{L_0 [\nu(1 + z)]}{4\pi R^2 (1 + z)^3} \tag{9}$$

We assume that $L_0(\nu_0) = C\nu_0^{-\alpha}$ for each source (C may differ from source to source). The relation between the monochromatic apparent magnitude m and the proper monochromatic absolute magnitude M is:

$$m = M + 5 \log R + 2{\cdot}5 \log [(1 + z)^{3+\alpha}] \tag{10}$$

Let $n(m,z)dm dz d\Omega$ be the number of objects with apparent magnitude in $[m, m + dm]$ and with red-shift in $[z, z + dz]$ in the solid angle of sky $d\Omega$. Let $N(M,z)dM dz$ be the number of objects with absolute magnitude in $[M, M + dM]$ and with red-shift in $[z, z + dz]$ in a unit volume of space. Thus,

$$n(m,z)dz d\Omega = \left[\int_0^\infty N(M,z)R^2 dR \right] dz d\Omega \tag{11}$$

From equation (10):

$$R = 10^{0.2(m-M)} (1 + z)^{-(3+\alpha)/2} \tag{12}$$

and

$$dR = -0{\cdot}2 \times 10^{0.2(m-M)} (1 + z)^{-(3+\alpha)/2} \times \ln 10 dM \tag{13}$$

Thus

$$n(m,z) \, dz = \left[\int_{-\infty}^{\infty} 0{\cdot}2 \, N(M,z) \, 10^{-0{\cdot}6M} \times \ln 10 dM \right] 10^{0{\cdot}6m} (1 + z)^{-\frac{3}{2}(3+\alpha)} dz \tag{14}$$

Let $\Phi(M)dM$ be the number of objects per unit volume of space with absolute magnitude in $(M, M + dM)$. Then

$$N(M,z)dM dz = \Phi(M)dM \, P(z)dz \tag{15}$$

and equation (14) becomes:

$$n(m,z)dz = \left[0{\cdot}2 \int_{-\infty}^{\infty} \Phi(M) \, 10^{-0{\cdot}6M} \ln 10 \, dM \right] 10^{0{\cdot}6m} (1 + z)^{-\frac{3}{2}(3+\alpha)} \frac{2 (1 + z_m)^2}{(1 + z_m)^4 - 1} (1 + z) \, dz \tag{16}$$

The quantity in brackets is a constant; thus the form of $n(m,z)$ is independent of the luminosity function $\Phi(M)$. Note also that $\log [n(m,z)]$ is proportional to $0{\cdot}6m$.

The ratio of the number of blue-shifted objects to red-shifted objects at apparent magnitude m is

$$\frac{n(m)_{blue}}{n(m)_{red}} = \frac{\int_1^{(1 + z_m)^{-1}} n(m,z)d(1 + z)}{\int_1^{(1 + z_m)} n(m,z)d(1 + z)} = (1 + z_m)^{\frac{3}{2}(3+\alpha)-3} \tag{17}$$

for a single ejection speed corresponding to z_m. It should be noted that our final assumption minimizes the ratio n_{blue}/n_{red}, because it enhances the probability of seeing red-shifted objects. If, however, the objects were emitted uniformly in both space and time with an infinite lifetime, n_{blue}/n_{red} would be increased by a further factor $(1+z_m)$. The coefficient α is in the vicinity of 0·7 for most of the quasi-stellar radio sources in the radio region and varies around 0·7 in the optical region[4]. Taking $\alpha = 0{\cdot}7$ we have:

$$\frac{n(m)_{blue}}{n(m)_{red}} \sim (1 + z_m)^{3\cdot 55} \qquad (18)$$

If $z_m = 2$, the ratio is 50; even for z_m as small as 0·5, the ratio is 4·2. Red-shifts for quasi-stellar radio sources as large as 2 have been observed[5], and most of the observed ones are larger than 0·5; thus one expects at least of the order of five times as many blue-shifts as red-shifts. So far, no blue-shifted quasi-stellar radio sources have been observed while many have measured red-shifts. It seems unlikely that any of our initial assumptions are violated to an extent sufficient to change our result appreciably; we thus conclude that if the red-shifts are Doppler, the quasi-stellar radio sources have not been ejected from peculiar galaxies. If, however, the quasi-stellar radio sources have been so ejected, the red-shifts are not of Doppler origin.

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Determination of Direction, Frequency and Polarization of Radio Emission from Galactic OH

A MAJOR problem in the study of galactic radio emission from OH is to understand how it is that the emissions appear to be localized in very small bright regions each characterized by a particular frequency[1]; in addition, it has been found that emissions can show both linear and circular polarization[2,3]. The localization of emission at a particular frequency can readily be explained on the hypothesis of stimulated emission[4] if there are regions in which the velocities of the OH molecules are correlated over large distances. Should the velocities vary considerably along a line of sight, so will the Doppler shifts relative to some local standard and stimulated emission will not occur because the optical depth at any particular frequency would be inadequate. For a sufficient optical depth, the velocities along the line of sight must be correlated over a distance of about 10^{19} cm. In the absence of shifts of wave-length from any other cause, the velocity must be constant along the line of sight over such a distance. Only in those directions along which there is such a systematic motion will intense emission be observed. The frequencies of peaks in observed spectra indicate that velocities in different parts of a source may differ by 100 km/s (ref. 1).

Whereas linear polarization might arise from some directional effect such as could be associated with the outward movement of an ionization front bounding an H-II region, circular polarization seems to require magnetic fields. The situation does not correspond, however, to simple Zeeman splitting, because just one particular frequency is observed along a particular line of sight, instead of a complete symmetrical Zeeman pattern[1]. The observations can be accounted for, however, by a magnetic field combined with a velocity field. If the magnetic field is directed along the line of sight and is proportional to the OH velocity, and if both vary along the line of sight, then at any point, the wave-length shift for one sign of circular polarization can be just balanced by the Doppler shift, but not for the other sign nor for the linearly polarized unshifted component. In a similar way, a magnetic field at right angles to the line of sight may give rise to linearly polarized radiation.

A magnetic field proportional to the velocity is consistent with the magneto-hydrodynamic equations for a fully ionized gas, for the field is then frozen into the gas and

$$\frac{\partial \mathbf{H}}{\partial t} = \text{curl}\,(\mathbf{v} \wedge \mathbf{H})$$

If $\mathbf{v}$ is always proportional to $\mathbf{H}$ and in the same direction, $\mathbf{v} \wedge \mathbf{H}$ is zero and the situation is static.

Accordingly, let it be assumed that the OH molecules have the same velocity as the ionized hydrogen at the edge of an H-II region. The present suggestion has a number of implications. The Zeeman splitting patterns of OH (ref. 2) show that for the lines at 1,665 and 1,667 Mc/s, the velocity and magnetic field must have the following relations:

$$1{,}665\ \text{Mc/s}: \quad \mathbf{v} = \pm\, 3{\cdot}0 \times 10^2\, \mathbf{H}$$
$$1{,}667\ \text{Mc/s}: \quad \mathbf{v} = \pm\, 1{\cdot}8 \times 10^2\, \mathbf{H}$$

where $\mathbf{v}$ is in km/s and $\mathbf{H}$ is in gauss.

The Zeeman patterns of the lines at 1,612 and 1,720 Mc/s are more complex and Zeeman shifts would compensate the Doppler shifts for the relations

$$\mathbf{v} = \pm\, 0{\cdot}6 \times 10^2\, \mathbf{H}$$

as well as for each of the relations for the 1,665 and 1,667 Mc/s line.

Circular polarization would therefore not be expected to be observed along the same line of sight at both 1,665 and 1,667 Mc/s, but might be seen at 1,612 and 1,720 Mc/s if seen at either 1,665 or 1,667 Mc/s. The observed spectra seem to require velocities of the order of 100 km/s and fields of the order of 0·1 G; however, it may be that they do not have to be so closely related, nor the fields so large, for, as has previously been pointed out[4], when stimulated emission occurs, relatively small differences of population can give large differences of intensity.

Such a picture of the velocities and magnetic fields in the neighbourhood of an H-II region is much more complex than that in which an ionization front moves with a predominantly radial velocity, for the tangential velocities required by the present ideas are comparable with the radial velocities on the simple model. It is difficult, in fact, to see how such a scheme of large-scale circulation can be avoided, for if the tangential velocities are only correlated over distances much less than 10^{18} cm, no net stimulated emission will occur along any line of sight.

This communication has been concerned with the problem of the determination of the frequency, line of sight and polarization of stimulated radio emission from OH; to account for the observations it is also necessary to understand how the populations of the Λ-doublet levels

may be inverted[4] and how the intensities may change rapidly with time[5].

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The X-ray Source *Sco X-3*

TAMMANN[1] has advanced the suggestion that a supernova observed by Albumazar in A.D. 827 may be connected with the X-ray source *Sco X-3* in the H II region No. 114.

Dr. Tammann depends on Lundmark[2] for the report of Albumazar. It has been shown, however, that Lundmark's notice depends on a long chain of references far removed from the original report[3]. The original report is in Ali ibn Ridwan's commentary to Ptolemy's *Tetrabiblos* and refers to an apparition seen by Ali and clearly dated April 30, 1006. Ali gives the celestial longitude of the observed object as Scorpio 15° (which need not refer to the constellation Scorpio). In fact, on the basis of a variety of medieval reports of this supernova, many of them contemporary with the event, the position of the supernova has been determined. Gardner and Milne[4] report that, on the basis of radio evidence, the remnant of this supernova is located at 14^h59^m6, $-41°42'$ (epoch 1950.0).

Thus, *Sco X-3* (17^h23^m, $-44·3°$ (1950)) is not related to the supernova to which Tammann referred.

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PLANETARY SCIENCE

"Westward Drift" of the Satellite Geoid

RECENTLY, Dr. Tuman[1] compared the mean free air anomaly as calculated by Jeffreys[2] with the satellite geoids calculated by Izsak[3] and Kaula[4], and on the basis of this suggested that the satellite geoid may have a westward drift. If I were to admit that Jeffreys's geoid of 1943 was adequate for a comparison with the satellite results I might accept that Dr. Tuman's work was meaningful, yet I would still maintain that it is remarkable that Dr. Tuman failed to use better solutions of satellite geoid than Izsak's 1963 solution (many have since been published, for example, by Izsak[5], by Anderle[6] and by Guier and Newton[7]). But I must reject that attitude which maintains that Jeffreys's solution of 1943 is adequate for such a comparison. The problem that Jeffreys dealt with was a statistical one. Without observations in vast oceanic areas at the time of his analysis, it was not possible for him to be certain of how much of the gravity anomaly should be attributed to terms of degrees 2 and 3 and how much of it should be attributed to those of higher degrees. The uncertainties in his longitudinal terms were thus considerable. It is significant that the general features of Jeffreys's geoid are compatible with those of the satellite geoid, but the reliability of its detailed features—for example, the longitudinal positions of the maxima and minima of the geoidal height—requires considerable care.

The longitudinal positions of the maxima and minima of satellite geoids determined by various authors are not consistent among themselves. For purposes of comparison, a few of the available results are listed in Table 1.

Table 1

Approximate locations	Jeffreys (ref. 2)	Izsak (ref. 3)	Izsak (ref. 5)	Guier and Newton (ref. 7)	Kaula (ref. 8)	Gaposchkin (ref. 9)
Gulf of Calif. (min)	—	125° W.	115° W.	135° W.	135° W.	130° W.
Andes (max)	—	90° W.	75° W.	75° W.	—	70° W.
Guinea Basin (min)	80° W.	45° W.	50° W.	70° W.	65° W.	85° W.
South Africa (max)	20° E.	20° E.	55° E.	50° E.	50° E.	50° E.
Indian Ocean (min)	85° E.	65° E.	70° E.	75° E.	70° E.	75° E.
New Guinea (max)	165° E.	180°	155° E.	150° E.	145° E.	155° E.
West Europe (max)	10° E.	5° E.	35° W.	10° W.	0°	15° W.

Besides statistical reasons, the close agreement among the last three columns also supports the implication that they are more accurate than the others. "Drifts" of these geoids from Izsak's 1963 one are observed of 5°–30° (both in eastwardly and in westwardly directions). A comparison between Gaposchkin's solution and that of Jeffreys shows that a geoidal minimum near Guinea Basin and a maximum near South Africa "drift" eastward by 15° and 30°, respectively.

Dr. Tuman used Vogel's[10] and Egyed's[11] works to support his observations. I have already questioned[12] Vogel's work as the assumptions used in his calculation of the undulations at the core-mantle boundary are not consistent with his results. Egyed supported Vogel's hypothesis by using a much simplified model. Serious objections to Egyed's work were raised by Coode and Runcorn[13] and myself[12]. A comparison of modern information on geomagnetism with satellite geoid shows no obvious correlation such as was suggested by Vogel.

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Arc Quench Gap in Vacuum

THE purpose of these preliminary experiments was to determine the effect of vacuum pressure from 10^{-1} torr (100μ) to 10^{-3} torr (0·01μ) on the stability of a d.c. arc. A pressure of 100μ is obtained in space at an altitude of 40 miles, while 0·01μ is attained at 70 miles.

During the launch phase of unmanned instrumented artificial satellites or space probes, vibration of the grounding cables interconnecting electronic modules can become severe enough for high-voltage arcing between improperly shielded copper conductors and parts of the spacecraft structure or the shroud to occur. In a number of cases, because of their advantageous strength to weight ratio, magnesium alloys are used as a structural material in a number of spacecraft applications[1].

In this preliminary laboratory investigation of d.c. arc stability, electrodes of oxygen-free high-conductivity copper and the magnesium–thorium alloy *HM21-A* were chosen as a possible combination of materials that have been used. For example, the shrouds protecting the

Ranger and *Mariner* space probes during the ascent phase of the mission were constructed of the *HM*21-*A* alloy.

As the spacecraft system with its first- and second-stage boosters travels through the thinning atmosphere during the ascent phase before jettisoning the shroud and before separation of the first stage occurred, pressure under the shroud can rapidly decrease. However, it may also reside at pressure regions that are critical for the generation of glow discharge or electrical arcing and high-voltage breakdown phenomenon for periods during which a grounding bus cable under vibration could come into too close proximity to the shroud skin and cause high-voltage arcing. This event could seriously damage spacecraft circuitry. In order to determine the separation distance for arcing of a 28-V, 20-amp d.c. current between typical spacecraft materials and the influence of vacuum pressure on their behaviour, these preliminary experiments were conducted.

The equipment used for these experiments consisted of a vacuum system with a 4-in. oil diffusion pump, a liquid-nitrogen trap above the diffusion pump, high-vacuum valve, base-plate and lead through adaptor ring and a bell jar. Electrical power, mechanical, calibrated leak, and vacuum gauging lead through into the bell jar were provided in the base-plate adaptor ring. The d.c. power supply provided 28-V, 20-amp current through a resistive load to the test specimens.

The test device is shown in Fig. 1. The test specimens were 0·5 in. square and 0·75 in. long. The upper specimen of magnesium alloy *HM*21-*AT*8 was the cathode, the lower specimen of oxygen-free high-conductivity copper, the anode. The device was made of polymethylmeth-acrylate ('Plexiglass'). A lead screw and nut provided the electrode separating motion of 0·0015 in./sec, through bevel gears, a stub shaft and magnetic drive.

The system was pumped to high vacuum ($\sim 10^{-5}$ torr), the bell jar was cut off by a valve, and dry air was admitted through the calibrated leak while throttling the pumping system valve to achieve the pressure required (for example, 3×10^{-2} torr or 30μ). With the electrodes touching, power of 28 V, 20 amp d.c. was applied, the electrodes were separated slowly (0·0015 in./sec), while observing the quenching of the arc. The moment the arc was quenched, separation was stopped, the power turned off, air admitted to the system, and after removal of the bell jar the electrode separation measured with a feeler gauge.

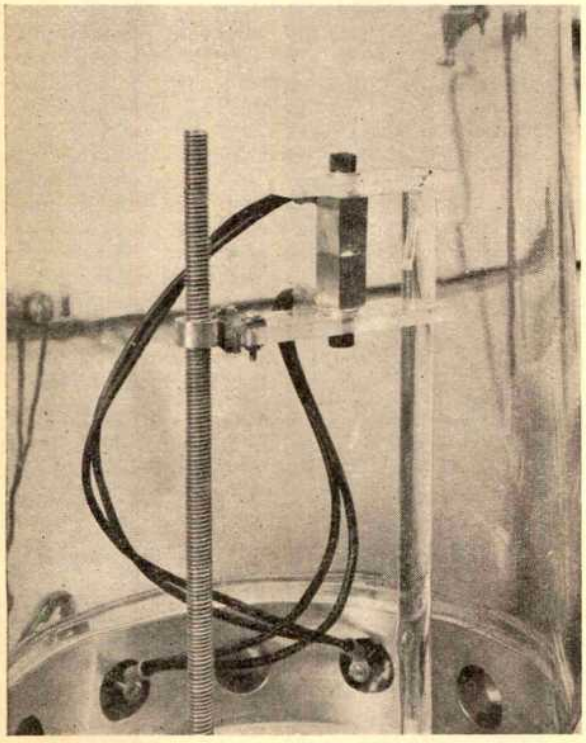


Fig. 1. Arc quench gap electrode separation device

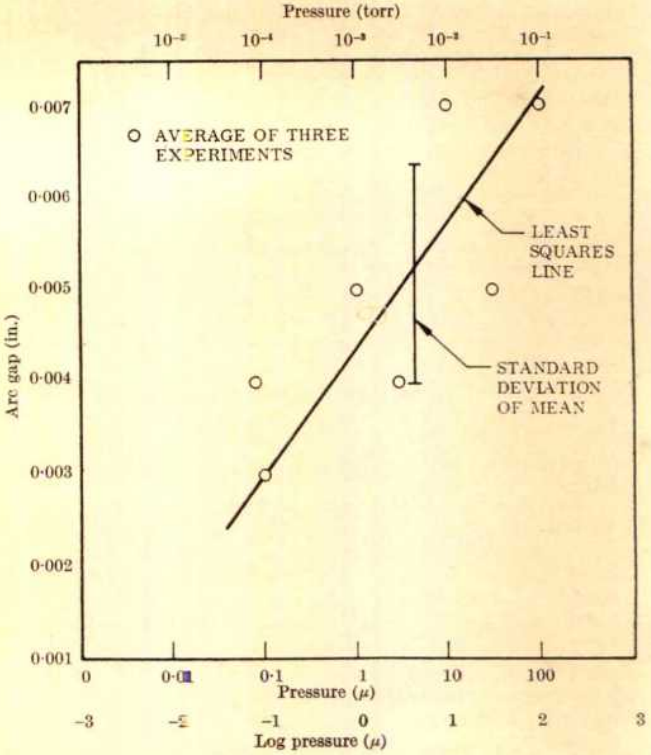


Fig. 2. Arc quench gap in vacuum, 28 V, 20 amp d.c., copper anode, *HM*21-*AT*8, magnesium alloy cathode

The surfaces of the electrodes were electrolytically cleaned after each test. Surface contamination was checked after each test. The contamination of the anode increased with decreasing pressure.

The electrode separations at arc quench are shown in Table 1. The maximum value was 0·008 in. at 10^{-2} torr (10μ), the minimum was 0·002 at 10^{-4} torr (0·1μ).

Three experiments were performed at each pressure in random sequence, for example, 10^{-4}, 10^{-2}, 10^{-1}, 10^{-3} torr, etc. The average of each of the three was plotted with arc gap as ordinate versus the log value of the pressure in

Table 1. Effect of Vacuum Pressure on D.C. Arc Quench Gap at 28 V-20 Amp

Test No.	Pressure (μ)	Pressure (torr)	Arc distance (in.)
4	100	10^{-1}	0·007
5	100	10^{-1}	0·007
6	100	10^{-1}	0·006
7	10	10^{-2}	0·008
8	10	10^{-2}	0·007
9	10	10^{-2}	0·006
19	3	3×10^{-3}	0·005
20	3	3×10^{-3}	0·004
21	3	3×10^{-3}	0·004
18*	3	3×10^{-3}	0·002
22*	3	3×10^{-3}	0·001
15	1	10^{-3}	0·003
16	1	10^{-3}	0·005
17	1	10^{-3}	0·005
18*	1	10^{-3}	0·002
12	0·1	10^{-4}	0·004
13	0·1	10^{-4}	0·002
14	0·1	10^{-4}	0·007
10	0·07	7×10^{-5}	0·004
11*	0·04	4×10^{-5}	0·001
23	30	3×10^{-2}	0·005
24	30	3×10^{-2}	0·004
27	30	3×10^{-2}	0·005
33*	1,000	1	0·002
34*	1,000	1	0·002

* In these tests the electrodes were separated, and the distance required to initiate the arc as electrodes were moved towards one another was measured.

microns, and a straight line was obtained (Fig. 2). A least squares estimate gave the equation:

$$A = 4{\cdot}39 \times 10^{-3} - 1{\cdot}36 \times 10^{-3}\ P$$

where A was arc gap inches and P the log of the pressure in microns.

A mean arc gap of $0{\cdot}0052 \pm 0{\cdot}0012$ in. was found at a pressure of 5μ.

Under the conditions of this experiment, an initiated arc can be sustained at an electrode distance of less than 0·004 in. and quenched at about 0·007 in.

The electrode separation required to initiate an arc was smaller than that to quench one. The average value over a range of pressure $1\text{–}4 \times 10^{-5}$ torr (1,000–0·04μ) was 0·0015 in. with a maximum of 0·002 in. and a minimum of 0·001 in.

The arc length at quench was measured by slowly separating a copper anode and a magnesium alloy (*HM*21-*AT*8) cathode, which were in contact in vacuum. Vacuum pressures were from 10^{-1} torr (100μ) to 10^{-3} torr (0·01μ).

The arc length at blow-out varied from a maximum of 0·007 in. (100μ or 10^{-1} torr) to a minimum of 0·001 in. (0·01μ or 10^{-5} torr).

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An Iron-bearing Nepheloid Layer near Key West, Florida

VERY little is known about the suspended matter contributing to those turbid zones in the sea which occur well above the bottom. Recently, however, Ewing and Thorndike[1] reported a layer containing 2·5 mg/l. of lutite at 4,030 m in the Sargasso Sea 160 m above bottom. They did not indicate any further characterization of their material.

In connexion with suspended-matter analyses in near-surface waters to the south of Key West, Florida, it was observed on both occasions when deeper casts were made that some of the water samples taken from depths below 150 m, but 275 m or more above the bottom, were yellowish in colour and contained substantial amounts of material in fine suspension. Chemical and spectrographic analyses of this material have shown that the suspended matter is predominantly iron.

The first samples were obtained from a Nansen cast in lat. 24° 13′ N., long. 81° 41′ W., on January 22, 1965. Turbid water was found in adjacent bottles at 180 m and 215 m with clear water above and at 230 m (the deepest sample taken). The bottom was at about 490 m. The turbid water samples left an orange deposit on membrane filters which were later made available to us for analysis. Preliminary tests showed that a large amount of iron was present. An emission spectrogram of the washed suspended matter was then obtained which confirmed the predominance of iron and revealed the presence of lesser amounts of manganese and vanadium. Other elements were either absent or only occurred in trace amounts. No quantitative analyses were made.

On March 22 a cast to 305 m in lat. 24° 11′ N., long. 81° 52′ W., again resulted in yellowish turbid water samples at 245 m and 305 m, this time with clear water intervening at 275 m. The bottom at this point was about 642 m.

Under the microscope, the particles in both turbid samples were bright golden yellow and seemed to consist of loose aggregations of small spheroids uniformly less than 1μ in diameter. Material of similar appearance was obtained from the January membranes on suspension of the precipitates in distilled water. The spherules in the March samples often occurred singly but tended to aggregate loosely in what appeared to be a soft, gelatinous matrix when left undisturbed. The 245-m sample settled out only slowly over a period of many days, whereas the 305-m sample was more flocculent and appeared to contain more suspended matter. The *p*H of both turbid samples was 7·9, whereas that of the other samples from the cast was about 8·1 (8·3 at the surface). A few particles with a clear, plate-like appearance were found among the yellow material. The January membranes contained many rod-shaped bacteria, and the turbid samples were reported to have a strong organic odour (not hydrogen sulphide). No such odour was noted in the March samples, and no particles that could be identified as bacteria were seen in the turbid samples from that cast.

Volume limitations prevented extensive analyses being made of any of the samples. Two-hundred ml. of the 245-m sample was filtered on to a tared 'Millipore *HA*' membrane filter (effective pore size 0·45μ), and the dry weight of suspended matter was obtained after washing with distilled water. This amounted to 1·8 mg/l. 'Reactive' particulate and soluble iron were also determined on portions of this same sample using the procedure of Strickland and Parsons[2]. This was found to be 1·1 mg/l. in the particulate fraction, or 61 per cent of the total mass, and essentially zero in the filtrate. Total iron was later determined in a portion of the 305-m sample using the method of Lewis and Goldberg[3] but omitting the preliminary separation into particulate and soluble fractions. Total iron was thus found to be 2·0 mg/l. Total 'reactive' iron was also determined in the same sample and was found to be 1·2 mg/l., or 60 per cent of the total iron as determined after digestion in perchloric acid.

An X-ray powder analysis was undertaken of a small quantity of the washed solids from the 245-m sample. The material was mounted in a 0·3-mm-diameter silica capillary and patterns were obtained using the standard 'Norelco' 114·59-mm camera with manganese-filtered iron radiation. Analysis revealed that the material was mostly amorphous but indicated the presence of minor amounts of quartz and a plagioclase feldspar. The presence of another mineral was also indicated, but it could not be identified because of the absence of sufficient analytical data; determination of the plagioclase composition was not possible for the same reason. There was no evidence of carbonate minerals.

It is concluded that the suspended matter in this turbid water consisted almost entirely of hydrous iron oxide(s). No quantitative analysis of manganese was made, but the emission spectrogram indicated that manganese was present at a concentration of about 0·1 per cent. The concentration of vanadium was slightly less than this. The nature of the sampling operations was such that certain fundamental data, notably a temperature profile, salinity, and dissolved oxygen content, which might have provided clues to the origin of the suspended matter, were not obtained. The question of origin was further complicated by the presence in the vicinity of two abandoned dumping grounds which might, conceivably, have enhanced significantly the iron concentration in the surrounding water.

The casts themselves provided little information on the extent of clear water between the depth at which turbid water was obtained and the bottom. In the January cast, one clear sample (at 230 m) was obtained from beneath the turbid samples, but this might, of course, represent only a clear layer in an extensive turbid zone. In the March operation, however, experimental sonar gear designed for acoustic reverberation studies on suspended matter in the sea was used in conjunction with the cast. This equipment gave good indication of a layer 30–50 m thick coinciding in depth with the nepheloid zone as

sampled and with no indication that water with similar scattering characteristics lay beneath it.

The occurrence of large amounts of iron in an extensive nepheloid zone located well above the bottom is noteworthy regardless of the location of the water mass. Further sampling and characterization of this and other turbid zones should produce important information bearing on the origins of suspended inorganic matter and marine sediments.

We thank A. J. Hiller of the U.S. Naval Research Laboratory (NRL) for both the January and the March samples; R. C. Beckett of NRL was in charge of sampling operations on both occasions. We also thank S. H. Cress of NRL for the spectrogram, H. N. Giles of the U.S. Naval Oceanographic Office for the X-ray powder analysis, and L. C. Ricalzone of NRL for the acoustic information.

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Late Weichselian Glaciation in the Cheshire–Shropshire Basin

RECENTLY, Boulton and Worsley[1] have interpreted the huge composite morainic suite which occurs in the Wrexham–Bar Hill area of the Shropshire–Cheshire Basin as an end-moraine marking the southern limit of the advance of an ice-sheet in the Weichselian Glaciation. This interpretation is based on a radiocarbon dating of about 28,000 years B.P. obtained from only two molluscan shells found beneath the Upper Boulder Clay in the Sandiway district of Cheshire to the north of the morainic suite. It ignores the fact that the Upper Boulder Clay is cut into and is therefore older than the highest stages of the Lake Lapworth sequence which was formed during the retreat of the Upper Boulder Clay ice-sheet. These lake stages at 330 ft. and 305 ft. have long been correlated with the Main Terrace of the River Severn and its equivalent terrace on its tributary, the River Salwarpe near Droitwich. From this terrace a radiocarbon dating of about 40,000 years B.P. was recorded[2] and, because there seems no reason to doubt this correlation, this clearly demonstrates that the Wrexham–Bar Hill morainic suite is considerably older than 40,000 years and cannot be a product of the last or late Weichselian Glaciation.

At the time of the 330 ft. and 305 ft. Lake Lapworth stages, the position of the retreating Upper Boulder Clay ice-sheet was marked by a series of meltwater channels at various points along the Mid-Cheshire Ridge (ref. 3, Fig. 4). The Peckforton Hall moraine was produced at this time—a small boulder clay moraine which contrasts markedly with the huge ridges of the earlier Wrexham–Bar Hill suite. These features provide indisputable proof of two glaciations with two completely different modes of retreat. Full details of these features found in the critical Nantwich district (*One-Inch Geological Sheet* No. 122) will be published shortly[4].

There is no definite evidence to show that the Wrexham–Bar Hill suite marked the limit of an advancing ice-sheet at any time, but on the contrary within this suite three separate ridges may be distinguished marking the successive halt-stage positions of an ice-sheet which split into two lobes across the Mid-Cheshire Ridge and retreated northwards. Similar morainic ridges in the Delamere area of Cheshire and the Chorley area of Lancashire show further pauses in this retreat northwards. At a later stage all these ridges were overridden by the Upper Boulder Clay ice-sheet which continued far to the south and overlies large areas of the outwash plain of the Wrexham–Bar Hill morainic suite in the Wem district.

Different carbonate leaching properties discovered in the Upper Boulder Clay which occur to the north and south of the Wrexham–Bar Hill morainic suite are not necessarily indicative of age differences between these deposits as suggested by Boulton and Worsley[1], but may have arisen because at the time of the 260–250 ft. Lake Lapworth stage the boulder clay to the north of the morainic suite was almost completely covered by ice and water while that to the south of the ridge was exposed to the elements in the normal way (ref. 3, Fig. 5).

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Age of the Aliwal Shoal, South Africa

SPECIMENS of calcareous sandstone containing foraminifera from the submerged Aliwal Shoal, about 5 km east of Green Point on the Natal coast, 50 km south of Durban, were received from Mr. M. J. McCarthy of the University of Natal. They have been investigated with a view to determining the age of the rock. The first specimen was obtained from the damaged bow of the ship *Aimee Lykes* following a collision between this ship and the Shoal. Later, a larger specimen was collected by a diver. The specimens were gently crushed to free the grains and, from the −28, +100 Tyler mesh fraction, all the foraminifera were picked out and mounted.

The foraminifera in these samples were all severely abraded before consolidation of the rock occurred. Consequently most of the identifications are not made to species level. Of the 211 particles recognizable as foraminifera, eighty-six are not identifiable other than to record them as calcareous foraminifera—this high proportion in itself being an indication of the degree of abrasion of all the particles in the rock. The foraminifera found in the samples are recorded as follows:

Cibicides refulgens (Montfort)	16 specimens
Cibicides sp.	6 specimens
Elphidium crispum (Linné)	40 specimens
Miliolids	63 specimens
Unidentifiable foraminifera	86 specimens

The absence of *Ammonia beccarii* (Linné) from these samples, including the 'unidentifiable' specimens, is noteworthy and will be commented on later. In so far as the Aliwal Shoal samples themselves are concerned, this absence of *A. beccarii* is of doubtful stratigraphical value and this assemblage of foraminifera otherwise gives no direct indication of the age of the rock other than that it has a late Cainozoic aspect.

In order to gain an estimate of the age of Aliwal Shoal by correlation with rocks on the adjacent mainland, the samples were compared with samples from Cainozoic calcareous sandstones from various localities in the vicinity of Durban and with some Recent samples from Durban Bay and the adjacent ocean (see Fig. 1). These comparative samples were provided partly by Mr. McCarthy and partly by Prof. J. J. Frankel.

(*a*) *Fossil samples*

(1) Aliwal Shoal. *Cibicides refulgens* and *Elphidium crispum* together are about equal in number to miliolids (mainly *Quinqueloculina* spp., but too abraded to be identified). No specimens of *Amphistegina radiata* (Fichtel and Moll) have been found here.

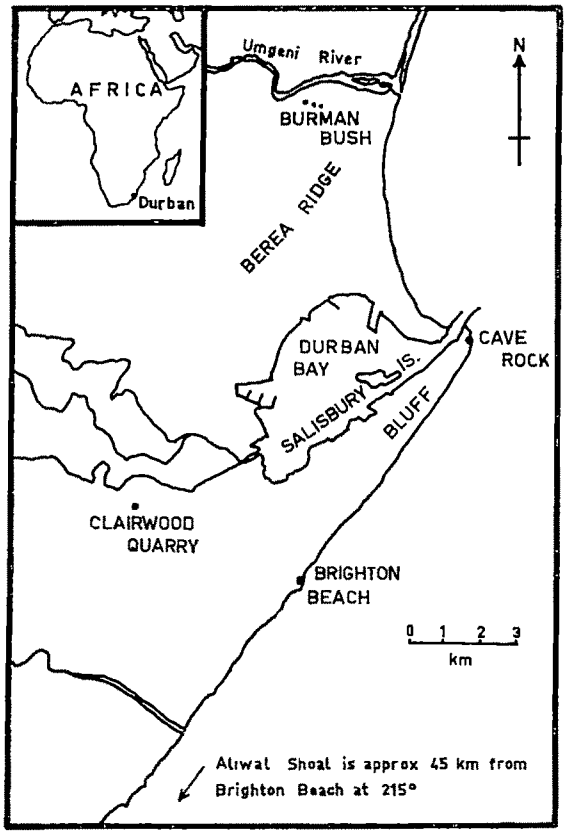


Fig. 1

(2) Berea Ridge, equivalent to sample 365 of W. J. Parr[1]. *Cibicides refulgens* and *Elphidium crispum* are common—*C. refulgens* being more numerous than *E. crispum*, whereas the reverse is the case at Aliwal Shoal. However, at Berea Ridge, miliolids are uncommon, but *Amphistegina radiata* is abundant.

(3) Burman Drive. *A. radiata* and *C. refulgens* predominate here.

(4) Cave Rock, at the Bluff, Durban—'above conglomerate'. *Elphidium crispum* predominates—only a few other species occur as single specimens, poorly preserved.

(5) Cave Rock, at the Bluff, Durban—'below conglomerate'. A large, severely abraded and unidentifiable miliolid equals *E. crispum* in abundance. Another species of *Elphidium* is also present (compare with *E. craticulatum* (Fichtel and Moll)) as well as a moderately large *Discorbis*-like form, also too poorly preserved for further identification.

(6) Clairwood Quarry. *Cibicides refulgens* predominates slightly over miliolids. No other species are present in the sample.

(7) Brighton Beach. *C. refulgens* and *E. crispum* are about equally abundant, miliolids are scarce and other species negligible.

(*b*) *Recent samples*

(8) Durban Bay, sample *DB*.1–2, collected by H. R. Belderson. Of the species relevant to this appraisal, *E. crispum*, *C. refulgens*, *Amphistegina radiata* and *Elphidium craticulatum* are all common and so is *Ammonia beccarii*. Large miliolids, *Triloculina* sp. and *Quinqueloculina* sp. are present, but less common.

(9) Durban Bay, north of North Pier, sample *D2A*, collected by H. R. Belderson. *E. crispum* is the commonest species, accompanied by *C. refulgens*, *Amphistegina radiata*, *Ammonia beccarii* and miliolids in lesser but about equal numbers.

(10) Durban Bay, harbour dredge sample from near Salisbury Island[2]. In the harbour dredging from near Salisbury Island, an entirely different facies of accessory species is present, but the commonest forms are *Elphidium craticulatum*, *Ammonia beccarii* and *Cibicides refulgens* with *E. crispum* quite common. No specimens of *Amphistegina radiata* are present. The sediment is grey mud.

(11) Sample from 11–13 fathoms off Durban Heads, collected by H. R. Belderson. *C. refulgens* predominates, with *Amphistegina radiata*, *Ammonia beccarii* and miliolids also quite common.

Within the limits of variation shown by the series of fossil samples, the samples from Aliwal Shoal have faunal and sedimentary features which link them with the calcareous rocks of localities 2–7, the chief faunal difference being the absence of *Amphistegina radiata* from some samples. This and minor lithological variations are attributed to differences in the sedimentary environments in which the rocks accumulated or from which they were derived. It is important to emphasize that all the foraminifera in the samples from these seven fossil localities are abraded and recrystallized. The Recent foraminifera are well preserved.

The foraminifera of the fossil samples also closely resemble those of the four Recent samples with which they have been compared except for one feature—the absence of *Ammonia beccarii* from all the fossil samples and its occurrence in all the Recent samples. In view of the ubiquitousness of this species in near-shore, shallow-water deposits of Recent age, its absence from the fossil samples apparently has some stratigraphical significance.

A. beccarii appears in the Tertiary sequence in south-eastern Australia in the Lower Pliocene[3]. The time of appearance of *A. beccarii* in the Tertiary of South Africa is not accurately known because of the very incomplete stratigraphical sequence of Tertiary deposits in that country. Because *A. beccarii* attained an almost world-wide distribution in the Late Cainozoic it is to be expected that it would be approximately contemporaneous in two regions of comparable southern latitude. The evidence of *A. beccarii* therefore suggests an early Pliocene or slightly older age. However, in the samples examined, two common species, *Elphidium crispum* and *Cibicides refulgens*, and one less common, *Elphidium craticulatum*, appear in south-eastern Australia in the late Pliocene and Quaternary.

From this evidence, a Pliocene age is favoured for the rock of the Aliwal Shoal and lithologically similar calcareous sandstones at the six localities near Durban with which it is correlated.

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PHYSICS

Further Tests on the Ice Nucleation Potential of Meteoritic Material

SCHAEFER[1] was unable to detect any trace of ice-crystal formation when he vaporized a sample of iron–nickel meteorite by electric spark in a supercooled cloud—even in clouds as cold as −25° C. Mason and Maybank[2], who ground up and vaporized three samples of stony meteorites, and Mason[3] with three further samples also reported that they were unable to detect any production of ice-crystals at temperatures above −17° C. This communication records the results of tests on the nucleating properties of a further thirteen samples of meteoritic materials. These comprised iron–nickel types (siderites),

stony type (aerolites), plus two samples of the intermediate (siderolite) variety (all obtained from the American Meteorite Laboratory, Denver).

A fragment of each of these meteoritic samples was crushed in a steel mortar so that at least a portion of each was reduced to particles of a few microns in diameter. The crushed material was stored in small polythene bottles, from which a 'spray' of particles could be obtained by shaking and squeezing. Our refrigerator box was fitted with felt-covered sides, and the actual test chamber was a 5 l. glass flask with three outlet tubes. The temperature within it was determined by means of a copper-constantan thermocouple connected to a sensitive galvanometer. The temperature variation within the chamber was found to vary by less than 0·3° C from one place to another. A small lamp covered with a water-soaked gauze served as a source of vapour within the chamber. The chamber air, when required, could be flushed out by means of a vacuum cleaner—the suction caused clean air to enter the flask through a dust filter.

Detection of ice crystals was accomplished by means of a soap film, supported on a small copper loop which could be lowered into the test chamber by way of the central outlet tube. Owing to the rapidity with which this froze after nucleation, sugar was added to the soap solution to the extent of about 25 per cent by weight. Using this mixture, a nucleated film took 50–60 sec to freeze completely at −14° C, and slightly more than half this time at −20° C. Even at the lower temperature, film nucleation by the copper ring seldom occurred, so that it was not necessary to cover it with a plastic coating.

Before testing each substance, several observations of background ice nucleation occurrence were made. In general, fewer than one or two active nuclei were detected within a 0·5-min period of observation, but about twice this level was reached at temperatures below −19° C.

The testing technique at a particular temperature involved the production of a supercooled water droplet cloud within the chamber, and this was accomplished by turning on the vapour source lamp for 15 sec. Following this, a fresh soap film was introduced on the wire ring. The bottle containing the material to be tested was shaken and a small sample of the particles sprayed into the flask directly above the film. The threshold temperature was considered to be that at which four or five ice crystals (above background level) were observed to land on the film within 1 min of seeding the flask with the meteoritic dust sample. This was estimated to correspond to about 1 active nucleus in 10^4 inert particles. The testing sequence was carried out at least three times at any one temperature setting and the averaged result was taken as indicative of the behaviour of the specimen concerned.

The thirteen samples were first tested for activity at temperatures of −14° C to −16° C (between each sequence of trials the chamber was carefully flushed). Those that indicated activity levels above the threshol[illegible] set aside for investigation at warmer temp[illegible] that initially gave no ice crystals were test[illegible] lower temperatures until either th[illegible] was reached. Great care was [illegible] cross-contamination. [illegible]

A technique similar to that used in Mason and Maybank[2] was used to determine whether vaporization of meteoritic material by an electric spark could produce effective ice nuclei. A small fragment of an iron–nickel meteorite (*S*-5), previously observed to be inactive at −19° C, was placed between a pair of electrodes. This assembly was held at the top of the test chamber directly above a supercooled water droplet cloud and soap film detector. A 20 μF condenser, charged to 400 V, was discharged through the electrodes and meteorite. No ice crystals were observed by the film following this procedure, at least down to −19° C. The entire operation was repeated with a sample of an inactive stony, or aerolitic, meteorite (*A*-1) substituted for the iron–nickel specimen. Again, no ice nuclei were produced by the spark discharge at temperatures of −18° to 19° C.

In summary, it would seem that two of the thirteen meteorite specimens tested—one a siderite, the other an aerolite—had threshold temperatures for the onset of ice nucleation activity higher than −15° C. These may thus be considered as fairly effective sources of ice nuclei on the basis of this arbitrary division, although not so efficient as some soil components such as kaolinite and halloysite[2]. The other eleven specimens must, on this basis, be classified as ineffective. Finally, we have shown that vaporization by an electric spark is not effective in producing ice nuclei from inactive meteoritic material.

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A Computer Study of the Grouping of Spheres in a Random Three-dimensional Assembly

As a part of a fundamental research programme it became necessary to investigate spatial relationships in a three-dimensional assembly of particles. Since computing time has become more readily available, there have been a number of reports on work dealing with this topic. However, these have concentrated mainly on flocculated or close-packed models. Typical studies are those of [illegible] Sutherland[1], Levine and Chernick[2] and [illegible] Lewinson[3]. In contrast the simple [illegible] basis for this computer study was as [illegible] interacting spheres was [illegible] only random contacts

Table 1

N	D	$\bar{X}$	σ^2	Frequency distributions of group size X 1	2	3	4	5	6	7	8	9	⩾10
200	1,100	1·163	0 171	147	22	3							
				(148)	(15)	(6)							
	1,200	1·299	0 404	121	22	9	2						
				(122)	(21)	(7)	(4)						
	1,350	1·724	1·683	78	16	9	6	4	2	1			
				(72)	(24)	(10)	(5)	(2)	(1)	(1)			
	1,500	2·632	3·706	31	16	7	7	8	4	3			
				(30)	(17)	(10)	(7)	(4)	(3)	(5)			
300	1,100	1·293	0 371	179	42	7	4						
				(183)	(30)	(10)	(3)						
	1,200	1 523	0 839	136	32	19	7	3					
				(135)	(36)	(14)	(6)	(5)					
	1,350	2 273	3 698	68	24	14	11	7	3	1	2	2	
				(64)	(31)	(17)	(10)	(6)	(3)	(2)	(1)	(2)	
	1,500	3 297	11·523	25	10	7	7	8	6	2	5	0	6
				(20)	(15)	(11)	(8)	(6)	(4)	(3)	(3)	(2)	(5)
300	1,200	1 667	1 233	119	25	19	13	3	1				
				(135)	(36)	(14)	(6)	(3)	(2)				
	1,350	2 460	4 445	63	17	16	8	3	5	6	2	0	2
				(64)	(31)	(17)	(10)	(6)	(3)	(2)	(1)	(1)	(1)
	1,500	3 659	12 201	33	10	9	6	9	1	2	2	1	9
				(20)	(15)	(11)	(8)	(6)	(4)	(3)	(3)	(2)	(5)
400	1,100	1·482	0·832	191	44	22	11	1	1				
				(183)	(47)	(18)	(8)	(3)	(3)				
	1,200	1 878	1·891	129	30	28	14	4	6	2			
				(116)	(46)	(22)	(11)	(6)	(3)	(4)			
	1,350	3·101	6 912	51	20	17	8	14	7	2	2	1	7
				(42)	(27)	(18)	(13)	(9)	(6)	(4)	(4)	(2)	(3)
	1,500	5 333	21 209	16	11	8	7	4	5	6	2	3	13
				(10)	(9)	(8)	(8)	(6)	(5)	(4)	(3)	(3)	(12)

random sequential manner. The selection was termed 'random sequential', because a centre which caused the sphere around it to overlap with other spheres already selected was automatically rejected and another centre tried. The final assembly of selected centres was then examined to isolate the groups of spheres associated with the predetermined contact diameter. In each model the numbers of groups having X values of 1, 2, 3, etc., were then recorded.

The model volume was taken to be a cube of side 10^4 units and each sphere was 10^3 units diameter. Three sets of models having 200, 300 and 400 spheres respectively (that is, having volume concentrations of 0·105, 0·157 and 0·209) were used. Each model was investigated at effective diameters 1,100, 1,200, 1,350 and 1,500 units. These diameters were centre–centre distances and corresponded to surface–surface separations along the line of centres of $0{\cdot}1d$, $0{\cdot}2d$, $0{\cdot}35d$ and $0{\cdot}5d$, where d was the sphere diameter.

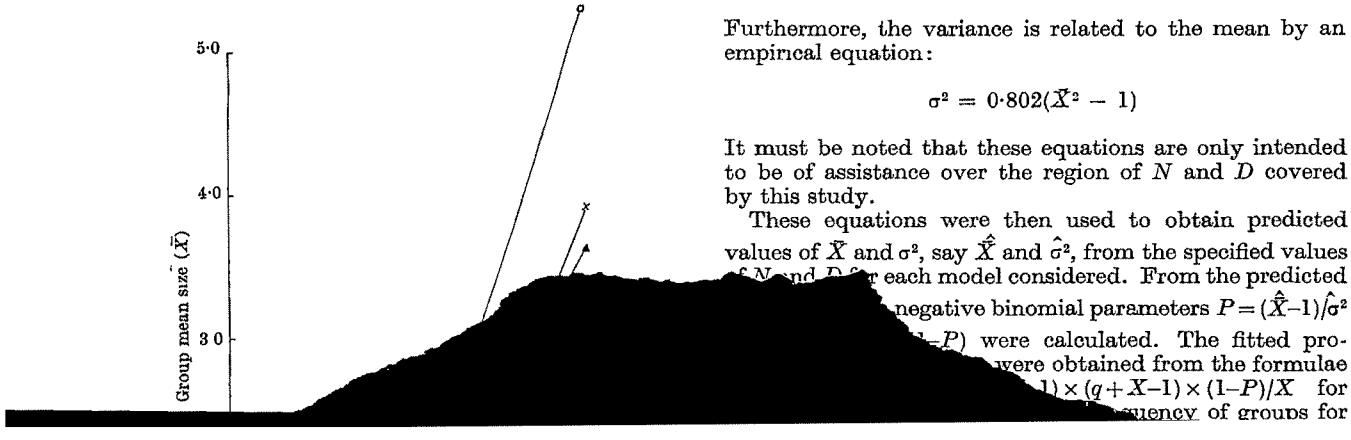


Table 1 shows the observed frequency distributions of group size X ($X = 1,2,3,..$) for all the sets of model conditions considered. The mean size $\bar{X}$ and variance σ^2 are also shown. Duplicate results are shown for the 300 sphere model and agree fairly well. Fig. 1 is a graph of the group mean size ($\bar{X}$) against contact diameter (D) for the 200, 300 (two samples), and 400 sphere models. The high computer costs of creating these types of models preclude very much replication.

The negative binomial distribution has been found to fit the observed frequency distributions of the variable $(X-1)$ quite well. This distribution is often a good fit to distributions of counts of particles. It is found that the observed mean $\bar{X}$ of a group is related to the number of spheres in the model N, and to the contact diameter, D, by the following empirical equation:

$$\bar{X} - 1 = \exp\left[10^{-3}(5{\cdot}4\,N + 5{\cdot}6\,D - 8{\cdot}96 \times 10^3)\right]$$

Furthermore, the variance is related to the mean by an empirical equation:

$$\sigma^2 = 0{\cdot}802(\bar{X}^2 - 1)$$

It must be noted that these equations are only intended to be of assistance over the region of N and D covered by this study.

These equations were then used to obtain predicted values of $\bar{X}$ and σ^2, say $\hat{\bar{X}}$ and $\hat{\sigma}^2$, from the specified values of N and D for each model considered. From the predicted negative binomial parameters $P = (\hat{\bar{X}}-1)/\hat{\sigma}^2$ $(1-P)$ were calculated. The fitted pro- were obtained from the formulae $\times (q+X-1) \times (1-P)/X$ for quency of groups for

result of random processes such as those assumed in this study or of simple flocculation under attractive forces.

The computer programme for this study was written in ALGOL and the computer used was Atlas. The programme has been extended to permit the generation of assemblies of different-sized spheres and also to consider not only the introduction of single spheres into the model but also aggregates of spheres.

It may be of more general interest to note that this computer model is a numerical attempt to solve an unsolved problem in geometrical probability, that is, the determination of the clustering properties of solid spheres distributed at random in three dimensions. Furthermore, the programme can be easily modified to consider the one-dimensional case of non-overlapping randomly placed intervals on a line or the two-dimensional case of non-overlapping randomly placed circles on a plane.

We thank Dr. Dunderdale, Mr. R. M. Driver and Mr. Hewson for their assistance both in discussions and with some of the calculations.

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Radium Content of Rainwater

ATMOSPHERIC radioactivity is generally attributed to the following: (1) escape of radon and thoron into the air from radioactive material in the surface of the Earth; (2) spallation products of atmospheric gases produced by cosmic radiation; (3) radioactive fall-out as a consequence of nuclear detonations.

Most of the natural activity in the troposphere is generated by the first. It is generally believed that after formation in the ground, radon and thoron diffuse into the atmosphere. Their decay products are heavy metals which soon become attached to natural aerosol particles and eventually return to the ground by way of rain or by some other process. The presence of radium-226 in the atmosphere is not likely to arise from (1). However, in an isolated measurement, Damon and Kuroda[1] detected radium-226 in rainwater, with a specific activity of 10^{-12} c./l. Chatterjee *et al.*[2] also detected it among other α-emitting radioactive fall-out elements. It was therefore considered worthwhile to search more carefully for radium-226 in rainwater.

During the last monsoon season in Calcutta, the radium content of about fifty samples of rainwater was measured. These were collected in wide-mouthed polythene vessels at appropriate intervals (the local times of collection being noted). On average, each sample consisted of about 15 l. of rainwater. In order to avoid any possible sources of contamination, barium sulphate and other materials such as nickel or platinum crucibles were not used in the preparations. Instead, the samples were treated with pure calcium chloride (2·0 g/l. of water) and sodium potassium carbonate, so that radium, if present, could be coprecipitated with calcium carbonate. (The possibility of such a separation was envisaged by Hahn[3] and quantitatively verified by Kropf[4]). Finally, the acid-soluble carbonate was dissolved in dilute hydrochloric acid and transferred to a bubbler for estimation of radon in the usual manner. In order to eliminate any background ionization that might arise from cosmic-rays or local contamination, the measurements were carried out using the differential ion-chamber arrangement as suggested by Kropf[4].

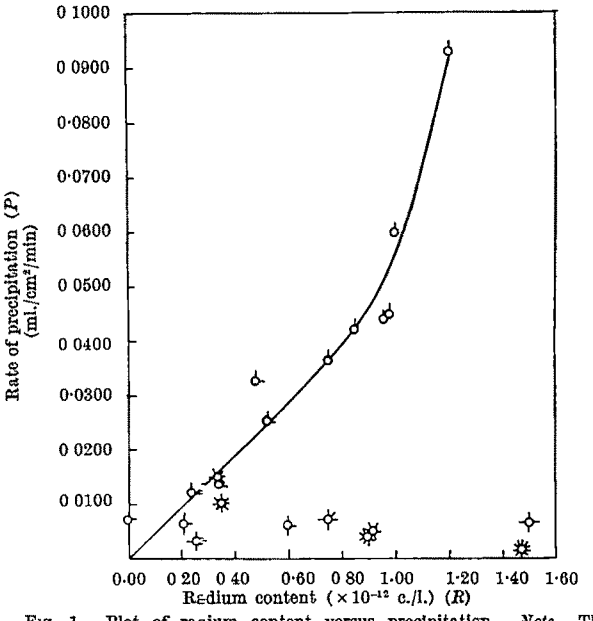


Fig. 1. Plot of radium content versus precipitation. *Note*.—The number of lines emanating from the plotting indicates the duration of rain in hours of the sample

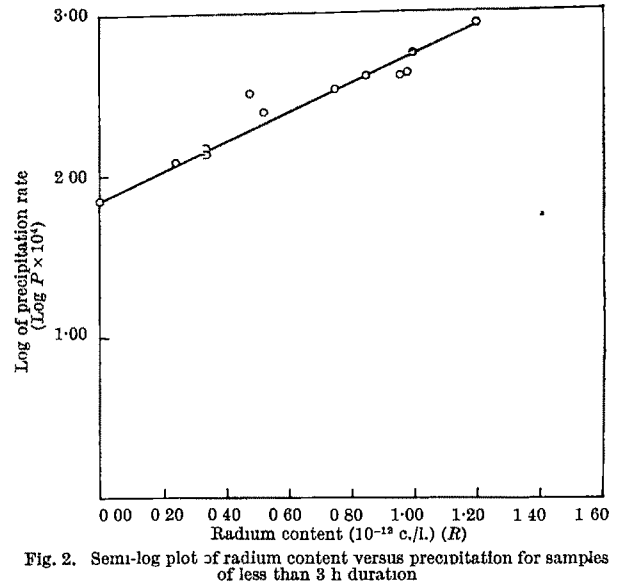


Fig. 2. Semi-log plot of radium content versus precipitation for samples of less than 3 h duration

Some twenty-five first samples were collected from separate rainfalls, while the remaining samples formed subsequent fractions. The rate of precipitation P as well as the duration of rainfall, D, corresponding to the former samples varied in individual cases. Fig. 1 is a plot of radium content (F) versus precipitation (P). If we consider all the points in the figure, there does not seem to be any decisive correlation between them. However, if we consider only samples of short duration, say for $D = 1$ or 2 h, there is a relationship which shows that as the precipitation rate increases, the radium content also increases. The relationship is not linear and a tentative curve fitting these small duration first sample data is shown in the same figure.

Fig. 2 is a plot of the same set of data with R in arithmetical scale and P in logarithmic scale. It can be seen that the points align themselves fairly closely. The linear relationship can be expressed as:

$$\text{Log } P = 0{\cdot}93\ R - 2{\cdot}15 \qquad (1)$$

An analysis of data relating to measurements of samples collected at hourly intervals during continuous rain-

falls indicates an exponential time-decay of the radium content. The negative exponent increases with the rate of precipitation, as the scavenging action becomes greater. The maximum radium content recorded was $1{\cdot}5 \times 10^{-12}$ c./l. When continuous rainfall was recorded, however, in most cases the last portion carried negligible activity ($< 10^{-14}$ c./l.).

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Cyclic Pattern of Metallic Crystal Structures

THE three principal crystal structures of the metallic elements, the face-centred cubic (f.c.c.), the body-centred cubic (b.c.c.) and the hexagonal close-packed (h.c.p.), follow a cyclic pattern as sub-shells are filled with electrons. The preferred structure changes from f.c.c. to h.c.p. to b.c.c. for the half-filled sub-shell. As the sub-shell continues to fill, the trend is reversed.

When a sub-shell is either empty or full, atoms pack as spheres and the f.c.c. structure predominates. The rare gases (p^6), calcium and strontium (s^2), the cobalt, nickel and copper group metals (d^{10}) and ytterbium (f^{14}) adopt this structure. The high packing density and high symmetry (low entropy) aspects of the f.c.c. structure produce the lowest energy environment for the packing of spherically symmetric atoms. The major exceptions to this occur among the alkaline earth metals where the effect of losing an s-electron to the conduction band is most pronounced.

As the s, d and f sub-shells start to fill, atoms gain a net electron spin. The crystal structure distorts to permit adjustment of interatomic distances. The crystal energy is lower if two sets of interatomic distances are provided, and atoms with antiparallel spins are permitted to maintain a closer approach than atoms with parallel spins. Classical examples of this type of spin interaction are the formation of the hydrogen molecule and the sign of the Slater[1] exchange integral. The f.c.c. structure does not provide two sets of interatomic distances. Every atom has nearest neighbours which are nearest neighbours of each other. No assignment of spins can satisfy the requirement of maintaining different interatomic distances for parallel and antiparallel interaction. The h.c.p. structure, when c/a is less than the theoretical value for closest packing 1·633, permits nearest neighbours to have antiparallel spins and next nearest neighbours to have parallel spins. Six nearest neighbours occupy the corners of a trigonal prism at a distance less than the lattice parameter 'a'. Next nearest neighbours occupy the corners of a hexagon at a distance equal to the lattice parameter 'a'. The h.c.p. structure with c/a less than 1·633 is found where metal atoms have a weak net spin. The energy gained in providing two sets of interatomic distances makes up for the energy lost in a lower packing density and lower symmetry. Weak net spins occur when shells just begin to fill and when they are almost filled. The scandium and titanium groups of metals have this structure. They represent the low net spin as the d-shell begins to fill. It occurs again in the almost filled d-shells with the metals technetium, ruthenium, rhenium and osmium. The structure is also observed among early $4f$ metals lanthanum to samarium and the late $4f$ metals gadolinium to thulium. All these h.c.p. metals have c/a ratios less than 1·633. Two metals, zinc and cadmium, have c/a ratios greater than 1·85. This large departure has been explained on the basis of dp^2 hybrids[2].

The net spin reaches its maximum value when the sub-shell is half-filled. The alkali metals, lithium to caesium, the refractory metals of the titanium, vanadium and chromium groups and europium have half-filled s, d and f shells, respectively. These metals crystallize in the b.c.c. structure. Zener[3] observed the tendency of the d sub-shell metals of high net electron spin to crystallize in a structure which permits nearest neighbours to have antiparallel spins. He explained how the b.c.c. structure fulfilled this requirement. Each atom in the b.c.c. structure is surrounded by eight nearest neighbours located at the corners of a cube. Next nearest neighbours are located at the centre of six adjacent cubes sharing the cube faces. The packing density is now about 9 per cent less than in the f.c.c. structure. The energy saved in providing two sets of interatomic distances for parallel and antiparallel electron spin interactions more than compensates for the lower packing densities.

It is a matter of conjecture whether or not the requirement of two sets of interatomic distances to accommodate parallel and antiparallel spin interaction requires a spin ordering in the structure. Neutron diffraction investigations of such systems have been discussed extensively[4]. Briefly, attempts to see spin ordering in b.c.c. elements by neutron diffraction have failed. However, the question remains whether the resonance frequency of exchange is too rapid to be resolved by neutron diffraction techniques.

In summary, the crystal structures of metallic elements possessing a net electron spin have their lowest energy when in a structure which provides a closer approach for atoms with antiparallel net electron spin than for atoms with parallel spin. As the s, d and f sub-shells fill, the equilibrium crystal structures follow the cyclic pattern f.c.c.–h.c.p.–b.c.c.–h.c.p.–f.c.c. When the sub-shells are empty or completely filled and the net spin is zero, the f.c.c. structure is preferred. When the net electron spin reaches a maximum, metallic elements tend to assume the b.c.c. structure. For intermediate values of net spin, they prefer the h.c.p. structure with c/a less than the theoretical value of 1·633. Exceptions to the rule occur among the alkaline earth metals, the actinides, and the lighter elements lithium, sodium, manganese and iron. In general, however, metals follow the cyclic pattern as closely as the anions follow the Hume-Rothery 8-N rule.

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METALLURGY

Formation of Intergranular Voids and Cracks in an Irradiated Austenitic Steel tensile-tested in the Temperature Range 650°–850° C

THE elevated temperature embrittlement induced in austenitic steels by neutron irradiation is now well known[1,2]. Characteristic features are the intergranular mode of fracture in a tensile test and the marked reduction

in the non-uniform elongation. Barnes[3] has reported work by Pfeil and Barton which suggests that the (n,α) reaction of thermal neutrons with the boron-10 in the steel is responsible.

In the case of beryllium containing irradiation-induced helium in an atomic concentration of $\sim 10^{-5}$, the bubbles which precipitate at elevated temperatures can grow by vacancy absorption if a hydrostatic tensile stress is applied and maintained in excess of some critical value which depends on the bubble size[4]; those bubbles sited on grain boundaries grow most readily into holes, linking up to form interconnected fissures, and in this way lead to intergranular failure at reduced elongation in a hot tensile test.

Despite the low atomic concentration of helium in irradiated austenitic steels (about 10^{-7}), Barnes[3] has now deduced that the same mechanism would account for the extent to which irradiation reduces their elevated temperature ductility, assuming that the helium produced from boron-10 can reach the grain boundaries. Rowcliffe[5] has observed bubbles 60 Å in diameter at the grain boundaries of 20Cr–25Ni–Ti steel irradiated at 625° C, and found that in subsequent tensile tests at 750° C those in the gauge-length grew up to 600 Å in diameter.

However, there has so far been no direct indication of the way in which irradiation promotes intergranular parting in austenitic steels, primarily because the characteristic metallographic features occur very close to (within 0·1 mm of) the fracture surface. It has now been possible to reveal these by careful attention to the preparation of specimens.

I have examined 20Cr–25Ni–0·7Nb strip tensile specimens which had been irradiated at 750° C for 1,300 h to a thermal neutron dose of $3{\cdot}5 \times 10^{20}$ *nvt*, and pulled to rupture in argon at 3×10^{-4} sec^{-1} (at 750° C and 850° C) and $1{\cdot}7 \times 10^{-5}$ sec^{-1} (at 650° C and 750° C). In each instance the ductility of the irradiated specimen was reduced compared with that of the corresponding unirradiated thermal control. Electron microscopy using thin foils showed that the unstrained portions of the tensile-heads from the irradiated specimens contained gas bubbles at grain boundaries. Most of these were 50–100 Å in diameter, but occasionally 100–200 Å diameter bubbles were observed (Fig. 1); the number of bubbles per unit area of grain boundary surface was estimated to be of the order of 10^8 cm^{-2}. If the bubbles contain helium at a pressure $p = 2\gamma/r$, and the surface tension (γ) is assumed to be 10^3 dynes cm^{-1} and the mean bubble radius (r) 50 Å, then with a mean grain size of 30μ the number of bubbles could account for only about 1/100 of the gas produced by the boron-10 (n,α) lithium-7 reaction. A search for bubbles was made in thin foils prepared from the gauge lengths, but the high dislocation density made observation difficult, and the presence of gas bubbles was not established. (The area immediately adjacent to the fracture could not be studied by this method.)

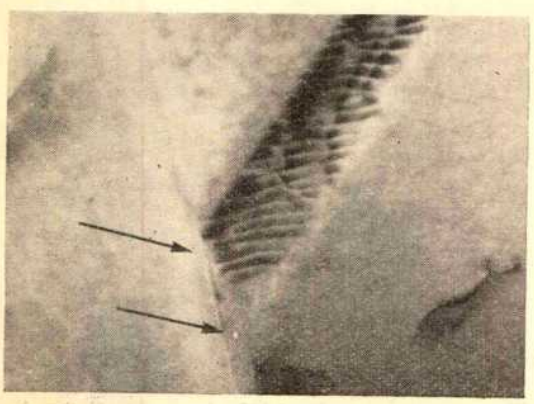


Fig. 1. Thin foil from tensile-head of specimen irradiated at 750° C. Note bubbles at grain boundary. (×50,000)

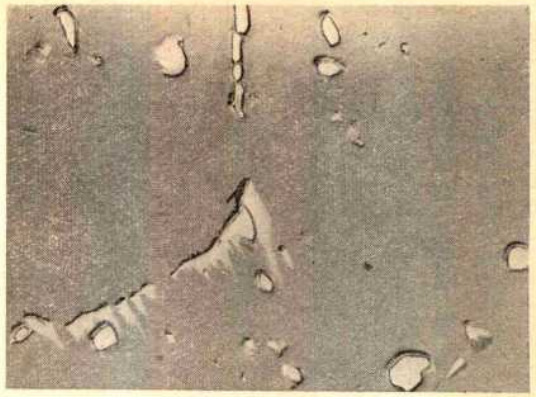


Fig. 2. Replica of electro-polished surface, irradiated specimen slowly strained at 650° C, area within 0·10 mm of fracture. (×5,000)

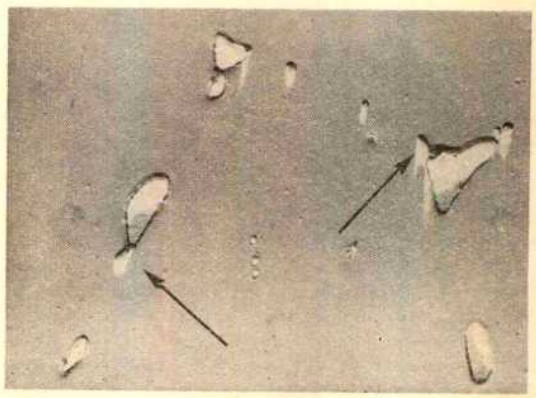


Fig. 3. Replica of electro-polished surface, irradiated specimen slowly strained at 750° C, area within 1 mm of fracture. (×8,000)

Little or no cracking was observed optically in the fast-strain specimens, except in the irradiated specimen tested at 750° C, which showed intergranular cracking limited to within 0·1 mm of the fracture. Intergranular failure was more marked in the slow-strain specimens. Briefly, the irradiated specimen tested at 650° C showed little plastic deformation and a few narrow intergranular cracks within 0·1 mm of the fracture, whereas the corresponding control showed plastic deformation and wide cracks up to 4 mm from the fracture. The irradiated specimen slowly-strained at 750° C showed plastic deformation near the failure but negligible cracking; the corresponding control showed marked necking and wide intergranular tears up to 0·5 mm from the fracture.

A light electro-polishing technique was developed for revealing the presence of intergranular precipitates, fine cracks and holes in the tensile specimens. The specimens were mechanically polished and then electro-polished in chromic-acetic electrolyte[6] at 18 V for 5 sec, which removed the surface layer to a depth of about 0·25μ; this treatment leaves the carbides in relief and the sigma FeCr slightly depressed below the surface.

Two-stage replicas taken from the irradiated fast-strain tensile specimens revealed the presence of holes of 0·1–0·2μ diameter at grain boundaries, sometimes attached to precipitates, together with micro-cracks, about 1μ long, in the region within 0·1 mm of the fractures. These features were less evident further away from the fracture, and were absent in the tensile heads.

The slow-strain irradiated specimen tested at 650° C showed similar holes and micro-cracks near the fracture, but in this instance the process of intergranular failure

was more clearly seen. Micro-cracks occurred at grain boundary precipitates, together with chains of discrete holes which were elongated in the direction of the boundary (Fig. 2). The slow-strain specimen tested at 750° C again showed grain boundary holes, some extending from precipitates along the boundary (Fig. 3), but no micro-cracks were seen. These features were not seen in the tensile-heads of the irradiated specimens or in any of the thermal controls.

The observations of grain boundary holes 0·1–0·2μ diameter are direct evidence that irradiation has affected the failure mechanism and suggest that, in the irradiated material, stress-induced growth of gas bubbles is responsible. The initial radius r_i of a helium-filled bubble which can grow indefinitely by vacancy absorption under a given critical uniaxial stress σ_{xc} is directly dependent on the surface energy γ of the material[3,4]. For a bubble within a grain:

$$r_i = 4\gamma/\sqrt{3}\sigma_{xc} = 2{\cdot}31\ \gamma/\sigma_{xc} \text{ (ref. 3)}$$

Furthermore, a bubble at a grain boundary is also acted on by the tension of the grain boundary. The application of a tensile stress σ_x normal to the boundary will tend to alter the bubble shape and the equilibrium of forces at the periphery of the bubble. The peripheral grain boundary tension on a bubble of radius 10^{-6} cm is of the same order of magnitude as that of the tensile stress acting on the bubble; the effective hydrostatic component of the tensile force is therefore greater than $\sigma_x/3$ (for a bubble within a grain) and tends towards σ_x, that is, $r_i \rightarrow 0{\cdot}77\ \gamma/\sigma_{xc}$ (ref. 4).

If the surface tension at the helium bubbles is as low as 10^3 dynes cm^{-1} and σ_{xc} is approximately equal to the tensile strength (as little necking occurred), then the calculated values of $2r_i$ for grain boundary bubbles range from ~ 80 Å (for the slow-strain test at 650° C, ultimate tensile strength = 1,950 kg cm^{-2}) to ~ 230 Å (for the fast-strain test at 850° C, ultimate tensile strength = 678 kg cm^{-2}). This implies that stress-induced growth of the bubbles observed is feasible, assuming the foregoing conditions.

The observation that micro-cracks form near the fractures of the irradiated material, instead of blunt fissures as in the slow-strain controls, suggests that the grain boundary cohesion of the material is lowered by irradiation, as would result from segregation to the boundary of impurity atoms which lower the energy of free surfaces (compare with the behaviour of sodium in aluminium–magnesium alloys[7]). Both helium and lithium ions are produced in steel irradiated with thermal neutrons, and Higgins and Roberts[8] have recently shown that at concentrations of 10^{-5} these elements can separately cause embrittlement in this steel at 750° C, the helium exhibiting the larger effect. The combination of helium and lithium together may be more effective than either element alone in causing embrittlement, if the lithium can segregate to the free surfaces of helium-filled bubbles and reduce the stress required for these to grow into cracks.

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CHEMISTRY

Interior Morphology of Bulk Polypropylene

As the result in recent years of the growth and identification of lamellar single crystals of various polymers, produced both by dilute-solution methods and by melt crystallization of thin films, older concepts of the structure and texture of bulk crystalline polymers have been undergoing change[1]. Indications that bulk samples of partially crystalline polymers also contain lamellar-like crystals have come from electron microscope examinations of fracture surfaces[2], and of fragments[3,4] of bulk polymers dispersed by means of a nitric-acid digestive treatment[5].

Striking evidence in favour of the concept that bulk polymers may also consist largely of lamellar-like crystals has been obtained from a study of the crystallization behaviour of isotactic polypropylene. Samples of this material were first compressed in a high-pressure mould to 60,000 lb./in.² and then held at a temperature of 325° C for 3 h. This temperature is about 30° C higher than the melting point of polypropylene at the pressure of 60,000 lb./in.². The pressed sample was brought to a temperature of 248° C (about 47° C supercooling) and held at this temperature for 140 h. The temperature was then slowly reduced to room temperature, the pressure returned to atmospheric, and the sample removed. The sample, in the form of a solid rod 0·25 in. in diameter and 3·5 in. long, appeared to be in a highly brittle state and some powdering occurred during its removal from the mould.

The sample was cooled to liquid nitrogen temperatures and later fractured at these temperatures in order to inhibit plastic flow. After fracture, the fracture surfaces were examined by light microscopy, then replicated, and the replica examined by transmission electron microscopy. The light microscope examination showed a spherulitic structure with the size of the spherulites being of the order of 100μ. It was also observed that both inter- and intra-spherulitic fractures had occurred.

An electron micrograph of a replica of a portion of the fracture surface is shown in Fig. 1. Numerous, somewhat



Fig. 1. Electron micrograph of replica of fracture surface of a bulk polypropylene sample moulded at 60,000 lb./in.² and held for 140 h at 248° C



Fig. 2. Higher magnification electron micrograph of portion of fracture surface of polypropylene sample moulded at 60,000 lb./in.² and held for 140 h at 248° C

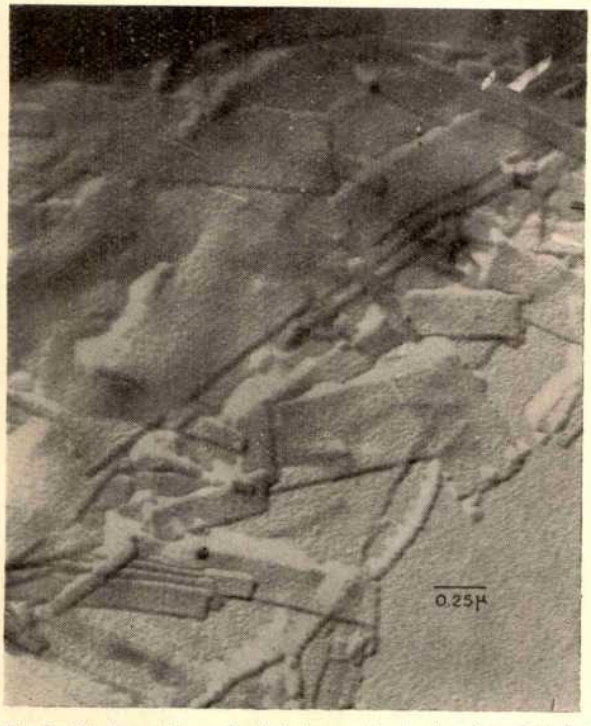


Fig. 3. Electron micrograph of single crystals at edge of quadrite for polypropylene sample isothermally crystallized in 0·01 per cent solution of α-chloronaphthalene at 115° C

interwoven, lath-like lamellar crystals are visible. Similar crystals are clearly delineated in the higher magnification electron micrograph of Fig. 2, which represents another portion of the fracture surface. The rectangular crystals appear to be about 3–8μ in length, 0·2–0·5μ in width, and about 125–150 Å in thickness. They are remarkably similar to the crystals which have been recently obtained[6] from dilute solution crystallization of isotactic polypropylene in α-chloronaphthalene (αCN). This is seen from Fig. 3, which shows a group of lamellar single crystals found at the edge of a quadrite on isothermal crystallization at 115° C of a 0·01 per cent solution of polypropylene in αCN. Note the marked morphological similarity of these crystals with those of Figs. 1 and 2. Also, in both the bulk sample as well as the dilute solution sample, the lamellar surfaces are smooth, the edges are well defined, and there are no indications of spiral growth.

The bulk sample crystallized at high pressures was also examined by X-ray diffraction and by differential thermal analysis. These data indicate that the sample had crystallized in the γ-modification of polypropylene[7]. The sample prepared by dilute-solution crystallization is known, however, to be in the α-modification[6]. Thus, despite the common morphology of the crystals of the bulk sample and the crystals of the dilute-solution crystallized sample, the crystal structure is triclinic in the first instance and monoclinic in the second.

These results show that crystallization in the form of lath-like lamellar crystals can occur both for the α-form and the γ-form of isotactic polypropylene. Also the marked similarities in morphology of the bulk-crystallized and the dilute-solution crystallized samples strongly suggest that the chain molecules in the bulk sample are folded and that restricted chain folding, as already noted for the α-form crystals[6], probably also occurs in the γ-form. Examination of the suggested unit cells of the two different crystal structures shows they are closely related. It would seem that one could be converted to the other by partial slip along fold planes. Turner Jones *et al.*[7] have, in fact, shown that the γ-form is converted to the α-form by mechanical deformation, while Kardos *et al.*[8] have shown that the γ-form can be converted to the α-form by thermal treatment.

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Structure Studies on Ternary Vanadium–Molybdenum Oxides

A BRIEF report on the crystal structures of some of the phases observed in the ternary vanadium–molybdenum–oxygen system[1] is presented below.

A phase of the composition $VMoO_5$, which has been reported recently[2], has been found to have a tetragonal unit cell with the dimensions $a = 6{\cdot}6078$ Å, $c = 4{\cdot}2646$ Å, and the space group $P4/n$. A single crystal study, including least squares refinement of atomic parameters to an R-value of 0·054, has revealed that the structure is isotypic with that of $MoOPO_4$ (ref. 3). The vanadium and molybdenum in $VMoO_5$ are structurally equivalent with the molybdenum and phosphorus, respectively, in $MoOPO_4$ and, accordingly, the formula of the compound may be written $VOMoO_4$. The structure is built up of strings of distorted VO_6 octahedra running parallel to the c axis. These strings are mutually connected by quite regular MoO_4 tetrahedra. The vanadium–oxygen distances within the octahedra are 1·68, 2·59, 1·97 (4x) Å, and the four molybdenum–oxygen bonds are 1·76 Å.

Another phase of the approximate composition V_2MoO_8 has been reported by Magnéli and Blomberg[4]. In this study, the powder pattern of this phase was indexed on

the basis of a monoclinic unit cell with the dimensions $a = 19{\cdot}32$, $b = 3{\cdot}621$, $c = 4{\cdot}107$ Å, $\beta = 90{\cdot}3°$, which is in close agreement with the values published recently by Freundlich and Pailleret[5]. Weissenberg photographs of several crystals invariably showed the presence of twinning with (001) being the twin plane. The single crystal studies revealed also that the true b axis is doubled, the layer lines with k odd (crystal rotated around [010]) showing only continuous streaks along the bows parallel to a^*. This behaviour indicates one-dimensional disorder—a kind of stacking fault—which can be explained readily in terms of the structure details.

The structure of the cell corresponding to the lattice parameters listed above, the stacking lattice[6], has been determined and refined to $R = 0{\cdot}065$. The structure, a distorted variant of the Nb_3O_7F-type[7] with vanadium and molybdenum statistically distributed over the two metal atom positions, exhibits $C2$ space group symmetry. The oxygen atoms are co-ordinated (octahedrally) around the metal atoms with two short, three intermediate, and one long molybdenum–oxygen bond. Distortion towards five-co-ordination is thus appreciable. The co-ordination can be considered intermediate between that in vanadium pentoxide and molybdenum trioxide.

Molybdenum may replace vanadium in the V_2O_5 structure to a considerable extent, thus forming a range of solid solution $(Mo_x,V_{1-x})O_{2\cdot5}$, where $x_{max} \approx 0{\cdot}30$[1]. This finding differs slightly from the results of Magnéli and Blomberg[4], who reported a solid solution of molybdenum trioxide in V_2O_5 vanadium pentoxide. Over the solid solution range, unit cell dimensions change continuously from the values $a = 11{\cdot}514$, $b = 3{\cdot}562$, $c = 4{\cdot}371$ Å for vanadium pentoxide to $a = 11{\cdot}809$, $b = 3{\cdot}650$, $c = 4{\cdot}174$ Å for the composition $Mo_{0\cdot30}V_{0\cdot70}O_{2\cdot50}$. Single crystal photographs reveal that the symmetry of the molybdenum-rich phase is, in fact, monoclinic with $\beta = 90°$ and that the systematic extinctions are consistent with space groups $C2/m$, Cm, and $C2$. The symmetry has thus changed from orthorhombic to monoclinic somewhere in the solubility range ($0 < x < 0{\cdot}30$). Powder data obtained for the composition $x = 0{\cdot}30$ are in general agreement with the d values listed by Freundlich and Pailleret[5] for "$MoV_2O_{7\cdot5}$". The compound $Mo_4V_6O_{25}$ reported by Munch and Pierron[8] may also be identical with this phase.

Andersson has shown[7] that the structure of Nb_3O_7F can be regarded as the second member ($n = 3$) of a homologous series, M_nO_{3n-1}, in which the first possible member ($n = 2$) corresponds to the ideal V_2O_5 structure. Thus, there is a close relationship between the structure of V_2MoO_8 and V_2O_5, even though they are distorted in different ways from the ideal regular octahedral arrangements. But when molybdenum is substituted for vanadium in V_2O_5 the observed symmetry changes indicate that the structure gradually adopts the distortion exhibited in V_2MoO_8.

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Orientation-induced Nucleation in Polymer Crystallization

IT is a well-known fact that the crystallization kinetics of polymers crystallizing from the melt are influenced by the mechanical history of the sample[1,2]. Strain-free samples show a larger crystallization half-time than samples in which the strain has not been completely removed by relaxation at a suitable temperature[3,4]. A larger number of nuclei has been assumed to be present in strained samples than in strain-free ones. This has been called "orientation-induced" or "stress-induced" nucleation.

This communication presents a morphological description of the phenomenon and of the underlying type of nucleation. My observations were made with a microscope (Reichert 'Zetopan-Pol') equipped with a hot stage and 24-mm camera, and mainly with the polarizers crossed. For most of my experiments I used polypropylene, but similar phenomena were also observed with several other polymers.

Polypropylene, when melted at 250° C and cooled to 140° C, crystallized slowly at this temperature, and only a few spherulites were formed. Application of a small shear stress (about 0·1 kg/cm²), however, resulted in abundant nucleation of small spherulites in long rows parallel to the direction of shear.

Heavy strains could also be introduced when the polymer was milled at 180° C on a two-roller mill and later quenched. A microscopic specimen was made at 200° C from a piece of about 2 mg weight cut from the quenched milling sheet. When the specimen was allowed to cool slowly, sharp birefringent lines developed, which gradually increased in number and birefringence (Fig. 1). Sometimes these lines were visible above the normal melting point of the polymer (172° C). The sign of the birefringence indicated that the polymer chains were



Fig. 1. Nucleation lines in a polypropylene melt at successive stages of cooling. (×*c.* 135)

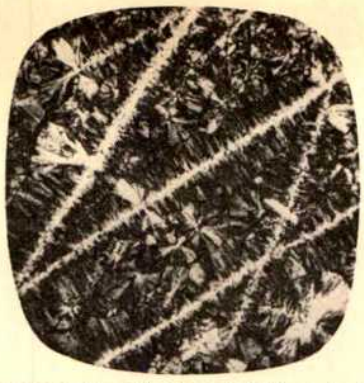


Fig. 2. "Spherulitic" outgrowth after orientation-induced nucleation. (×c. 135)

parallel to these lines. Where the density of these lines was low—namely, in a polymer sample made at small shear stress—normal fibrillar outgrowths could be seen and these proved to be similar to those of the spherulites (Fig. 2). I have called these lines "nucleation lines", and the type of nucleation "orientation-induced nucleation" although the latter is not a new term.

Provided the temperature was kept below 210° C, the phenomenon observed in Fig. 1 could be repeated at will by heating and cooling—the nucleation lines invariably appearing in the same place. After heating above 210° C the density of the nucleation lines observed on cooling decreased. When I heated my sample (intrinsic viscosity$_{\text{dec. }135^\circ\text{C}}$ = 3·1) at 240° C for 1 min, the lines completely disappeared (see ref. 4, Fig. 2). For a material of higher intrinsic viscosity a higher temperature was necessary in order to bring about the complete disappearance of the orientation-induced nucleation.

Unlike stretched rubbers, sheared polypropylene does not crystallize as a whole above its normal melting point; this is probably because the ratio of viscosity to elasticity of the molten polymer is not sufficiently high to maintain a recoverable strain sufficient for crystallization. On the other hand, at the locus of the nucleation lines the melting point of the polymer has probably increased considerably owing to a certain degree of alignment of polymer chains in the melt. A peculiar feature is that the strain is not homogeneous but culminates in lines.

Because a normal fibrillar outgrowth was found to be typical of lamellar crystallization in bulk polymers, the phenomenon of the nucleation lines and the subsequent growth bears a close relationship to the morphology found in crystallized stretched rubber[5] and even in the crystallization of a stirred polymer solution[6], where long strands of short lamellae were found, connected by a central string.

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Electron Spin Resonance of Nickel in Synthetic Diamonds

IN addition to the ordinary nitrogen hyperfine lines in diamond[1,2], an isotropic line was observed at 77° K in certain polycrystalline samples of synthetic diamond. This line, which does not appear in natural diamond, has a g-value of 2·0310 ± 0·0005 and a width of ~6 gauss. Samples from Japan, the General Electric Company (U.S.A.) and from the Adamant Research Laboratory, South Africa, were investigated.

The ratio of the intensity of the new line relative to the intensity of the central line of the nitrogen spectrum varied from sample to sample, being the greatest (about 2·4) for the Japanese diamonds. The line width of the central line of the nitrogen spectrum in the Japanese diamonds is also about 6 G. The new line was also observed in a single crystal of synthetic diamond, and no anisotropy was observed.

In an effort to identify the line, synthetic diamonds were made containing no nickel, as most synthetic diamonds are known to contain nickel[3]. For example, diamonds were made containing cobalt and iron, and in both these cases the additional line was absent. Measurements were also made at 20° K and ~4° K, but no splitting of the line was observed.

Ludwig and Woodbury found an isotropic line in silicon[4] belonging to nickel, with a g-value of 2·026. They could explain its behaviour by assuming that the nickel was present as Ni^+ (spin $\frac{1}{2}$) in an interstitial site. The line was motionally narrowed and below ~2° K the spectrum split and several geometrically non-equivalent sites could be distinguished.

With nickel in germanium[5], they concluded that the nickel was present as Ni^- in a substitutional site. Above 20·4° K the Ni^- centres in germanium re-orient so rapidly that the anisotropic set of six lines collapses into one line.

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Reaction with Propane of $I(5^2P_{1/2})$, produced by Photolysis of Iodine in the Continuum of the $B^3\Pi^+_{ou} - X\,^1\Sigma^+_g$ System, and by Collisional Release inside the Banded Region

FROM an analysis of the kinetics of the formation of i-propyl iodide in irradiated mixtures of iodine and propane, we have recently shown that electronic excitation of atomic iodine to the $5^2P_{1/2}$ state greatly enhances its chemical reactivity; in respect of hydrogen atom abstraction (activation energy ~ 5 kcal/mole) its behaviour is similar to that of atomic chlorine. The $B^3\Pi^+_{ou}$–$X^1\Sigma^+_g$ electronic transition of iodine exhibits a banded region with a convergence limit at 4995 Å, and a continuum at shorter wave-lengths[1]. Dissociation of molecular iodine in the continuum produces one atom in the $5^2P_{3/2}$ ground state and one atom with the $5^2P_{1/2}$ spin-orbit orientation corresponding to an excitation energy of 21·7 kcal/mole. The occurrence of $I(5^2P_{1/2})$ reactions has been established by a study of the inhibition caused by addition of various species which deactivate $I(5^2P_{1/2})$ to the ground state:

$$I^* + M \rightarrow I + M$$

There is a general correspondence between efficiencies measured directly by flash spectroscopy[2,3] and those determined from the isopropyl iodide inhibition. The production of $I(5^2P_{1/2})$ also occurs well inside the banded region above 4995 Å; collisional release resulting from long-range attractive interaction with any added gas, and the kinetic energy available during collision, effectively shifts the continuum threshold to longer wave-lengths.

Quantum yields were measured by focusing light from a compact-source, quartz–iodine lamp (100 W), through narrow band interference filters, into the reaction cell.

The transmitted light-intensity was measured by chemical actinometry, and the *i*-propyl iodide was determined by gas chromatography, after separation of the unreacted propane on a low-temperature still. The quantum yield is independent of the area of the cell window that is illuminated, which rules out the possibility of complications arising from reaction on the glass surfaces. Most of the kinetic measurements were made in a reaction system in which a 1,000 W quartz–iodine lamp, length 15 cm, was placed parallel to the axis of a cylindrical reaction vessel, the whole being enclosed in an aluminium reflector. A variety of filters was used to determine the dependence of the kinetics on the wave-length of the radiation.

The yield of *i*-propyl iodide is accurately proportional to the duration of exposure, and to the light intensity. At 60° C in a mixture of 0·20 mm of iodine with 100 mm of propane, the quantum yield for the formation of *i*-propyl iodide is $1{\cdot}5 \times 10^{-4}$ at 4835 Å. Inside the banded region at 5020 Å, the quantum yield is $0{\cdot}7 \pm 0{\cdot}1$ of the yield at 4835 Å. The yield falls off above 5200 Å, and is very small at 5480 Å, where ground state atoms are still produced[4].

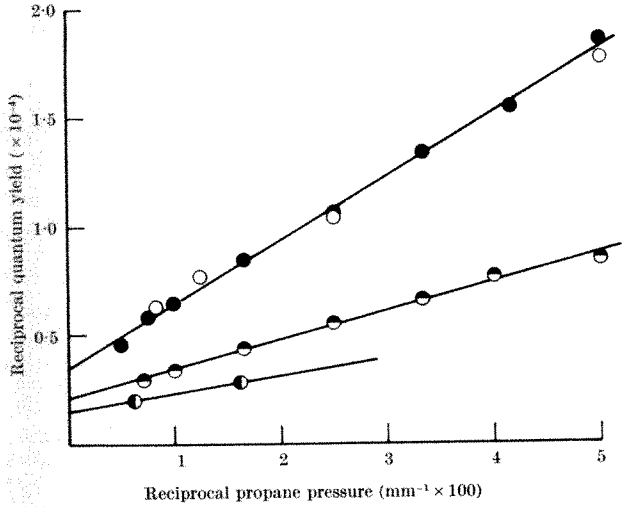


Fig. 1. Linear relationship between reciprocal quantum yield and reciprocal pressure of propane. Continuum: ●, 0·2 mm iodine + propane (30° C); ◐, 0·20 mm iodine + propane (90° C); ◓, 0·20 mm iodine + propane (60° C). Banded region, 0·20 mm iodine + propane + nitrogen (30° C) (○)

Fig. 1 shows the linear relationship between reciprocal quantum yield and reciprocal pressure of propane. The quantum yield for the banded region refers to 5020 Å, and has been divided by 0·7 to illustrate the invariance of the slope to intercept ratio with wave-length. In the latter experiments, nitrogen was added to a total pressure of 500 mm, to avoid any significant changes in pressure broadening. For a particular temperature, the ratio of slope to intercept is the same on either side of the 4995 Å threshold, and there can be no doubt that essentially the same reaction occurs in the two wave-length regions. Assuming the scheme:

$$\begin{gathered} I_2 + h\nu \rightarrow I + I^* \\ (\text{or } I_2 + h\nu \rightarrow I_2^*,\ I_2^* + M \rightarrow MI + I^*) \\ I^* + C_3H_8 \rightarrow HI + i\text{-}C_3H_7 \\ I^* + M \rightarrow I + M \\ i\text{-}C_3H_7 + I_2 \rightarrow i\text{-}C_3H_7I + I \end{gathered}$$

the rate coefficients for deactivation of $I(5^2P_{1/2})$ relative to propane may be derived from plots similar to those of Fig. 1; in Table 1 these are compared with rate coefficients measured by flash spectroscopy. We consider that as the general pattern of deactivation efficiencies is the same, the occurrence of hydrogen atom abstraction by $I(5^2P_{1/2})$ is established from these measurements.

Table 1

	H_2	I_2	CO_2	Ar	N_2
Donovan and Husain (~20° C)	1·5	88	0·0023	v-small	0·0035
This work (30° C)	0·55 ± 0·02	430 ± 10	< 0·03	< 0·05	< 0·03

Filseth and Willard[5] have reported the formation of *n*-propyl and *i*-propyl iodide in equal yield from the same reaction, and have derived a different mechanism. In our investigation, the ratio of *i*-propyl to *n*-propyl iodide was always 40 : 1, except for the first few experiments in freshly cleaned vessels, when significant amounts of *n*-propyl iodide seem to be produced by heterogeneous reaction.

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Bond Character in Germanium Selenides by X-ray Spectroscopy

X-RAY spectroscopy provides a direct and powerful method of studying the electronic structure of solids[1]. It has been applied so far mainly in the investigations of energy band structure of solids[2–4] (mostly metals and alloys) and in the study of complexes[5–7]. This communication, which reports our work on the *K* absorption spectra of selenium and germanium in GeSe and $GeSe_2$, shows that this method can also be useful in determining the nature of chemical bonding in intermetallic compounds which are of special interest because of their semiconducting properties.

The absorption spectra were recorded photographically using a bent crystal X-ray spectrograph 40 cm in diameter. The experimental technique has already been described[8,9]. The intermetallic compounds were prepared in stoichiometric ratio by suitable heating and annealing. Table 1 gives the wave-lengths and corresponding energies of the germanium *K* absorption discontinuity in pure metal and in the two compounds. Table 2 gives similar data for the selenium discontinuity.

Table 1. DATA ON GERMANIUM *K* ABSORPTION DISCONTINUITY

Absorber	λ in X.U.	ν/R	Energy (*E*) eV	Δ*E* eV
Ge metal	1114·35 ± 0·05	817·7	11101·1 ± 0·4	
GeSe	1114·50 ± 0·05	817·5	11099·6 ± 0·4	−1·5 (ΔE_1)
$GeSe_2$	1114·32 ± 0·05	817·8	11101·4 ± 0·4	− − − ($\Delta E'_1$)

Table 2. DATA ON SELENIUM *K* ABSORPTION DISCONTINUITY

Absorber	λ in X.U.	ν/R	Energy (*E*) eV	Δ*E*
Se metal	977·82 ± 0·07	931·94	12651·1 ± 0·5	
GeSe	977·60 ± 0·07	932·14	12653·8 ± 0·5	2·7 (ΔE_2)
$GeSe_2$	978·02 ± 0·07	931·75	12648·5 ± 0·5	−2·6 ($\Delta E'_2$)

It is generally believed that the inflexion point on an X-ray absorption edge represents the top of the outermost filled band. For germanium and selenium it can be taken to represent the top of the valence bands which consist, respectively, of $4p^2$ and $4p^4$ electrons. In GeSe some of the $4p^2$ electrons of germanium are transferred into the conduction band of selenium because of the higher electronegativity[10] of the latter (2·4 for selenium and 1·8 for germanium). This results in the lowering of the energy of the germanium edge in the compound giving the shift ΔE_1. However, in the band structure of selenium the bonding and the non-bonding $4p$ bands are

completely occupied[11] and the germanium electrons can only go into the antibonding $4p$ band, which in pure selenium is at 1·8 eV from the valence band[12]. We obtain, therefore, in the opposite direction, a considerably higher shift ΔE_2 for the selenium K edge. On the assumption that the distribution of the transferred electrons remains more or less of the same type in the compound, E_g for GeSe is $(2{\cdot}7-1{\cdot}5)=1{\cdot}2$ eV. This is in good agreement with the value 1·0 eV obtained by Asanabe and Okazaki[13] when they used the normal resistivity variation method. The electron transfer in GeSe shows that the bonding in this compound is largely of the ionic type. The NaCl type structure[14] of this compound further supports this contention.

However, the situation in $GeSe_2$ would seem to be entirely different. The germanium edge in this compound remains practically at the same position while the selenium edge is shifted towards lower energies by 2·6 eV. The change in energy of the selenium edge most probably indicates strong covalent bonding in this compound, which lowers the energy of the top of the selenium valence band. It would seem that the lone germanium atom in $GeSe_2$ is unable to satisfy the electronegativity requirements of two selenium atoms and thus this compound 'prefers' covalent linkage.

We thank Dr. K. P. Sinha of the National Chemical Laboratory, Poona, for helpful discussions and the Directorate of Industries, Government of Maharashtra, Bombay, for financing the research scheme under which a part of this work has been done. One of us (R. N. P.) also thanks the University Grants Commission, New Delhi, for the award of a junior research fellowship.

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S-Demethylation catalysed by the Formation of Metal Complexes

S-ALKYLATION has been observed to occur *in situ* when certain metal complexes of β-mercaptoethylamine and other thiols are treated with alkyl halides in dimethylformamide solution[1]. We have observed *S*-demethylation reactions with certain metal chelates of thioethers.

8-Methylthioquinoline (N-SMe) forms complexes with several transition metal ions in which it functions as a neutral bidentate chelate group[2]. However, it reacts with sodium tetrachloroaurate (III) in acetone solution to yield yellowish-green crystals of dichloro-8-quinolinethiologold (III), $Au(N\text{-}S)Cl_2$ (I), which is a non-electrolyte in nitrobenzene and nitromethane. The infra-red and nuclear magnetic resonance spectra of (I) show no peaks that can be attributed to a methyl group. The compound (I) was also prepared from 8-quinolinethiol and sodium tetrachloroaurate (III).

If solutions of $M(N\text{-}SMe)Br_2$ (M = palladium or platinum) in dimethylformamide are heated for 10 h and alcohol is then added, the thiolo-bridged complexes $M_2(N\text{-}S)_2Br_2$ separate when the solution cools. Similarly, when solutions of $Pd(As\text{-}SMe)X_2$ (As-SMe = dimethyl-*o*-methylthiophenylarsine[3]; X = chlorine or bromine) in dimethylformamide are heated, the thiolo-bridged complexes $Pd_2(As\text{-}S)_2X_2$ can be obtained. Similar metal-ion catalysed dealkylation reactions of ether groups have recently been reported to occur with copper (II) complexes of certain Schiff bases[4].

It is possible that these thioethers could, in the presence of metal ions, effect transmethylation analogous to what has been observed with *S*-adenosylmethionine in living systems[5,6].

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Rapid Voltammetric Determination of Methionine with a Platinum Electrode

THE presence and concentration of methionine in aqueous media can be quickly determined by a simple voltammetric method. The solution for electrolysis must be acidic and must contain a moderate amount of chloride ion. Using a platinum microelectrode as the working anode and a saturated calomel electrode (SCE) as the cathode, anodic current–voltage curves with limiting currents proportional to the methionine concentration are obtained. The voltammetric waves are well developed between +0·7 and +1·0 V against the SCE.

The usual voltammetric technique was used. Electrolysis was carried out at 25° C in an H-shaped cell. Solutions of methionine were made in distilled water to which had been added known amounts of hydrochloric acid and sodium chloride. The methionine was a Dow product of 99·9 per cent purity. For methionine concentrations smaller than 100 p.p.m., a platinum microelectrode about 3 mm long and 0·6 mm in diameter was used as the working anode. The platinum wire was sealed into the end of a glass tube containing some mercury for electrical contact, as usual. For concentrations greater than 100 p.p.m. a much smaller electrode was used. This was made from the same platinum wire; after sealing, the wire was cut and filed flush with the glass surface. Current–voltage curves were automatically recorded with a Sargent 'Model XV' polarograph. The voltage was applied at a rate of 0·05 V/min.

The anodic wave obtained under the experimental electrolysis conditions is believed to involve the thio group of the methionine molecule. Micro-scale complete electrolysis indicated a two-electron oxidation step. Because the electrolysis products have not yet been identified, no plausible electrode reaction mechanism can be proposed. In Fig. 1 the waves develop between +0·7 and +1·0 V. In order for such waves to appear, the solution must be acidic and must also contain a moderate amount of chloride ion. Apparently both the hydrogen and chloride ionic species are necessary for the wave to develop in good form. Concentrations of hydrogen and chloride ions can be widely varied with practically no change in

either the half-wave potential or the limiting current (Fig. 2). For methionine concentrations of 1–500 p.p.m., the best waves are obtained with 1–2 M solutions in chloride, provided the solutions are definitely acidic (pH ~2 or less).

In neutral solutions very poor waves may appear; in basic solutions there is no evidence of a wave at all. This is shown in Fig. 3. Methionine solutions acidified with sulphuric acid, and containing no chloride, produce waves at a more anodic potential than that required in the presence of chloride ion. The form and reproducibility of these waves are not satisfactory for analytical work.

In Fig. 1 the limiting currents are proportional to methionine concentration. They are reproducible to within ± 5 per cent for concentration levels of 5–500 p.p.m.

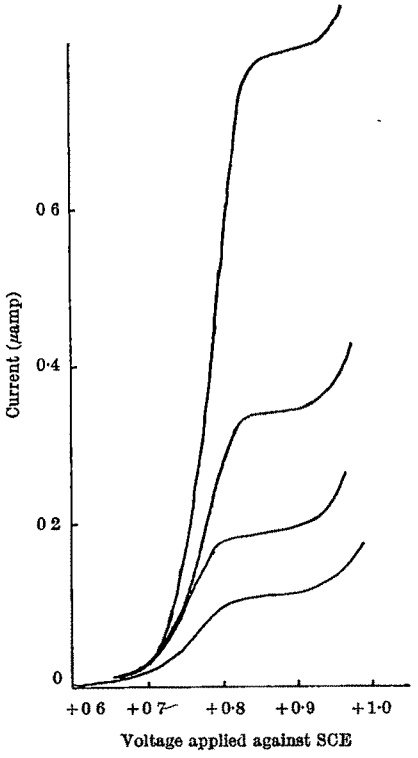


Fig. 1. Current–voltage curves of methionine, showing proportionality of current to concentration of methionine. Curves obtained with the smaller electrode. Chloride concentration, 1 M; hydrogen ion concentration, 0·1 M. The curves represent concentrations of methionine of 50, 100, 200 and 500 p.p.m.

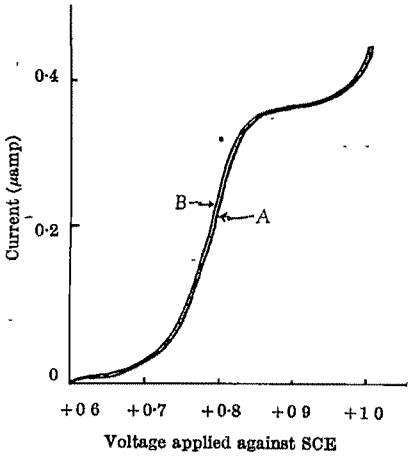


Fig. 2. Current–voltage curves of methionine (200 p.p.m.) with different chloride and hydrogen ion concentrations. Curve *A*: chloride, 1 M; hydrogen, 0·1 M; curve *B*; chloride, 2 M; hydrogen, 0·3 M

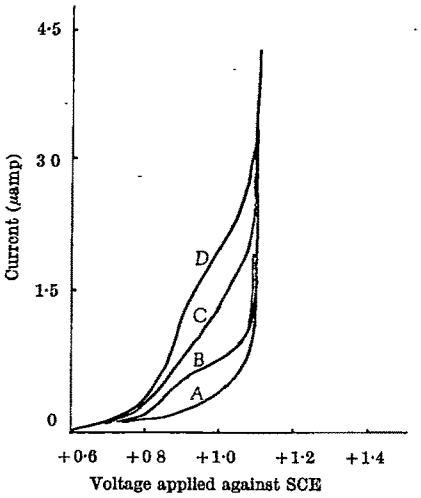


Fig. 3. Current–voltage curves of methionine in neutral solution. Methionine concentration *A*, 0 p.p.m; *B*, 50 p.p.m.; *C*, 100 p.p.m.; *D*, 150 p.p.m.; sodium chloride concentration, 1 M. Curves obtained with the larger electrode. In basic solutions a curve like *A* is obtained, irrespective of methionine concentration

The waves start to form ahead of the chloride wave such as to suggest the possibility of a methionine-electrode complex; this complex can apparently be electro-oxidized at a less positive potential than the chloride, thus making possible development of a wave before discharge of chloride ion. If iodide or bromide ions are present instead of chloride, no waves appear; a slight shift in the decomposition potential of these ions to less positive values occurs, which probably indicates depolarization of the halide ions by methionine. It is probable that methionine–electrode complexes with iodide or bromide also form, but these complexes may decompose so near to the decomposition potential of the halide ions that distinct methionine waves cannot develop.

Regarding the platinum electrode, the occasional difficulties arising from oxidation of the electrode in chloride media[1], and the maxima that may form with stationary electrodes[2], should be considered. Sometimes the platinum electrode behaved very erratically, especially when the applied potential exceeded +1·1 V. In such cases the electrode was made the cathode at −1·9 V for a few minutes while it was dipped into the solution to be electrolysed; the potential was then reduced to −0·6 V and the polarity was reversed; after a waiting period of about 10 min, current–voltage curves were obtained. The use of two electrodes, a smaller one for methionine concentrations greater than 100 p.p.m. and a larger one for smaller concentrations, practically eliminated the undesirable formation of maxima. Although a stationary platinum micro-electrode requires a few minutes to give a steady current with successive increments of applied potential, satisfactory reproducibility of the waves was attained by applying the potential continuously at a rate of 0·05 V/min (ref. 3). There was no particular advantage in manually obtaining point-by-point current–voltage curves. When the electrode was rotated at rates of 200–1,000 r.p.m., very steep current plateaux were produced.

Determination of methionine in various hydrolysates was made either from standard calibration curves or by the standard addition method. In the analysis of unknown samples possible interferences should be considered. Organic sulphides are likely to produce anodic waves at the same potential. For example, the hydroxy analogue of methionine yields identical waves with those of methionine. Bromide ion definitely interferes. Advantage can be taken of the fact that most organic sulphides are more or less soluble in ethyl ether (methionine is not) and can be easily removed by extraction with the ether.

The merit of the present method lies in its simplicity and rapidity. Like most voltammetric methods, it can be used satisfactorily with relatively small sample volumes; if necessary, only 0·1 ml. of sample solution can be used. The sample is not destroyed—an advantage which is not obtained with most of the chemical methods used for methionine analysis.

I thank Edgar Elkins and Dr. Dalton MacWilliams for their interest and useful discussions during this work.

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Evaporation Reduction of Water

SINCE Langmuir explained the nature of monomolecular films, efforts have been made to use them as economically feasible reductants of evaporation of water. Apart from practical engineering difficulties in encountering breakage of the film due to winds, dirt deposits, etc., there are two built-in difficulties: (1) Reduction of evaporation causes a warming up of the water which corresponds to an increase of the vapour pressure. Thus the advantage of evaporation reduction is lost at the slightest break of the film[1]. (2) Fatty alcohols used to obtain the monolayer are attacked by bacteria, namely *Pseudomonas* and *Flavobacterium*[2].

I found that spreading solid paraffin to which a white reflective pigment has been incorporated on the surface of water overcomes these difficulties, as paraffin is biologically inert and the incorporated pigment reflects direct sunlight.

Paraffin without pigment is an excellent barrier against liquid water and water vapour. Its refractive index, however, is too near that of water and therefore does not sufficiently reflect direct sunlight.

Table 1 shows how the temperature rises as a result of efficient reduction of evaporation. The experiments were carried out in class "*A*" pans on the roof of the Water Planning Authority building in Tel-Aviv.

Table 1

Substance	Daily evaporation (mm)	Evaporation compared with untreated water (per cent)	Surface temperature (° C)
Nil	2·1	100	20
White polystyrene balloons	1·32	63	18
Limna	1·86	88·5	19
Magnesium stearate powder (87 g/m²)	1·37	65·0	17
Zinc stearate (91 g/m²)	1·44	68·5	18
Polyurethane foam covering 85 per cent of the water surface	1·17	55 7	19
Paraffin powder (150 g/m²)	0 17	8 1	21 5

A second experiment was carried out with paraffin containing 10 per cent (by weight) of titanium dioxide and 20 per cent (by weight) of calcium carbonate (precipitated). The temperature measured 1 cm below the surface was lower than the temperature of the untreated water (20° C and 19° C) and the saving in evaporation was 33 per cent, for paraffin treated with titanium dioxide, and 35 per cent, for paraffin treated with calcium carbonate.

The pigment containing paraffin was prepared as follows: 450 g paraffin (melting range 58° C–60° C) was melted at about 90° C and 50 g titanium dioxide added with constant stirring. After resolidification of the titanium dioxide-containing paraffin it was milled (under cooling with liquid air) and the powder passed a sieve of 40 mesh. 55 g/m² were broadcast.

The same technique was used for the incorporation of a number of pigments like calcium carbonate, zinc oxide, barium sulphate, etc. For obvious reasons (lightness, physiological inertness, etc.) calcium carbonate is best.

Results similar to the ones with pigmented paraffin were obtained with white polypropylene powder and with white high-density (Ziegler) polyethylene powder.

However, the synthetic plastic materials were not exposed for a long enough time for any degradation due to the combined effect of ultra-violet rays and water vapour to become apparent. The degradation is first signalled by a yellowish–brownish tinge, and defeats the reflective power of the undegraded white material.

Fox[3] recently reported similar results using an oil-wax surfactant system. Unfortunately he did not report the temperature readings.

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Interference with the Determination of Aldrin and Benzene Hexachloride Residues by Gas Chromatography

WE wish to report an artefact which has occurred in our laboratory during the analysis of plant materials for residues of organochlorine pesticides by gas–liquid chromatography, with the electron capture detector. It occurs as a peak in a position very close to that of aldrin, when using a silicone elastomer on 'Celite' packed column, and at the same position as β-benzene hexachloride (BHC), when using an 'Apiezon *L*' on 'Celite' packed column.

Solvents, as purchased, often give this peak, but the contaminant causing the peak can be removed by distillation through a short fractionating column. Distilled solvents and water are recontaminated if kept in contact with polythene, for example, storage bottles and pipework. The contaminant can be extracted, by redistilled hexane, from absorbants such as 'Florisil', alumina and cellulose powder; and from clean glassware, glass-wool, cotton-wool and filter papers, especially after these have been in our laboratory for a few days. It was recovered in relatively large quantities from hexane swabbings of cupboard-door windows and from a hexane-extract of laboratory dust.

Column chromatography on alumina of a hexane-extract of several grams of laboratory dust, using gas–liquid chromatography with electron capture detector to monitor the eluate, gave a yellow oily fraction producing only the artefact peak. The infra-red spectrum of this fraction suggested a phthalate ester.

Di-*n*-butyl phthalate has the same retention characteristics as the contaminant and is strongly electron capturing—a solution of 1×10^{-6} g/ml. giving about the same response as a solution of 2×10^{-8} g/ml. of aldrin.

We have been unable to determine the source of the contaminant in solvents delivered in steel drums, or in glass bottles with loose aluminium foil disks separating the polythene cap-insert from the bottle contents. We believe that the main source of the contamination in the laboratory is the polyvinyl acetate-based paint, containing dibutyl phthalate as plasticizer, which was used to paint the walls and ceilings. Polyvinyl acetate latex-based adhesive is often used to secure aluminium foil coverings to cardboard inserts in bottle caps, and the adhesive on tapes

used to seal capped bottles often contains dibutyl phthalate; these are possible sources of the contaminant found in absorbants from newly opened bottles. This contamination is so widespread in our laboratory and, even with the most stringent hygiene, so difficult to control that care must always be taken when identifying a peak with β-BHC or aldrin, especially when the residue level is too low to confirm the identity by some other technique, such as thin-layer chromatography.

β-BHC is unlikely to occur as a residue in crops because its concentration in the mixed-isomer of BHC is very low and is virtually zero in the insecticide γ-BHC (lindane). Therefore, a peak, tentatively identified as β-BHC, when using 'Apiezon *L*' columns, can usually be regarded with suspicion.

The amount of the contaminant varies unpredictably and makes the quantitative determination of aldrin in crops virtually impossible by the silicone column method. Because the contaminant peak tails into the aldrin peak position, quantitative aldrin determinations are also difficult using the 'Apiezon *L*' column when the contaminant peak is greater in height than the aldrin peak.

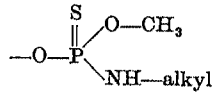
We thank Dr. N. T. Crosby, of the Laboratory of the Government Chemist, for running and interpreting the infra-red spectrum.

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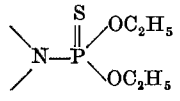
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Biological Activity of Phosphoramidothionates

IN the course of our work on this class of compound an animal-anthelmintic[1] and a selective herbicide[2] had previously been discovered. Common to the structures of the two compounds was the linking of the phosphoramidothionate group (I) to a chlorinated phenyl ring by an oxygen atom. Our speculations on biological activity led us to replace this atom by nitrogen and to investigate the effects of a different type of toxophoric phosphoramidothionate group (II):



(I)

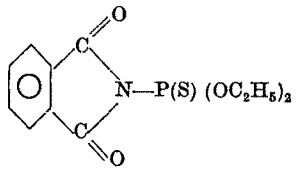


(II)

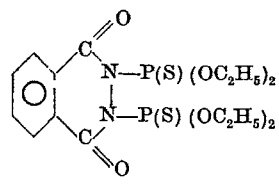
We were particularly interested in the biological effects resulting from an incorporation of the moiety,

$$-C(O)NP(S)(OC_2H_5)_2,$$

into heterocyclic structures. These we were able to synthesize when the inherent nucleophilicity of certain amide-type phosphorothionate esters first revealed itself in our own investigations, before 1960[3,4]. Among the new types of structure that thus became available were (III) and (IV).



(III)

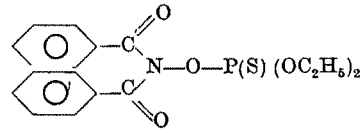


(IV)

It was known that certain compounds containing the groups, $-CH_2\ O/S-$ and $-C_2H_4\ O/S-$, between N and P in (III) were insecticidal. Quite unexpectedly, we found (III) to be an excellent fungicide, controlling powdery mildew completely at concentrations of about 10 p.p.m. Because (III) was the first organic phosphorothionate to be highly fungicidal and as it contained a peracylated nitrogen atom at phosphorus, its mammalian toxicity became of practical as well as of theoretical interest. This toxicity was found to be uniquely low for a phosphorothionate pesticide—much lower than the toxicity of any other heterocyclic phosphorus derivatives reported[5]. Compound (III) proved to be a very weak esterase inhibitor and had the following acute, oral toxicity in mg/kg body-weight.

Animal:	Rats		Cavies	Chicks
Sex:	Male	Female	Male	Male
$L.D._{50}$	5,660	4,930	5,660	~4,500

The actual causes of this remarkable biological behaviour of (III) have not yet been experimentally established. They may involve the well-known fact that five-membered imide rings are readily opened by the action of basic reagents, and the fact that in phosphorus pesticides a carboxyamido group is more readily hydrolysed than the phosphorothionate group[6]. Thus, the metabolic fate of (III) may primarily arise from interaction with amidases instead of esterases. Also, there may be a distinct difference in the reaction rates involving the rupture of the carbon–nitrogen bonds and the phosphorus–nitrogen bond. The rate of attack on the trigonal, electron-deficient carbonyl-carbon of the planar phthalimide group should be relatively high. The rate of 'backside' attack on the less electron-deficient, tetrahedral phosphorus-atom may be decreased by steric interference of the phthalimide substituent. Reduction of this interference by insertion of an oxygen atom between nitrogen and phosphorus should lead to easily detectable, cholinergic activity. This conjecture is supported by the acute, oral toxicity of the insecticide, Bayer 22408 (V), on rats ($L.D._{50}$, 500 mg/kg). On the molecular $L.D._{50}$ basis, (V) is fourteen times as toxic to rats as is (III).



(V)

For a more complete description of our new fungicide, 0,0-diethyl phthalimidophosphonothionate (III), the following details should be mentioned. The compound consists of white, flat crystals that melt at 83°–84° C; it has a boiling point of 99°–101° at 7×10^{-4} mm and shows the following solubility (in moles per 100 moles of solvent at room temperature):

C_6H_6	CCl_4	$CH_3COOC_2H_5$	C_2H_5OH	S	*n* C_6H_{14}	H_2O
33·8	25	23 3	1 1	0·74	0 38	< 0 01

The symmetrical structure of (III) has also been confirmed by proton magnetic resonance. The phosphorus-31-chemical shift relative to 85 per cent phosphoric acid has a value of −56·6 p.p.m. The infra-red absorption spectrum shows the following intensities:

cm^{-1}	1,785	1,740	1,715	1,370	1,300–1,285	1,065–1,025	971
intensity	m	s	s	m	vs	vs	m

Interestingly, the compound does not undergo the thiono-thiol isomerization characteristic of compounds containing the group, $>P(S)(OC_2H_5)$. This stability evidently exists with all compounds which contain the moiety, phthalimido-$P(S)(OC_2H_5)$, but we do not know

of any other phosphoramidothionate alkyl esters possessing the same kind of stability. A preparation method for compound (III) has been published elsewhere[7].

Fungicidal activity and mammalian toxicity were investigated by D. R. Mussell and K. J. Olson, respectively. Nuclear magnetic resonance was investigated by E. B. Baker and J. P. Heeschen, and infra-red absorption by R. A. Nyquist.

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An Estimate of the Hammett Reaction Constant for the Basicities of Substituted Benzophenones

HAMMETT reaction constants ρ for the basicities of several series of carbonyl compounds are known and have been found to correlate linearly with the infra-red frequencies of the carbonyl bond[1]. This constant is not available for monosubstituted benzophenones (that is, substituted in one ring only), because the basicities of only a few of them have been measured[2,3] and the data obtained give a very poor Hammett plot. A knowledge of this constant would, however, be of interest in connexion with the linear correlation with infra-red frequencies. Therefore an attempt to estimate it indirectly is reported here.

Use can be made of the known fact that groups inserted between the benzene ring and the reaction centre produce an attenuation in the transmission of substituent effects, reflected in a reduced reaction constant ρ′, the fall-off factor ($\pi = \rho'/\rho$) being independent of the reaction centre and characteristic of the bridging group only[4]. Thus, for example, the ρ′ value for the acidities of substituted trans-cinnamic acids[5] is 0·46 (when *p*K's are plotted against σ) as compared with $\rho = 1$ for substituted benzoic acids, giving a fall-off factor of 0·46 for the vinylene group. The reaction constant for the basicities of substituted trans-chalcones[6] must be similarly reduced relative to the value for substituted benzophenones. The acidity constants of protonated substituted chalcones (pK_{BH^+}) correlate well with the σ^+ substituent constants[6], giving a ρ^+ value of 1·60 (in obtaining this value the *p*K's were corrected to the acidity function scale of Jorgenson and Hartter[7]). Using the fall-off factor of 0·46, this leads to $\rho^+ = 3{\cdot}5$ for the acidities of protonated benzophenones. This is fairly close to the value that can be obtained from the data[3] on the basicities of benzophenone and its *p*-chloro-derivative (~4·3), but is much higher than the value that may be obtained from the data[2] on the basicities of benzophenone and its *p*-methyl-derivative (~1). It is also higher than the value (~2) guessed by Yates and Scott[8]. It is remarkable that this indirectly estimated ρ^+ falls excellently on the linear plot of the ρ^+ values for the acidities of other protonated carbonyl compounds against infra-red frequencies of the carbonyl bond[9], as shown in Fig. 1.

One might suppose that using the fall-off factor for the methylene group ($\pi = 0{\cdot}489$), obtained from the acidities of phenyl-acetic acids[4], and the ρ value for the acidities of protonated substituted benzyl phenyl ketones[2], an equally satisfactory estimate of the ρ value for the acidities of protonated benzophenones could be made. The pK_{BH^+} values of protonated benzyl phenyl ketones, also corrected to the new acidity function scale[7], correlate with the σ substituent constants giving a ρ value of 0·72.

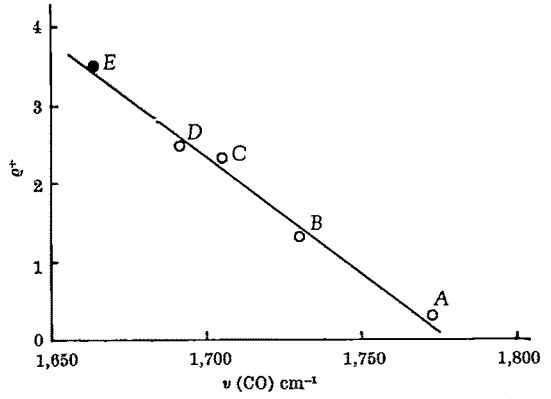


Fig. 1. Hammett reaction constants for the acidities of protonated substituted carbonyl compounds versus the carbonyl vibration frequency. *A*, Benzoyl chlorides *B*, benzoic acids; *C*, benzaldehydes; *D*, acetophenones; *A-D*, experimental values (taken from ref 1), *E*, estimated value for benzophenones (this work)

This, together with the fall-off factor for the methylene group, leads to a ρ value of only 1·43 for protonated benzophenones, which does not correlate at all well with the infra-red frequency of the carbonyl bond. The mere fact that the basicities of substituted benzyl phenyl ketones correlate with σ substituent constants, and not with σ^+, as do the basicities of other substituted carbonyl compounds with a direct link between the benzene ring and the carbonyl group, suggests that the methylene bridging group significantly alters the nature of the interaction of the substituent with the carbonyl group. It cuts out the conjugative interaction, which is transmitted effectively by the vinylene group. On these grounds the estimate of ρ for the basicities of monosubstituted benzophenones from the basicities of substituted chalcones should *a priori* be expected to be better than that from the basicities of substituted benzyl phenyl ketones.

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On the Enthalpy–Entropy Relationship

THE changes in the enthalpy (ΔH^0) and the entropy (ΔS^0) for a given process are frequently calculated from the temperature-dependence of a parameter (k) that characterizes the process. k may be a rate coefficient, a partition coefficient or even a saturation concentration of a chemical species in a solvent. This calculation is usually equivalent to the determination of both ΔH^0 and ΔS^0 from the one single equation:

$$\ln k = \Delta S^0/R - \Delta H^0/RT \qquad (1)$$

so that the two quantities ΔH^0 and ΔS^0 are not *a priori* mutually independent.

This leads to difficulties when a linear correlation is found between the values of ΔH^0 and the values of ΔS^0 in a family of measurements, because such a correlation may have no theoretical significance. If, for example, in a series of measurements on similar systems the values of ln k do not vary very much, then there may be a linear correlation between the values of ΔH^0 and the values of ΔS^0. The slope of the straight line that correlates ΔH^0 and ΔS^0 in such an instance is directly related to the

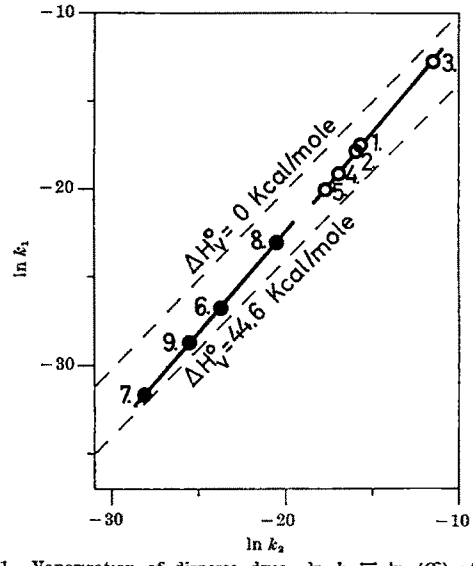


Fig. 1. Vaporization of disperse dyes. ln $k_1 \equiv$ ln (C_V) at 60° C; ln $k_2 \equiv$ ln (C_V) at 80° C. ○, Majury (ref. 3); ●, Jones *et al.* (ref. 4)

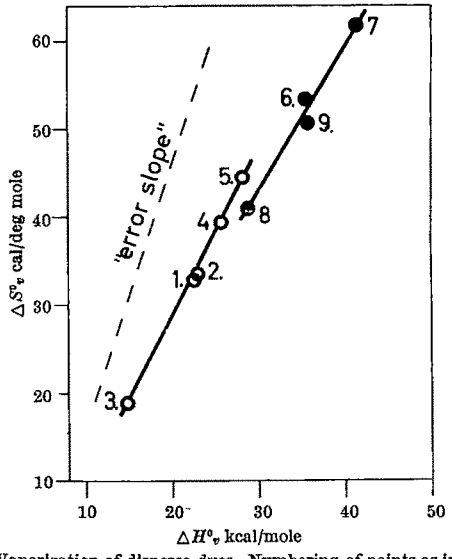


Fig. 2. Vaporization of disperse dyes. Numbering of points as in Fig. 1. Broken line is the "error slope"

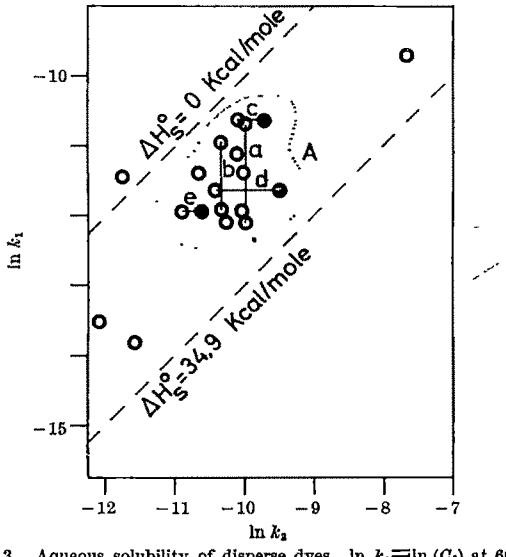


Fig. 3. Aqueous solubility of disperse dyes. ln $k_1 \equiv$ ln (C_S) at 60° C; ln $k_2 \equiv$ ln (C_S) at 80° C. ○, Patterson (ref. 5), maximum variation given by ○——○; ● Bird (ref. 6), deviation from Patterson given by ○——●. *A* denotes the "scatter area"

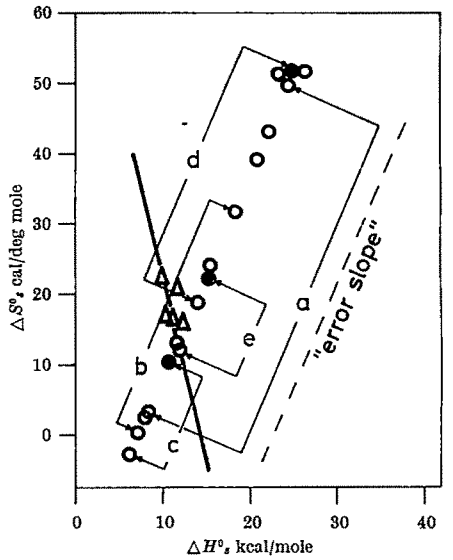


Fig. 4. Aqueous solubility of disperse dyes. The relative positions, in the new co-ordinates, of the points directly linked in Fig. 3 are indicated by the linked arrows. The correlation in Fig. 5 is here indicated by the heavy line. The broken line gives the "error slope". The symbols are as in Fig. 3, with the addition of △ (Majury) (ref 7)

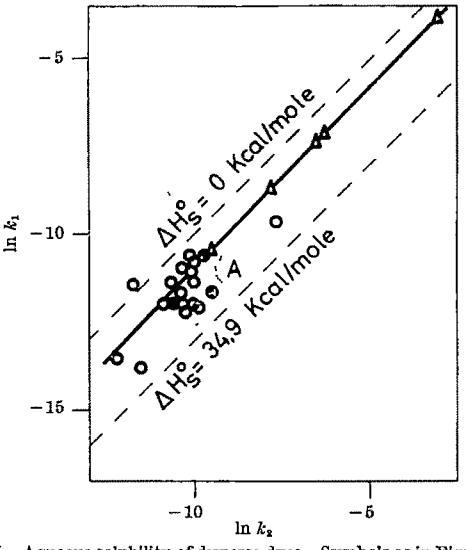


Fig. 5. Aqueous solubility of disperse dyes. Symbols as in Figs. 3 and 4. Range of ln k increased by inclusion of Majury's results (ref. 7)

mean temperature of the experiments and has been referred to as the "error slope" because this correlation may arise purely from the errors in determining ln k (refs. 1 and 2).

The problem of distinguishing the genuine from the spurious correlations between ΔH^0 and ΔS^0 is important and has been thoroughly discussed by Exner[1,2], for systems in which chemical reaction rate constants have been measured, in terms of a transformation of co-ordinates. If k_1 is the value of the parameter k when the temperature $T = T_1$, and if k_2 is the value when $T = T_2$, then a linear relationship in the co-ordinates $x^1 \equiv \Delta S^0/R$ and $y^1 \equiv \Delta H^0$ should also correspond to a linear relationship in the co-ordinates $x \equiv \log k_2$ and $y \equiv \log k_1$ (refs. 1 and 2). The problem of distinguishing the genuine correlations from the fallacious ones is therefore simplified.

In an attempt to analyse and compare the vaporization of disperse dyes with the dissolution of disperse dyes in water, we have obtained results that illustrate Exner's approach rather well, and which in addition supply measurements for a system that is completely different from the reaction-systems which he has discussed in detail[1].

Fig. 1 shows experimental results for the vaporization of disperse dyes[3,4], which have been recalculated to give the saturation vapour-phase concentrations of disperse dye (C_v, moles/l.) at 60° C and at 80° C. We have ln $k_1 \equiv$ ln (C_v) 60° C and ln $k_2 \equiv$ ln (C_v) 80° C. The linear correlations in the co-ordinates ln k_2 and ln k_1 remain as linear correlations in the co-ordinates ΔS_v° and ΔH_v° as is clear from Fig. 2. The correlation in Fig. 2 therefore appears to be genuine; in support of this the "error slope" is also given and it can be seen that it is quite different from that of the correlation lines.

In contrast to this, Fig. 3 shows some results for the saturation aqueous solubilities (C_s, moles/l.) of disperse dyes[5,6] and now ln $k_1 \equiv$ ln (C_s) 60°, while ln $k_2 \equiv$ ln (C_s) 80° C. The results form a scattered area A and the experimental uncertainty seems to be rather large. There is no clear correlation between the results in Fig. 3 in the ln k_2 and ln k_1 co-ordinates. A clear-cut correlation occurs, however, between the ΔS_s° and ΔH_s° co-ordinates. The error-lines from Fig. 3 have been transformed to Fig. 4 together with the "error slope". The "error slope" correlation in Fig. 4 is for this reason of doubtful significance.

When the range of variation of ln k is increased, however, by including aqueous solubilities measured by Majury[7], a correlation between the ln k_2 and ln k_1 co-ordinates becomes apparent, in addition to the area of scatter A (Fig. 5). In the ΔS_s° and ΔH_s° co-ordinates, the correlation in Fig. 5 is so masked by the effect of the "scatter area" A as to be difficult to detect (Fig. 4).

It would seem that these results support Exner's analysis of these problems[1]; they will be discussed in more detail elsewhere in the context of, for example, the Barclay and Butler rule[8,9].

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BIOPHYSICS

Platelet Aggregation in the Variable-frequency Thromboviscometer

The importance of platelet aggregation in the initiation of thrombosis is now established, as is the variation in the quantity of these platelet masses with the conditions of flow at the site of deposition[1,2]. The morphology of an arterial thrombus consists of large amorphous masses (composed of platelet aggregates) surrounded by leucocytes and embedded in a fibrin network. A venous thrombus contains fewer platelet masses, whereas the glass tube clot has no visible masses on light microscopy and is made up entirely of a network of fibrin with adherent red cells. A quantitative relationship between the degree of aggregation of platelets and the velocity gradient *in vitro*, using a rotational cone-in-cone viscometer, have recently been demonstrated[3,4] and it was suggested that this apparatus[5] might provide a more physiological method of investigating coagulation in relation to thrombosis, as at high rates of shear the coagulation produced was identical with an arterial thrombus.

The use of a thrombelastograph[6,7], to study the blood in thrombotic states, has led to the demonstration of characteristics said to be typical of such states; namely, a shortened clotting time (r), and a rapid appearance of the point of maximal deflexion (or amplitude) of the curve (MA), producing a "tuning fork" appearance. Hartert's thrombelastograph uses a constant and low frequency of oscillation, and has a low amplitude of deflexion. The morphology of the clot obtained is a fibrin network with no visible platelet masses, and is thus characteristic of a glass tube clot[7].

Recently, one of us (L. D.) devised an oscillatory viscometer and applied it to the investigation of clotting time and viscosity of blood during coagulation[8]. It was possible to show that (*a*) the clotting time of blood decreases with the increased frequency of oscillation and (*b*) the consistency of blood clots decreases progressively with the increased frequency of oscillation applied during the process of coagulation.

This instrument, now called a variable-frequency thromboviscometer oscillates over a range of frequencies, from 1 to 300 c/min, the amplitude of oscillation of the external cone being 70°. The temperature is kept at 37° C using a mercury-contact thermometer and relay, while the measurement of deflexions of the torsion strip and thus of the inner cone is carried out in the manner previously described for the cone-in-cone viscometer[4,5,9]. Blood, obtained by venepuncture from human donors without a haematological disorder or a thrombotic state, is poured into the gap between the cones and the clotting time is observed at certain fixed frequencies of oscillation. In the present experiments, three samples were taken from each donor and usually tested at frequencies of 6·6, 33 and 166 c/min. The blood coagula obtained in each experiment were then fixed in formalin, paraffin sectioned, and stained with haematoxylin and eosin. The structure can be determined microscopically, and a quantitative estimation of the platelet masses can be carried out by means of a graduated eyepiece. This eyepiece is divided into squares which correspond to a surface area of 0·01 mm² on the specimen. A systematic scan of each slide allows determination of the number of squares occupied by platelet masses per ml. of original blood sample, and thus gives an estimate of the quantity of platelet aggregation in each experiment.

A typical record of the coagulation results obtained in this investigation is shown in Fig. 1. It is clear that the curves obtained resemble those described in the standard thrombelastograph[6]. However, in a manner analogous to coagulation in the rotational cone-in-cone viscometer[4], the clotting time decreases as the frequency (or mean rate of shear) increases. Simultaneously, the viscosity or consistency of clots falls as the frequency to which they are subjected rises. The morphology of the coagula obtained also bears a similarity to our previous findings. The number of squares representing surface area of platelet masses obtained from 1 ml. of blood is shown for each frequency in Table 1. There is a rise in area of platelet aggregates corresponding to the rise in frequency of oscillations (or mean rate of shear) at the time of the experiment. This is further shown in Fig. 2 where a gradual change and increase in platelet masses are observed, eventually producing a structure, similar to arterial thrombosis, at high oscillatory frequency. While the increase of irreversible platelet agglomeration with the rise in velocity gradient appears of great physiological interest, some questions still remain unanswered. The reduced clotting time associated with an increase in velocity gradient cannot be explained, although three suggestions might be given: first, that more effective diffusion of clotting factors might occur because of the high velocity gradient; secondly, that this effect might be due to the decreased viscosity of the original blood at higher velocity

Table 1. COAGULATION AND DEGREE OF PLATELET AGGREGATION DETERMINED BY MEANS OF THE VARIABLE-FREQUENCY THROMBOVISCOMETER

Case	Frequency (c/min)	r	MA	Viscosity (poises)	Platelet aggregation (No. of squares)
1A*	6·6	11 m 30 s	26	115	27
1B*	33	7 m 15 s	13	11·2	32
2A*	6·6	9 m 30 s	28	124	10
2B*	66	6 m 0 s	4·8	2·15	96
3A*	132	12 m 0 s	2·4	0·55	52
3B*	232	3 m 0 s	2·3	0·34	56
4A	132	4 m 0 s	2·2	0·50	126
4B	232	2 m 0 s	2·8	0·41	116
5A	6·6	5 m 0 s	39·2	173	3
5B	33	3 m 40 s	32·0	27·7	18
5C	166	1 m 15 s	—	—	—
6A	6·6	5 m 0 s	28·8	127	4
6B	33	3 m 40 s	21·4	18·5	21
6C	166	1 m 35 s	14·4	2·5	149
7A	6·6	5 m 15 s	34·0	150	20
7B	33	2 m 40 s	30·0	26	25
7C	166	1 m 30 s	18·0	3·1	108
8A	6·6	5 m 15 s	27·5	121	13
8B	33	2 m 15 s	17·0	14·6	17
8C	166	1 m 40 s	11·8	2·0	70
9A	6·6	6 m 40 s	18·0	80	39
9B	33	2 m 45 s	17·0	14·6	62
9C	166	2 m 0 s	5·4	0·9	153
10A	6·6	7 m 0 s	35·0	154	14
10B	33	3 m 0 s	24·0	20·7	23
10C	166	2 m 15 s	3·0	0·52	84
11A	6·6	5 m 5 s	28·4	125	7
11B	33	2 m 30 s	26·0	22·5	16
11C	166	2 m 30 s	17·4	3·05	117

r, Clotting time, measured from the moment when the first drop of blood appears in the syringe during venepuncture to the moment when blood viscosity starts to increase; MA, maximum amplitude, arbitrary units.
* The first few cases were investigated at room temperature; all the others were tested at 37° C.

gradients; and thirdly, that some unidentified mechanochemical transformation within platelets might contribute to the higher reaction rate at high velocity gradients. The latter point might not be of paramount importance, as decrease of clotting time at higher velocity gradients has also been observed[4] in platelet-poor blood.

Hartert[6] noted that the maximal amplitude of the curves obtained in thrombelastography depends on the platelet concentration, a decrease in amplitude being related to a fall in platelet count. We cannot comment on the correlation between amplitude and platelet concentration in the multiple-frequency thromboviscometer. The amplitude was found to be greatly sensitive to changes in the frequency and rate of shear. It has been suggested that the fall in consistency with increasing frequency or

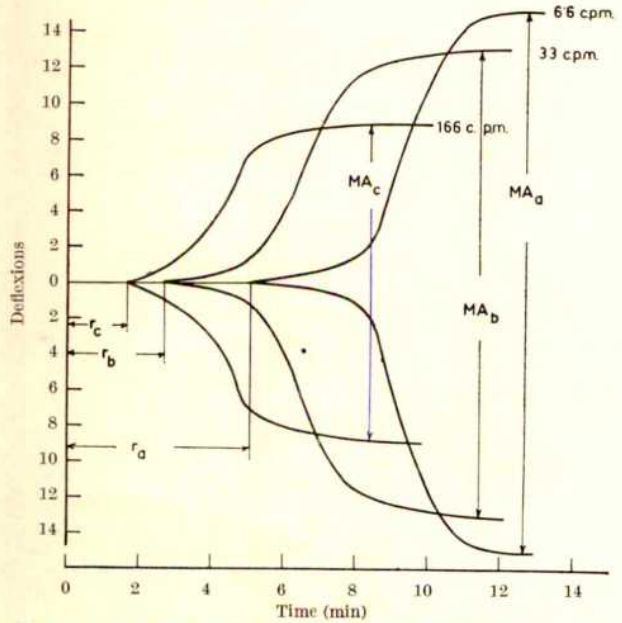


Fig. 1. Consistency of coagulating blood determined by means of the variable-frequency thromboviscometer at 37° C. MA, Maximum amplitude; r, clotting time; subscripts a, b, and c refer to the blood samples coagulating at frequencies of oscillation 6·6, 33 and 166 c/min, respectively. Please note that increasing frequency reduces both the consistency of blood coagula and the clotting times

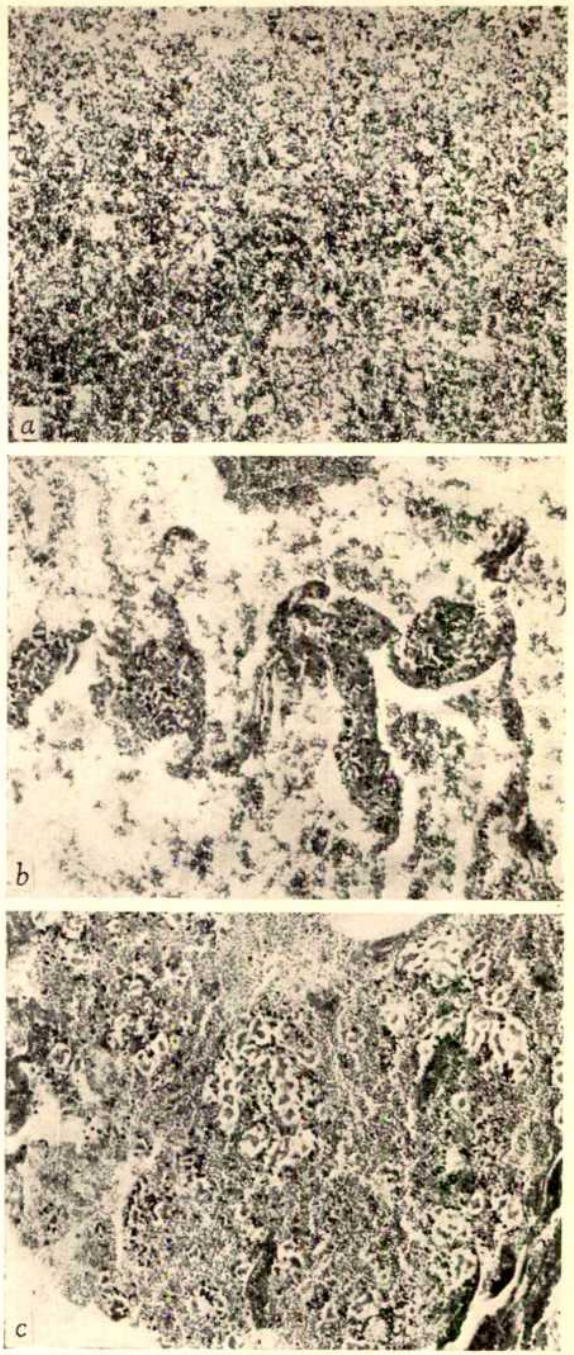


Fig. 2. (*a*) Section of coagulum obtained in thromboviscometer at 6·6 c/min. The structure consists of a fibrin layer with red cells and white cells in random distribution. The morphology is identical with a glass tube clot (× 60). (*b*) Section of coagulum obtained in thromboviscometer at 33 c/min. The fibrin network is still evident, but small amorphous masses consisting of platelet aggregates and surrounded by leucocytes are now evident in the centre of the field (× 60). (*c*) Section of coagulum obtained in thromboviscometer at 166 c/min. Amorphous masses surrounded by leucocytes are abundant and the fibrin network is reduced in quantity. This structure resembles *in vivo* thrombosis (× 60)

velocity gradient[8] reflects the variable orientation of fibrin during polymerization. This process will require further elucidation. The role of platelets in the rheology of blood coagula is rather obscure. Neither free platelets nor platelet aggregates contribute significantly to the viscosity of blood or consistency of blood coagula, respectively.

It could be added that as thrombosis is a process occurring in the body in flowing blood, any investigations related to this process should attempt to simulate these

conditions. As the morphology, consistency and clotting time in coagulation have been shown to depend on the frequency and rate of shear, the future investigation of blood coagulation in the oscillatory viscometer (variable-frequency thromboviscometer) would appear of definite value.

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RADIOBIOLOGY

Radiation-induced Inhibition of Thymidine Incorporation by Mammalian Cells *in vitro*

THE influence of ionizing radiation on the metabolism of DNA has frequently been studied using radioactive precursors such as tritium- or carbon-14-labelled thymidine. In many systems the uptake of tracers into DNA was clearly suppressed according to a biphasic dose-effect curve[1–3]. However, effects of pool size have been observed as well, that is, the observed effect is reverted in increasing concentrations of the labelled thymidine. This phenomenon, first observed by Hell *et al.*[4] in ascites cells *in vitro*, was tentatively explained by the authors as a lowering of the specific activity of the tracer by DNA breakdown products. In HeLa S_3 cells, a similar concentration dependent effect was noted by Painter and Rasmussen[5], but here the effects were attributed to an impairment of the rate of DNA synthesis by high thymidine concentrations, masking some of the effects of X-irradiation.

In view of the importance of the aforementioned pool effects in elucidating the influence of ionizing radiation on DNA metabolism, the incorporation of ^{14}C-thymidine (^{14}C-TdR) into DNA of control and irradiated cells was re-investigated in graded concentrations of the tracer. The cell line used was a bovine liver strain isolated in our laboratory[6] and cultivated as a monolayer in small Carrel flasks. The medium consisted of 25 per cent calf serum and 0·5 per cent lactalbumin hydrolysate in Hanks's balanced salt solution. X-irradiation was performed with a 30 m.amp, 50 kVp unit. With filtering by the glass covering of the vessels (uniformly 1 mm thick), a dose rate to the cells was achieved of 405 r./min at a distance of 20 cm. Based on previous experience[7], thymidine incorporation was investigated within a 2 h period following irradiation with 500 r. In these circumstances, observed effects all fell within the range of the steep component of the well-known biphasic dose-response curve (denoted as the S_1 effect according to Lajtha *et al.*[1]).

Cultures containing $5 \cdot 10^6$ cells each were incubated in medium with various concentrations of ^{14}C-TdR (specific activity 30 mc./mmole) for 2 h following irradiation or sham irradiation. At the end of the incubation period, cells were trypsinized from the vessels and their DNA isolated[8]. Trypsinized cells were washed consecutively in 5 ml. of each of the following solutions: 96 per cent alcohol, 70 per cent alcohol and twice 96 per cent alcohol–di-ethyl ether (3 : 1). RNA was removed by incubation for 18 h in 0·5 N perchloric acid (PCA) at 4° C, the resulting suspension was centrifuged and the pellet washed with cold 0·5 N PCA. DNA extraction was accomplished by incubation in 1 N PCA for 45 min at 70° C. After cooling, an equimolar amount of potassium hydroxide was added and cell debris, together with insoluble potassium perchlorate, were spun off. Equal portions of the supernatant were used for the estimation of DNA using Burton's modification of the diphenylamine test[9] and for the measurement of radioactivity by means of a Geiger–Müller type counter. A comparison of the specific activities of DNA extracted with hot acid with that of DNA digested with DNase I showed that no part of the radioactivity in the acid extract originated from other sources than DNA after incubation of cells in either ^{14}C-thymidine or phosphorus-32. Incorporation rates were expressed as the specific activity of the isolated DNA. The results of a typical experiment are given in Fig. 1.

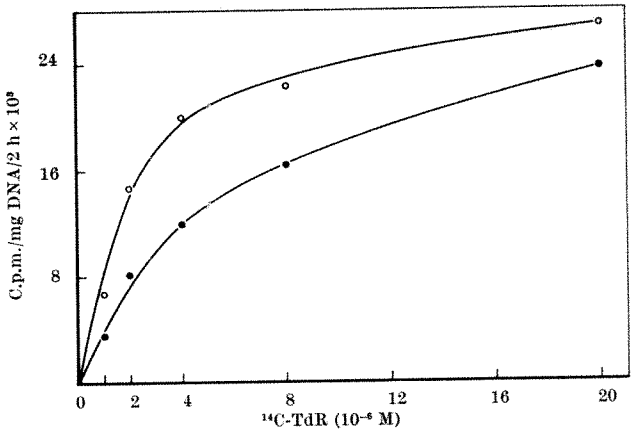


Fig. 1. The incorporation of ^{14}C-TdR at different concentrations of the tracer in control (○——○) and irradiated (●——●, 500 r.) cultures. Data are based on measurements of specific radioactivity of DNA isolated after 2 h of incubation. Each point is the average of from three to five individual determinations

Table 1. DNA SPECIFIC ACTIVITY IN CONTROL AND IRRADIATED (= 500 R.) CULTURES AFTER A 2 H PERIOD OF INCUBATION IN EITHER $^{32}P_i$ (765×10^{-6} M) OR IN ^{14}C-TdR (10^{-6} M)

Tracer used	Specific activity (c.p.m./mg DNA) control	500 r.	Change (per cent)
$^{32}P_i$, 765×10^{-6} M, 0·5 μc./ml.	1,178* ± 71†	1,203 ± 94	+2
^{14}C-TdR, 10^{-6} M, 0·03 μc./ml.	6,326‡ ± 289	3,248 ± 181	−49

* Average of seven individual determinations.
† Standard deviation.
‡ Average of six individual determinations.

As expected, irradiation results in a decrease in thymidine incorporation from the medium, but it is also evident that this decrease is progressively less pronounced in series of media with increasing concentrations of exogenous thymidine (that is 50 per cent decrease at 10^{-6} M but only 10 per cent at 2×10^{-5} M). If data of Fig. 1 are represented in a Lineweaver–Burk plot[10], no significant difference can be observed between the "V_{max}" of controls and cultures receiving 500 r. (Fig. 2). The effect of irradiation in these circumstances seems to be mainly competitive inhibition in the uptake of exogenous thymidine into DNA, leaving the enzymes involved in thymidine incorporation unaffected. In addition, if we keep in mind that cells *in vitro* do not need exogenous thymidine for sustaining their synthesis of DNA, there is considerable doubt as to whether X-rays affect this synthesis at all under our experimental conditions. Therefore, instead of labelled thymidine, phosphorus-32 as inorganic phosphate (P_i) was used for studying the rate of DNA synthesis after

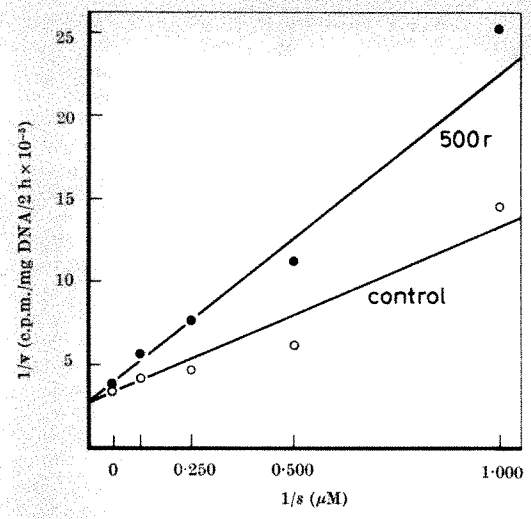


Fig. 2. Data of Fig. 1 taken as indices of ^{14}C-TdR incorporation rates and represented in a Lineweaver–Burk plot. Control, $V_{max} = 4{\cdot}0 \times 10^4$ c.p.m./mg DNA/2 h. 500 r., $V_{max} = 3{\cdot}9 \times 10^4$ c.p.m./mg DNA/2 h

500 r. The label was added as carrier-free phosphorus-32-labelled potassium dihydrogen phosphate to obtain 0·5 μc. per ml. culture fluid. Total concentration of inorganic phosphate in this medium was 765×10^{-6} M, high enough to swamp out any pool effect. For this reason, $^{32}P_i$ is considered to be a more accurate tracer for determining DNA synthesis. As can be seen in Table 1, no decrease of phosphate incorporation is observable after 500 r. as compared with the incorporation of labelled thymidine (at 10^{-6} M concentration).

These observations are consistent with our assumption of an unchanged rate of DNA synthesis after irradiation (so far as the S_1 effect is concerned). In explaining their finding of a thymidine dependent reversal of the effect of irradiation, Painter and Rasmussen[5] attributed this effect to an influence of high thymidine concentrations on the rate of DNA synthesis in HeLa S_3 cells. For my cell line, however, this possibility could be ruled out. The uptake of $^{32}P_i$ into DNA was investigated at various concentrations of non-radioactive thymidine up to 30×10^{-6} M over 2 h, but I was not able to detect any decrease in the rate of incorporation. My results then, namely, a competitive inhibition of thymidine incorporation together with an unchanged rate of DNA synthesis, can be explained by assuming that the endogenous (and thus non-radioactive) pools of thymidine are augmented after irradiation as a result of DNA breakdown according to the hypothesis of Hell *et al.*[4]. These breakdown products will compete with labelled substrate for incorporation into DNA and decrease the uptake from the medium in lowering concentration gradients.

The questions arise as to why so few reports on pool effects exist and why Hell *et al.*[4] were able to detect them only in ascites cells and not in bone marrow cells *in vitro* and in mouse thymus cells *in vivo*. Though far from suggesting that this effect is a general one, one nevertheless could offer a reason for the inability to detect pool effects in some cases. First of all it will be clear that exogenous thymidine concentrations have to be varied within a range where there exists some proportionality between concentration and incorporation rate. From the data of Marin and Bender[11] it can be deduced that in HeLa S_3 cells *in vitro*, maximum incorporation rates already occur at thymidine concentrations of 2×10^{-6} M. Using these HeLa S_3 type cells, Painter and Rasmussen detected concentration dependent effects at 0·15 and 10×10^{-6} M thymidine[5], but no differences[12] in a range of $15–40{,}000 \times 10^{-6}$ M, which might be explained by assuming that in the latter series the maximum rate of incorporation had already been reached at the lowest concentration tested. Furthermore, in my cell strain, maximum incorporation rates only occur at concentrations of 20×10^{-6} M or higher (see Fig. 1), which suggests that there are considerable differences between the abilities of cell strains to use exogenous thymidine in the synthesis of DNA. In view of these differences it seems appropriate to investigate whole "Michaelis–Menten" curves[10] in detecting (or excluding) pool effects after irradiation.

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Distribution of Caesium and Rubidium in Human Blood

DURING an investigation of the total body burden of caesium-137 in human beings[1], the caesium-137 content of various samples of blood was analysed. About 100 ml. of blood taken from male subjects was heparinized and centrifuged for 10 min at 3,000 r.p.m. Twenty mg of carrier caesium was added to the plasma and erythrocyte fractions, and the content of caesium-137 determined radiochemically[2]. The results (Table 1) for two subjects on three occasions show a fairly consistent ratio of 1 : 3 for the caesium-137 content of the plasma to that of the erythrocytes.

The sodium and potassium metabolism of erythrocytes has been extensively studied and it is known that the greater part of the latter, more than 90 per cent, is located in these cells. Rubidium and caesium are members of the heavy alkali metals and resemble potassium in many of their chemical and biological properties. Nevertheless, the distribution pattern found for caesium-137 (a fall-out product of nuclear explosions which becomes incorporated in man) differs from that of potassium.

From *in vitro* tracer experiments, Love and Burch[3] found that rubidium and potassium were incorporated in human erythrocytes. To confirm the possibly different distribution in blood *in vivo* of caesium to potassium and rubidium, stable caesium and rubidium were measured by the neutron activation followed by gamma spectrometry[4] in blood fractions taken from subjects by the method described previously. The results are shown in Table 2. Ninety-eight per cent of the rubidium present in blood is located in the erythrocytes. This supports the findings of Love and Burch, but disagrees with those of Bertrand and Bertrand, who reported that the erythrocytes contained about three times as much rubidium as did plasma[5].

The whole blood concentration of rubidium was found to be fairly constant in four individuals (1·6–2·0 mg/kg) and came within the range reported by Bertrand and Bertrand (1·63–4·95 mg/l.)[6]. The relative variation of the whole blood concentration was much greater for caesium than for rubidium, and came to 1 : 10, and the range of the rubidium : caesium ratio in individuals is from 5 to 39. The average ratio of the contents of caesium in the plasma to that in erythrocytes is 1 : 3, which is in good agreement with that found for caesium-137.

Table 1. DISTRIBUTION OF CAESIUM-137 IN HUMAN BLOOD

Time of collection	Subject	Concentration in Whole blood (pc./kg)	Plasma (per cent)	Erythrocytes (per cent)
3/65	Ku	49·7	26·7	73·3
3/65	Iw	63·7	20·6	79·4
4/65	Ku	45·7	29·3	70·7
4/65	Iw	56·5	28·5	71·5
6/65	Ku	29·5	25·4	74·6
6/65	Iw	35·0	20·3	79·7
			Av. 25·1 ± 3·8	74·9 ± 3·8

Table 2. DIFFERENT DISTRIBUTION OF STABLE CAESIUM and RUBIDIUM IN HUMAN BLOOD

	Subject	Concentration in Whole blood (p.p.m.)	Plasma (per cent)	Erythrocytes (per cent)
Rubidium	Ku	1·8	< 2·3	> 97·7
	Ya	2·0	3·2	96·8
	Ar	1·6	< 2·3	> 97·7
	Iw	1·8	< 2·1	> 97·9
Caesium	Ku	0·046	26·0	74·0
	Ya	0·40	40·4	59·6
	Ar	0·042	24·3	75·7
	Iw	0·13	11·8	88·2

It is generally accepted that potassium, rubidium and caesium are univalent ions of intracellular importance, which is in contrast to sodium, which is distributed extracellularly throughout the body. However, it has been pointed out by numerous workers that while rubidium closely resembles potassium in many of its chemical and biological properties and metabolic pathways, caesium does not. The difference between rubidium and caesium cannot be attributed to differences in ionic radii, ion-exchange and adsorptive properties. An example of this is the distribution study of the heavy alkali elements in soil and native plants[7]. A greater variation in the ratio of caesium : potassium was found in plants as compared with that for rubidium : potassium. The existence of any correlation between the caesium and potassium contents of plants is doubtful ($r = 0{\cdot}18$); however, in contrast, a good correlation is found between rubidium and potassium ($r = 0{\cdot}72$).

The present investigation has been confined to the static aspect of the distribution in human blood. A dynamic study by means of radioactive tracers *in vivo* in human beings is being carried out in the hope of demonstrating metabolic difference between caesium and rubidium or potassium.

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BIOCHEMISTRY

Interaction of Actin and Myosin during Clearing

SUPERPRECIPITATION of actomyosin has long been thought to involve both binding of the actin to the myosin and activation of the myosin adenosine triphosphatase (ATPase) by actin in the presence of magnesium[1]. The clearing response, on the other hand, has been reported to result from dissociation of the actomyosin with a concomitant loss of this ATPase activation[2]. In both situations, the physically observable binding and the activation of the myosin ATPase by actin were thought to change in parallel[3], but some recent observations have been difficult to reconcile with this conclusion. In investigating the effect of tropomyosin *B* on actomyosin, A. M. Katz[4] noted that actin can activate the myosin ATPase without superprecipitation occurring. In other investigations, Leadbeater and Perry[5], and Perry and Cotterill[6], found that actin activates the ATPase of heavy meromyosin under conditions where viscosity measurements indicated that the two proteins might be dissociated. Extrapolating these data to the actomyosin system, Perry *et al.* argued that the interaction of actin and myosin which results in activation of the myosin ATPase is not accompanied by physical interaction as strong as that which occurs between the two proteins in the absence of adenosine triphosphate (ATP). Maruyama and Gergely[2], however, noted that actomyosin undergoes a brief viscosity increase simultaneously with the rise in ATPase just before superprecipitation, which suggests that strong physical interaction does accompany the activation of the myosin ATPase by actin.

In the present investigation we compared the turbidity, ATPase, and viscosity of actomyosin at various ionic strengths to determine, first, to what extent clearing represents dissociation of the actin from the myosin, and second, whether it is possible to demonstrate activation of the myosin ATPase by actin in the absence of measurable physical interaction between the two proteins.

Myosin was prepared by the method developed by Szent-Györgyi[1] and actin by the method of Ulbrecht *et al.*[7], with the extraction from the acetone-dried powder carried out at 0° C in order to avoid contamination with tropomyosin[8]. Turbidity was measured as absorbance at 675 mμ[9], ATPase activity was recorded with a *p*H-stat, and viscosity was determined with an Ostwald viscometer having an outflow time for water of about 1 min. In all cases, the reaction was initiated by the addition of myosin and the reported measurements were taken within three minutes thereafter. For all measurements the temperature was regulated at 25° C. The absorbance, ATPase, and viscosity (log $\eta_{rel.}$) of the actomyosin samples are presented in Table 1 as multiples of the theoretical values which would be expected in the absence of interaction between the two proteins. This theoretical value for each parameter is calculated as the sum of its values for actin and myosin individually[1], which in turn were measured under conditions identical to those used in the corresponding actomyosin experiments.

Table 1. TURBIDITY, ATPASE AND VISCOSITY OF ACTOMYOSIN*

Conc. of potassium chloride (M)	Clearing time (min)	Multiples of theoretical values† A_{675}	ATPase	Log $\eta_{rel.}$
0·50	—	1·0	1·0	1·01
0·13	31	1·1	3·5	1·07
0·12	18	1·0	4·5	1·20
0·105	12	1·0	5·5	1·30
0·0925	6	1·0	9·5	1·53
0·0925 (after superprecipitation)	—	~ 8·0	~ 20·0	—
0·50 (no ATP)	—	—	—	2·62

* All samples contained 2 mM ATP, 1 mM magnesium chloride, 10 mM imidazole buffer (*p*H 7), 2 mg/ml. myosin, 0·5 mg/ml. actin, and potassium chloride as indicated, in a total volume of 7·5 ml. Myosin was added last and all measurements were made within 3 min after the start of the reaction. Clearing times were determined as the time from the start of the reaction until half-maximal turbidity was reached.

† The theoretical values were approximately as follows: $A_{675} = 0{\cdot}10$; ATPase = 0·01 μmole/min.mg myosin; log $\eta_{rel.} = 0{\cdot}23$.

It can be seen in Table 1 that the turbidity of the actomyosin suspension is in every case equal either to the theoretical value for no interaction, which represents clearing, or to a value seven to eight times higher, which represents superprecipitation. However, as the ionic strength is reduced under conditions where the actomyosin remains completely cleared, the ATPase activity rises to a value considerably higher than that of the myosin alone under identical conditions. This confirms the observation of A. M. Katz[4] that actin can activate the myosin ATPase without superprecipitation, and furthermore, our data indicate that as much as ten-fold activation can occur during clearing as compared with the approximately twenty-fold activation which occurs during superprecipitation.

To determine whether physical interaction of the actin and myosin accompanies this ATPase activation, the viscosity of the cleared actomyosin was measured. As shown in Table 1, the increase in the myosin ATPase was accompanied in all cases by a parallel increase in log $\eta_{rel.}$ to values considerably higher than that expected theoretically. As these measurements were taken within 3 min of the start of the reaction, it is evident from the clearing times that the elevated viscosity is not limited to the period just prior to superprecipitation. Furthermore, this viscosity increment during clearing can reach an appreciable fraction of that observed at high ionic strength in the absence of ATP.

From these observations it can be concluded that, although actomyosin can be completely dissociated during clearing under certain conditions[2], under other conditions both physical binding of the actin to the myosin and activation of the myosin ATPase can occur with the system remaining completely cleared. Furthermore, ATPase activation during clearing was always accompanied by the formation of bonds between the two proteins sufficiently strong to give rise to a parallel increase in viscosity, thus suggesting that the interaction which leads to ATPase activation is comparable in strength to that which causes the viscosity increase in the absence of ATP at high ionic strength.

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Acetylcholine and the Incorporation of [^{32}P] Phosphate into Phospholipids and Phosphoproteins of Nerve Endings of Developing Rat Brain

IT has been shown that acetylcholine stimulates the incorporation of [^{32}P] phosphate into the phospholipids of slices, homogenates, and microsomal fractions of guinea-pig cerebral hemispheres under conditions of oxidative phosphorylation[1,2]. Maximal stimulation of phosphorus-32 incorporation was found to occur in phosphatidic acid and phosphoinositide[2].

In a previous communication on the *in vivo* incorporation of phosphorus-32 into the lipids and proteins of the subcellular fractions of developing rat brain, the phospholipids of the nerve endings fraction were found to have the lowest specific activity[3].

In the present *in vitro* experiments, investigations were undertaken to determine the influence of exogenous acetylcholine, in the presence of physostigmine, on the incorporation of phosphorus-32 into both phospholipids and phosphoproteins of the nerve endings of the adult and developing rat brain. It was found that concentrations of the order of 10^{-4} M–$4{\cdot}75 \times 10^{-4}$ M acetylcholine inhibited the incorporation of phosphorus-32 into both lipids and proteins.

Nerve ending fractions were isolated from the conventional mitochondrial preparations by means of density gradient centrifugation in a Ficoll medium as was previously described[3,4].

Incubations were carried out in a medium containing: 10 mM pyruvate, 1·22 mM malate, 0·54 mM adenosine triphosphate, 0·5 mM cytidine diphosphate, 0·5 mM guanosine triphosphate, 0·1 mM nicotinamide-adenine dinucleotide, 0·006 mM cytochrome *c*, 6·7 mM magnesium chloride, 6 mM nicotinamide, 6·7 mM potassium fluoride, 3·7 mM glucose, 12·5 mM potassium phosphate buffer (*p*H 7·5), 0·11 mM ethylenediamine tetraacetic acid, 100–350 μc. of phosphorus-32. When added, acetylcholine and physostigmine were each $4{\cdot}75 \times 10^{-4}$ M or 10^{-4} M. All incubations were carried out at 37° C for 1 h with shaking in a final volume of 3·2 ml., and contained the nerve endings fraction, which corresponded to 0·3 g of wet brain. The Ficoll was washed off with distilled water before adding the nerve endings to the reaction mixture. This step probably removed the labile acetylcholine from the synaptosomes[5].

At the end of incubation 2 ml. of chilled 10 per cent trichloroacetic acid were added to the incubation mixture. Analytical techniques, and the techniques used for the removal of contaminating phosphorus-32, extraction of lipids, nucleic acids, thin-layer chromatography, paper electrophoresis, autoradiography, and determination of specific activity have already been described[3].

Results on the effect of acetylcholine on the incorporation of phosphorus-32 into phospholipids and phosphoproteins are shown in Table 1. In the presence of $4{\cdot}75 \times 10^{-4}$ M acetylcholine, there was an inhibition of phosphorus-32 incorporation up to 40 per cent into phospholipids and up to 42 per cent into phosphoproteins.

At a lower concentration of acetylcholine (10^{-4} M), inhibition of phosphorus-32 incorporation into phospholipids, but not phosphoproteins, was increased (Table 1). Physostigmine alone (10^{-4} M or $4{\cdot}75 \times 10^{-4}$ M) had no effect on the incorporation of phosphorus-32. In a previous investigation[4] of the developing rat brain, the presence of nerve endings was demonstrated in the embryonic stage. Because the nerve impulse appears 10 days post partum in rat brain[6], the present finding that acetylcholine has similar effect on the incorporation of phosphorus-32, regardless of age, could suggest a regulatory function for this neurohormone on phosphate metabolism in nerve endings, in addition to that of synaptic transmission. It is interesting to note that Hokin *et al.*[7], working with guinea-pig adrenal medulla, observed an inhibitory effect of acetylcholine (10^{-3} M) on the incorporation of phosphorus-32 into phospholipids.

Table 1. ACETYLCHOLINE EFFECT ON LABELLING OF LIPIDS AND PROTEINS OF NERVE ENDINGS WITH PHOSPHORUS-32 [PHOSPHATE]

	Specific radioactivity of lipids					Specific radioactivity of proteins × 10^{-3}				
Age in days	Control	Acetylcholine $4{\cdot}75 \times 10^{-4}$ M (c.p.m./μmole P)	10^{-4} M	Δ	Inhibition (per cent)	Control	Acetylcholine $4{\cdot}75 \times 10^{-4}$ M (c.p.m./mg N)	10^{-4} M	Δ	Inhibition (per cent)
Embryo*	3,790	2,291	–	1,499	39·6	73·5	62·2	–	11·3	15·3
12	5,567	3,935	–	1,632	29·3	126·3	95·6	–	30·7	24·3
12	5,820	–	2,616	3,204	55	74·2	–	56·8	17·4	23·4
Adult	4,500	2,960	–	1,540	34·3	115·1	67·2	–	47·9	41·4

* 14–16 days gestation.
The results are averages of two separate experiments in the embryo, and three separate experiments in the 12-day and adult, and the values agreed within 5–12 per cent.

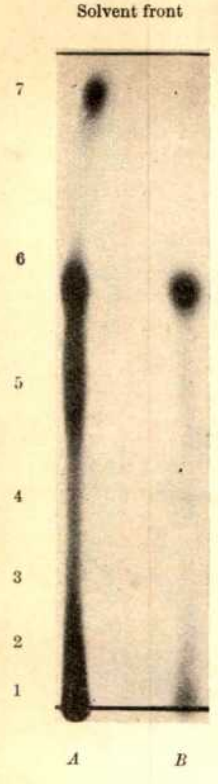


Fig. 1

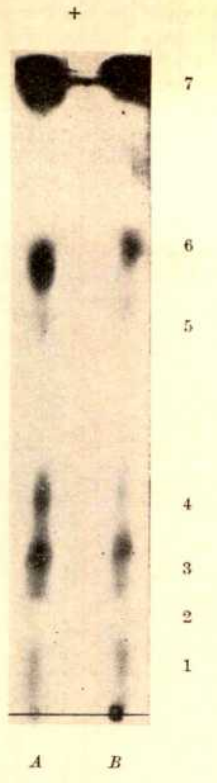


Fig. 2

Fig. 1. Autoradiograph of a thin-layer chromatogram of total phospholipids; solvent system: chloroform–methanol–acetic acid–water (25 : 15 : 4 : 2, by volume). Plates were exposed to iodine vapour, and the spots counted. *A*, *B*, Acetylcholine omitted and present respectively. 1, Phosphorus-32 and proteolipids (*A*, *B*, 864 c.p.m., 180 c.p.m., respectively); 2, lysophosphatidyl choline (776, 132); 3, sphingomyelin (756, 132); 4, phosphatidyl choline (916, 148); 5, phosphatidyl inositol (1772, 328); 6, phosphatidyl serine (242, 48); 7, phosphatidic acid (160, 44)

Fig. 2. Autoradiograph of an ionopherogram of a phosphoprotein hydrolysate of nerve endings of 12-day-old rats. The phosphoprotein was hydrolysed in 12 N hydrochloric acid at 38° C for 24 h. Electrophoresis was run at *p*H 3·3, pyridine–acetic acid–water buffer, 6,000 V-h. *A*, Acetylcholine omitted; *B*, acetylcholine added. 1, 2, 3 and 4, Phosphopeptides; 5, phosphothreonine; 6, phosphoserine; 7, phosphorus-32

Acetylcholine, in concentrations of the order of 10^{-4} M or $4{\cdot}75 \times 10^{-4}$ M, had no effect on the incorporation of serine uniformly labelled with carbon-14 serine into phospholipids or phosphoproteins.

When the phosphorus-32-labelled phospholipids were run in one-dimensional thin-layer chromatography[8], phosphatidyl inositol had the highest activity in the presence or absence of acetylcholine (Fig. 1). Ionophoresis of phosphoprotein hydrolysates (Fig. 2) at *p*H 3·3, together with phosphoserine and phosphothreonine controls, revealed the presence of phosphorus-32-labelled phosphoserine and phosphothreonine as well as phosphorus-32-labelled phosphopeptides. There was little labelling of phosphoproteins and phospholipids when the ingredients of oxidative phosphorylation were omitted. This labelling could arise from the fact that nerve endings contain a high concentration of 'stable' ATP (ref. 9).

The inhibitory effect of acetylcholine on this formation of ATP as measured according to the procedure of Crane and Lipmann[10] was negligible, and ATP had a specific activity 7–10 times as high as that of phosphoproteins. Also acetylcholine and physostigmine ($4{\cdot}75 \times 10^{-4}$ M) had no effect on the phosphorus : oxygen ratio[11].

The foregoing results confirm previous *in vivo* observations[3], which indicated that nerve endings incorporated phosphorus-32 at a lower rate than the other subcellular fractions. Thus, it is possible that the inhibitory effect of acetylcholine observed in the present *in vitro* investigations could occur *in vivo*.

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Low Molecular Weight Carbohydrates in Urine from Secretors and Non-secretors of Different Blood Groups

IT has been shown that gel-filtration is a useful method for the fractionation of ultrafilterable substances in normal urine. At least six carbohydrate fractions have been obtained from concentrated urine ultrafiltrates by means of gel-filtration on 'Sephadex *G*-25'[1]. The general pattern of these fractions from pooled urine was found to be fairly constant, but some variations were observed in individual urines. Recently, it was shown that secretors of A-, B- and H-substances show a considerably higher excretion of fucose in gastric mucus and urine than non-secretors[2,3]. This communication describes some differences in the excretion of low molecular weight fucose-containing components that have been detected in the urine of secretors and non-secretors belonging to various blood groups by a comparison of the gel-filtration patterns of their urine ultrafiltrates.

Urine produced during 4 h in the morning after starvation for 16 h was collected from seventy-two healthy male individuals, selected according to their blood group and secretor status. Phenylmercuric nitrate was added to a final concentration of 1 : 50,000 in order to prevent bacterial growth. The urine samples were filtered at +4° C and analysed for fucose and hexosamine. Secretors belonging to blood groups A_1, B and O were found to have a higher fucose excretion than non-secretors. The difference was of the same magnitude as was previously shown for individuals belonging to A and O groups[3]. There was no significant difference, however, in the hexosamine excretion between secretors and non-secretors. Four-hour urine samples from three or four individuals of each blood group and secretor status were then ultrafiltered through Visking dialysis tubing (23/32 in flat width). On average, 80 per cent of the hexosamine and fucose material could be ultrafiltered. The ultrafiltrate from each individual was concentrated ten times by means of a rotating flask evaporator. At the most, only 10 per cent of the fucose and hexosamine material was lost during the concentration. Seven ml. of each urine sample was then applied to a 'Sephadex *G*-25' column (2·9 × 140 cm), and eluted with distilled water. The eluates were analysed for total hexose, fucose, sialic acid, hexosamine, uronic acid and protein. The methods used were those described earlier[1].

Individuals of various blood group and secretor status gave very similar sialic acid-, hexosamine-, uronic acid- and protein-curves. These curves closely resembled the earlier ones obtained for pooled urine[1]. Examination of the distribution of the total hexose and fucose materials, however, revealed four types of patterns, as can be seen from Fig. 1. There was one non-secretor pattern, obtained

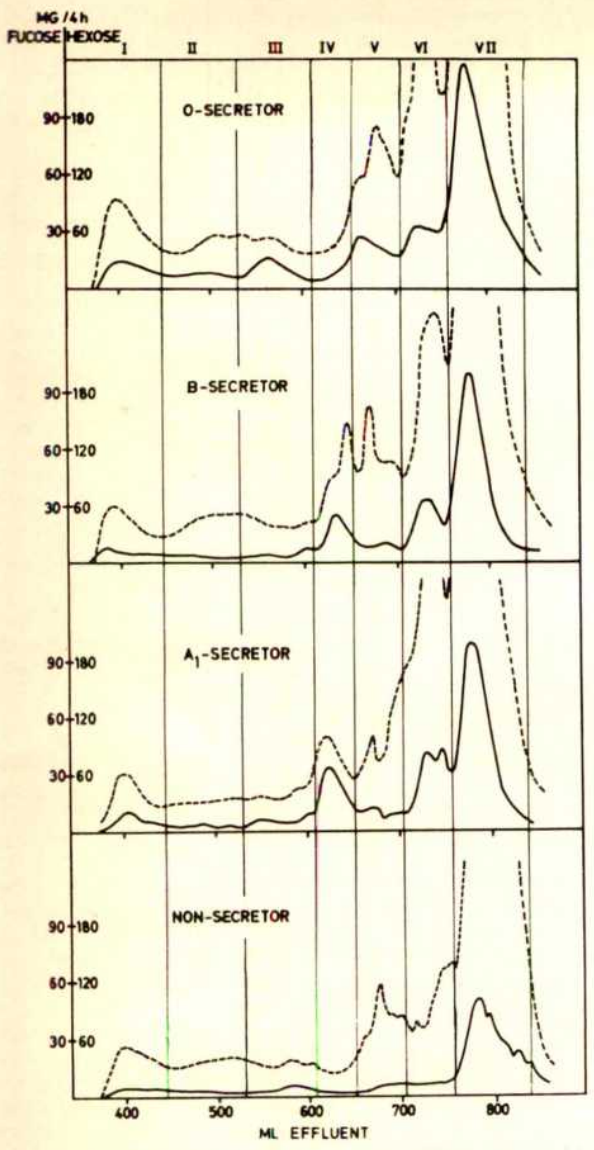


Fig. 1. Fucose (——) and total hexose (– – – –) patterns obtained by gel-filtration on 'Sephadex *G*-25' of urine ultrafiltrates from individuals of various blood groups and secretor status. The eluant was distilled water

from individuals of blood groups A_1, B and O. Individuals having the same blood group and secretor status displayed almost identical patterns. The non-secretor pattern had a low fucose level and a high and broad peak in region VII, whereas the spectra obtained with urine from secretors were all characterized by distinct fucose peaks in both the VI and VII regions. An additional fucose peak was found in region V from O-secretors and in region IV from A_1- and B-secretors; however, the peak from B-secretor urine was eluted somewhat later than that from A_1-secretor urine. Small elevations of the curve were found in regions I and III from both secretors and non-secretors. The total hexose curve followed the fucose curve except in region V in A_1- and B-secretors and regions V and VI in non-secretors, where a high hexose-level occurred without a corresponding elevation of the fucose curve. Preliminary investigations concerning the material in region IV from A_1-secretors showed that the main part of the carbohydrate material in this region was neutral. After paper chromatography, the material did not react with ninhydrin.

So far, only a few serological tests have been performed with the fractions obtained. A slight inhibition of anti-A-serum was observed with the material in region IV from A_1-secretors, whereas anti-B and anti-H were not inhibited by this material. Material in region V from O-secretors inhibited anti-H very slightly, whereas material in region V from A_1-secretors did not show this property.

A more detailed characterization of the various carbohydrate fractions described here is being carried out.

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HAEMATOLOGY

Human Serum Enzymes in Electrophoretic Fractions

SOME of the electrophoretic components of human serum have been shown to contain certain enzyme activities[1,2].

As human serum is known to contain a number of enzymes such as cholinesterase, lipase, transaminases, amylase and phosphatases, it was decided to find the positions of the different serum enzymes after electrophoretic separation of the protein components.

Electrophoretic separation of normal human serum was carried out using 0·5 per cent agar (Difco Laboratories) in 0·05μ veronal buffer, *p*H 8·6, for 6 h with a current of 1 mamp/cm. After electrophoresis one strip was stained with amido-black 10*B* (saturated solution in solvent; composition: methanol, distilled water, acetic acid; 50 : 50 : 10, v/v)[3]. On the other strip, 1 per cent of soluble starch in the same veronal buffer was applied uniformly by a filter paper soaked in the starch solution for 5 min. The electrophoretic strip was incubated at 30° C in a water-saturated cabinet for 1 h. After this, the electrophoretic plate was carefully sprayed with dilute iodine solution in potassium iodide. Blank areas showing the digested starch appeared against the blue background of the undigested starch. The results are given in Fig. 1.

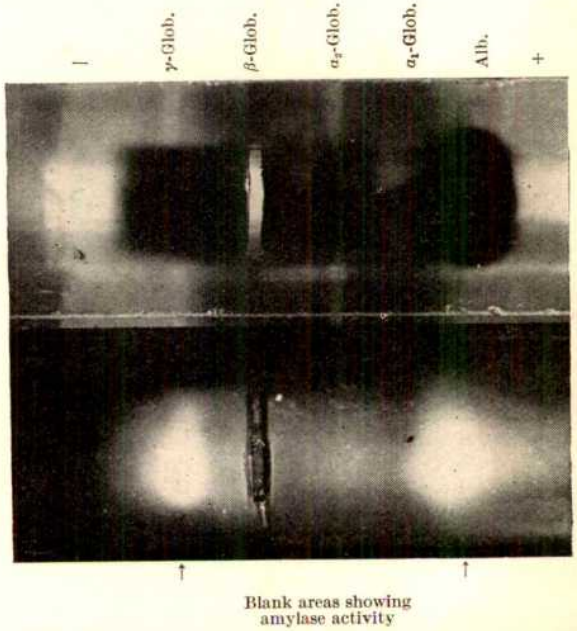


Fig. 1. Agar electrophoresis of human serum and detection of amylase. Upper strip stained with amido-black 10*B*, and lower strip stained with starch solution

It can be seen that there are two very intense zones of amylase activity in the albumin and γ-globulin fractions; faint bands can also be seen in α_2- and β-globulin fractions. Human serum thus seems to contain more than one component with amylase activity.

It is interesting to note that M. Ataur Rahman[4] has detected serum amylase activity in the globulin region only, whereas McCeachin and Potter[5] found serum amylase mostly in the albumin region. The present communication shows amylase activity both in the albumin and in the γ-globulin regions.

In another experiment serum glutamate oxalacetate and serum glutamate pyruvate transaminases were found to appear in sodium chloride (0·9 per cent) eluates of 1-cm segments of electrophoretic strip corresponding to the albumin, α_2- and β-globulin fractions. The estimations of these enzymes were carried out according to the method of King[6].

All these experiments were repeated several times and the same results were obtained. More detailed investigations of the electrophoretically distinct amylases of normal human serum and other enzymes will be reported elsewhere.

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Cytogenetic Studies in Twins, one with Chronic Granulocytic Leukaemia

CHROMOSOMAL investigations of a patient with chronic granulocytic leukaemia and his normal identical twin were reported by Goh and Swisher[1]. While some cells cultured from the peripheral blood of the patient contained the Philadelphia chromosome, those from the peripheral blood and the bone marrow of the normal twin did not show the abnormality. The authors comment on the extreme rarity of this finding; however, we now record a second example.

E. F., a male born in 1928, a few minutes before his identical twin F. F., was diagnosed as having chronic granulocytic leukaemia in May 1963. In December 1962 the patient had a severe haemorrhage following removal of his tonsils. After the operation he did not regain his usual good health and in May 1963 he was referred to hospital for investigation. Pallor and hepatosplenomegaly were recorded on physical examination, and a blood test indicated chronic granulocytic leukaemia. Cytogenetic investigations were positive for the Philadelphia chromosome (Table 1). The patient has since been responding well to radiotherapy and chemotherapy.

Further investigations were envisaged when we became aware that he had a twin brother. The twin (F. F.) consented in May 1964 to a blood examination but not at that time to a bone marrow aspiration. Cytological and cytogenetic investigations revealed no abnormalities in his blood, and a comparison of his groups (ABO, Rh, MNS and Kell) with those of our patient confirmed the clinical and family impressions that they were identical twins.

No significance could be attached to the absence of the Philadelphia chromosome in the cells cultured from F. F.'s peripheral blood as it contained no primitive myeloid cells. Recently, however, he consented to a bone marrow aspiration and twenty-five clear metaphases have been investigated. They do not contain the Philadelphia chromosome. This is significant and confirms the fact, already deduced from other data, that the Philadelphia chromosome is not present in the zygote but must develop later. It also suggests that environmental factors are more important than hereditary factors in the development of this chromosomal abnormality.

Table 1. CYTOGENIC EXAMINATIONS

Subject	Date	Material	Metaphases No. examined	Metaphases No. Ph¹ + ve
E. F.	6.6.63	Peripheral blood culture (92 h)	42	21
	9.12.63	Peripheral blood culture (72 h)	23	8
	27.8.64	Peripheral blood culture (74 h)	28	3
	28.6.65	Bone marrow direct	10	10
F. F.	29.5.64	Peripheral blood culture (96 h)	25	0
	25.6.65	Bone marrow direct	25	0

The possibility, should the disease become acute, of treating our patient with high doses of total-body radiation has been made practicable by his twin's offer to donate marrow.

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Oxygen Dissociation of Sheep Haemoglobins A and B in Solution

Two distinct electrophoretic types of sheep haemoglobin have been identified by Harris and Warren[1]. They designated the fast-moving component which travelled towards the anode at *p*H 8·6 'haemoglobin A', and the slow-moving one 'haemoglobin B'. Similar electrophoretic differences were found with adult haemoglobins in Dutch sheep by Helm *at al.*[2], which they named HbI and HbII, corresponding to HbB and HbA respectively. Further differences in chromatographic behaviour, in resistance to denaturation by alkali, in solubility in concentrated salt solutions and in amino-acid composition were demonstrated for HbI and HbII[2]. The oxygen equilibrium curves of these haemoglobins were also found to be different[3,4], HbII(A) having a higher affinity for oxygen than HbI(B), and it has been suggested that HbII(A) originated in sheep living at high altitude.

The kinetic dissociation constant, k_4, of the reaction $Hb_4O_8 \rightarrow Hb_4O_6 + O_2$, can be measured by the carbon monoxide displacement method of Gibson and Roughton[5], though in practice it is inconvenient as four or five experimental determinations have to be made, and the result obtained by an extrapolation procedure. At *p*H 9·1, this constant can be more easily measured by mixing a dilute solution of oxyhaemoglobin with 0·4 per cent sodium hydrosulphite dissolved in a buffer solution equilibrated with 1 atmosphere of carbon monoxide[6]. Kernohan[4,7], using the latter method, has attributed the difference in oxygen affinity between HbII(A) and HbI(B) to variation in their rate of dissociation—the respective mean values of k_4 being 40·6 ± 2·5 sec^{-1} and 57 ± 3 sec^{-1} at *p*H 9·1 and 20° C. Though a comparison of the sodium hydrosulphite method with the displacement technique gave good agreement at *p*H 9·1 (ref. 6), hydrosulphite is known to attack haemoglobin[8,9] and is not applicable to the lower *p*H range. Recent work has also

suggested that errors may arise from changes of light transmission and refractive index when hydrosulphite is dissolved in solution.

An alternative method, primarily developed for measuring the rate of dissociation in red blood cells, has been used to examine this problem further. A buffer solution of oxyhaemoglobin at *p*H 9·1, in equilibrium with oxygen at a pressure of 10–40 mm mercury, is rapidly mixed and at the same time diluted 1 : 20 with a similar buffer solution equilibrated with carbon monoxide at 800 mm mercury. Dissociation of the HbO_2 occurs with rapid uptake of carbon monoxide, under conditions similar to the displacement method of Gibson and Roughton[5]. The relative ratio of the concentrations, $[O_2]/[CO]$, after mixture, however, is now such that the rate is determined by $k_4/4$ to within 2 per cent. In practice this is within the experimental errors and no significant difference in the rate of egress can be detected with a four-fold change of oxygen tension or a three-fold change of carbon monoxide concentration. A comparison of this method with the hydrosulphite technique has revealed no significant error. The method can also be applied to measurements at lower *p*H.

With HbII(A), the electrophoretically fast component, the value of k_4 at 20° C and *p*H 9·2 was 40·8 ± 7 per cent by the sodium hydrosulphite method, and 38 ± 7 per cent by the dilution technique. At *p*H 7·4, k_4 was 44·5, higher by a factor of 1·1 in accord with the observations of Gibson and Roughton[5]. The activation energy at both *p*H values was 19·5 kcal ± 7 per cent. Similar measurements on an electrophoretically pure form of HbI(B), at *p*H 9·2, gave a value of k_4 = 45 at 20° C by both methods. The activation energy in this case was 16·5 kcal ± 7 per cent. At *p*H 7·4, using the dilution technique, k_4 at 20° C was 54·8 and the activation energy had increased to 19 kcal.

The values of k_4 for HbII(A) are, within experimental error, the same as those obtained previously[4,5]. In the case of HbI(B), however, an interesting discrepancy has occurred. The value at *p*H 9·2 is not the same as that obtained by Kernohan[4], though the value at *p*H 7·4 is only slightly lower. On the other hand, it had been assumed that the values of k_4 obtained by Gibson and Roughton[5] were those of HbII(A), though while at *p*H 7·1 only activation energies of the order 20 kcal were obtained, at *p*H 9·1 two distinct values occur one at 16·5 kcal and another at 20 kcal. It now seems probable that the 16·5 kcal measurements were made on HbI(B) samples. A similar difference in the rate of egress with *p*H change has been observed for this haemoglobin within intact red blood cells, so that the discrepancy is unlikely to be associated with the method of preparation of the haemoglobin solution.

The differences at *p*H 9·2 do not affect Kernohan's conclusion that the shift of the oxygen equilibrium curves observed by Huisman *at al.* is associated with differences in k_4, but the question arises as to whether HbI and HbB are identical, or whether there are two forms of the electrophoretically slow HbI(B) sheep haemoglobin.

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HISTOLOGY

A Simple Dye Test for Skin Graft Viability

PATENT blue V dye (alphazurine 2*G*), which was previously used in evaluations of cutaneous burns[1], has also been found useful in the evaluation of the viability of allografted skin before and including the process of rejection. In small animals, dye injected intravenously provides immediate colouring of the skin over an intact vasculature.

The autograft after revascularization will be coloured light blue, the same as the surrounding skin. With increased vascular permeability as occurs in second degree burns or early rejection, the dye is deposited extravascularly as a dark blue stain, enabling the observer to locate the area of vascular disruption. With coagulation necrosis and thrombosis as found in third degree burns, or total allograft rejection, no tissue colouring occurs, and this demarcates the non-viable skin.

In very small animals, the dye can be injected intraperitoneally, but a 5–10 min lag period must be allowed for the full dye pattern to develop. Dye patterns should be read shortly after the skin has reached its maximum colour, as patent blue will in time diffuse into non-vital areas. This latter phenomenon provides a "reciprocal pattern" for confirmation while the intravascular dye is being rapidly metabolized by the liver and excreted through the kidneys.

This test may be repeatedly carried out without disturbing the graft to follow the process of rejection or its suppression. The histological changes occurring during rejection are not delineated in detail by this technique, but sequential information concerning the vascularization of the graft is obtained, and this bears directly on survival.

There are many possible clinical applications of this technique, but care should be taken in using this experimental agent in debilitated patients.

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PHYSIOLOGY

Structure and Function of the Sarcolemma of Skeletal Muscle

THE functions of the sarcolemma of skeletal muscle are still uncertain, because of our lack of knowledge of its structure and detailed distribution over the entire surface of the muscle cell. Most of our knowledge is derived from electron microscope investigations and the sarcolemma is regarded as a semi-permeable unit membrane about 100 Å thick, which directly encloses the contents of the cell. This membrane is probably bimolecular and lipoprotein in character, and similar to that which is thought to invest most animal cells and many of their cytoplasmic organelles. Immediately external to the sarcolemma is a layer about 500 Å thick; this is an extracellular deposition of material of moderate density (the so-called basement membrane[1]) which gradually diminishes in density as it extends from the sarcolemma into extracellular space and merges with the ground substance of the endomysial connective tissue. Because of its extreme thinness, the sarcolemma is difficult to observe in its longitudinal section; on the other hand, its characteristic unit membrane structure can usually be recog-

nized in transverse section and occasional thickenings and invaginations of the sarcolemma are known to occur[1] at certain regions of the cell. However, using thin-section techniques it is not easy to follow the detailed variations in its course over large areas of the cell surface. For structural investigations of this type the surface replication methods, such as the layer-stripping method developed by Reed and Rudall, are probably far more useful, as the various levels exposed in the tissue may be examined over wide areas[2]. Early investigations using the layer-stripping method revealed the sarcolemma as a continuous elastic sheet, capable of accommodating itself to the structures it encloses, since it often bears undulations corresponding to the striations of the myofibrils[3].

Recently in our work on the post-mortem changes in muscle tissue, particularly as they affect the properties of meat as a foodstuff, we have re-examined the sarcolemma-basement membrane system of pig rectus femoris muscle, using such surface replica techniques. In tissue taken immediately after slaughter and before rigor mortis has been allowed to develop, the myofibrils are almost completely covered with the sarcolemma-basement membrane, so that the striations of the myofibrils are mostly obscured (Fig. 1). However, these membranes do not have a uniform appearance, but show a system of regularly spaced bars indicating an intrinsic periodic structure. This indication that the sarcolemma-basement membrane may possess a regular structure is confirmed by the appearances shown by tissue which is examined after rigor. Here the membranes are observed not as continuous structures but as sheets containing holes of various shapes and sizes or as a system of bars arranged in a very definite pattern (see Figs. 2 and 3). Moreover, this pattern seems related to that of the underlying striations of the myofibrils.

These observations suggest that the sarcolemma-basement membrane is a very labile system and one which may change very rapidly after the death of the animal. More significantly, however, it points to some degree of definite interaction between the sarcolemma and the myofibril, in that there seems to be a specialized mode of attachment of the membranes to different parts of the myofibrillar surface. Although the sarcolemma-basement membrane originally invests the whole surface of the adjacent myofibrils, the portions adhering most tenaciously to the myofibrils (since they remain attached during the rigor process) appear to be at regions between the *H* zone and the *Z* line. These are regions where the actin and myosin filaments interdigitate and lock together during contraction. Possibly, therefore, the sarcolemma-basement membrane, in addition to enclosing the myofibrils of the cell, may be a highly dynamic system in the sense that it can take part in, respond to and conceivably initiate changes in the physiological state of the myofibrils. At certain times the membrane could be quite free from the

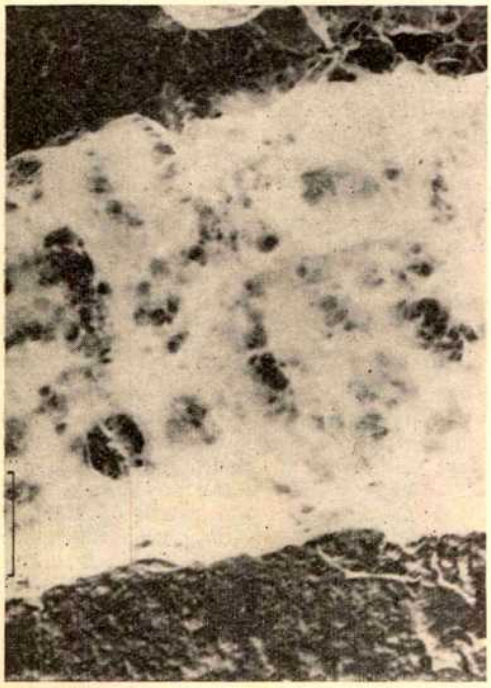


Fig. 1. Surface replica of sarcolemma-basement membrane almost completely covering myofibrils in pre-rigor condition

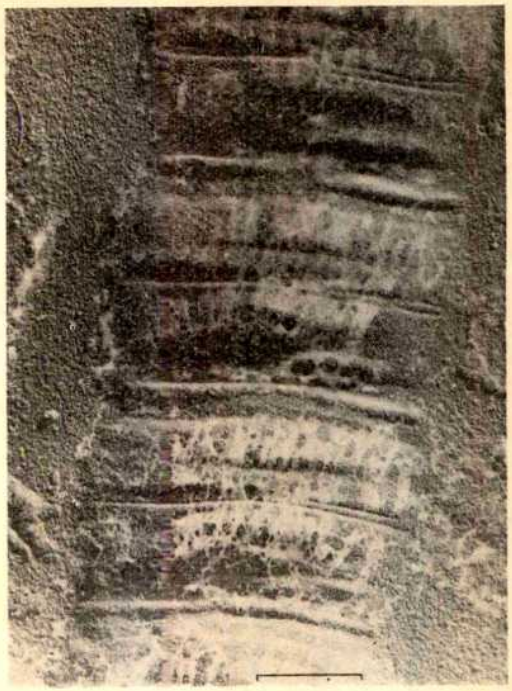


Fig. 2. Surface replica of sarcolemma-basement membrane partially covering myofibrils in post-rigor condition

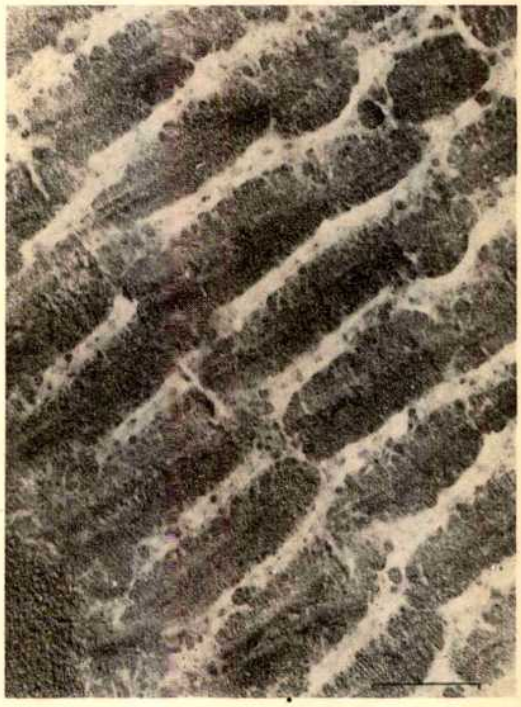


Fig. 3. Surface replica of sarcolemma-basement membrane, with regular arrangement of bars, partially covering myofibrils in post-rigor condition

myofibrils, that is, with no firm links of adherence to the latter. At other times certain regions of the myofibrils could become firmly attached to the sarcolemma. This dynamic view of the sarcolemma forming specialized links with myofibrillar regions could help in assessing its function during the contractile process of living muscle. It could help in understanding how muscle cells act in concert, while it could provide a basis for explaining why regions of the sarcolemma, under electrical stimulation, lead to differing responses in the contractile pattern of the myofibrils[4]. Perhaps most important is the possibility that it could provide a basis for the transmission of the contractile force of the myofibrils to the endomysial and perimysial connective tissue and ultimately to the muscle tendon. Majority opinion at present[5] holds that there is no permanent structural continuity between sarcolemma and the myofibrils, because of the ease with which the sarcolemma may be induced to separate from the muscle substance by a wide variety of methods. What is suggested here is that, depending on the contractile state of myofibrils, a definite pattern of adhesion may occur between the sarcolemma and the myofibrils; further, that this adhesion may be involved not only in transmitting electrical impulses to the myofibrils, but also in transmitting the contractile force of the latter to the connective tissue structures.

Apart from its possible significance in physiological states of muscle, the sarcolemma-basement membrane may play an important part in post-mortem effects, that is, it may determine the rate of release of sarcoplasm and other materials from the muscle cells. Once the integrity of this membrane system has been impaired, it is likely that the endomysial connective tissue will then be concerned in determining the rate of drip or exudation from meat. Work at present in progress in these laboratories, to be reported in full elsewhere, suggests that the drip characteristics of meat depend markedly on whether the tissue is in the pre- or post-rigor condition and therefore on the state of the sarcolemma-basement membrane system.

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Effect of Peroxides on the Hydration Elongation of Rat Tail Tendon

IT has been shown that physico-chemical changes occur when connective tissue is treated with water that has been subjected to ultrasonic vibrations[1]. The suggestion has been advanced that chemically active substances are formed through the ionization of the water in such ultrasonic fields, and that peroxides are probably involved[2].

Collagen fibres were used to investigate the proposed action of these substances. The fibres were obtained from rat tails (Wistar males, 3 months old) according to the method described by Elden[3]. Before each experiment was carried out, the fibres were dehydrated until a constant ratio of weight and length was achieved. The individual fibres were fixed lengthwise to the bottom of a 2 ml. 'Perspex' cell. The fibres were measured microscopically and divided into three classes according to their diameter. The fixed fibres were set to the mark of a measuring eyepiece (the magnification was × 150). The time was measured from the moment the solution was injected into the cell until the fibre jerked off the setting mark. This time interval served as a measure of the beginning of longitudinal elongation of the fibre due to hydration. Deionized water (*p*H 6·6–6·8) was used as the control solution, while hydrogen peroxide in deionized water at concentrations of 1, 0·1, 0·01 and 0·001 per cent were used as experimental solutions. The concentration of peroxide was controlled by polarography and the solutions were applied at a temperature of 21° C. Table 1 presents the results of our experiments.

Table 1

		I	II	III
	Control	21·40 ± 1·58	27·35 ± 1·97	29·70 ± 1·63
Percentage of hydrogen peroxide	0·001	8·95 ± 0·50	15·40 ± 1·34	20·60 ± 1·89
	0·01	11·90 ± 0·27	13·50 ± 0·64	16·30 ± 0·56
	0·1	13·50 ± 0·71	16·80 ± 0·78	20·10 ± 1·10
	1·0	17·40 ± 0·75	23·40 ± 2·33*	23·61 ± 1·57

Mean time in seconds (± *S.E.*) of the onset of hydration-elongation of the three classes of collagen fibres of various diameter (I, 84–126μ; II, 126–168μ; III, 168–210μ) under the influence of a control solution and various concentrations of hydrogen peroxide. In each group ten fibres were measured. With the exception of one value (*), all the experimental (– peroxide) values are significantly different from the control.

In Table 1 it can be seen that the peroxides influenced the onset of elongation, but that the extent to which they were effective depended on the concentration and diameter of the rat-tail tendon. The beginning of elongation was accelerated most in the fibres with smallest diameter and with the lowest concentration of hydrogen peroxide in the solution; however, in the fibres with larger diameters the maximum acceleration is shifted towards higher concentrations.

These results can be explained in the following way. Either the chemically active substances, formed through the decomposition of hydrogen peroxide, facilitate the penetration of water through the membrane enveloping the rat-tail tendon[4], or they directly influence the hydration rate of the collagen proteins[5]. Of special interest is the decrease in the efficiency of peroxides with increasing concentrations, which is analogous to the haemolytic effect of peroxides[6]; however, for the time being, this effect must remain unexplained.

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A Re-investigation of the Effects of 'Nilevar' on the Right Gonad of the Pullet

IN the female chicken, as in most female birds, only the left gonad develops into a functional ovary. Surgical removal of this left ovary is followed by hypertrophy of the right rudimentary gonad, indicating that the ovary exerts an inhibitory effect on the development of the right gonad (see refs. 1 and 2 for reviews). Kornfeld[3] and Taber *et al.*[4,5] demonstrated that physiological amounts of oestrogen inhibit the hypertrophy of the rudimentary gonad following sinistral ovariectomy. This inhibition does not appear to be due to suppression of pituitary gonadotrophins.

Kornfeld[6] administered 'Nilevar' (17α-ethyl-19-nortestosterone), a synthetic progestational compound and a reported "anti-oestrogen", to intact pullets. He reported a four-fold increase in the weights of the rudimentary

right gonads and interpreted this hypertrophy as due to the anti-oestrogenic effects of the drug, releasing the rudimentary gonad from the inhibition of endogenous oestrogens.

Our experiments with ovariectomized birds[7] have provided evidence to support the hypothesis that the avian ovary produces another, non-oestrogenic substance which also inhibits the development of the rudimentary gonad. If our hypothesis is correct, the right gonad of a pullet treated with 'Nilevar' should remain inhibited if 'Nilevar' is acting only as an anti-oestrogen. For this reason, it was decided to re-investigate the effects of 'Nilevar' on the rudimentary right gonad of the chicken.

(*A*) *Normal pullets.* Twenty-three 60-day-old intact brown Leghorn pullets received subcutaneous injections of 3·0 mg of 'Nilevar' (dissolved in olive oil and 5 per cent benzyl alcohol) each day for 40 days; eighteen pullets of identical age served as controls and received only the vehicle of the drug. New feathers of both groups were feminine, providing evidence of circulating oestrogens and indicating that 'Nilevar' had no anti-oestrogenic effect at the feather target organ. The combs and wattles of the birds treated with 'Nilevar' were stimulated and, at the time of autopsy, the combs were more than four times the size of those of the controls (height × length = 54·7 cm^2 as compared with 12·6 cm^2). In addition, the treated pullets were more aggressive and occasionally crowed. These observations are evidence for the androgenic action of 'Nilevar'.

At the time of autopsy, control pullets revealed no grossly visible right gonadal tissue, whereas hypertrophied tissue was observed at the site of the right gonad in each experimental bird. However, when tissues from control and treated birds were examined histologically, it was found that the right gonads of both groups were inhibited and were indistinguishable from each other. The hypertrophied tissue which had been observed grossly in pullets treated with 'Nilevar' consisted of enlarged Wolffian bodies. These were estimated to be from three to four times the size of those in controls and the tubules were markedly larger and hyperplastic.

(*B*) *Sinistrally ovariectomized pullets* (*poulards*). Twenty-four pullets were ovariectomized at the age of 21 days and eighteen of these received 0·4 mg of 'Nilevar' per 100 g body-weight beginning the day of operation and continuing for 8 weeks. Again, the combs of the birds receiving 'Nilevar' were greatly stimulated (57·0 cm^2 as compared with 19·5 cm^2, at the time of autopsy).

At the time of autopsy, the right gonads of untreated poulards were hypertrophied and averaged 85·8 mg. The average weight of the gonads from poulards treated with 'Nilevar' was 53·9 mg, which does not differ significantly from that of controls. However, because the weights of the tissue from the experimental group include the greatly enlarged Wolffian bodies, it is a reasonable assumption that treatment with 'Nilevar' produced a significant retardation in the development of the right gonad.

Histologically, the gonads of poulards treated with 'Nilevar' showed considerable variability. In some, the general structure was similar to that of the inhibited right gonad of pullets although some proliferation of medullary tissue had occurred. The larger gonads were less well differentiated, but otherwise resembled those of untreated poulards.

These experiments failed to demonstrate any anti-oestrogenic effect of 'Nilevar' and the right gonads of treated pullets were inhibited to the same degree as those of untreated controls.

The right gonads of poulards treated with 'Nilevar' were retarded in size and degree of differentiation when compared with those of controls. Undoubtedly, this resulted from the androgenicity of 'Nilevar', which even on a conservative estimate must be about one-sixteenth as potent as testosterone propionate[8]. On the basis of this estimate, our poulards received the equivalent of 25 μg of testosterone propionate daily, a dosage known to inhibit or retard the growth of the right gonad in poulards[5].

Evidence for the androgenicity of 'Nilevar' (comb stimulation and the hypertrophy of the androgen-sensitive Wolffian body) was observed in both intact pullets and poulards. Kornfeld[6], basing his conclusions entirely on gross observations, reported hypertrophied right gonads in pullets treated with 'Nilevar'. Our observations show that the hypertrophied tissue observed by us and probably by Kornfeld was due to the hypertrophy of the Wolffian body and not to the hypertrophy of right gonadal tissue.

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Plurivesicular Nerve Endings in the Pulmonary Artery

PREVIOUS electron microscopic studies of blood vessels have failed to reveal "nerve endings" or synaptic regions within the muscularis[1–4] although small bundles of myelinated and unmyelinated axons have been clearly seen in the adventitia. The specific histological fluorescence method of Falck and Hillarp[5] for catecholamines has provided morphological evidence for the intraneuronal, varicose distribution of the adrenergic transmitter in blood vessels[6]. While electron microscopic details are available on the terminal autonomic innervation of non-vascular smooth muscle[7–10], the identification of comparable neuro-effector relationships in blood vessels is lacking, apart from the preliminary observations of de Robertis and de Iraldi[11].

In recent light microscopic observations of the isolated pulmonary artery-nerve preparation, in which there is direct, unequivocal evidence for postganglionic sympathetic vasoconstrictor innervation of the vascular smooth muscle[12], Verity *et al.*[13] demonstrated the existence of an unmyelinated nerve plexus of small diameter located primarily in the adventitio-medial zone of the artery.

In view of the unequivocal pharmacological and physiological evidence for the existence of a motor system in this vessel, we attempted to identify the adrenergic transmitter termina.

Pieces of tissue from the region of the bifurcation of the pulmonary artery were rapidly excised from cats previously anaesthetized with sodium pentobarbital. The tissue was washed in cold, phosphate buffered 3 per cent glutaraldehyde and prefixed for 1 h. Subsequent fixation was carried out in phosphate buffered 2 per cent osmium tetroxide[14] for 2 h, followed by dehydration in ice-cold ethanol and embedding in 'Epon 812'. Sections were double stained with uranyl acetate and lead citrate and examined with a Hitachi *HU*-11 electron microscope.

No axon-like profiles or synaptic regions were identified within the inner muscularis after examination of many grid squares, representative of twenty-eight 'Epon'-embedded blocks. In general, the structure and arrangement of the smooth musculature was similar to that of the aorta[15,16], and will not be considered further here.

Winding, varicose, unmyelinated axons of 0·2–0·5μ diameter were identified with some difficulty, presumably because of their low distribution density, in the adventitio-medial junction, and outer smooth muscle-elastic lamellae of the muscularis. Typical neurotubules were identified in the axoplasm[17,18]. Vesicle-containing "synaptic nerve endings" were noted in many preterminal axons. The predominant vesicular population was granular and composed of Type 1 and Type 3 vesicles (after Grillo and Palay[19]) with diameters of about 1000 Å and 450 Å respectively (Fig. 1). While typical agranular synaptic vesicles were occasionally observed, they never accounted for more than 15 per cent of the vesicular population in any one ending, and in many instances were considered to represent inadequately stained Type 3 structures. Longitudinal fragments of the neurotubular system, 75 Å in diameter, were present in some vesicular regions, accompanied by mitochondria and elongate granular (Type 1) and agranular vesicles (Fig. 2). The plasma membranes of smooth muscle cells were never in close proximity to the nodose axon expansions.

The identification of a plurivesicular "synaptic nerve ending" in the adventitio-medial zone of the pulmonary artery provides confirmatory evidence for the presence of an adrenergic neuroeffector system, previously suspected from *in vitro* pharmacological observations[12,13]. A plurivesicular component has been recognized in what are presumed to be adrenergic nerve endings innervating the pineal gland, in fine branches of the splenic nerve penetrating the adventitia of splenic blood vessels[11] and in the anterior hypothalamus of the rat[20]. Evidence for the association of granular vesicles with catecholamines is very strong. The plurivesicular endings of this study are unusual in containing a large number of granular (1000 Å in diameter) vesicles with very few synaptic (presumed cholinergic) vesicles. The mixed, heterogeneous nature of the granular vesicular population allows for the use of the term "plurivesicular", as coined by de Robertis and Pellegrino de Iraldi[11], but the relative lack of agranular vesicles is in marked contrast to their observations on the adrenergic axons and endings in the pineal gland and splenic nerve. Also of note was the complete lack of a junctional relationship between the adrenergic nerve endings and the smooth muscle membranes.

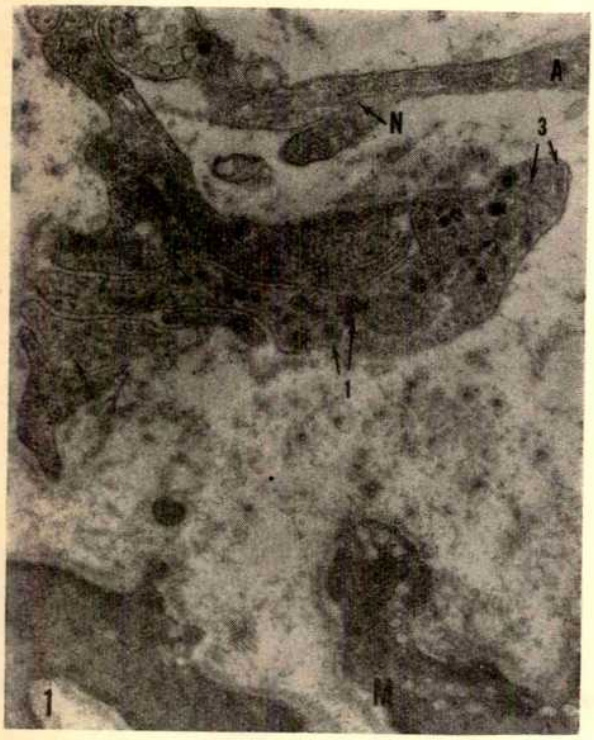


Fig. 1. Granular and agranular vesicle-containing axonic expansion in the medio-adventitial zone of the pulmonary artery of the cat. Large (~1000 Å diameter) light and dense osmiophilic granular vesicles surrounded by unit limiting membrane (Type 1) are interspersed with smaller granular vesicles (Type 3) with light osmiophilic cores. *A*, Adjacent, small-diametered axon with neurotubules (*N*). *M*, Smooth muscle. (×*c.* 21,000)

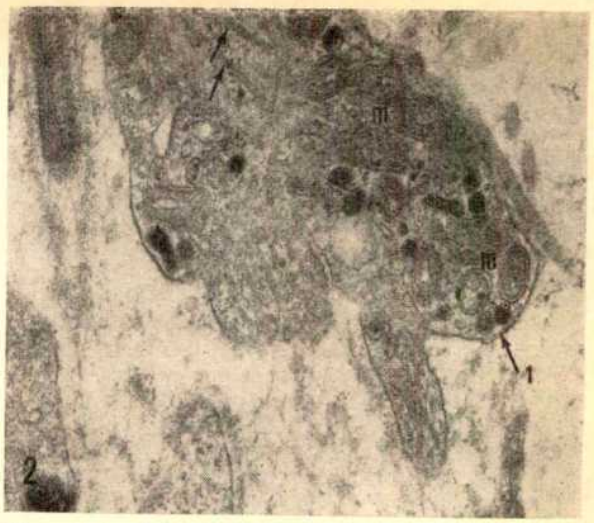


Fig. 2. Preterminal vesicle-containing structure with numerous mitochondria (*m*). Neurotubules (arrows) approach the vesicle aggregation, some of which appear elongate. (×*c.* 33,750)

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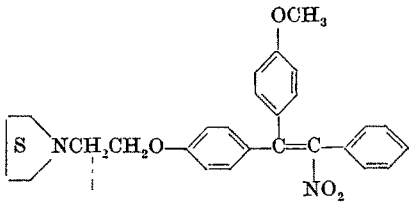
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PHARMACOLOGY

A New Class of Orally Effective Antifertility Agents with Hypocholesteraemic Activity

THIS communication describes the synthesis and some of the relevant pharmacological properties of a typical member (I) of a new class of agents with potent antifertility and hypocholesteraemic effects in experimental animals.



(I)

1-[2-[*p*-[α-(*p*-Methoxyphenyl)-β-nitrostyryl]-phenoxy] ethyl]pyrrolidine.

A convenient synthesis of I from commercially available starting materials is illustrated by the following sequence of reactions: (*A*) Treatment of a mixture of β-bromophenetole and anisic acid with polyphosphoric acid at 80° C gave a 97 per cent yield of crude *p*-(β-bromoethoxy)-*p'*-methoxybenzophenone (II) (melting point of 111·5°–113° (from methanol)). Analysis calculated for $C_{16}H_{15}BrO_3$: carbon, 57·33; hydrogen, 4·51; bromine, 23·84. Found: carbon, 57·28; hydrogen, 4·43; bromine, 23·57. (*B*) Treatment of II with excess pyrrolidine for 4 h in a refluxing solution of tetrahydrofuran gave, after recrystallization of the crude product from aqueous methanol, an 87·5 per cent yield of pure *p*-methoxy-*p'*-[β-(1-pyrrolidinyl)ethoxy]benzophenone (III) (melting point 97°–98·5° C). Analysis calculated for $C_{20}H_{23}NO_3$: carbon, 73·82; hydrogen, 7·12; nitrogen, 4·31. Found: carbon, 73·95; hydrogen, 7·28; nitrogen, 4·18. (*C*) Reaction of III with the benzyl Grignard reagent gave a 78–86 per cent yield of 1-(*p*-methoxyphenyl)-2-phenyl-1-(*p*-[β-(1-pyrrolidinyl)ethoxy]phenyl)-ethanol (IV) (melting point 121°–122° C) from *cyclo*hexane-ethyl acetate. Analysis calculated for $C_{27}H_{31}NO_3$: carbon, 77·66; hydrogen, 7·48; nitrogen, 3·36. Found: carbon, 77·61; hydrogen, 7·42; nitrogen, 3·35. (*D*) Conversion of IV to I (free base) was effected in yields of 75–79 per cent by treating IV in glacial acetic acid with 2 equivalents of 90 per cent nitric acid at 25°–30° C for 1·5–2·0 h. Recrystallization of the resulting crude product from methanol gave pure I, despite the characteristically indefinite melting point; 103°–106° C (softens) 120° C (liquid phase). Analysis calculated for $C_{27}H_{28}N_2O_4$: carbon, 72·95; hydrogen, 6·35; nitrogen, 6·30. Found: carbon, 73·15; hydrogen, 6·16; nitrogen, 6·27. The ultra-violet spectrum obtained in methanol was λ_{max} (mμ), 282, 239; $E^{1\%}_{1cm}$, 348, 485. The nuclear magnetic resonance spectrum of this material showed it to be essentially a 50 : 50 mixture of two isomers. Separation of the isomers resulted by recrystallization from methanol: isomer *A*, melting point 136°–138° C, nuclear magnetic resonance methoxy hydrogen at 3·75 δ; isomer *B*, melting point 117°–119° C, nuclear magnetic resonance methoxy hydrogen at 3·70 δ. Optical crystallographic studies showed that I-*A* is orthorhombic and isomer I-*B* monoclinic. The variability in melting range of I may thus be attributed in part to the presence of two crystalline modifications, as well as to possible variability in isomeric composition. Both isomers possess identical hypocholesteraemic and antifertility activity. All the pharm[illegible] cological data that follow refer to the mo[illegible] of the 50 : 50 isomer mixture. This [illegible] prepared by mixing warm acetone [illegible] anhydrous citric acid (5 per ce[illegible] crystalline I citrate that s[illegible]

suspension in 4 per cent aqueous acacia once daily for 7 days by oral intubation led to plasma total cholesterol depressions of 26–52 per cent relative to the response of an equal number of control animals.

Another controlled experiment in rats, in which I citrate was incorporated in the diet at doses of 0·5 mg/kg to 10 mg/kg, gave 55–67 per cent plasma total-cholesterol depressions at the end of a 104-day treatment period. Analysis of the liver sterol pattern in the 104-day treated animals by the method of Rodney *et al.*[1], with particular attention being paid to desmosterol (24-dehydrocholesterol), showed that I is without significant effect on the sterol synthetic sequence in that organ.

Administration of I citrate (60 mg/kg/day) by oral intubation to one male and two female monkeys produced, by the end of the 90-day period of treatment, plasma cholesterol depressions of 44–50 per cent relative to the pre-treatment control values. Oral treatment of one male and two female dogs with I citrate (20 mg/kg/day) for the same period produced depressions of 27–60 per cent, also relative to pre-treatment control-levels. Doses of this size in the monkey and dog exerted a slight (monkey) to moderate (dog) desmosterogenic effect on the liver sterol synthetic sequence in these species: in partial compensation, however, the plasma cholesterol depression produced by I in the monkey was accompanied by a parallel depression of all plasma sterols, so the net effect was not simply a shift in the desmosterol/cholesterol ratio.

Species in which the antifertility activity of I has been demonstrated include the mouse, the rat, and the dog. In the prophylactic mouse test, a fertile male was introduced to mature female mice in groups of four who were being fed the drug continuously in a diet of ground grains and powdered milk. After 2 weeks, the male was withdrawn and the females were carried to term. The minimal effective dose for complete protection (ED_{100}) was about 50 μg/kg/day. In the post-coital mouse test, the drug, suspended in 0·25 per cent methylcarboxycellulose, was administered in a single dose by gavage to females previously caged with a fertile male for 72 h. A single dose of about 500 μg/kg was required to prevent pregnancy.

In the rat, day 1 of pregnancy was established by the identification of sperm in the vaginal smear. On day 8, the females were killed and the uteri examined for implantation sites. Administration of compound I orally by gavage at 25 μg/kg daily was 100 per cent effective in inhibiting implantation. A single oral dose of 500 μg/kg on post-insemination day 1, 2 or 3 was equally effective. The $E.D._{100}$ of I in the dog lies between 250 and 500 μg/kg/day when given orally by capsule continuously for 15 days beginning on the day of the first of three alternate-day matings. The compound caused a weak uterotrophic response in the rat, but when given concomitantly with oestradiol, an anti-oestrogenic effect was observed.

Acute toxicity studies of I gave an oral $L.D._{50}$ of 832 ± 32 mg/kg in the mouse and 1,778 ± 53 mg/kg in the rat. [illegible] tolerate chronic doses in the diet exceeding one [illegible] es the therapeutic levels [illegible] $_{100}$ values in the rat [illegible] c and anti-oestro- [illegible] for his crystallo- [illegible] d his staff [illegible] for their

Role of Catecholamines in the Amphetamine Excitatory Response

WHETHER amphetamine exerts its behavioural excitatory action in the brain directly or indirectly by interference or synergism with the catecholamines (noradrenaline and dopamine) is open to debate[1,2]. The advent of α-methyl *para*-tyrosine[3] (α-MPT), which inhibits the *in vivo* synthesis of 3,4-dihydroxyphenylalanine (DOPA)—the physiological precursor of the catecholamines—offers a new way of investigating this problem.

Spector *et al.*[3] showed that after treatment with α-MPT the content of noradrenaline in the brain and other organs gradually decreased, and after repeated doses it finally became impossible to detect noradrenaline. We found, using twelve rats, that even 3–5 h after one dose of α-MPT (75–200 mg/kg subcutaneously), amphetamine (3 mg/kg subcutaneously—referring to the *d*-amphetamine base) elicited little motor activity. The rats sat quietly and performed some grooming actions; with the lower doses, however, they also walked forwards. After repeated doses of α-MPT, even 6 mg of amphetamine/kg given subcutaneously was antagonized (Table 1).

Table 1. BEHAVIOURAL EFFECTS OF AMPHETAMINE AND *l*-DOPA IN RATS PRETREATED WITH REPEATED DOSES OF α-METHYL *para*TYROSINE

All rats received subcutaneously 100 mg/kg α-MPT 9, 6 and 3 h before treatment with amphetamine and DOPA

	Dose (mg/kg s.c.)	No. of rats	No. of rats showing stereotype behaviour*	Level of motor activity
Amphetamine	6	6	0/6	Low†
Amphetamine + *l*-DOPA	3 200	6	6/6	High‡
l-DOPA	400	6	0/6	Low§

* Constant sniffing, licking or biting of cage netting wire.

† Sat quietly most of the time. Performed some grooming and 50–70 min after amphetamine walked forward a little. Very little sniffing.

‡ Vigorous sniffing and biting for more than 30 min, walked forwards, backwards and circled about, but no grooming.

§ Sat or lay quietly, grooming, very little sniffing.

Amphetamine, when given alone, always produced marked hyperactivity and continuous, stereotype sniffing, licking or biting of the wire netting of the cage[2,4,5] (more than 200 rats were observed when doses of 1·5–30 mg/kg were given subcutaneously). With doses larger than 3 mg/kg, forward walking was completely abolished for a while; the rats performed their stereotype activity without moving, or they only walked backwards[4,5]. Grooming actions were not observed[5].

The experimental results in Table 1 strongly suggest that the antagonistic effect of α-MPT on amphetamine is due to the inhibition of DOPA-synthesis and not to some other action of the drug. Doses of up to 400 mg *l*-DOPA/kg given subcutaneously (nine rats treated) also had little effect on the gross behaviour of rats which had not received any pretreatment. A larger dose of *l*-DOPA (1,200 mg/kg given subcutaneously), however, produced hyperactivity and stereotype behaviour similar to that seen after amphetamine (ten rats)[6].

As inhibition of amphetamine induced behaviour would s[uggest] neuroleptic drugs[5], [the] activity. Here [it] perphenazi[ne] type a[ction] [7–10] days after the implantation of [p]eriod was considered suitable for the [im]portant degree of tolerance to and [al]kaloid[2]. Doses ranging [us]ed (Table 1). 3–4 min

We thank Merck, Sharp and Dohme for a generous supply of α-MPT.

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Intraventricular Administration of Nalorphine to Mice implanted with Pellets of Morphine

WHENEVER nalorphine is administered to white mice implanted with a pellet of morphine (base) an 'abstinence syndrome' is produced[1]. Its well-defined symptomatology and characteristics have been reported elsewhere[2–4]; the action of several drugs on the abstinence syndrome has also been tested[5–7]. However, its genesis is still not well understood. Because of this, it was thought of interest to determine whether nalorphine injected by the intraventricular route can elicit the abstinence syndrome in tolerant mice.

Male and female white mice weighing 25–30 g were divided into several groups of five animals each. Some groups were used as controls, while a morphine pellet was implanted in each mouse of the other groups; the daily absorption ranged between 90 and 130 mg/kg (ref. 1). Drugs were dissolved in physiological saline and administered in volumes of 0·025 ml. Intraventricular injections were performed according to the method of Haley and McCormick[8] as modified by Adler[9]. The correct location of the injected material was confirmed by (1) histological investigation after administration of indian ink; (2) by the appearance of the signs observed by Haley and McCormick immediately after a simple ventricular puncture or after the administration of saline and drugs (stupor, changes in the tail position, hyperexcitability and even slight convulsions)[8]. Observation of the mice was discontinued 15–20 min after the intraventricular injection.

In control mice, intraventricular injection of nalorphine (25 and 50 μg/kg) produced immobility or hypomotility during the first 5 min, followed by the appearance of exophthalmus, catatonia, and restlessness alternating with normal behaviour. The higher the dose, the more accentuated were the symptoms. Larger quantities (100 μg/kg) induced depression. Intraventricular injection of morphine (50 μg/kg) induced immobility in control mice; after 10 min some tail rigidity and restlessness were observed. Doses of 500 μg/kg elicited convulsions [fo]llowed by a typical morphine syndrome.

ml., results were obtained comparable with those for similar doses in more dilute solution, but recovery began sooner. In any event, two points must be emphasized: (*a*) an intense abstinence syndrome was never observed; and (*b*) the weak abstinence syndrome presented by some groups always appeared with a delay of 10–15 min (Table 1).

Table 1

Nalorphine, dose in μg/kg (1)	Time (in min) elapsed between the intraventricular injection of nalorphine and the appearance of signs: Dubious signs (2)	Weak abstinence syndrome (3)	Clear abstinence syndrome (4)
0·62	—	—	—
1·25	—	—	—
2·50	14	—	—
5·00	13	—	—
6·25	10	—	—
7·50	—	—	—
9·30	10	—	—
10·00	—	—	—
12·50	10–15	—	—
18·75	—	—	—
22·50	—	—	—
25·00	10	—	—
37·50	—	—	—
50·00	15–20	—	—
75·00	8	—	—
100·00	15	—	—
150·00	10–15	—	—
200·00	—	13–15	—
300·00	—	5–15	—

(1) In this series doses were administered dissolved in 0·025 ml. of physiological saline
(2), (3) and (4), see text.

The results reported here are in striking contrast with those obtained when nalorphine was administered intraperitoneally in mice implanted with morphine. In such cases, the manifestations of the abstinence syndrome were induced approximately 2 min after the administration of the drug; 3 min later, the symptoms increased significantly and in less than 10 min the abstinence syndrome actually reached a maximal intensity[1,2]. In order to exclude the possibility that the abstinence syndrome might be masked or hindered by the intraventricular injection of nalorphine, experiments were performed on various groups of mice: (*a*) 20 min after the intraventricular injection of nalorphine, 10 mg/kg of the drug was given intraperitoneally to all the animals in the groups shown in Table 1. Typical and intense abstinence symptoms appeared swiftly after the intraperitoneal injection, or the existing weak signs were intensified. (*b*) An intraventricular injection of saline was administered and immediately afterwards mice received a dose of nalorphine (10 mg/kg) intraperitoneally. (*c*) Nalorphine was injected intraperitoneally first, and saline was then injected intraventricularly. In the last two groups, a typical and intense abstinence syndrome could be seen after the symptoms that characterize the intraventricular injection.

Lockett and Davis[10] have shown that nalorphine given intracisternally is much less effective in antagonizing morphine than when it is administered via the systemic route. Nevertheless, the absence of precocious and definite symptoms of abstinence in the experiment reported here, even with high doses of nalorphine, was astonishing. These results may be interpreted as evidence that nalorphine itself is not the compound responsible for the production of the abstinence syndrome in mice that have received morphine chronically. The compound would trigger the abstinence syndrome if an 'activation process' were to occur. The 'active compound' may be derived from the injected nalorphine, from the morphine previously deposited in the animal or from some product that results from the chronic administration of morphine. This activation process (or processes) would take place slowly or incompletely in the central nervous system; for this reason, no abstinence syndrome or a delayed and weak abstinence syndrome is observed when nalorphine is injected into the ventricles. On the other hand, the 'activation process' may be fulfilled outside the nervous tissue; thus, when nalorphine passes into the systemic circulation, the 'active molecule' is set free and reaches the central nervous system to induce the abstinence syndrome. These possibilities are not opposed; on the contrary, they may enhance each other.

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VIROLOGY

Heterogeneity of Rabbit Serum Interferons

THERE is evidence that, in the rabbit, virus-induced interferon (VII) is induced differently from endotoxin-induced interferon (EII). The induction of VII is inhibited by actinomycin *D* (ref. 1) and by puromycin[2], while the induction of EII is not. Furthermore, Hallum *et al.*[3] demonstrated that, in the mouse, the molecular weight of EII is greater than that of VII. In view of these differences between EII and VII, we decided to study the molecular sizes of rabbit serum interferons induced by virus and by endotoxin.

VII was produced by injecting 5×10^9 P.F.U. of Herts strain Newcastle disease virus (NDV) intravenously into albino rabbits weighing, on average, 3 lb. and collecting serum after 7 h (ref. 4). EII was obtained from serum 2 h after an intravenous injection of 20 μg of *E. coli* endotoxin prepared according to the Boivin method[5].

The molecular sizes of VII and EII were compared by the gel-filtration method, as already described[6]. In brief, samples with phenol red incorporated as an internal standard were overlayed on a 'Sephadex *G*-100' column, then eluted with a phosphate buffered saline of *p*H 6·5. Fractions of constant volume were collected. The absorbancy of each fraction was determined spectrophotometrically at 280 mμ wave-length. Interferon activity was measured by the reduction of vesicular stomatitis virus (VSV) plaques in rabbit embryo cell cultures.

A typical set of results for VII and EII is presented in Figs. 1 and 2. As may be observed from Fig. 1, two major protein peaks were evident: one at fraction 18, the exclusion point of this column, the other at fraction 26·5, presumably globulin and albumin portions of the rabbit serum, respectively. Phenol red peaked at fraction 59·5.

Some phenol red was found to bind with albumin and post-albumin proteins which peaked at fraction 26·5 (coincidental with the 280 mμ peak), thus forming a convenient visual internal standard.

The interferon activity curve also showed two peaks, one being at the exclusion point, the other at fraction 29. Afterwards, fractions 14–22 were pooled, as were fractions 23–37. Interferon assays of these pools showed that about 32 per cent and 68 per cent of the total activity was associated with the heavy and light peaks, respectively. Repeated chromatography of VII produced by different individual rabbits showed that these peak positions were reproducible. Only the ratio of interferon activities distributed in the heavy and light peaks varied somewhat from sample to sample.

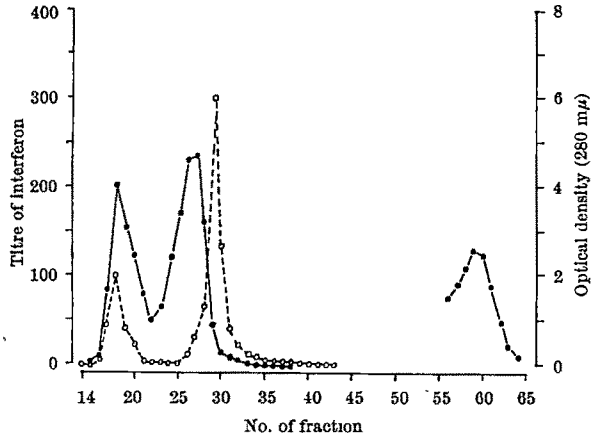


Fig. 1. Fractionation of VII on 'Sephadex G-100' column. O - - - - O, Titre of interferon, ●——●, O D (280 mμ)

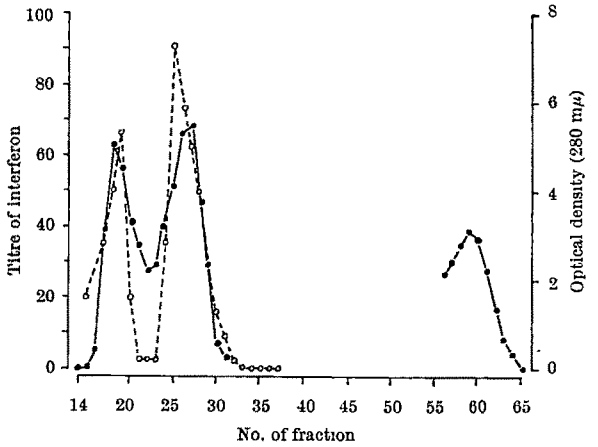


Fig. 2. Fractionation of EII on 'Sephadex G-100' column. O - - - O, Titre of interferon, ●——●, O.D (280 mμ)

The results concerning EII (Fig. 2) showed peak positions similar to those referring to VII, with the exception that the second interferon peak was at fraction 25·0. Interferon assays of the pooled peaks indicated that approximately 36 per cent and 64 per cent of the total activity were associated with the heavy and light peaks, respectively. Repeated chromatography of EII produced by different individual rabbits also showed reproducible peak positions, varying only in the ratio of interferon activity distributed in the heavy and light peaks.

When the lighter peaks of both VII and EII were separately pressure dialysed, and the concentrated materials afterwards mixed with normal rabbit serum, then re-chromatographed on the 'Sephadex G-100', no change of peak positions was observed.

Because both heavier species of the interferons peaked at the exclusion point of the column, they were roughly estimated to have molecular weights of more than 100,000[7]. The lighter species of VII was estimated to have a molecular weight of 40,000, whereas that of EII was 56,000[6].

The dual peak phenomenon observed here suggests that in the case of rabbit VII and EII, there are at least two molecular species of interferons.

This molecular heterogeneity of rabbit VII and EII is strikingly different from results obtained in mice[3]. It is probably unlikely that the heavier species represents a simple reversible binding of the lighter species to the heavier proteins of the serum as no such binding was found when the lighter species was incubated with normal rabbit serum. One possible explanation of these results is that the incubation of either virus or endotoxin triggers off more than one interferon-inducing mechanism each responsible for the production of a different molecular species; however, the predominant mechanism may vary.

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A Simplified Course of Immunization for the Antirabies One-day-egg Vaccine

ANTIRABIES vaccine produced from animal brains has been known to contain an encephalitogenic factor[1]. Attempts have been made, therefore, to remove this factor from brain tissue vaccines by physicochemical means[2–4], or to prepare vaccines from the brains of newborn animals[5–7], tissue cultures[8,9] or embryonated eggs[10,11]. Among these attempts, the use of chick embryos has incurred the difficulty of obtaining only a low virus yield in the embryo. However, it was discovered in our laboratory that the viral yield in the chick embryo markedly varied, depending on the strain of virus[12], its grade of adaptation to eggs[12] and the age of eggs inoculated[13]. Eventually we were able to adapt the rabbit-fixed Nishigahara strain of rabies virus to the 1-day-old chick embryo to such an extent that the resulting strain (named '*NOPM*') was able to yield a high concentration of virus in the embryo when inoculated into 1-day-old eggs[12]. A beta-propiolactone-inactivated vaccine prepared from this stimulated a good antibody response among volunteers who received it intradermally in 3–4 doses of 0·1 ml. spaced at 3–7 day intervals[14].

The next question to be solved was whether this course of injection could be simplified without lowering the efficacy of this vaccine so as to make feasible a mass prophylaxy against rabies in a population facing the possible risk of being bitten by rabid animals. Thus, in an attempt to determine the simplest schedule for this vaccine to confer a satisfactory immunity, we undertook to vaccinate two groups of persons in different schedules. The volunteers were students of the Yokohama City University School of Medicine who had never previously received antirabies immunization. The vaccine was prepared by the standard method described earlier[14]; its potency as expressed by the Habel index[15] was 4,900, and its nitrogen content was 520 γ/ml. One group of volunteers received only one intradermal dose of 0·1 ml., while the other group received the same injection twice with a one-week interval. Bleeding was effected just before and 1, 2, 4 and 6 weeks after the first immunization, and the test for neutralizing antibody was performed using mice in the manner recommended by the World Health Organization[16].

As indicated in Table 1, the one-dose group showed weak antibody responses, and only four of sixteen subjects developed appreciable levels of antibody, which averaged about 1 : 10. In contrast, in the two-dose group, ten of sixteen volunteers developed antibody higher than 1 : 10,

Table 1. EFFECTS OF ONE-DOSE AND TWO-DOSE IMMUNIZATIONS WITH THE *NOPM* ONE-DAY EGG VACCINE

Group	Volunteers*	Sex	Virus used in the serum neutralization test	Neutralizing antibody titre at week 0	1	2	4	6
One-dose			*L.D.*					
	F.N. (24) and O.S. (24)	M	25	<2†	<2	8	2	<2
	K.F. (22), H.T. (24), H.A. (23), K.H. (23)	M	25	<2	<2	<2	<2	<2
	T.H. (24), Y.M. (23)	M	25	<2	<2	<2	<2	2
	Z.U. (24)	M	25	<2	4	2	2	2
	K.O. (22), K.M. (21), A.W. (28)	M	25	<2	<2	2	<2	<2
	S.K. (23)	M	25	<2	<2	4 8	10	3 8
	T.S. (23)	M	25	<2	<2	2	2	<2
	S.I. (21)	M	120	<2	4 8	11	17	12
Two-dose								
	S.R. (22)	M	20	<2	<2	128	64	24
	H.S. (23)	M	20	<2	<2	91	11	18
	M.U. (24)	M	20	<2	<2	2	2	2
	N.H. (23)	M	20	<2	<2	<2	<2	<2
	J.O. (26)	M	50	<2	<2	18	3	7
	K.M. (22)	M	50	<2	<2	<2	3	4
	K.I. (24)	M	50	<2	<2	32	32	14
	I.S. (23)	M	50	<2	<2	43	13	6
	T.M. (23)	M	90	<2	<2	5 2	2	2
	H.Y. (23)	M	90	<2	<2	8	2 5	5
	N.K. (23)	M	90	<2	<2	47	16	11
	S.N. (23)	M	120	<2	<2	13	3	2
	Y.S. (24)	M	500	<2	<2	24	12	5
	T.S. (26)	M	500	<2	<2	36	11	13
	T.S. (23)	M	500	<2	<2	8	4	8
	S.S. (24)	M	500	<2	<2	12	56	12

* Age in years in parentheses.
† Reciprocal of end point dilution in the final virus–serum mixture.

and there was only one person who showed no antibody rise at all. Systemic reactions among these volunteers were not appreciable except in some of the two-dose group volunteers who complained of swelling and pain of the axillar lymph node following the second injection; however, this condition disappeared within a couple of days, and in none of them was a rise in body temperature observed to more than 37° C. As to local reactions, erythema averaging 1·1 cm in diameter was seen at the injection site after the first immunization, and in the two-dose group the second injection caused erythema and induration measuring on the average 3·7 and 2·0 cm, respectively, on the next day, but all these reactions disappeared within a week.

Another experiment was then undertaken to determine the effect of one-dose injection as a booster for eighteen persons, who had volunteered in the previous experiment carried out 21 months before in which the vaccine was given in three to four doses[14]. As controls, twenty-five new volunteers, who had not experienced any previous antirabies vaccination, were given one intradermal injection of the same vaccine simultaneously in an equal amount of 0·1 ml. The vaccine used showed a Habel index of 4,100, and contained 470 γ/ml. of nitrogen. As can be seen from Table 2, it was again found that the one-dose injection was insufficient to stimulate an antibody rise in the group of primary immunization, but the same dose of vaccine was enough to elevate the antibody levels in the booster group. In the latter group, fairly high levels of antibody were reached within one week, and in most of the subjects the maximum levels were about equal to those reached previously in their primary vaccination. The systemic and local reactions were similar to those in the primary vaccination group. Such a response is comparable with that usually obtained with the classical brain-type vaccine, and therefore the *NOPM* one-day egg vaccine can be used for mass prophylaxy of a population against rabies. The most satisfactory results will be obtained by the two-dose immunization as the primary course and giving one booster dose after every 2 years.

Table 2. BOOSTER EFFECT OF THE *NOPM* ONE-DAY EGG VACCINE ADMINISTERED IN ONE DOSE

Group	Volunteers*	Sex	Maximal antibody level reached previously in the primary vaccination	Virus used in the serum neutralization test	Neutralizing antibody titre at week 0	1	2	4	6
				L.D.					
Booster	H.S. (25)	M	19†	38	1·1†	420	210	—‡	—
	T.T. (27)	M	70	38	1	80	100	—	—
	Y.S. (25)	M	>512	38	<	94	128	—	—
	S.S. (24)	M	178	38	<	45	26	—	—
	N.N. (24)	M	64	38	<	23	78	—	—
	M.A. (25)	M	220	38	3	260	260	—	—
	N.K. (25)	M	64	38	5	180	85	—	—
	H.G. (24)	M	64	38	<	58	40	—	—
	M.S. (26)	M	3·2	38		11	21	—	—
	H.S. (25)	M	54	38	·5	26	128	—	—
	N.A. (25)	M	190	38		38	32	—	—
	M.U. (25)	F	512	38	2	150	230	—	—
	G.K. (26)	M	106	87		72	87	—	—
	Y.U. (26)	M	52	87	<	53	27	—	—
	Y.I. (24)	M	300	87	9	53	85	—	—
	T.Y. (24)	M	60	87		150	78	—	—
	K.F. (25)	M	18	87		12	8	—	—
	H.A. (24)	M	150	87	<	64	180	—	—
(Control) primary	K.K. (23), Y.S. (23), Y.M. (23), K.H. (24)	M		160	<	<2	<2	<2	<2
	Y.T. (23)	M		160	<	<2	2	<2	<2
	F.K. (22)	M		160	<	<2	5 2	<2	<2
	H.O. (34)	M		12	<	2 8	11	4·4	3 5
	K.H. (22)	M		12	<	<2	2 6	3·3	<2
	H.K. (22)	M		12	<	<2	10	4	2
	M.K. (23)	M		12	<	<2	2 6	10	17
	R.T. (24)	M		12	<	<2	<2	<2	<2
	K.U. (24)	M		12	<	<2	<2	<2	3
	S.Y. (23)	M		12	<	<2	<2	<2	2·8
	Y.N. (25)	M		12	<	<2	<2	<2	2
	H.S. (22), H.H. (24), M.T. (25), K.H. (24), Y.M. (23)	M		38	<	<2	<2	<2	<2
	M.Y. (25)	M		38	<	<2	2 8	3·1	<2
	S.N. (24)	M		210	<	<2	20	11	8
	H.T. (24), N.G. (24), Y.F. (25)	M		210	<	<2	<2	<2	<2
	K.T. (23)	M		210	<	2·5	2	2·7	<2

* Age in years in parentheses.
† Reciprocal of end point dilution in the final virus–serum mixture.
‡ Not tested.

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BIOLOGY

A Specific Midventral Gland in Gerbils

MAMMALIAN skin is rich in specific glands which perform various functions. Dambach[1] found a specific midventral gland in *Meriones unguiculatus* and gave a histological description of it. Other references exist in which attention is directed to the presence of such a gland in *Rhombomys opimus*[2]; however, its structure was not studied. The presence of similar glands in other gerbil species also remained uncertain, and thus we endeavoured to bridge this gap. Unfortunately, these glands cannot be distinguished in dry (museum) preparations, so that either living or alcohol and formalin-fixed animals were required. (Part of the material was placed at our disposal by the Zoological Museums of the Zoological Institute of the U.S.S.R. Academy of Sciences and of the University of Moscow, and by Mr. V. S. Lobachev (University of Moscow), whom we thank.)

As can be seen from Tables 1 and 2, the midventral gland was found in almost all the males inspected and also in many females; furthermore, the gland occurs in both adult and young animals (a well-developed gland was found in a 23-day-old male).

The gland is situated on the mid-line of the body, almost in the middle of the abdomen. In appearance it is a small thickening of the skin—two to three times greater than neighbouring areas (Table 3), and many ducts empty into this region (in *Rhombomys opimus* there are from forty to sixty-seven). The glandular field is irregularly oval, and elongated along the mid-line of the abdomen. The dimensions of the gland are presented in Table 2 (measurements were made from the internal surface of the skin).

As can be seen from Table 2, the glands in males are larger than those in females. It is of interest here that in *Rhombomys opimus*—the species of larger animals compared with other species—it turned out that the glands were even smaller than those in other gerbil species.

The midventral gland can easily be distinguished from the outside as the hair in the region is much shorter and, together with the surrounding hair, a dirty-white colour. When pressed, a dense white secretion with an odour of burnt horn exudes from the ducts.

A histological investigation of the midventral gland was undertaken. The gland was embedded in celloidin and cut along its longitudinal axis. Sections were stained with haematoxylin-eosin. Histologically, the structure of the gland was the same in all species of gerbils studied: the gland is formed by a complex of closely adjoining complicated alveolar glands with a holocrine type of secretion. The glands are considerably larger (in *Rhombomys opimus*: 0·38 × 1·10; 0·27 × 1·21; 0·88 × 3·41; 0·71 × 1·87 mm) than normal sebaceous glands in the hair sacs (in *Rhombomys opimus* the size of sebaceous glands is: 17 × 28, 46 × 52μ). (It is probable that the glands of the midventral glandular field developed from normal sebaceous glands.) The lobes of an individual gland (the size of these lobes differing widely) empty into a common duct which is widened at its base and which serves as some sort of reservoir for the secretion. In its upper regions, the duct narrows and empties into a small orifice at the surface of the skin. Each duct is situated laterally from the hair sac, but the size of the large duct and that of the tiny hair are so disproportionate that it is impossible to say whether or not the ducts empty into the hair sacs. It would be better to consider that the hair sac is an appendage of the duct.

The epidermis and its corneous layer in the region of the glandular field attain a somewhat greater thickness than in adjoining areas (Table 3).

The reticulate layer of the dermis in the region of the glandular field has in general a structure similar to normal skin, although it tends to be somewhat thinner (Table 3). The papillary layer formed by almost continuous aggregation of sebaceous glands is much thicker in the region of the glandular field than in normal skin.

The presence of the midventral gland enables us to suggest a new systematic character for the representatives of the sub-family Gerbillinae. However, a midventral gland resembling that of gerbils in its structure is also found in other animals systematically very remote from the gerbils (*Blarina, Neotoma, Cricetus, Peromyscus, Cricetulus, Masocricetus, Phodopus, Sigmodon, Martes zibellina, Rattus exulans*[3–8]). This finding indicates a convergence in the evolution of these glandular formations in various mammalian species. This character should therefore be applied with care in any elucidation of the relationship between such mammals—as was pointed out by Quay and Tomich[8].

Table 1. GERBIL SPECIES INVESTIGATED AND NUMBER OF ANIMALS WITH THE MIDVENTRAL GLAND

Species	No of animals inspected		No. of animals with the midventral gland	
	Males	Females	Males	Females
Meriones tristrami	4	6	4	2
M. persicus	3	2	1	1
M. meridianus	11	14	11	13
M. erythrourus	4	4	2	1
M. tamariscinus	2	2	2	2
M. unguiculatus	1	1	1	1
Rhombomys opimus	20	9	20	6

Table 2. DIMENSIONS OF THE MIDVENTRAL GLAND (IN MM)

Species	Sex	No. of gerbils inspected	Dimensions of the gland	
			Length	Width
Meriones erythrourus	♂	4	21–33; *M* 29	6–7, *M* 7
	♀	1	21	5
M. tamariscinus	♂♀	2	24–35	6–7
M. tristrami	♂♀	2	14–17	3–4
	♂	1	25	6
	♀	1	21	6
M persicus	♂	1	19	6
	♀	1	13	3
M. meridianus	♂	7	11–14; *M* 13	3–7; *M* 5
	♀	8	4–9; *M* 7	2–3, *M* 2, 7
Rhombomys opimus	♂	7	17–27; *M* 22	5–10, *M* 7
	♀	6	14–18; *M* 15	3–6, *M* 4

Table 3. MEASUREMENTS OF THE SKIN AND OF THE GLANDULAR FIELD IN *Rhombomys opimus* (MEAN DATA)

Sample	Sex	No of animals studied	Thickness (μ)		
			Skin	Epidermis	Reticulate layer of the dermis
Midventral gland	♂	7	997	29	36
	♀	3	648	21	41
Adjacent skin	♂	5	343	21	150
	♀	2	214	20	74

The biological function of the midventral gland seems to be to demarcate territory. In the wild, *Rhombomys opimus* can often be seen to rub its abdomen against the soil. The presence of the gland in both sexes and in very young animals, as well as the fact that the gland functions throughout the year (observations were carried out on *Rhombomys opimus* maintained in the laboratory), indicates that it plays no part in the sexual function of gerbils.

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Water Content in the Tissues of Desert Animals

WHEN studying *Gerbillus piramidum* and *Jaculus jaculus*, Khalil and Abdel-Messeih[1] claimed that the tissues of desert mammals contained less water than did those of non-desert ones. Several other workers (for example, the Schmidt-Nielsens[2]) found that desert animals lost less water through their skins. The data obtained by Khalil and Abdel-Messeih[1] suggest that the low water content in the tissues of desert animals (including the skin) contributes to the decreased loss in water and thus represents an important adaptation of animals to the environmental conditions of the desert.

I endeavoured to study the water content of the tissues of wild *Rhombomys opimus*, *Meriones meridianus*, *Spermophilopsis leptodactylus* and *Lepus tolai* in the Kara-Kumy desert. Samples were taken from freshly killed adults in winter (January) and in summer (June), excluding *Rhombomys opimus*. Samples of the skin (thoracic back without its hair), of the bone (femur) and of muscles (thigh muscles) were taken. Dehydration was performed in vials in a thermostat at 103° C and the water content of the tissues was calculated for the initial weight. The results of these analyses are given in Table 1.

The data obtained reveal a regularity noticed by all previous workers: the bones contained the least amount of water while the muscles contained the greatest. I was unable to confirm the data of Khalil and Abdel-Messeih[1] concerning the lower water content in the tissues of desert animals. A comparison of desert animals with non-desert ones shows that the water content of their skin and muscle is almost the same (see Table 1); however, the water content of bones of desert animals is somewhat less.

Table 1. WATER CONTENT IN THE TISSUES OF SOME MAMMALS
(The mean data in per cent)

Species	Season	No. of specimens	Percentage of water content in Skin	Bones	Muscles
Rhombomys opimus	Summer	5	65·2	28·6	79·1
Rhombomys opimus	Winter	10	56 9	33 7	76 7
Meriones meridianus	Winter	3	61·3	28 8	74·1
Spermophilopsis leptodactylus	Summer	6	65 7	29 1	80 8
Sphermophilopsis leptodactylus	Winter	7	61 6	16·9	75·6
Lepus tolai	Summer	4	65 6	—	75·8
Lepus tolai	Winter	2	66 2	—	75·9
*Gerbillus piramidum**	—	10	39 3	27·1	74·9
*Jaculus jaculus**	—	6	48 7	26 8	74·8
Cow, sheep, pig*	—	—	—	—	78–79
Dog*	—	—	55–77 3	58	68–87 3
Albino rat*	—	—	58 1–64·7	—	74·4–76 5
Rabbit*	—	—	75	—	80
Monkey*	—	—	70	61	79
Man*	—	—	58 9–72	50–55	75–78

* Data are taken from refs. 1 and 3–13.

No significant seasonal differences were revealed when winter and summer animals of the same species were compared (see Table 1). It is of interest here that a somewhat higher water content was found in both the muscles and skin of *Rhombomys opimus* and *Spermophylopsis leptodactylus* in summer.

These findings suggest that small water losses through the skin in desert animals taken for investigation cannot be explained by a difference in their water content from that of non-desert animals. Histological investigations[14] show that no morphological adaptations are present in the skin of desert animals for decreasing their water losses. On the basis of these data, it can be concluded that decreased water loss through the skin in desert animals is related to peculiarities in their endocrine and biochemical systems.

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Phenomenon of Swarming in Nematodes

THE phenomenon of swarming has been observed by one of us[1] in thirteen species of plant-parasitic and free-living nematodes embracing all sub-classes of the class Nematoda. Additional reports of swarming of plant parasitic species have come from Germany[2], Kenya[3,4], and Florida[5]. Staniland's original use of the term "swarming"[6] for massing of a *Rhabditis* sp. in response to light has priority. However, in view of the unique character and general nature of the phenomenon investigated at Louisiana State University Laboratories[7–10], it is proposed to retain for it the term "swarming" and to refer to Staniland's "swarming" as Staniland's phenomenon.

Work on swarming is being continued in our laboratory, and this communication presents evidence for the induction and specificity of swarming in the genus *Tylenchorhynchus* (Tylenchida).

Dependence of swarming induction in *Tylenchorhynchus martini* Fielding 1956 on rapid and abundant host plant development[10] was confirmed by results of nutrient-sand culture experiments with rice. Swarming, after 60–75 days, occurred only where host plants were supplied with inorganic nutrient solutions containing all essential elements. Under these conditions, the use of organic supplements—asparagine, glutamine and amino-acids from acid-hydrolysed casein—had no effect on the induction of swarming, although development of rice plants was stimulated. Inasmuch as induction of the anabiotic state in nematodes occurs in unfavourable environments[11], these results reaffirm the distinctions between swarming and anabiosis. The basic outwardly visible distinction between the two phenomena is that swarmers must pull apart at a point of contact in directions perpendicular to the plane of contact; anabionts can separate by gliding motions parallel to the plane of contact.

Further evidence on specificity of swarming reactions comes from the demonstration that *T. martini* and *T. claytoni* Steiner 1933 swarm together. These results, combined with the fact that *T. martini* and an undescribed

species of *Tylenchorhynchus* from sugar-cane at Grand Isle, Louisiana, swarmed separately in mixtures[8], indicate that both species and sub-generic (group or section) specificity of the swarming reaction exist in the genus *Tylenchorhynchus*. Morphologically, *T. martini* and *T. claytoni* are close relatives, whereas the Grand Isle *Tylenchorhynchus* is distinct from these species. The combined evidence suggests that swarming specificity lies at the sub-generic group level of morphological distinctions.

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Picrolonic Acid as a Hatching Agent for the Potato Cyst Nematode, *Heterodera rostochiensis* Woll.

Calam, Todd and Waring[1] reported that almost as many eggs of the potato cyst-nematode, *Heterodera rostochiensis*, were stimulated to hatch by a 3 mM aqueous solution of anhydrotetronic acid as by potato root diffusate; other tetronic acid derivatives they synthesized did not stimulate hatching. Marrian, Russell and Todd[2] synthesized various compounds based on an α-arylidene-$\Delta^{\beta\cdot\gamma}$-butenolide skeleton and reported that two of these, α-(*p*-hydroxybenzylidene)-γ-methyl-$\Delta^{\beta\cdot\gamma}$-butenolide and α-(3-methoxy-4-hydroxybenzylidene)-γ-methyl-$\Delta^{\beta\cdot\gamma}$-butenolide, "showed definite activity" at a concentration of 0·1–0·5 mM, whereas the rest were inactive or only slightly active. That anhydrotetronic acid is a good synthetic hatching agent has been confirmed[4–6], and in our tests, at 3 mM concentration, its hatch rating[3] was 90 (potato root diffusate = 100, water = 0), whereas 0·1–0·05 mM α-(*p*-hydroxybenzylidene)-γ-methyl-$\Delta^{\beta\cdot\gamma}$-butenolide was only slightly active (hatch rating 7).

Fenwick[7] and Doliwa[8] reported, respectively, that picric acid and potassium permanganate hatched eggs of *H. rostochiensis*. We tested these but found that picric acid was inactive (hatch rating 4) and potassium permanganate hatched only few eggs (hatch rating 9), whereas both hatched eggs of the beet cyst nematode, *H. schachtii*, well (hatch ratings >200 and 86, respectively)[3].

We tested the ability of more than four hundred compounds to hatch eggs of *H. rostochiensis*, using techniques already described[3,9,10], and, except for anhydrotetronic acid, found only one compound, picrolonic acid[11] (4-nitro-3-methyl-1-*p*-nitrophenylpyrazolone), which, at concentrations of 0·4–4 mM, hatches eggs as effectively as potato root diffusate (hatch rating 102). By comparison, zinc salts, which hatch eggs of several *Heterodera* spp.[9], have a hatch rating of 50 for *H. rostochiensis*. Other tetronic acid derivatives, pyrazolones and closely related compounds were inactive.

Picrolonic acid, like anhydrotetronic acid, is formed of two linked rings and has an acidic, enolic group as part of a potentially tautomeric system of two polarizable atoms separated by an extended chain.

Picrolonic acid also hatched many eggs of the soybean cyst-nematode, *H. glycines*, and some of the tobacco cyst-nematode, *H. tabacum*, but none of the cabbage cyst-nematode, *H. cruciferae*. We also confirmed that anhydrotetronic acid does not hatch *H. cruciferae*[6].

Unlike anhydrotetronic acid, picrolonic acid is a commercial product, so it is the first easily obtainable, efficient, synthetic hatching agent for *H. rostochiensis*. By contrast, more than fifty such compounds are known to hatch eggs of *H. schachtii*.

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Esterase Activity in the Arachnida

DESPITE the immense amount of work which has been carried out on the physiology of the insects, there is little comparative information available for the Arachnida. The present note is a report on current studies of the comparative biochemistry of these animals.

Using polyacrylamide gel electrophoresis the esterase activity of eight species belonging to seven genera of Arachnida has been investigated. Following homogenization, supernatants containing 300γ protein/0·05 ml. were run for 24 min at a constant current of 5 m.amp per tube. The tubes were made of soda-glass and were 65 mm long with an internal diameter of 5 mm; the gels were prepared according to the method of Ornstein and Davis[1]. After electrophoresis, the gel columns were removed and incubated in the appropriate substrate media.

The reaction mixtures used were those developed by Holt and Withers[2], Laufer[3], and Gomori[4]. The distribution of protein bands was indicated by staining for 1 h in 1 per cent amido-black in acetic acid, followed by the standard de-staining procedure. The association between total esterase activity and the protein bands was demonstrated by incubation in 5-bromo indoxyl acetate for 2–4 h at 21° C; and also α-naphthyl acetate together with either fast blue *RR*, or fast garnet *GBC*, for 2 h at 21° C. Incubation in solutions of acetylthiocholine iodide and butyrylthiocholine iodide demonstrated the presence of enzymes capable of hydrolysing these substrates, that is to say, specific and non-specific cholinesterases. Work is in progress in which selective inhibitors are being used to distinguish between these enzyme types. Table 1 summarizes the results obtained for the eight species

Table 1. OCCURRENCE OF ESTERASE ACTIVITY IN EIGHT SPECIES OF ARACHNIDA

	Dictynidae	Agelenidae	Argiopidae				Linyphiidae	Phalangida
	Ciniflo similis Blackwall	*Agelena labyrinthica* Clerck	*Meta segmentata* Clerck	*Meta merianae* Scop	*Araneus diadematus* Clerck	*Zygiella x-notata* Clerck	*Linyphia triangularis* Clerck	*Mitopus morio* Fab
Substrates								
5-Bromoindoxyl acetate	+	+	+	+	+	+	+	+
α-Naphthyl acetate								
+fast blue	non det	+	+	+	+	+	+	+
+fast garnet	non det	non det	+	+	+	+	+	+
Acetylthiocholine iodide	+	+	+	+	+	+	+	+
Butyrylthiocholine iodide	?	?	+	+	?	+	+	?

under consideration and provides a basis for comparison with the information available for other taxa.

I thank Dr. D. Boulter, Dr. M. E. Korn, and Dr. C. L. Smith for their advice on certain technical problems, the Medical Research Council for financial support, and Dr. R. G. Pearson for suggesting the problem and giving constant assistance.

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Mechanism of the Pasteur Effect

THE increase in rate of carbohydrate breakdown in peas in anoxia was earlier associated with decreases in adenosine triphosphate (ATP), oxidized diphosphopyridine nucleotide, 3-phosphoglyceric acid (3-PGA) and phosphoenolpyruvate (PEP) and with increases in adenosine diphosphate (ADP) and fructose-1,6-diphosphate (FDP)[1]. The decreases in 3-PGA and PEP were attributed to an acceleration of the metabolism of these compounds initially in anoxia; in consequence a faster rate of production of "glycolytic" ATP was inferred. We suggested that the sugar phosphorylating and glycolytic enzymes were located in an organized structure, which was effectively more permeable to ADP than to ATP. With such a structure the faster production of "glycolytic" ATP, mentioned above, should result in faster phosphorylations of both glucose and F6P than under aerobic conditions and so glycolysis would be hastened. Moreover, evidence quite different in nature from that just outlined, that is a discrimination in the use of ATP in air and in anoxia, could also be explained in terms of a glycolytic structure.

The occurrence of various important cellular processes in specialized structures, such as the nucleus, the chloroplast, the mitochondrion and the ribosome, provides a general argument for the existence of a glycolytic structure. The reason why a glycolytic organelle has not as yet been identified unequivocally could be that the structure is both smaller, and more easily disorganized by grinding, than, for example, the mitochondrion. In fact the glycolytic enzymes have hitherto been generally accepted as being "soluble" and located in the cytoplasm. Indeed in 1964 there was little evidence of the occurrence of a glycolytic organelle, but now data are accumulating which indicate that a glycolytic structure may exist.

Thus in "dried" yeast the glycolytic enzymes were not "soluble" but seemed to be located in an organized structure[2]. The enzyme carboxylase and about 25 per cent of the protein content was extracted by adding water, but the glycolytic enzymes were only extracted if the "dried" yeast was first homogenized by grinding. Moreover, the glycolytic activity of the "dried" yeast amounted to about 50 per cent of that of fresh yeast.

In other work yeast cells were ground in a mixer with glass beads and the glycolytic activity was found in fractions sedimenting at between 1,000*g* and 10,000*g*; no glycolytic activity was found in the supernatant solution[3]. But an active particulate fraction was obtained only from one strain of yeast cultured in a special manner.

In red blood cells and yeast the complete glycolytic system (including the sugar phosphorylating enzymes) was thought to exist in, or on, an organized structure, which was believed to be associated with the "membrane" of these cells[4–8]. With the procedures used the glycolytic enzymes were found both in the particulate and soluble fractions. Nevertheless, the view was favoured that the glycolytic enzymes existed *in vivo* in or on an organelle from which they were, in part, released during the preparation of the particulate fraction[7]. The "membrane" fractions were examined with an electron microscope[8]. Recently a fraction was obtained from yeast, sedimenting at 9,000*g*, which retained more than 50 per cent of the original glycolytic activity of the preparation of fragmented cells[38].

Thus our theory that the mechanism of the Pasteur effect was associated with a glycolytic structure is now supported by this evidence for red blood cells and yeast. Moreover, in yeast[9], rhododendron leaves[10,11], apples and potatoes[12] and pea segments[13,14], PEP decreased in anoxia, so that with these tissues, too, the mechanism of the Pasteur effect might be that described above. With brain in anoxia, however, when glycolysis increased from 4- to 7-fold, PEP did not decrease but rose by 2–2·5-fold, that is, proportionately less than for glycolysis[15]. Buckwheat seedlings, which do not appear to develop a Pasteur effect in anoxia[16,17], nevertheless showed the changes in phosphate compounds[18], already mentioned as characteristic of various plant tissues in anaerobiosis. This anomaly could be explained if buckwheat seedlings do not contain a glycolytic structure.

With animal tissues the Pasteur effect has been attributed to the activation of hexokinase (HK) and of phosphofructokinase (PFK) in anoxia[15,19–24] (in ref. 20 the tissue was treated with serotonin, which hastens glycolysis). Thus extracts of HK are inhibited by G6P (ref. 25) while PFK is inhibited by ATP and citrate[26], but activated by inorganic phosphorus, adenosine monophosphate, ADP, FDP and other compounds[15,21,23,27,28]. The Pasteur effect is thus attributed to the influence on HK and PFK of these various activators and inhibitors.

In the activation : inhibition theory, just mentioned, the observation that G6P decreases and FDP increases in anoxia is accepted as evidence that HK and PFK are activated in anoxia[15,20–21]. But other explanations can be given for the inverse changes in G6P and FDP in both plant and animal tissues in anoxia. Thus the high content of G6P in aerobiosis has been visualized as related to the high content of ATP in or near to the mitochondria[29–31]. With the decrease of "mitochondrial" ATP in anoxia a large decrease in G6P would be expected. The increase of FDP in anoxia has been attributed to a higher content of reduced diphosphopyridine nucleotide, this increase holding back the triosephosphate dehydrogenase and aldolase reactions[9,32]. Thus the addition of acetaldehyde to yeast, before anoxia, decreased the rise of FDP in anaerobiosis[9,32]. Moreover, in potatoes, FDP did not increase for 2 days of anoxia, while in apples this compound remained unchanged for 2 days of anoxia with one variety, but increased in a second variety; yet in both potatoes and apples the Pasteur effect developed during the first day of anoxia[12].

The time-relations of the observed changes in phosphate compounds are particularly important in relation to the mechanism of the Pasteur effect. Thus in peas the Pasteur effect was fully developed after 15 min or less of anoxia[33] and the contents of ADP, 3-PGA and PEP had decreased to new steady values in this period[1,31]; in contrast G6P did not change initially in anoxia in one experiment and may even have increased in another experiment. In rhododendron leaves[10,11] G6P increased for some 2 h in anoxia to about double the aerobic value so that in both peas and rhododendron leaves the acceleration of the HK reaction in anoxia may not be due to a decrease in G6P removing the inhibition of this enzyme by G6P, as postulated for animal tissues.

Other evidence against the validity of the activation : inhibition theory is given separately[31].

Thus for plant tissues the available evidence appears to indicate in general that the Pasteur effect cannot be explained by the activation : inhibition theory. However, because the evidence consists mainly of determinations of the total cellular contents of certain phosphate compounds, we must emphasize that both the contents and changes of the relevant substances at the enzymatic surfaces may

differ greatly from the total cellular amounts[34,35]; conclusions, based on such determinations, either for or against the validity of the activation : inhibition theory, must therefore be viewed with caution.

A puzzling feature of the Pasteur effect is that the rate of phosphorylation of sugar must be faster in anoxia than in air, although the cellular content of adenosine triphosphate is generally lower under anaerobiosis[1,9,31]. But this anomaly can be explained by the glycolytic structure theory, as an essential postulate is that the content of ATP is higher in the structure in anoxia than in air[1,31]. In contrast, according to the activation : inhibition theory, ATP would be lower in anoxia than in air, so that sugar phosphorylation would be retarded. This anomaly is generally not mentioned in descriptions of the activation : inhibition theory.

In bananas the climacteric increase in rate of respiration is associated with increases in the contents of G6P, FDP, pyruvate, α-ketoglutarate and oxaloacetate but with a decrease in the content of PEP[36,37]. Thus here, as in anoxia, the increase in respiration rate may be related to a faster rate of metabolism of PEP giving a faster rate of formation of "glytolytic" ATP.

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Antifungal Compounds in Apple Fruit infected with *Sclerotinia fructigena*

RECENTLY, Swinburne[1] reported the infection of apple fruits (var. 'Bramley's Seedling') by *Nectria galligena* and suggested that the production of an antifungal material after infection could account for an observed inhibition of the pathogen. During our investigations with fruits of a number of apple varieties we found, in agreement with Byrde[2], that the variety 'Edward VII' possesses resistance to infection by the brown rot organism, *Sclerotinia fructigena*. We have established that, when such fruits become infected with brown rot, antifungal compounds are produced which can be readily detected by biological and chemical tests.

When disks of *S. fructigena*-infected tissue from the 1963 crop were placed on potato dextrose agar, seeded with the conidia of *Alternaria brassicicola*, zones of inhibited fungal growth were found after incubation. Disks from healthy fruits gave no similar zones of inhibition (Fig. 1).

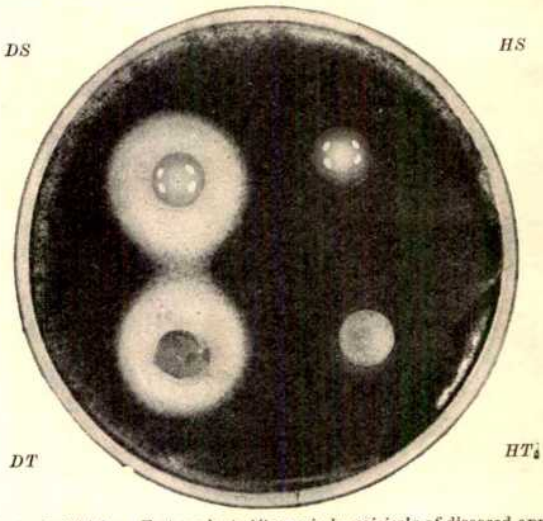


Fig. 1. Inhibiting effect against *Alternaria brassicicola* of diseased apple tissue (*DT*) and the syrup obtained from it (*DS*) compared with healthy tissue (*HT*) and its syrup (*HS*)

These observations were confirmed in experiments with apples harvested in 1964. 'Edward VII' apples (5 kg), visibly free from disease, obtained from trees which had not received fungicidal sprays, were wounded by sticking pins 2 mm long, at random, into their skins. They were then immersed momentarily in a suspension of conidia of *S. fructigena*. The inoculated fruits were kept in a humid atmosphere for eight days, by which time brown rot was well advanced and new conidial pustules were developing. After seeds and core tissue had been removed the fruits were macerated and the macerate

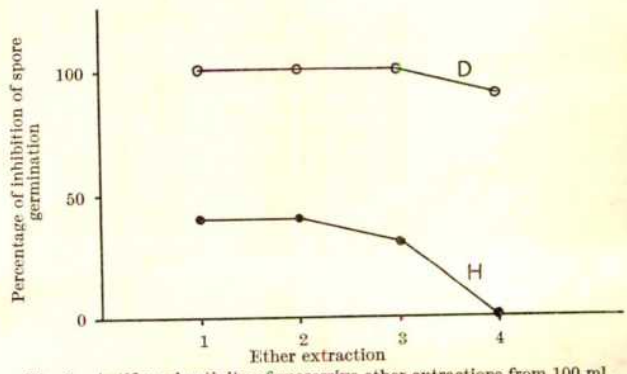


Fig. 2. Antifungal activity of successive ether extractions from 100 ml. of syrup from healthy (*H*) and diseased (*D*) apple. The residue from 0·05 ml. of each 200 ml. ether extraction was tested against *Botrytis cinerea* in 0·2 ml. of 0·2 per cent sucrose solution

Table 1. EXAMINATION OF ANTIFUNGAL MATERIALS *A* AND *B* FROM DISEASED APPLE BY PAPER CHROMATOGRAPHY ON WHATMAN NO. 1 PAPER WITH *n*-BUTANOL/PYRIDINE/WATER (1 : 1 : 1)

Chromogenic reagent (ref. 3)	Colour of spot: Material *A* $R_F = 0{\cdot}72$	Material *B* $R_F = 0{\cdot}26$
Diazotized *p*-nitroaniline	Red	None
Ammoniacal silver nitrate	Brown	White*
Potassium permanganate	White	White†
Bromophenol green	Pink	Pink‡

* On a grey background.
† On a magenta background.
‡ On a green background.

was filtered. The resulting liquid (about 4 l.) was rapidly concentrated to 400 ml. by evaporation under reduced pressure at 20°–25° C. The amber-coloured residual syrup (0·1 ml.) was placed in a cavity (1 cm in diameter) in a standard potato–dextrose agar plate seeded with the conidia of *A. brassicicola* and incubated. The antifungal effect is recorded in Fig. 1. Negligible antifungal effect was observed with the corresponding syrup obtained from healthy tissue.

Equal portions of the ether extract of the syrup from diseased apples completely inhibited the germination of spores of *Botrytis cinerea* while aliquots of the corresponding ether extracts from healthy apple syrup allowed germination (Fig. 2).

On shaking the active ether extract with sodium bicarbonate solution, most of the antifungal activity passed into the aqueous phase. This was acidified to *p*H 2·5 and re-extracted with ether. Malic acid, which possesses some antifungal activity, remained in the aqueous layer. The ether extract was dried over magnesium sulphate, filtered and evaporated to dryness under vacuum at 20° C. The pale yellow, tacky solid (0·75 g of crude material from 5 kg of apples) had an ED_{50} of about 60 μg/ml. against *A. brassicicola*. It was subjected to column chromatography on acidified silica gel in benzene/ether (1 : 1) and two antifungal materials were separated.

The chromogenic reactions (Table 1) and the infra-red spectra obtained with these materials indicate that *A* is a phenolic acid and *B* is an unsaturated aliphatic acid. These substances are readily detected by paper chromatography of ether extracts of the juice from diseased apples, but they are not detectable in the corresponding extracts from healthy apples. Further chemical investigations of these acids are now being made.

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GENETICS

'Kleine Liebling' a Haploid Cultivar of *Pelargonium*

IN the course of a comprehensive investigation of the chromosome constitution of *Pelargonium* species and cultivars, it has been discovered that the horticultural variety 'Kleine Liebling' is a true haploid with only nine somatic chromosomes. Kimber and Riley[1] list many records of the sporadic occurrences of haploids, both under natural conditions and in experiment, but this is the first time that the haploid condition has been so successful as to result in a vigorous and attractive cultivar which, although inevitably sterile, seems to lack any deleterious recessive genes which would naturally be exposed in a flowering plant possessing only a single set of chromosomes.

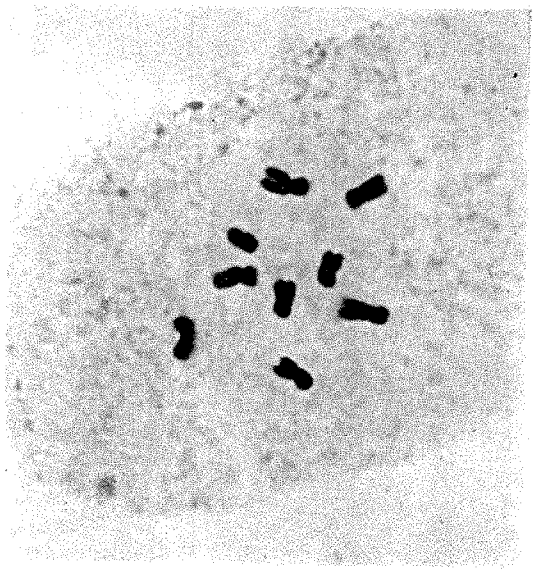


Fig. 1. Haploid root tip mitosis (× 2,000)

Pelargonium varieties are propagated almost entirely by taking stem cuttings, but the constant vegetative increase of plants carries with it the possibility of genetic variation by the conscious or unconscious perpetuation of mutants. For this reason, following the initial discovery of haploidy in one stock of 'Kleine Liebling', more chromosome determinations were made of plants from various sources and of the variegated form of this variety. The chromosome counts were also made from various parts of the plants such as roots, stem apices, and developing petals. It was found that whereas the aerial portions of the plants were invariably haploid, roots could be entirely haploid, haploid/diploid chimeras, or purely diploid, and each of these three types of root could occur on a single cutting. (Table 1 indicates the genetic constitution of both root and shoot systems for each plant examined.) This suggests that the variety is a haploid/diploid chimera, but it would be a strange chimera indeed if one of the genetically different tissues were consistently confined to the subterranean parts. If the plants were periclinal chromosome chimeras, evidence of this would be seen in the shoot apice squashes, which reveal the chromosome constitution of the *L* I, *L* II and *L* III layers; all, however, are haploid. For the same reason there is no evidence to suggest that the plants are mericlinal or sectorial chimeras in the generally accepted sense. There remains the possibility that the diploid cells are produced afresh when new plants are initiated by stem cuttings. When the cuttings were grown in water culture, it was observed that roots developed freely from the nodes. All such roots were found to be entirely haploid, but it is probable that under horticultural conditions callus tissue will form at the cut surfaces of the

Table 1. OCCURRENCES OF HAPLOIDY AND DIPLOIDY IN INDIVIDUAL PLANTS

Source	Root system: *n*	*n*/2*n**	2*n*	Total plants	Shoot system: *n*	*n*/2*n*	2*n*	Total plants
Non-variegated								
Kew, Royal Botanic Gardens	8	3	3	14	20	—	—	20
Telston Nurseries	—	1	—	1	1	—	—	1
Clifton Nurseries	—	1	—	1	1	—	—	1
Greybridge Nurseries	—	—	1	1	1	—	—	1
Variegated								
Telston Nurseries	1	—	—	1	1	—	—	1
Clifton Nurseries	—	—	1	1	1	—	—	1
Oxford Univ. Parks	—	1	—	1	1	—	—	1
Oxford Genet. Garden	1	—	—	1	1	—	—	1
Totals	10	6	5	21	27	—	—	27

* Included here are plants having both haploid and diploid roots, and also individuals with roots that are haploid/diploid chimeras.

stem, and it may well be that it is from this that the diploid roots arise.

Morphologically, 'Kleine Liebling' is characterized by the size of the leaves and flowers, which are distinctly smaller than in any other 'Zonal' *Pelargonium*; a factor that may well be attributable to the haploid condition. The origins of this cultivar have yet to be traced, but because pelargoniums are generally propagated by cuttings, it seems likely that all present-day plants have been derived from the same source, probably from a single genotype, initiated either as a bud mutation or through haploid parthenogenesis; its selection then being favoured by breeders on account of its miniature characteristics. The existence of a variegated form indicates that some gene or plastid mutation has occurred in the later development of this variety.

Further work is now in progress in an attempt to explain more fully the genetic constitution of this cultivar, but for the present it can be stated that there is little doubt that 'Kleine Liebling' owes its diminutive characteristics to the prevalence of a haploid condition which, in contrast to other monoploids, is highly successful.

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SOIL SCIENCE

An Improved Method of demonstrating the Distribution of Sulphides in Peat Soils

HYDROGEN sulphide is produced under certain conditions in soils and may be an important factor in governing the distribution of higher plant species in the field[1]. Burgeff[2] describes a simple method of demonstrating the presence of sulphides in peat soils. He obtained a visual measure of the distribution with depth from the formation of silver sulphide on silver-plated brass rods. Clymo[3] used polished silver wires for a similar purpose. Work at this research station on changes in redox potentials in depth and time at four peat bog sites has required better definition of sulphide distribution than either of these methods provides. The degree of discoloration of polished silver ranges from pale gold through brown to black, and it is difficult to observe the lighter end of the colour scale on a curved surface. Furthermore, there are often considerable horizontal variations in sulphide occurrence in the field and a large number of wires would be necessary to measure such variations. By using silver plates these difficulties can be overcome.

Polished copper sheets electroplated with silver (Johnson Matthey and Co., Ltd.), 30 cm square and 1·59 mm ($\frac{1}{16}$ in.) thick, were obtained. They were pushed vertically down into the soft peat after the surface mat of vegetation and roots had been sliced with a sharp knife. Before withdrawing, by a wire hooked through the two corner holes, the peat surface level was marked on them. Any material adhering to their surfaces was then carefully washed away and they were dried with soft paper tissues.

The images which formed on the plates were photocopied on to 'Ilfoprint' Contact Paper by exposure for 4·5 sec in a Dalcopier photocopy machine, and positives were prepared from these. This provided a permanent record of the distribution of sulphides at each site every month. The photocopies have the further advantage of eliminating the mirror effect of the plates and are consequently easier to examine. Two examples are shown in Fig. 1*A* and *B*.

Fig. 1*A* was from a *Sphagnum cuspidatum* pool on Striber's Moss, a relatively undisturbed estuarine raised bog in North Lancashire, and Fig. 1*B* was from the adjacent Deer Dike Moss. Deer Dike Moss has been extensively drained and burned, with the result that the surface is now dry and devoid of *Sphagnum*. Both plates were left in the peat for 1 h 30 min on July 9, 1965.

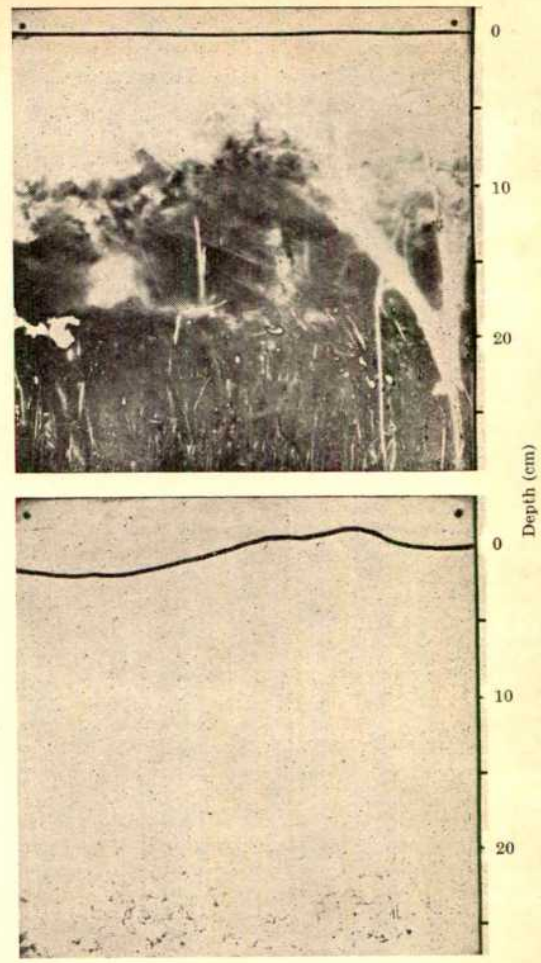


Fig. 1. Distribution of sulphide in the surface layers of an undrained (*A*) and a drained (*B*) raised bog peat profile. The profile surface is marked at the top of each figure

A striking feature of Fig. 1*A* is the silhouette of an *Eriophorum angustifolium* plant against which the plate had been deliberately placed. It is also apparent that large quantities of sulphides were present in this profile. *E. angustifolium*, however, would seem to be a plant species which is not inhibited by the sulphide. Armstrong found that oxygen diffused rapidly from the roots of this species, and the silhouette may indicate that oxygen was diffusing from the entire plant surface, and had locally prevented the formation of silver sulphide.

The irregular sulphide-free areas with intensely marked outlines on Fig. 1*A* are similar to those described by Burgeff[2] and probably arise from gas bubbles in the peat.

Fig. 1*B* illustrates the greater depth and very sparse local distribution of sulphide at Deer Dike Moss.

I thank Mr. A. J. P. Gore for his helpful advice and discussion.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS IN BIOCHEMISTRY (one in general biochemistry and one in biochemistry in relation to veterinary studies)—The Secretary of the University Court, The University, Glasgow (August 4).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF VETERINARY ANATOMY—The Registrar, The University, Liverpool, quoting Ref. CV/145/N (August 5).

ASSISTANT LIBRARIAN (with a good honours degree and preferably with a higher degree, professional diplomas and library experience)—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 5).

RESEARCH ASSISTANT IN AERONAUTICS to take part in the work directed towards the development of apparatus for a new high-speed aerodynamics laboratory, or alternatively, in the development of experiments on zero-gravity environmental studies—The Secretary of the University Court, The University, Glasgow (August 5).

HEAD (preferably interested in the inter-relationship between the mineral nutrition of plants and the feeding value of the forage produced) OF THE SOILS DEPARTMENT—The Secretary, Grassland Research Institute, Hurley, Maidenhead, Berkshire (August 8).

LECTURER or ASSISTANT LECTURER (preferably with research experience in fields other than soils or dairying, for example, food or taxonomy) IN THE SECTION OF FOOD AND AGRICULTURAL BACTERIOLOGY IN THE DEPARTMENT OF AGRICULTURE—The Registrar, The University, Leeds, 2 (August 8).

RESEARCH ASSISTANTS (with a degree or equivalent in biochemistry or chemistry) IN THE DEPARTMENT OF BIOCHEMISTRY for work on research projects in the following fields: muscle biochemistry, development and use of automation in amino-acid analysis, biochemical aspects of photosynthesis, and the metabolism of insect flight muscle—The Assistant Registrar (Science and Engineering), P.O. Box 363, University of Birmingham, Birmingham, 15 (August 8).

PHYSICAL CHEMIST or BIOCHEMIST (with a good honours degree in chemistry or biochemistry) IN THE N.E.R.C. MARINE BIOLOGY UNIT to investigate proteins associated with the settlement of marine organisms—The Registrar, University College of North Wales, Bangor, North Wales (August 10).

RESEARCH ASSISTANT (Non-medical) (with a B.Sc. with biochemistry as a main subject) IN THE UNIVERSITY DEPARTMENT OF PATHOLOGY, Western Infirmary, Glasgow, for research work on human autoimmunity and will consist largely of fractionation of tissues and serum proteins—Secretary of the University Court, The University, Glasgow (August 10).

ASSISTANT (with an honours degree in agricultural chemistry or biochemistry, and preferably postgraduate experience in agricultural chemistry) IN THE CHEMISTRY DEPARTMENT at Auchincruive for advisory investigational teaching duties—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (August 12).

LECTURER (preferably with experience in microbiological chemistry, microbial genetics or an applied field of microbiology) IN BACTERIOLOGY at Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (August 13).

SYSTEMS ANALYST (preferably with a degree or professional qualification and a knowledge of accountancy procedures) to develop new data processing systems for the University's administration and library—The Deputy Secretary, The University, Southampton (August 15).

COMPUTER PROGRAMMER (Technical Officer) (graduate with an ordinary degree in mathematics, computing and/or statistics an advantage) IN THE DEPARTMENT OF PUBLIC HEALTH AND SOCIAL MEDICINE for work which will be concerned with building up a suitable library of programmes in all aspects of medical research—The Secretary, University of St. Andrews, Queen's College, Dundee (August 19).

EXPERIMENTAL OFFICER and a TECHNICIAN (preferably with experience in mass spectrometry and/or electronics) to assist in the running of a unit for isotope geology being set up at the Reactor Centre—The Director, Scottish Research Reactor Centre, East Kilbride, Glasgow (August 20).

LECTURER or SENIOR LECTURER IN BOTANY at Fourah Bay College, The University College of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W C 1 (August 24).

ASSISTANT ADVISER (qualified man aged at least 45 with substantial practical experience of improving animal health and the livestock industries overseas, particularly in tropical conditions) based at the Ministry of Overseas Development London headquarters, but duties will involve travel to overseas countries to which H.M. Government furnishes assistance in the field of animal health—The Civil Service Commission, 23 Savile Row, London, W.1, quoting Ref. 6469/U (August 25).

COMPUTING ASSISTANT (with a good honours degree in mathematics or in a pure or applied science and preferably an interest in statistics and/or operational research)—The Secretary, The University, Leeds, 2 (August 26).

LECTURER/SENIOR LECTURER (with an interest and experience in one or more of the following fields: non-numerical computations, the use of computers in operations research, or the use of computers for control) IN THE BASSER COMPUTING DEPARTMENT, School of Physics, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 26).

LECTURER IN PHYSICAL or INORGANIC CHEMISTRY—The Assistant Registrar (Science and Engineering), P.O. Box 363, The University of Birmingham, Birmingham 15 (August 27).

PROFESSOR IN THE DEPARTMENT OF ZOOLOGY at the University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 27).

LECTURER (able to teach plant physiology to first-, second- and third-year students and supervise research for higher degrees) IN THE DEPARTMENT OF BOTANY; and a LECTURER IN CONTROL SYSTEMS IN THE DEPARTMENT OF ELECTRICAL ENGINEERING at University College, Nairobi (University of East Africa)—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 28).

LECTURER or ASSISTANT LECTURER (preferably with an interest in solid state physics or holography) IN PHYSICS at Queen's College—The Secretary, University of St. Andrews, Queen's College, Dundee (August 29).

SENIOR LECTURERS, LECTURERS, or ASSISTANT LECTURERS IN CHEMISTRY; a SENIOR LECTURER, LECTURER or ASSISTANT LECTURER (preferably with a special interest in applied mathematics) IN MATHEMATICS; and a SENIOR LECTURER or LECTURER (preferably qualified to advise on research design and on the use of computers) IN STATISTICS at the University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 30).

DIRECTOR OF THE INSTITUTE OF EDUCATION, University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (August 31).

LECTURER/ASSISTANT LECTURER (graduate with teaching and/or research experience in micrometeorology, environmental physics or cognate fields) IN ENVIRONMENTAL PHYSICS—The Registrar, University of Nottingham, Nottingham (August 31).

LECTURER (member of the Royal College of Veterinary Surgeons) IN THE DEPARTMENT OF ANIMAL HUSBANDRY AND VETERINARY PREVENTIVE MEDICINE—The Secretary of the University Court, The University, Glasgow (August 31).

LECTURER or ASSISTANT LECTURER IN MICROBIOLOGY IN THE DEPARTMENT OF BOTANY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (August 31).

READER (medically qualified) IN IMMUNOLOGY at the Institute of Cancer Research—The Academic Registrar, University of London, Senate House, London, W.C.1 (August 31).

READER or SENIOR LECTURER (with a registrable medical qualification, a higher degree or other higher qualification) IN MEDICINE at the University of Adelaide, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, August 31).

ASSISTANT LECTURER (with special qualifications and research interests in petrology, geochemistry or economic geology) IN THE DEPARTMENT OF GEOLOGY—The Registrar, The University, Nottingham (September 1).

CHAIR OF PHARMACOLOGY at Chelsea College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 1).

ASSOCIATE PROFESSOR OF PSYCHOLOGY (in the field of abnormal and clinical psychology) at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 5).

CHAIR OF PSYCHOLOGY and HEAD OF THE DEPARTMENT OF PHILOSOPHY AND PSYCHOLOGY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, September 15)

SENIOR LECTURER IN SOCIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (September 15).

CHAIR OF MATHEMATICS (Data Analysis) at the University of Canterbury, Christchurch, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 16).

LECTURER or SENIOR LECTURER IN THE DEPARTMENT OF STATISTICS, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 23).

LECTURER/SENIOR LECTURER IN VETERINARY MICROBIOLOGY IN THE DEPARTMENT OF VETERINARY PARACLINICAL SCIENCES, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 1).

RESEARCH ASSISTANT (graduate (or equivalent) in physics, engineering, or textile technology) IN THE DEPARTMENT OF TEXTILE TECHNOLOGY, to work on the development of woven, knitted and other fabrics—The Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1, quoting Ref. W.F. (October 1).

LECTURER/SENIOR LECTURER (with considerable chemical experience) IN PHARMACOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 14).

RESEARCH ASSISTANT (graduate (or equivalent) in physics, engineering, or textile technology) IN THE DEPARTMENT OF TEXTILE TECHNOLOGY, to work on unconventional weaving machinery—The Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (October 15).

BIOCHEMIST (B.Sc. or A.R.I.C.) in the Group Department of Biochemistry—The Hospital Secretary, Chase Farm Hospital, The Ridgeway, Enfield, Middlesex, quoting Ref. 191/66.

GEOLOGISTS (preferably with experience of structural investigation and drilling supervision or the computations of quantities) for coal prospecting in the United Kingdom—The Staff Manager, National Coal Board, Opencast Executive, Coal House, Lyon Road, Harrow, Middlesex, quoting Ref. S.V. 235(B).

MATHEMATICIANS to work on a wide range of problems arising from research into the utilization and distribution of electricity (topics include: arc physics, electro chemical reaction kinetics, solid state devices, magnetism, electrical distribution systems, heat transfer, and the application of mathematics to life science)—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Chester, quoting Ref. N/29.

ORGANIC CHEMIST (experienced in the synthesis and use of compounds labelled with radioactive isotopes) for the Chemical Research Team which is studying certain chemical aspects connected with tobacco smoking and human health—The Personnel Officer, Tobacco Research Council Laboratories, Harlow Hill, Harrogate, Yorkshire.

POST- or PRE-DOCTORAL RESEARCH ASSISTANT to assist in an investigation of Ziegler-Natta catalysed polymerization using radio-active isotopes—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

RESEARCH ASSISTANT (preferably with experience in one of the following fields: gas dynamics, electrical discharges, combustion) IN ELECTRODYNAMIC GENERATION OF POWER—Dr. J. Lawton, Chemical Engineering and Chemical Technology Department, Imperial College, London, S.W.1.

RESEARCH ASSISTANT (science graduate with a knowledge of microscopy) for laboratory work related to coronary thrombosis—The Secretary, Institute of Cardiology (University of London), 35 Wimpole Street, London, W.1.

RESEARCH ASSISTANT to take part in a study of the oxidation of organic compounds by high oxidation state transition metal halides and oxyhalides; and a RESEARCH STUDENT to participate in studies of free radical reactions of organic compounds—The Vice-Principal, Kingston College of Technology, Penrhyn Road, Kingston upon Thames, Surrey.

RESEARCH STUDENTS (with a good honours degree in physics) IN PHYSICS—The Registrar, The University, Sheffield.

RESEARCH STUDENTS (with a good honours degree or equivalent in physics or mathematics) IN THEORETICAL NUCLEAR PHYSICS—Prof. L. R. B. Elton, Department of Physics, Battersea College of Technology, London, S.W.11.

SENIOR LECTURER IN BIOCHEMISTRY; a SENIOR LECTURER IN PLANT PHYSIOLOGY; and a LECTURER IN MICROBIOLOGY—The Principal, Barking Regional College of Technology, [illegible]ge Road, Dagenham, Essex.

SENIOR LECTURER (with [illegible]n microbiological physiology/biochemistry) IN BIOCHEMIS[illegible]ipal, Bromley Technical College, Bromley, Kent.

SENIOR TECHN[illegible]chemical pathology) to work in the new Ren[illegible] with the Medical Unit—The House Go[illegible]n, London, W.2.

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SHOESTRING RESEARCH

THE tale of recent events at the British Food Manufacturing Industries Research Association on page 561 is not intended as entertainment. Those who hear of it will sympathize with all the parties concerned, the Council of the Association, the members of the Association, the laboratory staff and, most especially, the Director until October 11, Dr. C. L. Cutting. Most probably there have been mistakes on all sides. It remains unclear precisely how the future of the Research Association will work out, especially because a re-organization is having to be worked out at a time when British industrialists are scarcely likely to want to dig deeper into their pockets for a larger subscription. No doubt the Council will consider that it does not at this stage need to be embarrassed by unsolicited advice from outside. Though the Research Association may be comparatively small, its affairs raise issues of some importance.

The first thing to be said concerns the job of directing research in a laboratory of any size—in industry, government or a university. In one way or another, administration is an inescapable part of the job. The staff has to be kept happy, by being given a sense of purpose and in much more humdrum ways as well. Those who pay for the research have to be told what they are getting for their money—and persuaded to pay for more. Those who could make use of the work have to be shown how to do so. Then the broad strategy of the research programme has to be embodied in a host of small and apparently tedious decisions about the best kind of spectrometer to buy for the laboratory, or whether to send the laboratory assistants out for extra training. A director can, of course, be helped enormously by good advice, but in the last resort decisions must be his. To the extent that the Council hoped that they could operate a different system, it is to blame for what has happened. That, it is to be hoped, will be a comparatively easy lesson to learn.

It is much harder to see how the more enduring anomalies in the Association's organization are to be dealt with. Perpetual near-penury is a bad sign, and one that marks some other co-operative industrial research associations in Britain. Ironically, however, the food manufacturing industry is one of the largest, with a turnover of something like £5,000 million a year. Why is its co-operative research laboratory able to raise only a derisory fraction of this sum by subscription from food manufacturers? And how can the Association hold its head high when some individual member companies spend ten times as much in a year on their own food manufacturing research?

As at present organized, the Association is plainly trying to do too much with its meagre resources. The result is that it can neither command the respect of the big food manufacturers nor satisfy the diverse demands of the small members. And in trying to satisfy the needs of those who would pickle gherkins without loss of colour, and those concerned with the seeds in raspberry jam, the Association does not seem to have been able to pay as much attention as it should have done to the common and urgent problems of food manufacturers—the problems of hygiene and adulteration about which society is rightly becoming increasingly concerned. The best of all outcomes from the trouble at Leatherhead would be a determination by the food industry to see that it is not respectable to keep its research association so starved of funds that it is incapable of doing any job properly. The minimum subscription, at present fixed at a little more than £25, is, of course, derisory. It ought promptly to be multiplied by a factor between four and ten. If the result should be that some companies decide to cancel their membership altogether, nobody should worry. The doctrine of the co-operative research association is that the pooling of resources should make possible a more effective effort in research, but some of the food manufacturers appear to think that their association is just a way of getting research on the cheap.

The implications of what has happened at Leatherhead for research associations elsewhere cannot easily be spelled out. The signs are that the Food Manufacturing Industries Research Association is exceptional. Certainly the research associations concerned with iron and steel, or welding, have all the flair and freedom that good laboratories should possess. Scale is not crucial, for some quite small research associations are among the most effective. Yet the faults which are concentrated in the Food Manufacturing Industries Research Association are also those which tend to give the research associations as a whole a somewhat colourless and unattractive air. Members do often regard the associations as a complete substitute for research on their behalf, with the result that research programmes are needlessly fragmented. Councils do often exert too close a control on programmes of research, or keep too tight a hold on the moneybags. And staff tends to be down-trodden. Nobody should be surprised that the research associations—with some exceptions—find the recruitment of good people a perpetual headache. This is especially unfortunate in Britain at this time, for the associations are so well connected with industry that they should be able to do more than most organizations to help with the modernization of British industry.

TEACHING CHEMISTRY

THE Royal Institute of Chemistry has a splendid record in education. For several years it has worked hard on schemes to introduce modern ideas to teachers of chemistry in the schools. A year ago it broke new ground with a series of conferences for teachers, and if these were less successful than more permanent arrangements for teacher training would have been, the Institute is not the organization to blame for that. A measure of the Institute's educational ambitions is the fact that it is trying to raise £100,000 to finance a number of educational projects. One of the first things to be attempted is a study of the relationship between undergraduate and postgraduate courses at British universities, and this will provide an important part of the background to a conference on university education in chemistry due to be held in Manchester next January. That promises to be a valuable exercise.

With all this evidence of virtue, it is surprising that the Institute has so far done very little to remove the anomalous impediments to the transfer of bright young men and women from British schools to departments of chemistry at the universities. These are peculiarly British problems, and consequences of the bizarre practice of specialization in the schools. Like many other organizations, the Institute has been worried about them for some time. The most alarming symptom of what is wrong is the stagnation of so many of those who leave school at eighteen with a qualification in chemistry. In circumstances in which senior pupils must usually specialize in three subjects, chemistry has suffered more than physics, biology and mathematics from the attractiveness of newer studies such as economics. Yet British universities remain curiously inflexible in what they ask of intending entrants to chemistry departments. At most British universities, those with their eyes on a career in chemistry must present themselves with examination qualifications in mathematics, physics and chemistry. One immediate absurdity is that only a tiny handful of British graduates in chemistry have ever studied biology except at an elementary level. A more insidious evil is that young people inevitably suppose the rigidity of the regulations for entering a chemistry department to be a sign that chemistry itself is a kind of straitjacket. By requiring that young people wishing to take up chemistry at university should so resolve at sixteen, the same rigidity probably ensures that many potential chemists finish up in other disciplines.

These tendencies are probably a sufficient explanation of why chemistry has been lagging behind other scientific studies in the interchange between British schools and universities. All the Institute's work on the in-service training of teachers, and its willingness to spend liberally from its limited resources, can only be a palliative. It is like trying to cure measles by painting out the spots. Sooner or later there will have to be a direct attack on the problem of the demands made by the universities on would-be entrants to chemistry departments. As things are, the chemists are outdone in conservatism only by the mathematicians. The universities will have to recognize that they cannot rely indefinitely on the schools to supply them with stereotyped young chemists. If the alternative should be a fourth year at university, the chemists should not shrink from saying so.

SOBER SOCIAL SCIENCE

THOSE who may with justice have been afraid that the creation of the Social Science Research Council would be a device for subsidizing and perpetuating second-rate academic work at the universities should now be comforted. The first list of grants awarded by the Council is as respectable as anybody could ask. Altogether the Council has allotted £150,000 to eighteen projects. The recipients are as distinguished as they are well known. There is some straightforward social anthropology in Western Nepal and a study of kinship and support in pregnancy. Economics does well, and the study of the making of decisions in public enterprises which Prof. R. L. Meek is undertaking should be useful as well as interesting. The Council has done well to find a project on the development of cognitive skills in children, for too little work of that kind is being carried out in Britain, and there is a useful list of projects in what may properly be called industrial anthropology—the consequences of redundancy among the workers in a factory in South Wales, for example. It is particularly welcome that the Council should be supporting a study of the experiment at the Fairfields Shipyard in Glasgow whose ownership has been transferred to the British Government and certain trade unions.

Why, then, have academics been afraid of what the Social Science Research Council would attempt? And can they now relax, putting their trust in Dr. Michael Young and his committee? The difficulty, of course, is to know what is meant by social science. The several recent authorities on the subject, and particularly the Heyworth Committee, have been deplorably vague, with the result that too much attention has been drawn to pronouncements tending to suggest that any ill-considered set of concepts can be turned into a piece of science by a sufficient array of numerical information and a few applications of the chi-squared test. More seriously, except in fields such as economics, it is still uncommonly difficult to extract general conclusions from the kinds of studies to which social scientists appear prone. Having asked the wives of miners in Yorkshire how they spend their housekeeping money, there is often nothing to do but to put the same questions to miners' wives in Nottingham. Avoiding repetitious projects of this kind will be a constant headache. The temptation to lower standards will be great. The Social Science Research Council has begun well, but it will be helped by being watched closely.

NEWS AND VIEWS

Vienna Safeguards

THE International Atomic Energy Agency seems to have taken a still firmer grip on the problem of safeguarding the uses to which nuclear fuels are put. In the past few months it has been working out a procedure for extending the existing safeguards regulations, finally approved a year ago, to apply to reprocessing plants as well as nuclear reactors. The working group responsible for the original system was re-convened in February this year, and extensions to the safeguards system were approved by the Board of the Agency in June. This step, described in the annual report of the Board of Governors for the year ending on June 30, 1966, will go a long way to meet the criticisms of those who have argued that the control of re-processing plants is more important than the control of nuclear reactors in preventing the misuse of fissionable material.

An impressive number of nuclear installations now comes under the umbrella. By the beginning of 1967, it is expected that seventy-five installations with an electrical capacity of 4,500 MW will be concerned. The Agency is also planning to develop automatic monitoring devices which may make it possible for inspectors to follow some of the physical operations at reactors without being on the spot. Of the fifty-four reactors subject to safeguards at the end of June 1966, only seven were power reactors capable of producing more than 20 MW of electricity. The cost to the Agency of these activities is still relatively modest, and is expected to amount to just under half a million dollars in 1967.

The complete budget is a reminder that the Agency is still a relatively small organization. The total staff, from the doorman to the Director-General, will number 729 in the coming year. The estimate for 1967 of $11·9 million is actually somewhat less than for the preceding year. More than three-quarters of this sum will be spent on administration, but there is also room for some scientific work. The Vienna laboratory will take $200,000, and the International Centre for Theoretical Physics at Trieste a little more. The Monaco Laboratory will use $45,000, and the programme of technical assistance and training will cost nearly $2 million. As a whole, the record of good works is so respectable that it is all too easy to forget the troubles which attended the Agency's formation a decade ago, and which may yet break about its head.

Farmers' Weather

THE hope of a more trusting relationship between farmers and meteorologists was clearly to be inferred from a conference sponsored by Unesco and held last week at the University of Reading. It was the first international symposium on "Methods in Agroclimatology" and was attended by more than 120 people from thirty-six nations—meteorologists, agronomists, horticulturalists, foresters and even physicists. The purpose of the meeting was to survey the present contribution of meteorologists to agronomy, and to see what further benefits can be obtained.

It is not, of course, surprising that links between meteorology and agriculture are difficult to create. At Reading it was clear that efforts are now needed in almost diametrically opposed directions. First, there is a need to examine meteorological processes on the micro-scale so as to study the effect of the environment on the growth of plants, the productivity of animals and the epidemiology of pests and diseases. But, at the same time, there is also an urgent need for practical surveys on a macro-scale. Work of the second kind is indeed one of the essential needs of agricultural planners, particularly in developing countries where the rapid development of agriculture has to be guided by pure reason and not by the accumulation of unformulated tradition which determines agricultural practice in well-developed countries. Yet it was also plain, from the papers read at Reading, that experienced countries such as Japan and Great Britain have much to gain from the full exploitation of climatic potential. There was, for example, a detailed survey showing how more fruit could be grown in western counties of England.

A proof of the success of macro-surveys was the set of papers describing the surveys carried out by agencies of the United Nations, principally Unesco, FAO and WMO, in semi-arid areas. The general feeling at the symposium was that these surveys were a good beginning, and greatly to the credit of those who had carried them out. At the same time, it was now necessary to fill in some of the details of the relationship between climate and agricultural potential, particularly in the Levant and south of the Sahara.

The conference was also concerned with the modifications of the relationship between climate and agriculture which may be brought about artificially by irrigation and the provision of shelter, for example. There was also some speculation about different trends of climate. The conference was reminded that comparatively small changes of climate have in the past contributed to the downfall of nations, particularly around the Mediterranean, and that future plans for the development of land may be vitiated by similar alterations of the limits on agricultural practice. At the symposium there were also some suggestions as to how agriculturalists might codify their perpetual game with the weather along the lines spelled out by the games-theorists. Throughout, the mood of the conference was opposed to the view that it will be easy to make full agricultural use of climatic potential.

Fuel Enough

THE O.E.C.D. has taken a cautious but cheerful line in its most recent report on the balance between the supply of energy and the demand for it. The calculations and extrapolations of the group of officials responsible for the report suggest that between now and 1980 "resources are largely adequate to cover any foreseeable demand". More cheerfully still, it is thought probable that this comfortable balance can be achieved without moving too far from the present cost of energy.

Within the O.E.C.D. area as a whole, which includes North America and Japan as well as Europe, the increasing competitiveness of nuclear energy and of natural gas are given as the principal sources of price stability in the decade or so ahead. Nuclear energy, in particular, is said to be on the way to being "fully competitive in the near future". The authors of the report consider that nuclear energy will tend to set a

ceiling to the price at which fossil fuels can be sold for power production in the seventies.

The projections in the report are based on the assumption that the industrial production of the O.E.C.D. area will continue to grow without significant interruption. The demand for energy will march in step. It is expected that total consumption in the O.E.C.D. area will increase from the equivalent of 2,200 million tons of oil (in 1964) to 4,300 million tons of oil by 1980. One novel feature of these forward estimates, however, is a levelling tendency, for the rate of increase of demand is expected to be smallest in those countries in which energy is at present used most intensively—principally the United States. By 1980 it is reckoned that the United States will account for 60 per cent of the demand for energy within O.E.C.D., Europe for 30 per cent and Japan for the remaining 10 per cent. The total demand for energy in Eastern Europe and the Chinese Republic is expected to amount to 2,800 million tons of oil equivalent in 1980, or roughly 65 per cent of the then demand within O.E.C.D. The corresponding ratio in 1960 was roughly 51 per cent.

The forward projections for nuclear energy are more optimistic than previous forecasts by the O.E.C.D. By the mid-eighties nuclear power may account for 35 per cent of all electricity production in North America, and as much as 40 per cent in Europe. Then natural gas from the North Sea suggests at least the possibility that what has already happened in North America may also happen in Europe. The report says that this is an exciting prospect, and then goes on to suggest how individual nations should frame their policies so as to take advantage of the flexibility provided by a diversity of sources of supply. In passing, even the operators of coalfields can glean some comfort, for there is a solemn passage about the great size of the reserves of fossil fuel. This is a report to please everybody.

Who Needs Support?

LIKE foundations elsewhere, the Alfred P. Sloan Foundation of New York is hard at work trying to devise ways of keeping ahead of government support for science and for education. In his report for 1965, the President of the Foundation, Mr. Everett Case, says that the increased support from the U.S. Federal Government for scientific research at the universities and for students at college "has involved it in fields once largely the province of private initiative". Pointing to the scale on which the U.S. Government is at present supporting scientific research and education, Mr. Case says that "it is futile for private philanthropy to compete against the government". The report says that the federal government has not yet taken to supporting undergraduates and scientists in the early stages of their careers in precisely the way in which the foundation is anxious to do so, but clearly Mr. Case thinks it is only a matter of time before the government catches up. He is evidently hoping that a systematic appraisal of the outcome of the Foundation's grants in recent years may make possible an objective review of foundation policy, and then throw up suggestions of new fields for philanthropy to conquer.

Grants worth $13 million were awarded in 1965, mostly in scientific research and education. The Sloan-Kettering Institute is still high on the list of beneficiaries. The Foundation also seems to derive continued pleasure from open-ended grant-giving. Thus it has given $5 million to Stanford University as an expendable fund for research in the physical sciences, and has continued the programme by which university scientists, usually at the post-doctoral stage, are awarded fellowships detached from particular programmes of research. This is in line with the Foundation's view that too rigid a link between university research and the award of grants and contracts from outside may interfere with academic freedom. The Foundation also allocated $500,000 during 1965 to an imaginative scheme for supporting Negro male students at ten predominantly Negro colleges in the United States. The foundation stands out by its willingness to support schemes for the popularization of science, and is supporting the Scientists' Institute for Public Information and also the work of the Council for the Advancement of Science Writing in running training courses in science journalism.

Smithsonian 1965

THE annual report of the Smithsonian Institution for the year ended June 30, 1965, shows an appropriation of $15·54 million, of which $5·84 million was for the United States National Museum, $1·25 million for the Astrophysical Observatory, $319,601 for the National Air Museum, $179,640 for the Canal Zone Biological Area, $114,648 for the Bureau of American Ethnology, and $113,330 for the International Exchange Service. In his covering statement the Secretary, Dr. S. Dillon Ripley, refers to the establishment of an Office of Special Projects on the bicentenary of the birth of James Smithson, the founder, and says that one of the objects of that office is to examine the nature of knowledge and creative discovery as understood to-day.

The Museum of Natural History had a busy year. Members of its staff discovered the presence on Dominica of the snail *Planolina glabrata* (Say), the intermediate host of schistosomiasis. The close relation between agave and yucca has been proved beyond doubt in a study of yucca moths. An ancient connexion has been found between the fauna of Repa (in the mid-Pacific) and those of Australia, New Zealand and South Africa, and preliminary research in the Valley of Oaxaca, Southern Mexico, has verified the presence in many caves and rock shelters of abundant perishable remains of human occupation and shown that the corn cobs, cactus fruits and other associated artefacts came from the period 6500–2500 B.C.

The Radiation Biology Laboratory has developed instruments to measure the spectral quality of sunlight, in 100 mμ bands, from sunrise to sunset throughout the year. Further information was acquired on the chemical structure of polyphosphate compounds in marine organisms and algae. The Prairie network of automatic cameras for the simultaneous photography of very bright meteors went into full operation and the sensitivity and accuracy of the Harvard-Smithsonian Meteor Radar Network has been improved. New measurements have been made of radioactive and stable isotopes in meteorites and in dust samples from the Greenland Icecap and elsewhere.

Drop More Names

HAVE the bad old days when it was only possible to get on by knowing the right people gone for good?

Alas, no. An instrument recently introduced requires just this kind of intellectual nepotism, yet without the necessary personal acquaintance. Given a subject, a handful of top names and a library stocked with complete runs of some 20,000 top science journals, the *A–Z* of the subject is potentially at hand. The fundamental idea behind the *Science Citation Index*, of which the first quarterly volumes for 1966 have now become available, can be summed up in the phrase: "What has happened since ?" In other words, what has a man published, and who, in what publication, in any scientific field, has cited any of his works since they were published ?

Each quarterly issue consists of three massive volumes, two for the citations and one for recent sources. The former provide under name (or journal if anonymous), patent number or corporate body, every citation during the quarter in more than 1,500 journals, together with the names of those responsible for the citation, where and when. The companion *Source Index* provides chapter and verse of all new sources published in the quarter, and gives names of authors, titles of articles, journal, volume and page number, etc. It is estimated that when the 1966 *Index* is complete, 3·4 million cited references will have been listed from 300,000 new source items. At an annual subscription of $1,250 (educational rate) or $1,950 (all others), the *Index* is likely to be accessible only to those research institutions which already have, or are likely to obtain, a computer. Thus now that the searching technique by way of citation is being widely accepted, the compilers of the *Index*—the Institute for Scientific Information, Philadelphia—should be investigating the possibilities of putting it on tape.

Better Writing

THE report of the conference held earlier this year by what is known as the "Presentation of Technical Information Group" has now been published. The group seems to have recruited a company of speakers willing to say that scientific writing is bad and ought to be improved. It was apparently less successful in winning agreement about what should be done. Several speakers said that scientific appointments should go only to those able to demonstrate some skill in writing, but they were naturally somewhat reticent in suggesting how regulation should be imposed on the universities and the laboratories. The benefits of formal courses in language and writing for science undergraduates and postgraduates were extolled by some. Like most bodies that would change some aspect of society, the group seemed to think that the schools should be doing more, but there seems to have been some uncertainty about the need to encourage writing for self-expression in the schools. One opinion sent in after the conference was that "less emphasis could well be placed on self-expression and more on writing to supply information that somebody else needed to know".

Copies of the report may be had at 6*s*. from Mr. A. K. Parker, Godolphin Cottage, Gog Magog Hills, Babraham, Cambridge.

Abstracts on Tape

WHAT can be done with abstracts of the literature embodied on reels of magnetic tape ? The Chemical Society and the University of Nottingham are planning a research programme to explore the usefulness in a British context of the abstract service based on a computer system which is now being supplied by the American Chemical Society. The intention is that magnetic tapes from the United States will be processed by a unit under Dr. Anthony Kent, previously a lecturer in zoology at Nottingham, who will be the director of what will be known as the Chemical Society Research Unit in Information Dissemination and Retrieval. The title is evidently intended to suggest that both the Chemical Society and the university have in mind a continuing programme of research and development, and a statement by the Society says that its aim is to provide in Britain a comprehensive and mechanized information service comparable in every way with that now becoming available in the United States.

To begin with, at least, the Society is financing the project out of its own resources. By doing so it will put British chemists much where British medical research workers now find themselves as a result of the experiments being conducted from Newcastle as part of the MEDLARS programme. A related but necessarily more complicated programme of development is now being undertaken by the Institution of Electrical Engineers, which is responsible for publishing *Physics Abstracts*. That programme, which is being carried out in close collaboration with the American Institute of Physics, is financed by a grant from the Office of Scientific and Technical Information.

Virology Journal

THE Society for General Microbiology is to produce, and the Cambridge University Press is to publish in January 1967, a new journal called the *Journal of General Virology*. It will be edited by Dr. C. Kaplan of the Lister Institute of Preventive Medicine and Professor P. Wildy of the University of Birmingham. The Society for General Microbiology, which also publishes the *Journal of General Microbiology*, intends to divert virological papers to the new journal which will, however, not seek to publish clinical or epidemiological articles.

Podzol Jubilee

DR. H. KLINGE of the Max Planck Institut für Limnologie writes on the twenty-fifth anniversary of the publication by P. W. Richards[1] of "Lowland Tropical Podsols and their Vegetation":

"Before Richards's paper, podzols had been recognized and described from areas with cool-humid climates in temperate regions and on tropical mountains. From tropical lowlands of Borneo, white or bleached sand soils with a coal black or dark brown layer at some depth had been reported and the peculiar physiognomy and floristic difference of the vegetation from the surrounding normal equatorial rain forest mentioned[2,3]. Richards, however, was the first to realize that these soils are widespread in tropical lowlands in the Guianas, the Amazon and in Malaya, where they are always associated with a physiognomically peculiar vegetation and with blackwater rivers.

"Tropical podzols, being unsuited to profitable permanent agriculture, are considered important to forestry[4] and have therefore attracted much interest. This is borne out by the appearance of about four hundred publications concerning tropical podzols[5],

most of which deal with the soils themselves. Some hundred deal with tropical podzol vegetation and the remainder are concerned with tropical blackwater–podzol relationships. Of the seventy papers which appeared before 1941, a dozen were quoted by Richards.

"A considerable amount of information is now available on the distribution pattern of tropical groundwater and well-drained podzols; but no estimate is yet possible of the area covered by these soils, which mostly occur as scattered small patches. They are known to exist along the east and west coasts of Africa, at a few places in the Congo, and on the islands of Mafia, Pemba and Madagascar. Their distribution is wide in tropical South-East Asia and in tropical and subtropical Australia. They cover extensive parts in Florida and in the Guianas, and are also found in patches in the Caribbean region and in the Amazon basin. Despite the wide distribution in the tropical rain forest area, the actual proportion of the land surface occupied in the areas is rather small.

"Not all tropical bleached or white sands[6–8] are true podzols characterized by clay destruction[9], but immature soils on white sand deposits, occasionally overlying buried soils[10], or top layers of soils characterized by clay migration only.

"In his study of the tropical podzol vegetation, Richards referred to caatinga forests of the Amazon basin, Wallaba forests and Muri bush of the Guianas, heath forests (kerangas), padangs or mattangs of the Malayan region and noted that the forest types in the different regions were more closely related to each other than to the native mixed tropical rain forest. Podzols are now known to exist under grassy vegetation (Amazonian wet campos), campina forests of the Amazon, restinga forests of eastern Brazil, and coastal savannas of Africa. A feature of these more open plant communities is that they may include plant species of the genera *Drosera*, *Sphagnum* and *Cladonia* which also occur on temperate podzols.

"Richards saw the primary cause of podzolization, within the region of perhumid climate of the equatorial lowlands, in the low content of the parent material of bases and clay-forming materials. As a consequence, base-deficient soils develop on which, owing to this deficiency, raw humus accumulates. Raw humus accumulation is well known as a prerequisite for subsequent podzolization, so that the occurrence of this process in the tropical lowlands could be explained in this way. Richards's view has since been confirmed. The parent material on which tropical podzols predominantly develop are loose and consolidated sands. That these are mainly of marine origin would explain the frequent association of these soils with coastal plains, or fluviatic sands of river basins or outwash materials, or finally also acid volcanic rocks. Tropical podzols, however, have also been found to develop from latosolic parent soils[11]".

[1] Richards, P. W., *Nature*, **148**, 129 (1941).
[2] Beccari, O., *Wanderings in the Great Forests of Borneo* (London, 1904).
[3] Diels, L., and Hackenberg, G., *Bot. Jahrb.*, **60**, 293 (1926).
[4] Brünig, E. F., *Allg. Forst- u. Jagdztg.*, **128**, 156 (1957).
[5] Klinge, H., *Report on Tropical Podzols* (Paris, Unesco, 1965).
[6] Lang, R., *Jh. Ver. Vaterl. Naturk, Württ.*, **71**, 115 (1915).
[7] Mohr, E. J. C., *The Soils of Equatorial Regions*, translated by Pendleton, R. L. (Ann Arbor, 1944).
[8] van Steenis, C. G. G. J., *Tijdschr. K. Ned Aardrijksk. Genoot.*, **52**, 25 and 171 (1935).
[9] Mückenhausen, E., *Entstehung, Eigenschaften und Systematik der Boden der Bundesrepublik Deutschland* (Frankfurt, 1962).
[10] Vieira, L. S., and Filho, J. P. S. O., *Bolm Téc. Inst. Agron. N.*, **43**, 7 (1962).
[11] Bleackley, D., and Khan, E. J. A., *J. Soil Sci.*, **14**, 44 (1963).

Parliament in Britain

IN reply to questions in the House of Commons on July 28 regarding progress in establishing a University of the Air, the Parliamentary Under-Secretary of State for Education and Science, Miss Jennie Lee, said that the preliminary survey, including costs and television channels, had been completed. The figures were extremely encouraging and she considered that more could be done for more people at less cost in higher education once the project was under way than in any other part of the education field. The proposals would most certainly not be shelved: an open university had become an urgent necessity in Britain's circumstances to-day.

IN the House of Commons on July 26, Mr. H. Jenkins was given leave to introduce, under the Ten Minute Rule, a Bill "to restrain nuisance by aircraft noise; to restore freedom to pursue actions against aircraft owners and operators for nuisance by noise and vibration; to empower the President of the Board of Trade more effectively to limit and to restrain aircraft noise; and to empower the Parliamentary Commissioner to enquire into and report on all such questions". The Bill would seek to reduce the permitted level of noise from the present 110 perceived noise decibels by day and 102 decibels by night, to 100 noise decibels immediately and then to 90 noise decibels over 5 years. The Bill would also provide a defence against any actions against aircraft owners and operators if it could be proved that a noise level of no more than 100 decibels was being produced at the time.

IN a written answer in the House of Commons on July 18, the Minister of Technology, Mr. A. Wedgwood Benn, stated that, during the three years to March 1966, his department had spent £12,000, £6,000 and £77,000 towards the development of fuel cells. The National Research Development Corporation had spent £18,000 up to May 1966, and British Rail and the Gas and Electricity Councils were also giving attention to fuel cells as part of their research programmes. The Minister intended to seek better arrangements for co-ordinating this work. There was much to be done before manufacture of an economic working system became possible.

IN the House of Commons on July 28 the Tees Valley and Cleveland Water Bill was given a third reading after an amendment, moved by Mr. M. Kimball, had been rejected by 112 to 82 votes. The Bill, which authorizes the Tees Valley and Cleveland Water Board to construct a reservoir in the Cow Green Area, is strongly opposed by the Nature Conservancy and the National Parks Commission. The scientific argument, which was put forcibly by Mr. Kimball, Mr. A. Blenkinsop, Sir John Eden and Sir David Renton, was not met by the supporters of the Bill, nor has it been by spokesmen for Imperial Chemical Industries, Ltd. The Minister of Land and Natural Resources, Mr. F. Willey, who had already given reluctant approval to the proposal, admitted frankly that an area of unique scientific interest would be prejudiced or damaged, but chose to accept the advice of the Water Resources Board rather than that of the Nature Conservancy and to spend another £12 million over 60 years.

In reply to questions in the House of Lords on July 25, the Minister of Defence for the Royal Air Force, Lord Shackleton, said that the functions and powers envisaged for a National Council for Oceanic Resources were already vested in the Natural Environment Research Council, which was responsible for supporting and co-ordinating research in this field. The Council had set up two specialist sub-committees, for oceanography and fisheries and for geology and geophysics. Exploration for oil and gas was carried out under licences granted by the Minister of Power, and information obtained by the licensees in the course of their operations must be made available to the Natural Environment Research Council's Institute of Geological Sciences. Lord Shackleton said that the Council's Oceanography and Fisheries Committee included the Fisheries Departments, and also representatives of the Hydrographer, the Admiralty Research Laboratories, the British Ship Research Association, and the Ministry of Overseas Development. It was very strong indeed on the marine biological side, and real progress had been made. There was also much international co-operation in this field. The International Council for the Exploration of the Sea was mainly concerned with the North-east Atlantic and there was also the Inter-governmental Oceanographic Commission of Unesco as well as other special agencies, and Lord Shackleton was very encouraged by the progress being made.

In reply to questions in the House of Lords on July 28 about the National Reference Library of Science and Invention, Lord Hilton of Upton said that a Working Party of officials was set up early this year to determine the requirements of the Library. When these had been settled, which it was hoped would be before the end of 1966, detailed planning of the new building would begin. The recent decision to move the Patent Office out of London had affected the scheme, necessitating a revision of building plans, and had led to renewed discussions with the Greater London Council over the lease of the South Bank site. The Minister of Education and Science was well aware of the importance of obtaining a suitable site as soon as possible.

University News:

Cambridge

Following the appointment of Prof. F. P. Bowden to the chair of surface physics, the name of the laboratory he is directing, which was formerly Physics and Chemistry of Solids, has been changed to Surface Physics, Sub-Department of the Cavendish Laboratory, Free School Lane, Cambridge.

Edinburgh

Dr. P. M. B. Walker has been appointed to the chair of natural history in the Department of Zoology. The following have been appointed to personal chairs: Dr. M. Pickford (physiology); Dr. J. A. Strong (medicine); Dr. J. R. Greening (medical physics).

Liverpool

Mr. G. H. Lawson has been appointed to the newly established chair of business studies. Mr. J. A. Proudlove has been appointed to the newly established chair of transport studies. Dr. J. Stringer has been appointed to the chair of physical metallurgy in succession to Prof. D. Hull, who was recently transferred to the Henry Bell Wortley chair of metallurgy.

London

Prof. R. W. H. Sargent has been appointed to the Courtaulds chair of chemical engineering, tenable at the Imperial College of Science and Technology. Dr. W. L. L. Rees has been appointed to the chair of psychiatry, tenable at St. Bartholomew's Hospital Medical College.

Newcastle upon Tyne

Mr. J. R. O'Callaghan has been appointed to the chair of agricultural engineering. The following have been appointed to personal professorships: Mr. J. Shaw (zoology); Mr. D. N. Walder (surgical science).

Swansea

Prof. P. M. Davidson has been appointed head of the Department of Physics for the session 1966–67. Dr. J. Dutton has been appointed to a chair of physics. Mr. H. W. Gosling has been appointed to a second chair of electrical engineering.

Appointments

The new director of the Tata Institute of Fundamental Research, Bombay, is to be Prof. M. G. K. Menon. He is primarily known for his contributions to cosmic ray research, and indeed worked in the cosmic ray group under Prof. C. F. Powell at Bristol University between 1949 and 1955. Prof. Menon joined the Tata Institute in 1955 and became senior professor and deputy director (physics) in 1964.

The following have been appointed to the Medical Research Council: Prof. D. A. K. Black, University Department of Medicine, The Royal Infirmary, Manchester; Prof. W. T. J. Morgan, Department of Biochemistry, Lister Institute of Preventive Medicine. These members have been appointed in succession to Prof. G. M. Bull and Prof A. Neuberger. Prof. W. S. Feldberg has been appointed head of a new Laboratory of Neuropharmacology at the National Institute for Medical Research.

Mr. R. F. Turnbull, a senior principal research scientist in the C.S.I.R.O. Division of Forest Products, has been appointed Australian chief scientific liaison officer and scientific adviser to the Australian High Commissioner in London, in succession to Mr. W. Hartley.

Announcements

Under the auspices of the International Union of Pure and Applied Chemistry, the University of Michigan is now making publicly available the *Bulletin of Thermodynamics and Thermochemistry*—an unusual kind of abstracting publication. The *Bulletin* breaks new ground by printing lists, frequently accompanied by short summaries, of articles in the process of being published by the journals. It also includes a comprehensive bibliography of papers in thermodynamics and thermochemistry published in the past year (1965). The volume of the *Bulletin* now available is the ninth to have been produced, but the first to have been published. Because of increasing size and rising costs, the *Bulletin* has had to seek support from the National Science Foundation, which has provided capital to launch the enterprise.

The Association of Scientific Workers in Britain has taken a stand against the British Government's incomes policy. In a statement issued at the end of July, the Association says that it has consistently opposed the Government's policy and that it will "oppose with even greater vigour" the proposal that there should now be a "freeze" on wages in Britain. The statement says that the Association considers "the relative technological backwardness of some sections of industry, the need to expand rapidly in

education, health and housing and the need to increase exports" cannot be solved by a restraint on wages and salaries.

THE Ministry of Defence (Royal Navy) says that the time signal service, which since January 1, 1966, has been provided by Criggion VLF station (*GBZ*) on 19·6 kc/s while the Rugby station was modernized, will revert to Rugby (*GBR*) on 16 kc/s, as from 0001Z on September 1, 1966.

IT is planned to have early in 1967 a special issue of *Radio Science* devoted to the subject of the propagation of long radio waves. Experimental and theoretical workers in this field are invited to submit manuscripts for consideration, and further information can be obtained from the Editor, Radio Science, Environmental Science Services Administration, Boulder, Colorado 80302.

PROF. F. SONDHEIMER, who since 1964 has been holding a Royal Society research professorship at the University of Cambridge in the University Chemical Laboratory, will, from October 1, 1967, be holding his appointment in the Department of Chemistry at University College, London. The University has conferred the title of professor of chemistry on Prof. Sondheimer in respect of his appointment.

THE Canadian Society for Cell Biology was formed at a meeting held in the Queen's University, Kingston, Ontario, during May 19–20, with an executive consisting of: *President*, Dr. H. F. Stich (McMaster University); *President-Elect*, Dr. L. Simmovitch (University of Toronto); *Secretary*, Dr. S. T. Bayley (National Research Council, Ottawa); *Treasurer*, Dr. G. Setterfield (Carleton University); *Directors*, Dr. S. J. Webb (University of Saskatchewan); Dr. J. G. Lafontaine (Laval University); Dr. M. Barr (University of Western Ontario); Dr. P. Ford (University of British Columbia).

THE third international Congress of Nephrology will be held in Washington, D.C., during September 25–30. Further information can be obtained from the Secretariat of the Congress, 9650 Wisconsin Avenue, Washington, D.C.

A CONFERENCE on "Hazards in Foundries", organized by the British Occupational Hygiene Society, will be held in the University of Birmingham during September 13–14. Further information can be obtained from Mr. D. C. Murphy, Medical Department, Esso Petroleum Co., Ltd., Esso Refinery, Fawley, Southampton.

THE sixth international symposium on "Gas Chromatography and Associated Techniques", organized by the Gas Chromatography Discussion Group of the Institute of Petroleum, will be held in Rome during September 20–23. Further information can be obtained from the Institute of Petroleum, 61 New Cavendish Street, London, W.1.

A SYMPOSIUM on "Gastrointestinal Radiation Injury" will be held in Richland, Washington, during September 25–28. Further information can be obtained from Dr. M. F. Sullivan, Biology Department, Battelle-Northwest, P.O. Box 999, Richland, Washington.

THE third international meeting on "Organic Geochemistry" will be held at the Imperial College of Science and Technology during September 26–28. Further information can be obtained from Dr. G. D. Hobson, Department of Geology, Imperial College of Science and Technology, London, S.W.7.

A GENERAL discussion of the Faraday Society on "Colloid Stability in Aqueous and Non-Aqueous Media" will be held in the University of Nottingham during September 26–28. Further information can be obtained from the Assistant Secretary of the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

A SYMPOSIUM on "Bionic Models of the Animal Sonar System", sponsored jointly by NATO and the Office of Scientific Research of the United States Navy, will be held in Frascati during September 26–October 3. Further information can be obtained from Dr. R. G. Busnel, Laboratoire de Physiologie Acoustique, Domaine de Vilvert, Jouy-en-Josas, S. et O.

A CONFERENCE on "Energy Beams and their Uses", arranged by the Electronics Group of the Institute of Physics and the Physical Society, will be held in the University of York during September 28–30. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A RESIDENTIAL conference on "Integrated Process Control Applications in Industry", arranged by the Institution of Electrical Engineers, will be held at the Manchester Institute of Science and Technology during September 26–29. Further information can be obtained from the Secretary, Institution of Electrical Engineers, Savoy Place, London, W.C.2.

ERRATUM. Dr. B. G. Gowenlock has been appointed to succeed Prof. F. Bell to the chair of chemistry in the Heriot-Watt University, and not Dr. B. Glover as stated on p. 225 of the July 16 issue of *Nature*.

CORRESPONDENCE

Unpublished Literature

SIR,—Your trenchant criticism of the proposed Physics Information Exchange Scheme (PIE) (*Nature*, July 23, 1966) rather suggests that you do not find much wrong with the present system of publishing the results of scientific research. I do not share your Candide-like attitude.

In the first place publication is often far too slow.

Secondly, the practice of selection by referee is open to considerable objection. Many people have had the experience of having a paper rejected, or worse still rewritten in an unacceptable form, by one journal, and subsequently promptly accepted by another. Moreover, though it does not happen often and has certainly never happened to me, there do appear to be instances in which a referee has abused his privilege and started work based on an idea gained from a paper sent by a journal for his opinion before the article was published.

Thirdly, in order to keep abreast of progress even in one limited field it may be necessary to see regularly several dozen journals.

The Immunopathology Exchange Group (IEG), of which I am a member and which seems to be very non-exclusive, is to me of enormous value and I am most delighted both to read and to contribute to its scientific memos. Of course IEG has its critics and no doubt there is room for improvement in regard to distribution and perhaps in other ways, but it does represent a new approach to the quick dissemination of scientific information which goes far to overcome the disadvantages of conventional publication, some of which I have listed above. I know little about high energy physics, and nothing about PIE beyond the information in your editorial, but the scheme sounds as if it might help physicists in the way that IEG helps immunologists. One would hope that a journal of such high standing as *Nature* would have adopted a less timid and more constructive attitude to such an important experiment.

Yours faithfully,

MICHAEL WOODRUFF

Professor of Surgical Science,
University of Edinburgh.

ORGANIZATION OF A RESEARCH ASSOCIATION

RESIGNATIONS AT THE BRITISH FOOD MANUFACTURING INDUSTRIES RESEARCH ASSOCIATION

A PERIOD of dispute and misunderstanding about the management of the British Food Manufacturing Industries Research Association culminated on July 11 with the resignation of the Director of Research, Dr. C. L. Cutting, and the Secretary, Mr. D. H. Martin. For some months a firm of management consultants, Urwick Orr and Partners, has also been surveying the work of the organization, which is sited near a number of other co-operative industrial research associations at Leatherhead, Surrey.

Dr. Cutting has a distinguished record of work in the handling and processing of food. A physical chemist by training, he joined the Department of Scientific and Industrial Research in 1937 and worked at the Torry Research Station, Aberdeen. During this period he worked on the development of a mechanical kiln for the smoking of fish, and that device has since been widely used as the Torry Kiln. Dr. Cutting moved to the Humber in 1952, where he was responsible for building up a new laboratory concerned with fish technology, and where he also carried out a series of measurements of the temperatures at which fish were to be found at various stages of their journey from the North Sea to the fishmongers' slabs. Dr. Cutting became director of the British Food Manufacturing Industries Research Association on February 1, 1962.

A part of the cause of the disputes of the past four years seems to be the arrangements made for the management of the Research Association from the time of Dr. Cutting's arrival. It appears to have been decided that the new director should have as much time as possible to direct the scientific research of the Association, and that certain administrative responsibilities should be shouldered by the secretary of the Association.

These arrangements, designed though they may have been to assist the smooth running of the laboratory, seem to have been a source of friction. Sometimes it appears to have been difficult to decide whether the secretary of the association was responsible to the director of research or to the Council of the Association, possibly through the Finance and General Purposes Committee and its various sub-committees. Proposals for the purchase of new equipment had to be costed in such great detail that the director got the impression that he was not master in his own house. Though the director was responsible to the Finance and General Purposes Committee for recommending individual salary increases for his staff, he was not always present when decisions were made on matters like these.

The general level of salaries for research workers at the Association appears to have been another bone of contention. In 1962, the cost of salaries, superannuation and national insurance for the then staff of eighty-seven, thirty-seven of whom were scientifically qualified, amounted to £85,000. Since then there have been improvements in both salary and pension rights, and the most recent development is that the Association has adopted the salary scales of the Scientific Civil Service as a basis for the payment of its own research staff, with certain reservations concerning matters such as the timing of salary increases. Because of the proposal to increase salaries, the Association expects that income will not cover expenditure during 1966. Even so, the discontent of the staff seems not to have been fully allayed, at least in part because they were not fully informed about the reservations attached to the decision that the salary scale of the Scientific Civil Service should be adopted at Leatherhead. For one thing, the issue of salaries at the Food Manufacturing Industries Research Association has been taken to the Ministry of Labour by the Association of Scientific Workers, which claims the membership of a majority of the staff at Leatherhead. Another sign of continuing discontent is that the loss of staff from the laboratory during 1965 amounted to thirty-six (compared with fifteen in the previous year), or rather more than a third of the total staff.

These events have taken place in what seems to have been an atmosphere of impoverishment going back at least to the foundation of the Association in 1946 by the amalgamation of the British Association of Research for the Cocoa, Chocolate, Sugar Confectionery and Jam Trades and the British Food Manufacturers Research Association. Over the years the membership of the Association has steadily grown, and now amounts to close on 700, yet income by way of subscriptions lies between £90,000 and £100,000. The subscriptions of individual member companies range from £25 to £1,500. The Government, through the Ministry of Technology, contributed £47,000 in 1965—calculated as half the value of the subscription revenue from British companies. One of Dr. Cutting's contributions to the work at Leatherhead has been to increase the contributions from contract research for public authorities in Britain and elsewhere. The Association's work on cotton-seed oil and meat under contract with the U.S. Department of Agriculture was worth close on £12,000 in 1965. Work of this character for public authorities in Britain often ranked for grant from the Ministry of Technology.

The other outstanding feature of the work of the Research Association is the diversity of its scientific research. Inevitably the balance of effort reflects the interests of the subscribers, which means that confectionery and products made from meat and fish account for the greater part of the Association's effort. But it also attends to the problems of jam makers, picklers, bakers wishing to use materials such as ready-made icing, and food manufacturers concerned with edible oils. In its meat research, the Association has made some valuable contributions to the understanding and the control of bacterial contamination. Its programme of research is now being coordinated closely with that of the Meat Research Institute at Bristol, financed by a statutory levy on the meat industry.

In all the circumstances, it is more than probable that the Association will find itself having to make a drastic re-alignment of its policies in the months ahead, and in the light of the eventual report of the management consultants. A more secure source of a larger revenue is obviously a first objective, and there is no doubt that other research associations will take the greatest interest in the attempts that will no doubt be made to persuade member companies to pay larger subscriptions. It will also be surprising if the Association does not find itself looking for a more tightly-knit research programme. Getting staff—and keeping them—will not have been made easier by the events of the past few years. Going out of business cannot be altogether out of the question.

BIOLOGY OF SYNCHRONOUS FLASHING OF FIREFLIES

By DR JOHN BUCK and ELISABETH BUCK
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REPORTS of synchronous rhythmic flashing by fireflies in South-East Asia have been appearing for more than two hundred years. Smith's[1] description of displays along the Chao Phraya (Meinam) River south of Bangkok will serve to introduce the phenomenon: "Imagine a tree thirty-five to forty feet high thickly covered with small ovate leaves, apparently with a firefly on every leaf and all the fireflies flashing in perfect unison at the rate of about three times in two seconds, the tree being in complete darkness between the flashes . . . Imagine a tenth of a mile of river front with an unbroken line of *Sonneratia* trees with fireflies on every leaf flashing in unison, the insects on the trees at the ends of the line acting in perfect unison with those between. Then, if one's imagination is sufficiently vivid, he may form some conception of this amazing spectacle." The flashing, Smith continues, is confined to male fireflies and ". . . occurs hour after hour, night after night, for weeks or even months . . .".

This behaviour is strikingly different from that of most fireflies in other parts of the world: in fact, such large-scale, long-lasting, concerted rhythmic activity is seemingly unique in the animal kingdom. For this reason the phenomenon has been a perennial source of interest tinged with scepticism[2]. Yet the many accounts have not led to a consensus about certain important observational details of the synchrony, or to satisfying explanations of either the physiological mechanism of the mass flashing or of its biological significance.

On the basis of a recent visit to Thailand[3] and Borneo[4] we have reported elsewhere[5] some photographic and photometric measurements on a Thai species of firefly indicating that the synchrony of flashing varies less than 16 msec in a cycle of 560 msec, and have described observations on the build-up of synchrony under laboratory conditions which shed some light on the physiology of this firefly's "sense of rhythm". In this article we shall consider some more speculative aspects of Oriental concerted flashing related to its distribution and possible function. Two general premises are involved. First, the whole behaviour pattern, since it represents the product of countless millenia of evolutionary selection, must have a definite and important function in the life of the participating firefly species. Secondly, Oriental mass synchronism is a complex of behaviours (congregation *per se*, congregation in trees, choice of trees near water, flashing, synchrony) which may or may not be functionally related and which probably have quite different relative importances.

Riverbank Firefly Congregations

Morrison[6,7] and Smith claimed that the male Thai fireflies fly out from the adjoining jungle each evening ". . . for the purpose of engaging in this nightly display"[1]. This statement implies that the trees are populated anew each evening, an idea which we believe to be incorrect. Not only were riverbank trees near Bangkok full of fireflies in early evening, but we also found many of the insects present in mid-morning in a tree which we had studied the previous evening. Fireflies have also been seen by day in trees harbouring synchronizing swarms in New Britain[8] and in non-synchronous trees in Jamaica[9]. In Sarawak we found the tree inhabitants still flashing at midnight and at 6 p.m., as did Haneda[8] in New Britain. Resident fireflies thus account for at least part of the successive evening congregations. Fireflies were indeed seen flying to communal trees in both Thailand and Sarawak, but at the same time some were also seen flying away. The possible significance of these flights will be discussed later.

Practically nothing is known about the longevity of fireflies in Nature, but even at carefully controlled humidities and temperatures captive adults of most species die in a few days. This suggests that congregations that maintain themselves for weeks or months must be replenished constantly. Because a West African firefly species has been found to breed throughout the year[10], and the adults of several species in Jamaica[11] and in New Britain[12] are active in every month, tropical firefly trees could presumably be maintained for long periods, perhaps indefinitely, by recruits from a population in a more or less steady state of reproduction[13].

So far as it is known, all adult fireflies come from pupae in the soil—the pupae come, in turn, from larvae that live on or in the soil, or in water. Haneda found mating pairs and larvae on the ground in the Botanical Garden at Rabaul near silk-cotton trees in which fireflies were flashing synchronously. However, the mangroves bordering the swamp rivers of Thailand and Borneo stand in mud that is scoured by tidal currents so swift that it seems impossible that larvae, even if aquatic, could survive on the riverbank. Thus the swarms of fireflies in the trees must migrate from the interior swamp.

Good firefly trees were not common along the Chao Phraya River, but even in Sarawak, where the terrain is much closer to virgin swamp and firefly trees were more abundant, there were many mangroves of the same kind as those in which firefly swarms occurred, and similarly situated, which were completely free of the insects. The observed distribution of the firefly population therefore requires more than random flight from hinterland to the edge of a river. When watching male Thai fireflies in a darkroom we discovered that there is a period, before the flying specimens alight and synchronism begins to build up, when they are definitely attracted to each others' light[5]. This positive phototaxis suggests that male fireflies arriving at the fringe of riverbank trees would be attracted by flashing congregations there, or, if no previous swarm existed, might themselves form a nucleus by mutual photic attraction. Such assemblies might be expected to build up competitively, leading eventually to one or a few large swarms that had outdrawn nearby smaller centres because of higher mean light emission. Many potential display areas may therefore exist for each that develops to a spectacular level. The corollary that a good display tree would take some time to establish agrees with other indications that the assemblages are not renewed nightly.

The fact that flashing behaviour changes (from rapid twinkling to a steady tempo of two per second) after the male fireflies alight[5] is not a peculiarity of the particular Thai species or of the perching habit. Flash patterns in resting fireflies are usually quite different from those in flight[14]. What is unusual in tree fireflies is the maintenance of steady spontaneous flashing while perched—almost all roving fireflies flash irregularly, if at all, while at rest.

In sum, riparian firefly trees may be viewed as quasi-permanent assemblies, formed by way of photic attraction and maintained by recruitment.

Congregation Trees

An association of fireflies in South-East Asia with "certain", "favourite" or "particular" trees, especially mangroves, is widely recognized. In New Guinea the so-called "firefly mangrove" is *Sonneratia caseolaris* (which includes the former *S. obovata*, *S. acida* and *S. lanceolata*)[15]. The trees are well known to the natives and are said to be used in nocturnal navigation[16]. *Sonneratia* is also the "exclusive" firefly mangrove in Thailand, according to Morrison and to Smith (but see later). In Malaysia, although *Sonneratia* is common, the genus *Avicennia* must be considered an important firefly perch as its Malay name, "api-api", is also a name for firefly[17].

In Sarawak we found riverbank fireflies congregated in *Scyphiphora hydrophyllacea* and *Lumnitzera* sp. (near Kampong Senari), *Sonneratia alba* (Paya Paloh and Bako Park), *Avicennia marina* (Bako Park) and *Sonneratia caseolaris* (Paya Paloh)[18]. It was not uncommon to find *Sonneratia* and *Avicennia* trees growing side by side, each harbouring fireflies. Flashing was also seen in trees identified by our guides as mango and sago. Around Bangkok most assemblies were in *Sonneratia*, but one of our best displays in terms of population density and completeness of synchrony was in a small thorn bush (*Acanthus ilicifolius*). Synchrony was also seen in *Ziziphus mauritiana* and in another, unidentified, tree. The only instance of perch selection in either Thailand or Borneo was a negative one involving the ubiquitous nypa (nipa) palm (*Nypa fruticans*) on which it was rare to find even a single firefly.

Numerous other trees have been mentioned as firefly perches (see particularly Millard[19]), but even our own very limited experience is sufficient to show that there can be no causal relation between synchronous flashing, or even congregation, and particular kinds of trees. Nevertheless, the evidence confirms an essential involvement of trees in firefly behaviour. There has been no previous attempt to analyse this association aside from Morrison's vague suggestion that there may be ". . . something in the sap . . . which attracts the animals". This particular possibility seems very unlikely in view of the variety of acceptable trees and the very long duration of the displays, but in any event our samples of branches from firefly trees did not give any indication that the insects were being attracted by flowers or fruit. Fireflies in a Jamaican swarm have been seen possibly drinking from palm fruit scars, but the same species also congregates on acacia[9]. Furthermore, it is generally believed that most fireflies do not feed during adult life. On the whole, therefore, the evidence is very strong that trees are not an objective in themselves but are involved incidentally to some other firefly activity.

Aggregation and Mating

Since it is firmly established that male and female of most, if not all, roving-type fireflies are brought together for mating by individual-to-individual photic signalling, it is natural to enquire whether the flashing congregations in trees have any sexual significance. Both Morrison and Smith specifically denied this possibility but their stand was based on the misapprehension that female fireflies are always wingless and thus would be unable to reach the trees. The fact is that females of the riverbank species we studied in Thailand and Borneo are present in the trees, and in large numbers. The same situation was found in firefly trees in New Britain by Haneda. The females do not participate in synchronous flashing with the males, but this perhaps re-inforces the suspicion that they are in the trees for another purpose.

In the Jamaican trees large numbers of coupling pairs were found and the suggestion was made that the aggregating behaviour serves to promote mating[9]. Haneda similarly felt that the synchronous flashing of the New Britain male fireflies calls the females. In Thailand we unfortunately concentrated so hard on observing and recording the various kinds of flashes in the trees[5] that it was not until we had returned to the United States that we realized the significance of possible non-luminous activity (most fireflies cease flashing while mating). We did find a coupling pair on the gunwhale of our canoe after we had beaten the foliage with our insect nets in making a mass collection, and we observed that such beatings always flushed out many more animals than we would have expected from the density of flashing. Thus mating presumably does occur in the firefly trees of Thailand as in Jamaica and New Britain.

One further tenuous indication of mating habits comes from eye size. It is fairly well established that in firefly genera in which a roving male seeks a sessile female the eyes of the male are much larger than those of the female; but if the female takes a mobile part in the courtship the eyes are more nearly equal in size. In all the species of riverbank fireflies we have examined the eyes of the males and females are about equal. This finding is compatible with the idea that females as well as males are attracted to the congregations. Nets placed around a small firefly tree might give evidence of the postulated two-way (but not necessarily luminous) traffic and might make it possible to ascertain whether incoming females are primarily virgin and departing females mated[20].

Possible Rationale of Mass Mating

If fireflies do convene at the water's edge for mating, the utility of trees as fixed and dry sites for assembly is obvious, particularly since the insects do not mate on the wing. The assembly trees would presumably be selected by chance, and mangroves, being common, would be used often. However, it is not obvious why the riverbank should be chosen for assembly in the first place or why the fireflies in question should have evolved indiscriminate centralized mating instead of the system of individual courtships, which is not only the more usual plan among fireflies but would appear to be more efficient in terms of dispersal of the species.

In most species of roving firefly, males in an already widely distributed population search on the wing for stationary females. Recognition and homing depend on the pair being able to see each other nearly continuously in order to distinguish the signal code of their own species from those of other species patrolling the same terrain. Our experience with American, Caribbean and upland Malayan[21] forms indicates, as might be expected, that roving fireflies are found primarily on open ground, such as meadows, forest glades, road clearings, savannah and jungle trails.

There could scarcely be imagined a terrain less favourable for line-of-sight recognition than the flat, tangled, mangrove-nypa swamps of South-East Asia in their virgin state. A firefly tree on a watercourse, however, might provide a sufficiently bright and large beacon, perhaps enhanced by reflexions, to attract fireflies that wander out into the clear over the water, and might provide enough opportunities for mating to compensate for the (assumed) long flights required of the mated females for egg dispersal. We propose therefore that, in tropical swampland, mass mating has evolved instead of pair courtship because sustained photic communication between individuals is impossible.

Significance of Mass Synchrony

As reported elsewhere[5], not all the firefly trees which we saw in Thailand showed concerted flashing, and the trees we studied in Sarawak, where synchrony has been seen by professional biologists[22], showed only transitorily and incompletely co-ordinated flashing. We suspect that we failed to see well-developed synchrony in Sarawak because at the time of our visit the riverbank trees were harbouring approximately equal numbers of three species of fire-

flies (all of the genus *Pteroptyx*[23]), with consequent clashing of rhythms and breakdown of entrainment. Even in Thailand, where two other species of *Pteroptyx* were together in the trees, the synchrony of the dominant males only produced its wondrous effect because the numbers of the second species were very small. In Thailand we also felt that synchrony was more complete in more densely populated trees, in line with our "laboratory" finding that the visual feedback that brings two individuals into step operates only over relatively short distances[5]. It seems likely, therefore, that a species of firefly capable of flashing synchronously may produce displays ranging from apparently perfect unison to completely random sparkling depending on such factors as population density and purity.

There is little indication that synchronization of flashing is essential for either development or maintenance of arboreal congregations of fireflies. However, if the insect eye reacts like the human eye there are two reasons why synchronous emission would be more effective than disordered flashing as a beacon for assembly. An obvious effect would be to change a relatively continuous luminescence to an alternation of light and dark. (This principle is, of course, widely used in advertising signs in order to enhance their efficiency in catching attention.) The second advantage of coincidence of flashes would be to increase greatly the brightness of the tree, making it visible at greater distances.

Geography of Firefly Tree Aggregations

However reasonable the hypothetical rationale of riverbank aggregation and synchronous flashing may appear, it would be optimistic to suppose that observations made over a few evenings have settled major questions of communal behaviour of fireflies—even for the principal Thai species which we studied. For example, the basic postulate of mating in the trees still requires confirmation, as also do the proposed flights of male and female.

First-hand accounts of mass synchronism in the Philippines, Malaysia, New Guinea, Indonesia, Thailand, Burma and tropical India outnumber those from the rest of the world by thirty to ten[2]. The Oriental displays conform in general to the kind seen in Thailand: huge, long-lasting congregations in riverbank trees, flashing in nearly exact coincidence. Most of the occidental reports involve dispersed populations in flight, active for only short periods, and flashing in waves. The distribution of Oriental-type synchrony corresponds at least roughly with that of Oriental mangrove-nypa swampland, whereas the Western reports are almost all from open terrain.

There is, therefore, reason to regard the congregation of fireflies on trees as being linked to a specific type of topography. However, there is not a single display described in the literature that does not need further critical study of behaviour and habitat in specific relation to mating. Also, many more virgin Oriental swamplands will have to be sampled, as well as comparable areas in Africa and South America, before the nuptial device apparently used by *Pteroptyx* in Thailand can be considered the principal one evolved for coping with swamp conditions[24].

[1] Smith, H. M., *Science*, **82**, 151 (1935).

[2] Buck, J. B., *Quart. Rev. Biol.*, **13**, 301 (1938).

[3] We thank the American Philosophical Society and National Geographic Society for travel grants, and Professor Kloom Vajropala and Dr. Boonsong Lekagul for help in Thailand during October 11–20, 1965.

[4] We thank Messrs. John Dunsmore, John Turner, R. B. Jagat and Peter Ashton for innumerable favours and extensive assistance in Sarawak during September 25–October 1, 1965.

[5] Buck, J., and Buck, E., *Science* (in the press).

[6] Morrison, T. F., *J. Siam. Soc., Nat. Hist.*, Suppl. **7**, 71 (1927).

[7] Morrison, T. F., *Science*, **69**, 400 (1929).

[8] Haneda, Y., in *The Luminescence of Biological Systems*, edit. by Johnson, F. H., 452 (Amer Assoc. Adv. Sci., Washington, 1955).

[9] Buck, J. B., *Nature*, **139**, 801 (1937).

[10] Kaufmann, T., *Ann. Entomol. Soc. Amer.*, **58**, 414 (1965).

[11] McDermott, F. A., and Buck, J. B., *Trans. Amer. Entomol. Soc.*, **85**, 1 (1959).

[12] Dun, G. S. (personal communication).

[13] Moonlight is known to depress firefly activity (see also Smith (ref. 1) and Buck (ref. 9)) so there may be a monthly cycle of tree population density.

[14] Seliger, H. H., Buck, J. B., Fastie, W. G., and McElroy, W. D., *Biol. Bull. (Woods Hole)*, **127**, 159 (1964).

[15] Backer, C. A., and Van Steenis, C., *Flora Malasiana*, Ser. 1, **4**, 280 (1951). Brass, L. J., *J. Arnold Arboretum*, **19**, 174 (1938). White, C. T., *J. Arnold Arboretum*, **10**, 346 (1929). Rand, A. L., and Brass, L. J., *Bull. Amer. Mus. Nat. Hist.*, **77**, 341 (1940). We thank Dr. L. J. Brass for help in explaining the nomenclature of tropical mangroves.

[16] Watson, J. G., *Mangrove Forests of the Malay Peninsula*, Malayan Forest Records No. 6 (1928).

[17] Burkhill, I. H., *A Dictionary of the Economic Products of the Malay Peninsula*, 1, 273 (1935).

[18] Dr. B. C. Stone kindly identified the mangroves collected in Sarawak.

[19] Millard, W. S., *J. Bombay Nat. Hist. Soc.*, **16**, 520 (1905).

[20] Wynne-Edwards, V. C., in *Animal Dispersion in Relation to Social Behaviour* (1962) has discussed firefly synchronism as a type of conventionalized "epideictic" display or social aggregation aimed basically at population regulation rather than maximizing mating opportunity. If so, the firefly example would be unusual in being apparently a more or less steady-state affair rather than being confined to a breeding season. However, much more study of possible size limitation and spacing of swarms will be required before this interesting suggestion can be assessed.

[21] We thank Lord Medway for enabling us to collect at the Gombak Field Station of the University of Malaya, and Dr. John Bullock for arranging our stay at Fraser's Hill (Selangor).

[22] Anderson, J. A. R., Dunsmore, John, and Lord Medway (personal communications).

[23] We thank the late Mr. F. A. McDermott for identifying our fireflies.

[24] A species of *Colophotia* has been reported to be "the" synchronizing firefly of Thailand (Reinking, O. A., *Science*, **53**, 485; 1921) and we have also seen a large sample of *Luciola brahmina*, said to have been collected in the spring along the Chao Phraya River, and also supposed to have been flashing synchronously. Observational errors may well be involved in both reports, but the possibility of more than one synchronizing firefly species in the same area raises some very interesting questions and cautions against generalizations from limited geographical and seasonal experience.

For the convenience of readers, the following extracts are reprinted from Dr. Buck's communication "Flashing of Fireflies in Jamaica" (*Nature*, May 8, 1937, and ref. 9 above):

"DURING the past summer, the members of the Seventh Botanical Expedition of the Johns Hopkins University witnessed displays of firefly activity in the British West Indies as spectacular in their way as any reported from the Orient.

"In front of the expedition's laboratory at Chestervale, in the Blue Mountains of Jamaica, there was a thatch palm which bore below its whorl of leaves an inflorescence a metre in diameter. For about a week in June, and again a month later, this inflorescence was transformed nightly into a sphere of seething flame by the flashes of thousands of fireflies which gathered there. Later, other displays were discovered, particularly on two large acacia trees overhanging the Clyde Valley which harboured such prodigious swarms of fireflies that the nebulous glow was visible half a mile away.

"All the fireflies on these trees were of one species, *Photinus pallens*, and the females outnumbered the males in the ratio of 4 : 3. Each firefly flashed regularly about twice a second while walking along the twigs, and entirely independently of any other individual. There was no sign of synchronism or of response between any individuals or between different trees. The flashing was not inhibited by heavy rain, by lightning, or by the beam of a powerful flashlight, but did not occur on moonlight nights.

"Whatever the cause of the aggregation, it serves an important function in bringing the sexes together for mating, and many coupling pairs were observed on the palm tendrils. The mating, however, appears to be due entirely to accidental contact of the sexes during their peregrinations on the branches. The aggregation habit thus seems to take the place of the accurate systems of flashing signals which serve to bring male and female together in some species of firefly."

JOHN B. BUCK

BOOK REVIEWS

REMAINS OF THE REVOLUTION

Industrial Archaeology

An Introduction. By Kenneth Hudson. (University Paperbacks.) Pp. 183+59 plates. (London: Methuen and Co., Ltd., 1965.) 18*s*. net.

THE term "industrial archaeology" is little more than ten years old, yet the ideas behind it and the activities associated with it have acquired a remarkable popularity. Mr. Hudson's handbook, first published in 1963 and now appearing in a paperback edition, is a useful and interesting guide, which also includes a bibliography and a gazetteer. The last important chapter on the documentation and recording of industrial archaeology is of great practical value in itself.

The first chapter, on "What is Industrial Archaeology?", is somewhat over-preoccupied with terminology. Clearly industrial archaeology, as it has developed, is not so much a "subject" as a "field". Many of its practitioners would have got by in the past as economic and social historians, and large sections of subsequent chapters are straight economic and social history. At the same time, the term includes the history of technology—still a neglected subject and one which is certainly not exclusively dependent on an archaeological approach—and a sizable element both of architectural history (particularly the history of the "functional" tradition) and of historical geography. It also pulls together the work of railway and canal historians—a well-established and flourishing body—and of people interested in "houses" ("furniture historians" are a more recent group). Yet another element incorporated into the term is "pictorial history" of the kind developed so imaginatively by Hoskins and his school, although "archaeology" is only one side of this, and interest in industry—at least so far as Hoskins's own work is concerned—is only one aspect of an extended and more systematic interest in local history, examining landscape and environment as much as "things".

Hudson is anxious that "industrial archaeology" should be treated as a "subject" rather than that an archaeological approach should be used along with other approaches in inter-disciplinary work on the history (including the recent history) of industry. His healthy enthusiasm seems to me to lead him astray at this point, and he tilts at too many windmills. Who are the "economists, historians and archaeologists" who denigrate industrial archaeology "partly for reasons of sheer conservatism, partly from resentment against an upstart"? As for those who have "serious and genuine doubts that industrial archaeology can be made into a satisfactory academic discipline", one at least is more worried about what the term "a satisfactory academic discipline" means than about the name "industrial archaeology" which, so long as it attracts added interest and stimulates more thorough scholarship, is at least as good as any other term.

There seem to be various reasons for the very genuine interest. Britain's industrial past is being re-scrutinized from many angles, in the middle of the twentieth century, and references to the "industrial revolution" are becoming as frequent and as varied as American references have long been to the Wild West or to the "open frontier". Some critics have detected nostalgia in this, others the search for myth: at a more mundane level, the interest in old steam engines may be not all that different from an interest in vintage cars. It is not only the character or the range of the field, however, which attracts. The method itself—getting outside and around, digging (where relevant), measuring, photographing, keeping logs and record cards—is a source of attraction: it is a method which lends itself also to group activity, and it is not surprising that the growth of interest has been associated with extra-mural education. Some practitioners—and many of their friends and well-wishers—have also been inspired by a sense of urgency. "Things" are being rapidly destroyed—the theme of Hudson's second chapter. Can they be kept or, if they are not kept, can a full record of them be made? Hudson is a forceful and persuasive advocate of realistic proposals to "protect" Britain's industrial legacy, and he catalogues the various attempts already being made to maintain and develop records and to prevent both neglect and total destruction. His book is in places a manifesto as much as a handbook, and he is particularly and rightly concerned to get his "message" over to industrial firms. He quotes with approval, for example, an article by Maurice Barley in the *Journal of British Industry*, a publication where one would hope the lesson would strike home: "Only the most enlightened management realises that to preserve specimens of past machinery and products may create prestige, rather than damage the image of industrial enterprise".

Seven of the eleven chapters deal with particular aspects of the industrial legacy—a general background chapter on "the pace and pattern of the industrial revolution" and chapters (of varying length and depth) on coal and metals; power; textiles, pottery, glass, brewing and distilling (an oddly assorted group); railways, inland waterways and roads; building materials; and farm buildings. He does not have space to go into detail about disappearing evidence relating to more recent technology, although he has a section on cement and two interesting pictures (supplied by Shell-Mex and B.P., Ltd.) of a nineteenth-century "petrol filling point" (not located) and the oldest surviving railway tank wagon for petroleum, now kept at the Museum of British Transport, Clapham. Certain aspects of post-industrial revolution technology, to use conventional terminology, are of immense interest, and when archaeological methods are relevant or necessary to their study they should be encouraged and developed. Knowledge of the present shifting into the future is invaluable in this context. Just as Marc Bloch said that the master gift of the historian was to understand the living and that if you could not understand the living you could not understand the dead, so the most interesting "industrial archaeology" will be that which throws light on past technologies or on past societies so that both the technologies and the societies "live" again. In these terms, the archaeological approach is clearly relevant to much else which is modern besides industry. Urban studies are also beginning to boom, and there is ample scope for an archaeology of the Victorian city. The difficulty and fascination always come from the intelligent relating of the archaeological approach to other approaches. Very seldom do we have to rely on archaeological evidence alone.

For all other kinds of evidence and for understanding the relationships between the different kinds, industrial archaeologists, like others, will still have to rely on historians, historians with no qualifying adjective.

ASA BRIGGS

WILD NATURE

Conservation of Natural Resources

Edited by Guy-Harold Smith. Third edition. Pp. xi+533. (New York and London: John Wiley and Sons, Inc., 1965.) 75*s*.

RAYMOND DASMANN'S *Environmental Conservation* (1960) is probably the most useful text-book yet available on resource conservation viewed ecologically. Although concerned in the main with American situations, Dasmann's wide coverage provides not only a satisfactory basic appraisal of the subject for students—in a form suitable for elaboration by teachers—but also it presents clearly and concisely a readily understandable introduction to conservation acceptable to the general reader.

Guy-Harold Smith's *Conservation of Natural Resources* is concerned entirely with the United States. It is another, but very different, text, the usefulness of which for reference is well established. The first edition was published in 1950, the second appeared in 1958, and now we have this third and largely re-written edition of 1965. Both because its facts and figures have once again been brought almost entirely up to date and because it has sought to keep abreast of advances in conservation thinking and application, it will undoubtedly in the United States be a helpful complement to *Environmental Conservation* for university purposes. In Britain, however, it will be used mainly as a source of reliable factual information, and for an understanding of the American approach.

The book comprises twenty-three chapters, written by nineteen authorities, almost all of whom are university professors; it is arranged in eight parts. The first deals with conservation in the United States, with the public domain, and with economics and conservation; the second is concerned with soils, soil use and soil conservation, irrigation and wetland reclamation, grassland resources and agricultural land use. The five succeeding sections cover a variety of aspects of forest, water, mineral, wildlife and recreational resources, and the conservation of man; the final part summarizes the situation with regard to state and local planning, and national planning and the conservation of resources. There are numerous photographs, figures, tables and maps interspersed in the text, and each chapter is amply referenced. There is an adequate index.

Because of its immediate relevance to the situation in Britain, and perhaps particularly in Scotland, the following extract from the chapter on recreational resources may be quoted: "There are great opportunities for greater public use of private land for recreation. After all, more than two thirds of all land in the United States is privately owned and much of it has a large recreation potential. The large amount of the probable future demand for outdoor recreation makes it probable that public areas will not expand fast enough to satisfy the demand. Recreation could well develop as the dominant use for some private areas or as one use among several on larger areas". Partly militating against such development in the United States, as also in Britain, is the fact that so many public recreation areas are open without charge or for a very low fee. "Although the private providers often charge primarily for the service they render, rather than for their resources as such, the competition from public areas is certainly a factor. The American public, or at least a large part of it, has become accustomed to free or low-cost outdoor recreation and does not realize the value of the resources it so obtains." Surely, there is food for thought here.

Although it is entirely laudable that in a book of this nature controversies should be neither ignored nor dramatized, it is nevertheless remarkable that nowhere in it is there any reference either to Rachel Carson or to Robert L. Rudd's *Pesticides and the Living Landscape* (1964). It is notable that the word 'pesticides' occurs only once, coupled with 'insecticides'. The latter word has four index references but only twice in the text and in one caption is there any mention—and that, as it were, in passing—of the threats inherent, in the form of side-effects, in the widespread use of so many persistent killer chemicals—leading to accumulations of toxic levels.

W. J. EGGELING

A SUMMARY OF BEGINNINGS

Cybernetics and Development

By Michael J. Apter. (International Series of Monographs in Pure and Applied Biology. Division: Zoology, Vol. 29.) Pp. xi+188. (London and New York: Pergamon Press, Ltd., 1966.) 50*s*. net.

BIOLOGISTS interested in the theory of development have, in the past few years, had their expectations raised by the news that the physical and mathematical sciences are developing new bodies of theory concerned with processes of control and organization. These go under a variety of names, such as systems theory, information theory and the like; perhaps "cybernetics" is the most generally accepted way of referring to the whole range of these new developments.

Dr. Apter's short monograph is a summary of these recent developments in so far as they have been applied, or seem capable of application, to problems of biological development. In it can be found short accounts of most of the recent cybernetical work in this field, such as Turing's work on patterns, the application of the Turing machine to macromolecular processing by Stahl and Goheen, Maruyama's and Ulam's work on the algorithmic generation of patterns, Goldacre's model in terms of electrical circuits, discussions by Ashby, Pask, von Neumann and many others. This makes the work a valuable source-book for what might be called "new-fangled" approaches to developmental theory. As such it is to be welcomed and has a place on the shelves of anyone interested in theoretical biology.

The sceptical biologist is, however, likely to ask just what these new mathematical approaches really amount to. Have they produced definitely proved theorems and, if so, can these theorems be applied to biological entities? One is not very encouraged by some of the franker remarks in Apter's book. On page 2 we read: "It is difficult to say what cybernetics is. . . . There appear to be genuine differences of opinion among cyberneticians themselves as to what cybernetics is and what it should be". Or again, on page 71, "A developing system is closed to information, although not of course to energy, . . . (of course energy can also be said to possess information; indeed, information can be referred to almost anything . . .)".

The biologist, accustomed for a long time to analysing organisms into cells and such concepts as stimulus and response, knows already something of the weaknesses as well as the strengths of such an approach. When confronted with a mathematician who talks impressively about "axiomatization" which "means this: we assume that the elements have certain well defined, outside, functional characteristics; that is, they are to be treated as 'black boxes' ", he is tempted to reply: "Yes, fine, as far as it goes but now go on and say something new". After all, what Apter calls "Wiener's major insight that organisms can be discussed . . . in terms of 'homeostasis' and 'negative feedback' " had actually been applied by embryologists to developmental processes long before Weiner appeared on the scene; for example in ideas of the canalization of development. What one hopes for from the mathematicians is a clear-cut body of theory about the manner in which the individual processes are organized so as to result in homeostatic systems. Such a body of theory certainly exists in certain special systems,

such as electrical circuits, but does it exist in relation to the fundamental processes of embryonic development, such as gene activation, protein synthesis and the like? If it does, I do not think that any cybernetician has yet expressed it in a way that makes its relevance to embryology clear. Certainly Apter does not do so. His book is a summary of beginnings which always seem to be promising to lead to something important but have, as yet, scarcely done so. C. H. WADDINGTON

TREES IN BORNEO

Manual of the Dipterocarp Trees of Brunei State

By P. S. Ashton. Pp. xii+242+58 plates. (London: Oxford University Press, 1964.) 63*s*. net.

ONE of the major economic products of Borneo is timber, and this is largely produced by trees belonging to the family Dipterocarpaceae. It is therefore of considerable importance that this group should be well studied taxonomically. Many of the trees concerned are forest giants some 150–200 ft. high, so any taxonomic work based solely on herbarium specimens (often accompanied by inadequate field-notes) is obviously likely to be unsatisfactory. This applies especially to the Dipterocarpaceae, where considerable variation occurs between the vegetative characters of juvenile and mature leaves, and consequently a lack of suitable field-experience has led to misinterpretations of the differences between herbarium specimens, thus leading to wrong taxonomic decisions. The ideal circumstances for a taxonomist working on this group is for him to have first-hand knowledge of all the species in the field. This ideal has been achieved by Dr. Ashton in his investigations of the Dipterocarpaceae of Brunei. The major geographical centre of this family is Borneo, where more than half the species occur. Therefore, in spite of the small size of the State of Brunei the number of species which occur there is more than 150, which is just about the number of those which occur in the much larger area of Malaya. Nine genera are represented in Brunei, the largest being *Shorea* with 75 species; *Dipterocarpus*, *Hopea* and *Vatica* each have 20 or more species. Dr. Ashton was able to examine a total of some 1,500 Dipterocarp collections from Brunei, nearly half of which he collected himself. All this, in addition to his thorough knowledge of the trees themselves and their ecology, has provided the secure foundation of this excellent book. Its main purpose, as stated by the author, is to provide foresters with a work which will enable them to recognize all the species in the field, thus leading to a stable vernacular and trade terminology. This manual, furthermore, is intended as a basis for a complete revision of Bornean Dipterocarps.

Field-keys and systematic keys are provided both to the genera or main field-groups and to the species. The systematic keys include flower and fruit characters. As Symington's *Foresters Manual of Dipterocarps* is still widely used in Brunei and comprehensive generic descriptions were provided in it, these have not been repeated in this new publication, but full diagnostic characters of the genera are provided. The treatment of each species gives the synonymy, vernacular names, separate field and herbarium descriptions as well as notes on uses, distribution and regeneration. Herbarium specimens are also cited. The herbarium descriptions are very full and give much useful detail including seedling and sapling characters. Many of the species are illustrated by line-drawings of the leaves and fruits; there are also many photographs of trees, showing especially the appearance of their bark. In view of the number and diversity of species of *Shorea* involved, this genus receives a somewhat different treatment. Nine sections are recognized and some of these are further subdivided. The systematic key includes all the species occurring in Brunei. The treatment of the species is divided up into the four field-groups, each provided with a separate field-key; the species in each field-group are numbered separately, which is a disadvantage to the taxonomist, who will want to use this book as well as the forester. (The species in the complete systematic key are unnumbered.) It is noteworthy that although *S. albida* belongs to section *Anthoshorea*, which constitutes the White Meranti field-group, on gross field-characters it is a Red Meranti. A similar situation occurs in *Hopea*, where there are both four botanical groups and four field-groups, but these do not entirely correspond with one another.

Although intended primarily for foresters, the keys and detailed descriptions will also make this work most useful to taxonomists. This important book, which has been printed in Singapore, has been attractively and well produced. L. L. FORMAN

GRASSHOPPERS IN RUSSIA

Locusts and Grasshoppers (Catantopinae)

By L. L. Mishchenko. (Fauna of the U.S.S.R.: Orthoptera, Vol. 4, No. 2.) Translated from the Russian by B. Munitz. Pp. xii+560. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 135*s*.

A TAXONOMIC work published in 1952 and translated 14 years later cannot be expected to provide up-to-date information on the fauna of large parts of two continents, but its English version will be welcomed by specialists in this group of insects. They should, however, bear in mind the somewhat vague definition of the area covered, the author merely saying that he disregarded "species inhabiting western Europe, the Mediterranean region, southern Iran, Afghanistan, India, China and Japan, which are not found in U.S.S.R.". These limitations, moreover, are applied inconsistently, since the book includes the Mediterranean *Trapidopola longicornis*, *Pezotettix anatolica*, *Sphenophyme rugulosa* (and even a new subspecies of *Miramella* from Albania), Tibetan genera *Indopodisma* and *Kingdonella*, Indian *Pachyacris* and *Patanga*, some species from southern Iran and *Fruhstoferiola* of south-east China. This would be less objectionable, were it not that other genera and species from the same areas are omitted; thus, several species of *Kabulia* from Quetta are left out, but a *Conophyma* from that locality is included; two species of *Dericorys* from Baluchistan are in the book, but a third is not. two Iranian genera, *Lyrotylus* and *Dabba*, are mentioned in an introductory chapter, but ignored in the text.

The introductory chapters dealing with the morphology, development and ecology of Acrididae are brief. Anatomy is not even mentioned, except for a rough sketch of the male copulatory apparatus of *Conophyma*; this character has been used by taxonomists long before 1952, but it is ignored in keys and descriptions. A fairly long chapter deals with the geographical distribution of Catantopinae, but mainly in the form of long lists of tribes (illustrated by maps) and genera supposed to be characteristic of various regions; the fauna of the Palaearctic region and of the U.S.S.R. are dealt with in more detail, but in the same uninspired manner.

The bibliography is in two parts—papers in Russian, with their titles both transliterated and translated, and those in other languages.

The classification is still that of Brunner–Wattenwyl (1893), with a few recent modifications, but the author himself has introduced hardly any improvements. His main original contribution is a key to small apterous grasshoppers of the genus *Conophyma*. This is represented by sixty-eight species in the high mountains of Central Asia providing a remarkable example of speciation by isolation; most of the species have been described by 'Mishchenko' himself. They alone make up half the

section on U.S.S.R. fauna, which comprises 126 species of Catantopinae; the remaining 107 species dealt with in the book are from adjoining countries.

The initiative of the Israel Program for Scientific Translations is very welcome, but much greater care is essential in translating and editing highly specialized books than has been shown in this case. To begin with, the author's name is transliterated by himself in a paper published abroad (reference on page 50) as Mistshenko, not Mishchenko. When the English text is compared with the original, serious mistranslations can be found, such as "exoskeleton" instead of "armature" (page 10); tribe Podismini is "divided" into several genera, when the original says that it was considered to consist of a single genus *Podisma* (page 32). In the original it is said that there are no endemic forms in western Siberia, while there are many in the eastern, but the translation reads "In Siberia endemic forms are conspicuously absent" (page 35). In some cases the translator has invented new words, like "xerothermation" and "multiformity". Most ludicrous are his attempts to translate, instead of transliterating, popular Russian names for the insects. The Russian name for grasshopper is *Kobylka* which literally means "filly", in allusion to the resemblance of the head profile to that of a horse; the translator, following the dictionary, renders the name *polyarnaya kobylka* as "polar young mare grasshopper"; what is, in Russian, a "tree grasshopper" becomes "wooden young mare grasshopper". For some of these faults the responsibility falls on the editor who, although a specialist taxonomist, apparently had no knowledge of the original language; bilingual specialists in this group exist in Israel.

A very strange feature of the book is that the indexes at the end give references to the pages in the Russian edition (inserted on the margins of pages of the translation); this is mentioned, but in very small type, in a footnote, so that readers are likely to suffer annoyance before they discover the secret. The price of the book, even at present rates, is very high. BORIS UVAROV

REGULATION OF ADRENAL FUNCTION

The Neuroendocrine Control of Adaptation

By K. Lissák and E. Endröczi. (International Series of Monographs in Pure and Applied Biology. Division: Modern Trends in Physiological Sciences, Vol. 25.) Pp. xii+180. (London and New York: Pergamon Press, Ltd., 1965.) 70*s*. net.

THE main aim of *The Neuroendocrine Control of Adaptation* is to provide an account of the way in which the central nervous system controls the endocrine organs, and in particular the adrenal glands, concerned with the adaptation of the animal to its environment. The subject-matter may be divided into two main sections. The first half of the book deals with the regulation of the secretion of adrenocortical hormones by the pituitary, and in the second part the control of the production of adrenocorticotrophic hormone by the hypothalamus is discussed. Sections are also included on adrenocortical regulation during development and in relation to gonadal function.

This book was originally published in 1960 under the title *Die neuroendokrine Steuerung der Adaptationstätigkeit* by Verlag der Ungarischen Akademie der Wissenschaften, Budapest. The present edition, which is in English, represents a slightly revised version of the original work. However, the literature survey has not been brought up to date and the majority of the references quoted were published before 1960. For this reason, no account can be taken of the recent work in the general area of neuroendocrinology, much of which is germane to the subject-matter considered. Thus, recent studies involving the corticotrophin releasing factor, the use of synthetic adrenocorticotrophic hormone preparations, and new techniques for the estimation of adrenocorticotrophic hormone and of corticosteroids and their metabolites are not discussed. The book is therefore of little value in providing an up-to-date account of neuroendocrinology to workers in this field. However, a proportion of the references quoted are taken from journals such as the *Acta Physiologica* of the Hungarian Academy of Science and the *Bulletin* of the U.S.S.R. Academy of Science. Because of this, the international coverage of literature published between 1950 and 1960 which is presented is likely to be of value to investigators who do not normally have access to Eastern European and Russian journals.

In their survey of published work, the authors have in many instances presented conflicting results. Unfortunately, an assessment of the relative merits of the data described is not always given. It is therefore likely that a reader who has only a general knowledge of endocrinology may find this book rather indecisive. Summaries of the contents of each chapter are not provided and, particularly in the latter half of the book, subheadings in individual chapters have not been used. This means that, in order to use the book as a reference work, extensive use must be made of the subject index. However, this is unduly short, comprising only 2 per cent of the length of the book, while the index of authors represents 7 per cent. Two of the main omissions in the subject index are the absence of any reference to steroid hormones, such as hydrocortisone and corticosterone, referred to in Chapters 2 and 3, and to various animal species used in the experiments described in the second half of the book.

In spite of its limitations, this book is likely to be a useful addition to departmental rather than to general libraries. The main aims are certainly achieved and in particular a fairly readable account of some of the historical aspects of the subject is provided. Experiments in various animal species, as well as in humans, are described and care is taken to indicate the species differences which may occur in the study of the interrelationship between the hypothalamus, pituitary and adrenal glands. The book, which is printed on paper of high quality, is well laid out and the tables and diagrams are generally clearly presented. E. TREVOR BELL

ZONE ELECTROPHORESIS

Methods in Zone Electrophoresis

By John R. Sargent. Pp. 107. (Poole, Dorset: The British Drug Houses, Ltd., 1965.) 8*s*. 6*d*.

ZONE electrophoresis is a method extensively used for the investigation of substances of biological importance, but the worker is frequently faced with the problem of knowing the forms and conditions available and of evaluating which of them is the most suited for his particular problem. This book is therefore greatly to be welcomed, as it provides us with a much needed and up-to-date book covering with full working details the whole range of materials and conditions available for zone electrophoresis.

The book opens with a short chapter on general theoretical considerations and this is followed by chapters on electrophoresis on paper (low and high voltage), cellulose acetate, starch gel, agar gel and polyacrylamide. There is a chapter on block electrophoresis and the book closes with a useful section on the choice of media. The great merit of the book is that the abundance of information given is presented in an extremely concise manner and is divided into appropriate sections enabling details to be found at a glance. For example, for each medium discussed there is a description of the procedure, the types of apparatus which may be used, a selection of suitable

buffers and full details of staining for each type of substance. Where applicable, the author has described separately conditions for the electrophoresis of different classes of substances, such as proteins, amino-acids, carbohydrates and nucleotides. Although an empirical approach must generally be taken in establishing the best conditions for the analysis of a particular mixture of substances, especially with proteins, the author does suggest how this may best be done. There are numerous diagrams and plates to illustrate details of apparatus and types of pattern obtained, and a full bibliography is given for each method. It is perhaps unfortunate that there was a long delay in publishing (the bibliography extends only to September 1963) with the result that some developments before publication were excluded. Thus, there is only a brief mention of the thin-layer methods which are valuable in conjunction with chromatography for obtaining "fingerprints" of peptides. However, techniques used for proteins, carbohydrates and amino-acids have undergone relatively little modification since the book was prepared.

While the author points out some of the problems that arise in the interpretation of results, his book is essentially concerned with the practical aspects of the subjects and as such will be of great value not only to the novice but also to the experienced worker in this field.

E. R. SKINNER

NOT A HANDBOOK

Semiconductor Junctions and Devices

Theory and Practice. By William B. Burford, III, and H. Grey Verner. Pp. xx+328. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1965.) $12; 96*s*.

DURING the period 1950–60, when developments in semiconductor devices were rapid and often entirely novel, books on the subject quickly became, in part at least, out of date. There was, indeed, the risk that everyone interested in the devices would become so accustomed to frequent and major changes that they would continue to see them, potentially at least, in every small development. But although the effort devoted to research and development on the design, technology, characterization and applications of the devices continued to expand, the years since 1960 have been marked much more by evolution and consolidation, as witness much of the work on integrated circuits, which leans so heavily on the basic planar techniques. Important advances have still been made, such as the insulated-gate field effect transistor, the semiconductor laser and the Gunn effect oscillator, but these devices have supplemented rather than displaced earlier types.

Some consolidation must be welcomed by authors and teachers, for it will allow time to assess the relative importance of different parts of the subject, both in academic and industrial terms. It presents an opportunity for attempting the writing of a handbook which could be widely accepted for a few years. The subject has certainly become so big, both in coverage and in the number of scientists, technologists and engineers engaged in it, to offer enough incentive for several such attempts. Even if the subject were to break loose at its roots again, the time constant for large-scale industrial change has now lengthened to several years; new ideas must show a very clear-cut advantage in some important feature, while matching older ideas in other respects, before being quickly adopted.

Mention of a handbook here is misleading, for this book cannot be considered as such despite the claims of the publisher that it is a "comprehensive modern survey" whose pages of "hard-packed logically arranged information cover everything from general principles . . . to specialized processes of two-junction transistor synthesis". The authors are, however, more realistic as to the purpose of the book and its likely use. It is based on a teaching course, of considerable coverage, but was largely written during 1960–1 with re-editing during 1963. It seeks to cut across the boundaries separating university disciplines, in order to bring together physicists, electrical engineers and materials scientists, but it makes many concessions in doing so; anyone with a basic training in a sub-division of the subject will find some sections unduly elementary.

The order of presentation is sensible, with the first eight chapters running through the physical principles underlying junction behaviour to the structure and performance of devices with one, two or more junctions. Serious analysis or abstract ideas are restricted to a chapter on Fermi statistics; otherwise the writing is largely descriptive and will present no difficulties to engineers who are not mathematically minded. Some basic ideas are not, however, put over very well. In particular, the finite lifetime of minority carriers is insufficiently emphasized and the capacitance of a biased junction is based on Q/V rather than dQ/dV; the transport of carriers across the base region of a transistor, and the dependence of carrier density on position in that region, are not sufficiently explained.

The next four chapters describe, in a very elementary way, applications of transistors to amplifiers, generally using the simplest of equivalent circuits; they do not provide a basis for circuit design. The next chapter, on commercial transistor specifications, looks at first sight to be quite out of logical order, even assuming it has any place at all in the book. However, it is a serious attempt to explain to newcomers how device manufacturers express ratings and performance, but the parameters prominent in the previous chapters are largely missing. A descriptive chapter on switching applications follows. It is out of touch with modern practice and ignores both the classic analysis of Ebers and Moll and the charge control approach of Beaufoy and Sparkes. From this point onwards the book degenerates into a collection of descriptions, sometimes of devices long relegated to near oblivion; the commentary lacks any critical element. A chapter on metal-semiconductor contacts recognizes that these often form part of devices and makes the reader aware of the complications they can introduce, but the following chapter on point contact devices has little value to-day. The final chapter is largely devoted to device reliability, and the authors have usefully covered much ground in about twenty pages; the data they present may now be far from representative, but illustrate the subject adequately.

It is, frankly, difficult to see for whom, and for how long, this book will be a primary guide. It needs modernizing, much condensing and a sharpening up if it is to be even a substitute for the handbook which someone must surely by now be attempting.

J. R. TILLMAN

Annual Review of Nuclear Science

Vol. 15. Edited by Emilio Segrè, in association with Gerhart Friedlander and H. Pierre Noyes. Pp. 502. (Palo Alto, California: Annual Reviews, Inc., 1965.) $8.50.

OVER the years, the *Annual Review of Nuclear Science* has established a pattern of authoritative and up-to-date review articles, about half of which are on nuclear physics while the remainder range over the whole wide field embraced by the term 'nuclear science'. Volume 15 is no exception With such diversity, a reviewer can serve little purpose beyond indicating the subjects covered so that the reader can decide what may be in the mixture for him.

Probably the most noteworthy, and certainly the longest, article is that by Lee and Wu on weak interactions. This volume contains only the first part of the full review and

includes a comprehensive survey of the theory and that part of the section on experiments and phenomenological analysis covering β-decay, μ-decay, μ-capture and π-decay. The leptonic and non-leptonic decay of kaons and hyperons and parity-violating reactions will be included in next year's instalment. Other articles primarily of interest to those concerned with nuclear physics are those on photonuclear reactions, by Danos and Fuller; on the measurement of the masses of particles which undergo weak and electromagnetic decay and also of the mass of the antiproton, by Barkas; on non-spherical nuclei, by Rogers; on reactions between complex nuclei, by Greider; and on spin and parity determination of elementary particles, by Tripp.

Articles of more general interest are by Fleischer, Price and Walker, on particle track formation in solids and the use of solid-state detectors in nuclear science and geophysics; by Harbottle, on the chemical effects of nuclear transformations in inorganic solids; by Hart, on the radiation chemistry of aqueous solutions; by Blomeke and Roberts, on radioactive waste management, particularly that associated with reactor fuel processing; by Comar, on the movement of fallout radionuclides through the biosphere and man; and by Mole, on dose response relationships in mammalian radiobiology. J. E. TERRY

Information and Communication Theory

By A. M. Rosie. (Electronic User Series.) Pp. xii+175. (London and Glasgow: Blackie and Son, Ltd., 1966.) 32*s*. 6*d*. net.

THIS volume is one in a series ostensibly written for "qualified scientists and engineers for whom electronics is a fringe activity". The text is based on lectures given in final-year and postgraduate courses in a school of electrical engineering and is, therefore, potentially useful to those for whom electronics is much more than a "fringe activity". The author has tried to restrict the range of mathematical ideas used in presenting the theory and in two chapters has given brief introductions to such topics as the Fourier transform and probability theory. Nevertheless, the book is suitable for only two types of reader—those for whom these introductory chapters can serve as revision and those able to absorb new mathematical ideas quickly.

About two-thirds of the book is concerned with developing the ideas of information theory and applying them in the study of communication systems. The material is presented clearly and the examples and illustrations are well chosen. The chapters on "Sources and Channels" and "Ideal Communication Systems" are particularly good and these and two following sections—concerned with engineering applications of the theory—will be useful to undergraduates reading for degrees in electrical engineering.

The final chapter is concerned with the uses of information theory in biology, neurophysiology and psychology and with pattern recognition. This chapter is not up to the standard of the remainder of the text and the problems it attempts to treat cannot be dealt with in the space available. J. T. ALLANSON

The Plastic Methods of Structural Analysis

By B. G. Neal. Second edition. Pp. xiii+358. (London: Science Paperbacks and Chapman and Hall, Ltd., 1965.) 25*s*.

THE Steel Structures Research Committee published its final report on elastic design in 1936, and this date may be taken conveniently as that of the start of three decades of work on plastic theory. In the first decade Sir John Baker investigated simple beams and frames (and applied the findings to the design of the Morrison shelter); this phase closed in 1948, when a clause permitting plastic design of steel structures was inserted in British Standard 449. The second decade saw the formalization of the theoretical basis of the method; the theorems of the mathematical theory of plasticity were applied to the problem of the building frame, and immediate advances were made. For example, the powerful technique of combination of mechanisms was developed, with which Prof. Neal's name is associated. This period closed in 1956 with the publication by Baker *et al.* of *The Steel Skeleton II*; in the same year Neal's book was published (second edition 1963, now reprinted as a paperback). Since 1956 detailed problems of the design of structural elements have been investigated, but the main advance during the past ten years lies perhaps in the slow assimilation of plastic methods into the vocabulary of the designer. Plastic theory offers a completely different way of approaching the structural problem, and it is this new approach which may prove to be of the most lasting importance. Measured against this background, Neal's book betrays that it was first written 10 years ago. The first four chapters, laying the foundations of the theory, are clearly and elegantly written; written to-day, however, the first chapter (on stress distributions) would largely disappear, and virtual work would be introduced earlier than Chapter 3. The four chapters forming the second half of the book deal with particular topics such as deflexions, minimum weight design, and shakedown, this last reflecting one of Prof. Neal's own research interests. The book as a whole gives the impression of being slightly old-fashioned; the first half is not quite incisive enough in its treatment of the basic theory, and the second is too detailed in its treatment of topics which are seen now to be not of prime importance. Overall, however, the book is probably still the best introduction a student could have to the plastic design of steel frames.

The Habit of Authority

Paternalism in British History. By A. P. Thornton. Pp. 402. (London: George Allen and Unwin, Ltd., 1966.) 45*s*. net.

IN spite of an early reference to William Whewell, Prof. Thornton's study of paternalism in British history comes no nearer than Prof. Hogben's *Science in Authority* to examining the rival claims of authority and of intellectual initiative. The book might be described as an exposition of Governor Morris's observation to Washington that the exercise of authority depends on personal character, but while Prof. Thornton seems to accept the view that expertise of any kind is ancillary to the tradition of authority and in itself gives none, he never urges the scientist to accept office and is little concerned with how the scientist or other expert can qualify himself to exercise leadership or authority. To that extent the book disappoints, and it lacks the interest for the scientist or technologist that its opening might suggest. Prof. Thornton describes with perspicuity and candour the tradition of successful paternalism which characterizes British history, and the pragmatism which pervades the book suggests that Prof. Thornton himself is well qualified to expand this into a badly needed analysis of the personal aspects of the place of the scientist in the task of government. That task is not attempted: there are occasional pointers or warnings but it is left to the scientist himself to make the analysis. Accordingly, while the book is entertaining as well as instructive, its scientific interest is slight. The title alone points to the existence of a problem central and inescapable for our times, whether we approach it primarily from the political side or from the kind of consideration which prompted Prof. R. S. Kuhn to discuss the structure of scientific revolutions. Nevertheless, in these seven chapters, discussing in succession what Prof. Thornton terms the colonization of England, the Estates of the realm, English authority overseas, the dislocation of society, the survival of the ascendancy, Bonar Law's dynasty, and agents and patients, any one who attempts that task will find much that is instructive and suggestive. R. BRIGHTMAN

MEETINGS

MATHEMATICAL ASSOCIATION—1966

THE annual conference of the Mathematical Association was held in the University of Keele during April 13–16.

During the business meeting it was made clear that the Association, now with more than 6,000 members, is playing a full part in the present development of mathematical education in Britain and in the world as a whole: this has been achieved at no small cost to the Association, as the deficit of £3,000 on the year's working shows. Worthy of special comment is the fact, however, that members of the Association are anxious not only that the work should continue but also that it should be extended at additional cost to themselves, including an increased subscription.

Mr. J. T. Combridge, reporting generally on "Current Affairs", referred to the recent report of the Joint Mathematical Council on *In-Service Training for Teachers of Mathematics and Science*. It appears that mathematics is the only discipline which has put forward a blueprint indicating the costs of schemes for such training with its emphasis on local (school-based) mathematics centres. The Schools and Industry Committee of the Association continues to be influential and the part played by the Association in this field is recognized by and supported by other members of the Joint Mathematical Council. Of the Association's work in the past few years, Mr. Combridge said that the Association had followed its proper course, which is not one of sovereignty or slavery, but one of service: indicative of this was the support given by the Association to the Joint Mathematical Council and the Institute of Mathematics and Its Applications and the influence of members of the Association in many of the newer teaching projects. The meeting approved a change of rule which will allow overseas mathematical associations to become affiliated to the Mathematical Association. Such associations will continue to enjoy the facilities of the *Gazette* and other publications of the Association, but will also be assisted by an interchange of literature, thereby encouraging the development of such associations.

In her presidential address, Mrs. E. M. Williams, formerly principal of Whitelands College of Education, commented that it was only the fourth time a woman had been president of the Association, and recalled that in the more limited conditions of the nineteenth century at least two women demonstrated that new mathematical thinking was not alien to the feminine mind. Before the century closed, Maria Edgeworth at the beginning of the century and Mary Boole (wife of the founder of Boolean algebra) were foreshadowing some of the modern developments in the teaching of mathematics. Maria had given a hint of the concept of a set and recommended an approach through the handling of cubes; Mary had emphasized the logical basis of arithmetic as it should be taught to children and recommended the use of non-numerical situations to bring out the logical structures. Mrs. Williams considered that there were grounds for present optimism in the evidence of the lively interest among women teachers, particularly those in primary schools, in the more logical and structural approach to mathematics. Among all pupils there was a preference either for the kind of mathematics that could be seen to be useful or for those forms that gave satisfying patterns of thinking. In primary school experiments now being carried out it was recognized that children's own investigations were the material from which mental images and logical thinking emerged. The important task for a teacher was to lead pupils on from the intuitions derived from experience to the mental patterns which enabled them to understand the world around and to solve problems that occurred in new situations. If mathematics is supposed to concern patterns, structures and the building up of visual and symbolic imagery, varied practical experiences must be provided from which pupils can develop the power to relate the mental images with which their minds are stored. The essential role of mathematics for children in general seems to be the provision of a store of patterns of form and thought which enable an individual to recognize and to respond effectively to the scientific, aesthetic and social relations discoverable in their surroundings.

The Mathematical Association, with its 95 years of fostering improvements in the teaching of mathematics, had done much to help the present movement at home and overseas and will continue to do so. Mrs. Williams emphasized that the fact that the re-appraisal of school mathematics was world-wide drew attention to the nature of the subject itself. It was the outcome of man's systematized thinking during many centuries with contributions from all the great cultures. Through such thinking, developing now with great rapidity, man's scientific mastery continued to grow. New mathematics should represent not a break with the past but an enlargement in scope and depth. In this way mathematics could fulfil its true educational role of enabling each individual to enlarge his capacity for understanding and thinking about his complex world.

The first discussion of the conference was on the Joint Mathematical Council's report on the *In-Service Training for Teachers of Mathematics*, with the chair taken by Prof. W. A. C. Stewart, professor of education, University of Keele, a member of the Schools Council and Vice-Chancellor designate (1967) of the University of Keele. The discussion leaders were Prof. J. Wrigley, professor of education, University of Southampton, Mr. D. G. Crawforth, Dr. Challenor's Grammar School, Amersham, and Mr. W. M. Brookes, Department of Education, University of Southampton, all of whom were members, under Prof. Wrigley's chairmanship, of the working party responsible for drawing up the report on in-service training.

Prof. Stewart, in introducing the speakers, considered that the most pressing and important problem in the teaching of mathematics is that of those who teach and how they equip themselves. Prof. Wrigley described the report as almost inevitable: in this changing world of new syllabuses and new ideas, many of our previous procedures have been found wanting, if the needs of all teachers were to be served. The proposed Local Mathematics Centres have become an urgent necessity. In Prof. Wrigley's view it is imperative that the idea of class teaching as one teacher confined with thirty people should be dispelled. He spoke of much more team teaching, more use of aids, much more co-operation between different sorts of teachers, for example primary and secondary. A vertical interchange of teachers, he considered, was important.

Mr. Crawforth considered that there are a large number of children who are not effectively taught, however conscientious and dedicated the teacher may be. He described the efforts which he and his colleagues in the Amersham school and district have made to overcome this difficulty by the introduction of team teaching. Colleagues became used to working together in the same room and discussing work afterwards. They soon realized the mesh of activity in a conventional classroom situation of which they had previously been unaware or had ignored. The children also came to feel that there was something important to talk about in the mathematics of a situation: in time the children felt free to join the discussion when appropriate. Experienced teachers were discovering for the first time that children could perceive and generate mathematics out of their own awareness and their own strength in a way that had never been conceived possible

previously. Mr. Crawforth acknowledged the difficulties ahead, including the difficulties of head teachers with the many external and internal pressures on them, but said that in his view the children must be put first. The kind of activity which generates a neighbourhood of living and growing teachers in contact with living and growing children holds out the most hopeful prospect for effective mathematics learning that has been evolved so far.

Turning to the quality of the profession, Mr. Brookes, speaking of the present often self-imposed isolation of teachers, felt that, although the numbers of teachers are increasing, the quality is not sufficiently high to keep the mathematical body healthy. We may be faced with a redeployment of our mathematical skills. The present prejudices which exist between work at the levels of the university, the departments of education, the colleges, the schools (primary, modern comprehensive, layered, etc.), must be removed. Isolation will ensure that such prejudices are not removed.

After a useful floor discussion, Prof. Stewart spoke of similar problems to those of mathematics existing for all who are in any way concerned with teaching. He spoke of the complexity of the overall problem and the difficulty of disentangling the complexity to make it in any way manageable and yet of avoiding the setting up of little empires.

Modern Mathematics Projects

The second discussion of the conference dealt with modern mathematics projects, and took the form of a 'brains trust'. The members of the panel were selected as representative of different projects and consisted of Prof. J. D. Weston (University College, Swansea; Swansea Scheme), Mr. D. A. Quadling (Marlborough College; Schools Mathematics Project), Dr. G. Matthews (director, Nuffield Foundation Junior Mathematics Project) and Mr. A. J. Moakes (St. Paul's School; Mathematics in Education and Industry Project), together with Mr. F. J. Budden, Royal Grammar School, Newcastle upon Tyne, who represented the point of view of those not committed to any project or associated with any scheme. The questions ranged over the use of desk calculators, axiomatics, concepts *vis-à-vis* problems, the age for introducing sets, the needs of university science and engineering departments, and the place of group theory in school mathematics. The final question to the team was: "What do the members of the panel expect to find on Mount Ararat when the floods of modern mathematics subside?" The replies were summarized by the President, who likened the problem to the arrival of the ark with its preservation of all the valuable species. She hoped that all the essential mathematical ideas, which have always survived, would still be there on the top of Ararat when the ark has come to rest. But the arrival time was not a period of rest: it was the beginning of a developing culture and this was what could be looked forward to as, from this present stress and excitement, the new ideas were absorbed and, with their refertilization, school mathematics was taken forward along the expanding path of mathematics as a whole.

General Lectures

Prof. A. P. Robertson, professor of mathematics in the University of Keele, speaking on "Mathematics at Keele", said that postgraduate experience showed that Keele students could more than hold their own in the postgraduate field in mathematics, despite the somewhat wider base of their degrees. A questioner from the floor thought that the average of two mathematics graduates per year entering teaching was not a healthy sign. To this Prof. Robertson suggested that school teaching must be made a profession which attracts.

Dr. G. A. Garreau of the City University, London, who is in charge of the Association's Problem Bureau, described the work of the Bureau which mainly deals with problems at the university entrance scholarship level. Prof. E. C. Zeeman, professor of mathematics in the University of Warwick, spoke on "A Topological Theory of the Brain". Mr. G. Edgecombe, of Plymstock Comprehensive School, illustrated his lecture on Moiré Bands with carefully prepared patterns and drawings which illustrated the appearance of these bands in the very simplest of two-dimensional intersections of two sets of straight lines—up to three-dimensional effects. He mentioned many of the materials and conditions which lend themselves to Moiré effects and indicated the requirements which must be satisfied if a definite and strong pattern is to be produced. The final lecture of the conference was on "Gas Bearings: a Mathematical Problem in Engineering", and was given by Dr. M. D. Wood, director of engineering studies in the University of Cambridge. Dr. Wood used his lecture to illustrate how mathematics can and often must be used to solve engineering problems that have proved intractable to an empirical approach; and that engineering is a satisfactory and worth-while career for mathematical specialists from sixth forms.

R. G. Cross

STRONTIUM METABOLISM

The study of strontium metabolism illustrates the axiom that scientific progress creates more problems than it solves. A field of endeavour which was almost unknown 20 years ago is now the scene of persistent and growing effort by workers of diverse interests, including physicians, biochemists, veterinary scientists and physicists in many countries. A symposium was held at Chapelcross, Scotland, during May 5–6, which reviewed a variety of experimental enquiries, illuminating successes and—more often—failures in providing answers to the many important questions made topical by continuing interest in the biological consequences of radioactive fall-out from nuclear explosions, past and future. The symposium was sponsored jointly by the Medical Research Council and the United Kingdom Atomic Energy Authority.

The main areas of discussion were (*a*) the uptake and incorporation into body tissues of radioactive strontium present in food; (*b*) the somatic hazards of radioactive strontium; (*c*) the possibility of modifying the uptake and metabolism of radioactive strontium by the action of drugs. Progress in each of these areas is influenced by the difficulty of measurements based on the strontium-90 which occurs in fall-out.

The somatic hazards of radioactive strontium are difficult to assess because, at present levels of dietary and skeletal contamination, they are so small in relation to the notoriously elusive effects of ionizing radiation from the natural background or even from diagnostic radiology. Extrapolation from animal experiments is uncertain, partly because the dosage levels of strontium-90 commonly used are greater (by a factor of 10^6 or more) than those encountered in the human subject, and partly because of dietary and physiological differences between man and other animals.

The average personal daily intake of strontium-90 in Britain at the present time is about 25 pc. and the total amount of this nuclide contained in the body of a typical adult is no more than 2,000 pc. Because only β-radiation is emitted, it is not possible to obtain a numerical estimate of this contamination by external measurements—or, indeed, by internal measurements either. The investigator who is concerned with the human subject has three possibilities.

First, he may measure the strontium-90 concentration in bone samples obtained *post mortem*. In Britain, about 500 samples are analysed every year and similar programmes are under way in several other countries. Work

of this kind provides the bulk of our information about strontium uptake in children. In studying the processes involved in the passage of strontium-90 from atmospheric dust (or rain) into human bone it is useful also to examine the contamination of milk and other foodstuffs. Simple models allow reasonably accurate prediction (*a*) of dietary levels of strontium-90 and of radiation dose commitment (in terms of fall-out deposition on the ground) and (*b*) of strontium-90 levels in children's bones (in terms of dietary contamination). Using fall-out and dietary measurements, J. F. Loutit (Medical Research Council Radiobiological Research Unit, Harwell) found evidence of rapid turnover of skeletal strontium in childhood. During each of the first 9 years of life, about half of the existing body burden of strontium-90 is apparently lost.

Secondly, it is possible to measure the strontium-90 in urine, thus obtaining an indication of the metabolism of the nuclide in the living subject. At the Chapelcross meeting, Janet M. Warren (Regional Physics Department, Western Regional Hospital Board, Glasgow) reported studies on children in two age groups. In those less than 3 months old, living on an all-milk diet, the concentration of strontium-90 (expressed as pc. strontium-90/g calcium) was about the same in the urine as in the diet. For older children in the age range 2–13 years enjoying a normal mixed diet, the concentration of strontium-90 in the urine was about half the corresponding figure for the diet. C. L. Comar (Cornell University) observed that the ratio of urinary to dietary strontium is correlated with the daily output of calcium in the urine. In general, he advised, it is desirable to obtain data on both elements in simultaneous or parallel experiments. Earlier belief in the close similarity of calcium and strontium metabolism has been modified in the light of more refined experimental evidence now available, but the metabolism of strontium (stable or radioactive) is regulated more markedly by calcium levels in the body than by stable strontium levels.

Thirdly the experimenter may use radioactive isotopes of calcium and strontium in tracer experiments to illuminate metabolic processes. Useful information is still being extracted from an investigation begun at Harwell in 1962, when five adult male volunteers lived for periods of 3 or 4 weeks on a special diet consisting mainly of bread and milk. The bread was made from grain containing a relatively high concentration of strontium-90 and the milk came from a cow which had received an intravenous dose of strontium-85. Measurements of both isotopes were made in urine and faeces; the total retention of strontium-85 was estimated with a whole-body counter.

From this and similar studies it seems that the movement of strontium in the body may be described by a three-compartment model. Absorption through the gut supplies the first compartment, containing 64 per cent of the absorbed strontium. This freely exchangeable pool includes plasma, extra-cellular fluid and bone surfaces; the effective half-life of the strontium here is 2·7 days. The second compartment contains 18 per cent of the absorbed fraction and represents strontium more closely bound to the apatite crystals of bone, with an effective half-life of 19 days. The third, of the same size as the second, includes strontium attached to the apatite crystal lattice, from which it is released only by resorption of bone. The effective half-life of this portion of the bodily strontium is 750 days.

J. Shimmins (Regional Physics Department) described excretion and retention studies after administration of calcium-45 and strontium-85 over periods of several weeks, at various levels of dietary calcium. The skeletal retention of strontium-85 was less than that of calcium-45 at all levels of dietary calcium intake. Measurements on simultaneous plasma and urine samples showed the urinary excretion of strontium-85 to be more than three times that of calcium-45. It is not clear whether this discrimination arises at the glomerular filtration stage (because of a difference in the extent to which calcium and strontium are bound to plasma proteins) or at the tubular reabsorption stage. Nor is it clear whether the apparent difference in skeletal uptake between calcium and strontium denotes a genuine difference in mineral transfer rates at the bone surface—or is merely a consequence of the undoubted discrimination (favouring excretion of strontium) at the gut and the kidney.

In a review of this work, A. Catsch (Technische Hochschule, Karlsruhe) reported that no convincing successes had been obtained by methods so diverse as isotopic dilution (through addition of stable strontium to the diet), demineralization of bone (for example, by administration of parathyroid hormone or ammonium chloride), blocking of tubular reabsorption in the kidney (by administration of various diuretics) or chelation. Supporting evidence came from Herta Spencer (Veterans Administration Hospital, Hines, Illinois), who described extensive studies on human subjects in a metabolic ward, using calcium-45 and strontium-85 as tracers. When glycerophosphate was added to the diet, raising the daily intake of phosphorus from 600 mg/day to 1,600 mg/day, the absorption of strontium (as inferred from measurement of plasma levels of strontium-85) was reduced. Addition of calcium to the diet gave inconclusive results but administration of stable strontium, even in quite large amounts, appeared to increase the absorption of strontium-85.

Experiments with young animals have suggested that the absorption of both calcium and strontium from the small intestine is enhanced by the presence in the diet of certain carbohydrates (such as lactose) and amino acids (such as lysine); Spencer found no such effect in adult human subjects. Other substances, including oestrogens and corticosteroids, known to influence calcium metabolism in man, were also found to be without effect on the retention of strontium.

Naturally-occurring polyuronic acids are capable of complexing with Sr^{++} ions *in vivo*, so preventing transfer across the intestinal mucosa. Deirdre Waldron-Edward (McGill University) reported that the skeletal retention (in rats) of strontium-89 from a single dose was reduced (in some instances by a factor of five) when sodium alginate was administered with the strontium. Large variations in effectiveness were found among samples of alginate, whether obtained commercially or prepared in the laboratory from different species and varieties of seaweed; of the common seaweeds, *Laminaria digitata* was the most successful in this regard and *Fucus vesiculosis* the least successful.

This work is significant in the study of strontium metabolism, though its practical applications are limited. R. Hesp (U.K.A.E.A., Windscale) found that sodium alginate administered 2 min before a tracer dose of strontium-85 inhibited almost completely the uptake of the radiostrontium in the human subject; a smaller effect was observed if the alginate was given 20 min after the strontium-85. There is at present no method for reducing the body burden of strontium-90 acquired (from fall-out) over a period of time.

An unexpected association between strontium and fluorine was suggested by V. A. Knizhnikov (Institute of the Biophysical Ministry of U.S.S.R., Moscow), who reported that the concentration of radioactive and stable strontium in human teeth was lower in towns where the natural fluorine content of drinking water was relatively high. Animal experiments indicated that reduction in the bodily retention of strontium occurred also when the fluorine content was artificially enhanced. The skeletal uptake of calcium was not affected by the fluorine level in drinking water. Knizhnikov's observations were based on experience in several pairs of towns with differing levels of fluorine (but not of other mineral elements) in drinking water, but no substantial differences in dietary strontium intake, whether stable or radioactive.

J. M. A. LENIHAN

ORIGIN OF THE MICROWAVE RADIO BACKGROUND

By PROF. P. J. E. PEEBLES and PROF R. H. DICKE
Palmer Physical Laboratory, Princeton, New Jersey

RECENTLY, Miss Kaufman stated[1] that the newly discovered microwave radio background[2] at 7·3 cm wavelength may be emission from ionized intergalactic hydrogen, rather than primordial fireball radiation from the big bang[3]. In this new picture the intergalactic hydrogen would have a temperature of about 10^6 °K, and a present density of 2×10^{-30} g/cm³. It is assumed in the calculation that as the universe expanded the hydrogen temperature remained constant, at least as far back in time as we can see at 7·3 cm wave-length. In our opinion this assumption is not tenable; it requires that the intergalactic hydrogen temperature remains constant while the hydrogen has expanded by a factor of 10^{17} in volume. To keep the hydrogen temperature constant as it expands by this enormous factor would require a large amount of energy ($2{\cdot}5\times10^{14}$ ergs/g), and much of this energy must be provided in a period of a few years. The power requirement to keep the gas hot while the universe expands is as great as $2{\cdot}8\times10^5$ ergs/sec g, $1{\cdot}4\times10^5$ times the energy production rate of the Sun. No reasonable energy source would be adequate to prevent an adiabatic cooling of the medium during the rapid expansion of the early universe.

For any set of reasonable assumptions ($\rho\leqslant 2\times10^{-29}$ g/cm³, $T>10{,}000$ °K) there would be negligible microwave emission due to bremsstrahlung radiation (free–free transitions) in intergalactic ionized hydrogen in the present state of the universe. Thus, the question of whether or not the present universe contains any appreciable amount of ionized intergalactic hydrogen[4] is not relevant to the explanation of the microwave radio background. Contrary to Kaufman's arguments[1], we believe that the observed microwave background does not provide any appreciable observational restriction on intergalactic matter in the present universe. On the other hand, if ionized intergalactic hydrogen is present, the hydrogen must have been heated up at some time in the past, the energy source presumably being emission from galaxies, and we know that this heating could not have occurred at an arbitrarily early phase in the expansion of the universe. Otherwise, we could trace the expanding, adiabatically cooling universe back in time, to densities and temperatures so high that the material would have been opaque, and so in thermal equilibrium with radiation.

To estimate the emission from an intergalactic plasma we can use the approximate formula for the opacity due to free-free transitions:

$$\varkappa \sim 2\times10^{-23}\lambda^2N^2\,T^{-3/2} \qquad (1)$$

where C.G.S. units are used, and we have ignored the Gaunt correction. Here N is the number density of the intergalactic protons, T is the temperature of the plasma, and λ is the wave-length of the radiation.

We can neglect synchrotron emission processes in the plasma, because at the assumed temperature of 10^6 °K the electrons are non-relativistic, and so would radiate almost exclusively at the cyclotron frequency. The optical depth in the plasma is the integral of relation (1) along a line of sight, where we have to take account of the cosmological red-shift, so that λ varies directly as the expansion parameter $a(t)$, and $N\propto a^{-3}$. While the results do not depend sensitively on the details of the cosmology used, we have assumed for definiteness a matter-filled space with the present value of the acceleration parameter being $q=\frac{1}{2}$. We then have $a\propto t^{2/3}$ and the optical depth from a time t_0 in the past to the present is:

$$\tau=\int_{t_0}^{t}\varkappa c\,dt\cong 1{\cdot}2\times10^{-23}\,c\,t\,\lambda^2N^2\,T^{-3/2}\,[(1+Z)^{5/2}-1]$$
$$\cong 1{\cdot}1\times10^{-7}\,\lambda^2\,Z^{5/2}\,T^{-3/2} \qquad (2)$$

In this equation t is the present age of the universe, λ and N are evaluated in the present epoch of the universe, and Z is the value of the red-shift $\Delta\lambda/\lambda$ suffered by a photon in the time interval from t_0 to t. In the integration the temperature T is assumed constant. However, the main contribution to this integral occurs near the lower limit (t_0) and the physically significant assumption is that the temperature be constant during these early times. In the second line we have set $N=1{\cdot}2\times10^{-6}$ cm⁻³, following Kaufman[1], we have taken $t=\frac{2}{3}\,H^{-1}=6{\cdot}6\times10^9$ yr, where H is Hubble's constant, and we have assumed $Z\gg1$.

We can see back in time, and into the plasma, to about unit optical depth, that is, back to a red-shift:

$$Z\cong 6\times10^2\,T^{3/5}\,\lambda^{-4/5} \qquad (3)$$

Almost all radiation observed at present would have had its origin during these early times. Consequently, assuming that the hydrogen temperature has been constant, the radiation temperature T_r observed now at wavelength λ is

$$T_r\cong T/Z\cong 1{\cdot}7\times10^{-3}\,T^{2/5}\,\lambda^{4/5} \qquad (4)$$

Adopting a plasma temperature $T=1\times10^6$, we find from equation (4) that at $\lambda=7{\cdot}3$ cm:

$$T_r\cong 2\ ^\circ\mathrm{K} \qquad (4)$$

in agreement with the value $T_r=3{\cdot}7$ °K given by Kaufman[1]. For $\lambda=7{\cdot}3$ cm, equation (3) gives:

$$Z_1\cong 5\times10^5 \qquad (5)$$

In this calculation we have followed Kaufman[1] in ignoring energy transfer from matter to radiation due to scattering by free electrons. For electrons at temperature T the average energy transfer to a low-energy photon in each scattering is about $\delta E/E\cong 4\,kT/mc^2$. At a red-shift $Z=5\times10^5$ (equation (5)) in the past the maximum energy transfer to a low-energy photon in a characteristic expansion time of the universe is:

$$\delta E/E\cong 4\,kT\,\sigma\,Nc\,(\dot a/a)^{-1}/mc^2$$
$$\cong 1{\cdot}8\times10^{-11}\,(Z+1)^{5/2} \qquad (6)$$
$$\cong 3\times10^3$$

In computing the rate of the reactions we have ignored the correction for stimulated emission. Hubble's constant, $\dot a/a$, is assumed to be larger than the present value by

the factor $(Z+1)^{3/2}$. The energy density in radiation does not introduce a significant error here, because the mass density in radiation for a black-body spectrum at 10^6 °K would be only a factor of 10 larger than the assumed total matter density at this epoch. It is seen from equation (6) that energy transfer to the radiation due to free electron scattering is important until the red-shift has decreased to $Z=1\times10^4$. The effect is to move radiation quanta from lower energies to energies approaching kT. However, for the spectrum (4) the available number of low-energy quanta is so small that this could not substantially increase the radiation intensity at higher frequencies. This means that the red-shift required to explain the observed microwave background on the basis of this model could not be substantially less than the value given in equation (5).

According to equation (5), we would be receiving radiation emitted when the mean density in the universe was 10^{17} times larger than it is now. Evidently the present condition of the intergalactic material is of no interest for the radio background. One important question is whether the temperature could have been constant during the epoch in the past when emission from the plasma would have been appreciable.

It is a well-known result of relativistic cosmology that in the absence of internal energy sources a gas uniformly filling the space cools adiabatically with the expansion of the universe. Furthermore, this result does not even require relativity for its demonstration.

To discuss this problem it is convenient to limit our view at this early epoch to a small spherical volume V. We suppose that the hydrogen is expanding with the general expansion of the universe, and that V is expanding at the same rate, so that the mass within V remains constant. Assuming that the radius R of the sphere is much less than cH^{-1}, where $H_{(t)}$ is the value of Hubble's constant, the sphere would be expanding at a rate $\dot{R}=HR$ much less than the velocity of light. Thus, we can discuss the energy content of V in strictly classical, Newtonian terms[5]. In particular, the over-all structure of the universe appears to be isotropic, and this symmetry implies that the matter outside V cannot contribute to the gravitational field within V.

It is assumed that the material within the volume is expanding with the general expansion. The ordinary kinetic pressure resulting from the gas at the surface of V is $2NkT$, so that the material within the system is doing work at the rate:

$$-\dot{U} = p\dot{V} = 2NVkT\dot{V}/V$$

$$= 6NVkT\dot{a}/a \qquad (7)$$

If the temperature within V is to remain constant this work must be supplied by some energy source other than the thermal energy of the material. The only available sources are nuclear burning and the energy release accompanying the localized gravitational collapse of bodies inside V.

It is easy to see that nuclear burning in any known star could not be an appreciable energy source. It is conceivable that the most massive stars achieve 1 per cent conversion of mass to energy in a life-time of 10^6 years. This is a mass to energy conversion ratio of 3×10^{-7} in a characteristic expansion time of the universe ($H^{-1}=30$ yr). However, from equation (7) the energy requirement per unit mass of gas in the characteristic expansion time would be $6\,kT/m_pc^2=5\times10^{-7}$ if the temperature is $T=1\times10^6$ °K. Here m_p is the mass of a proton. Thus, for any reasonable assumption about the amount of material burned in such massive stars, this energy source is inadequate to keep the hydrogen temperature constant.

Energy is also released if material is collapsing to form bound systems. The most efficient established way to obtain energy in this way would be to form massive stars, with central temperature $T_*=3\times10^7$ °K. The temperature limit is set by the requirement that the nuclear energy release be sufficiently slow that the star does not explode. If the mass of such a star is M_*, we know that the energy which is radiated during the formation of the star is given by the formula:

$$U \cong 1{\cdot}6\,kT_*\,M_*/m_p \qquad (8)$$

The numerical factor follows from a polytropic model with polytropic index n in the range 2–3. If the rate of transformation of mass to stars within V is $\dot{M}$, the rate of energy release due to collapse is:

$$\dot{U} = \dot{M}U/M_* \cong 1{\cdot}6\,kT_*\,\dot{M}/m_p \qquad (9)$$

Comparing equations (7) and (9), we obtain the required rate of transformation of mass to bound systems:

$$\dot{M}/M \cong 4\frac{T}{T_*}\frac{\dot{a}}{a} \cong {\cdot}13\,\frac{\dot{a}}{a}, \qquad (10)$$

if $T=1\times10^6$ °K. Note that the result is independent of the mass M^* of the condensation. In order to preserve a constant temperature, stars or other more massive condensations must have been forming at a rate in excess of 10 per cent of the total mass available in hydrogen during each characteristic expansion time of the universe (about 20 years at this epoch). Notice also that the mean density here is 10^{12} times the mean density within a galaxy now, so that the galaxies could not have existed in their present forms. In this model an unreasonably large fraction of all the hydrogen in the universe would have been consumed before the galaxies could have formed.

It is concluded that the energy which would be required to hold the hydrogen temperature constant would not be available on any reasonably satisfactory picture for energy generation in the early universe. Apparently it is preferable to suppose that in the early, highly contracted universe matter and radiation were in thermal equilibrium[3]. When the temperature dropped to 4,000 °K the hydrogen would have recombined, and further expansion would have cooled any uniformly expanding hydrogen below the black-body radiation temperature. There is reason to suppose that the galaxies would have started to form at this epoch[6]. Later energy radiated from the galaxies might have heated up any remaining intergalactic hydrogen, but we have emphasized that this ionized hydrogen could not lead to any appreciable microwave background.

A direct experimental test of the model proposed by Kaufman[1] has been provided by the Princeton microwave experiment at 3·2 cm wave-length which has been performed by our colleagues D. T. Wilkinson and P. G. Roll[7]. According to equation (4) the radiation temperature would vary approximately as $\lambda^{4/5}$, while if the background is the primordial fireball, the temperature is, of course, independent of wave-length. It seems that the Princeton and Bell Laboratories' experiments are compatible with the black-body radiation spectrum but not with the spectrum predicted by equation (4).

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INTERPRETING THE COSMIC MICROWAVE BACKGROUND

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THE observation by Penzias and Wilson[1] of an isotropic microwave background, later confirmed by Roll and Wilkinson[2], has received two conflicting interpretations. Dicke, Peebles, Roll and Wilkinson[3] postulate a universe of the type considered by Gamow[4], in which the energy density of radiation exceeds that of matter near the beginning of the expansion, and interpret the observed radiation field as a remnant of the initial 'flash'. According to the second interpretation, the observed radiation was emitted between 10 and 10^5 years after the beginning of the expansion by ionized hydrogen at a nearly constant temperature in the range 10^4–10^6 °K. Miss Kaufman[6] has shown that this model[5] is consistent with the observations of Penzias and Wilson and of Roll and Wilkinson as well as with measurement of the radio-noise background between 10 and 400 Mc/s. At millimetre wave-lengths, where no measurements have as yet been made, the constant-temperature model predicts a much lower value of the background temperature than the model of Dicke and Peebles. The total energy density of radiation is also several orders of magnitude smaller in the constant-temperature model than in the model of Dicke and Peebles[7].

In the preceding article, Dicke and Peebles criticize the constant-temperature model, which they consider to be inconsistent with the cosmological energy equation:

$$\frac{\mathrm{d}}{\mathrm{d}t}(\varepsilon S^3) + p\frac{\mathrm{d}S^3}{\mathrm{d}t} = 0 \qquad (1)$$

Here S is the cosmic scale factor, ε is the internal-energy density, and p is the cosmic pressure. In a perfect gas of uniform density ε and p are both positive and are related by the usual equation of state. This is the situation envisaged by Dicke and Peebles. The constant-temperature model, however, describes a universe in which, owing to local non-uniformities in the distribution of matter, the internal energy is negative and the pressure is nearly zero. Thus the internal energy of a co-expanding volume element remains approximately constant in time.

Let us consider a statistically homogeneous and isotropic, but non-uniform, distribution of matter. The fluctuating part of the density field gives rise to short-range gravitational forces analogous to the screened Coulomb forces in a macroscopically neutral plasma. These forces are derived from a potential φ related to the fluctuating component of the density, $\tilde{\rho} \equiv \rho - [\rho]$, through Poisson's equation $\nabla^2\varphi = 4\pi G\tilde{\rho}$. (Brackets [ ] are herein used to denote ensemble averages. The ergodic theorem of Birkhoff and Khinchin ensures that these coincide with spatial averages if the scale of the local irregularities is finite.)

If the particle velocities are non-relativistic, the internal-energy density and the cosmic pressure may each be written as the sum of a kinetic, a gravitational, and a radiative contribution[8,9]:

$$\varepsilon = \varepsilon_k + \varepsilon_g + \varepsilon_r, \quad p = p_k + p_g + p_r \qquad (2)$$

where

$$\varepsilon_k = \tfrac{2}{3}p_k = \tfrac{1}{2}[\rho v^2], \quad \varepsilon_g = p_g = \tfrac{1}{2}[\rho\varphi], \quad \varepsilon_r = 3p_r \qquad (3)$$

Here v denotes the velocity of a particle relative to its instantaneous locally inertial frame of reference. It is given explicitly by the formula:

$$v = S(t)\frac{\mathrm{d}\bar{x}}{\mathrm{d}t} \qquad (4)$$

where the components of $\bar{x}$ are 'co-moving' spatial co-ordinates. Energy exchange between the radiation field and the matter is described by the equation:

$$\frac{\mathrm{d}}{\mathrm{d}t}(\varepsilon_r S^3) + p_r\frac{\mathrm{d}S^3}{\mathrm{d}t} = (q-r)S^3 \qquad (5)$$

where q and r represent, respectively, the mean rates of emission and absorption of radiant energy per unit volume. On inserting equations (2) and (5) in (1), we obtain:

$$\frac{\mathrm{d}}{\mathrm{d}t}(\varepsilon' S^3) + p'\frac{\mathrm{d}S^3}{\mathrm{d}t} = (-q+r)S^3 \qquad (6)$$

where

$$\varepsilon' = \varepsilon_k + \varepsilon_g, \quad p' = p_k + p_g \qquad (7)$$

Radiative processes always act to diminish the quantity $\varepsilon' S^3$, because in an expanding universe photons lose energy in flight. (The right-hand side of equation (6) may, however, assume positive as well as negative values. That is, the mechanical part of the internal energy of a co-expanding volume element may decrease less rapidly with the expansion than it would in the absence of radiation.)

The gravitational contribution to the internal-energy density may be expressed in the form[9,10]

$$\varepsilon_g = -2\pi G[\rho]\alpha^2\lambda^2 \qquad (8)$$

where

$$[\tilde{\rho}^2] \equiv \alpha^2[\rho]^2, \quad \int_0^\infty [\tilde{\rho}(\mathbf{x}+\mathbf{r})\,\tilde{\rho}(\mathbf{x})]\,r\mathrm{d}r = \lambda^2[\tilde{\rho}^2] \qquad (9)$$

The quantities α and λ measure, respectively, the amplitude and the scale of the local density fluctuations. From equation (8) and the relation $p_g = \varepsilon_g$ it follows that the gravitational contribution to the cosmic pressure is always negative. Thus, we cannot immediately conclude that the internal energy of a co-expanding volume element must decrease in the absence of energy sources. The cosmic expansion tends to decrease the kinetic contribution to the internal energy and to increase the (negative) gravitational contribution. The sum of the two contributions may increase, decrease, or remain constant.

Observational evidence strongly suggests that at the present time the part of the internal-energy density of the universe associated with structure of galactic and supergalactic scale is negative. (The part of the internal energy associated with stellar structure is not relevant to the present discussion.) The actual value of this part of $\varepsilon'/[\rho]$ is difficult to estimate because we have no reliable information about the density or temperature of intergalactic matter, but it may perhaps lie in the range -10^{12} to -10^{14} ergs/g. The corresponding range of kinetic temperatures is:

$$10^4 \lesssim T_k \lesssim 10^6 \text{ °K} \qquad (10)$$

It has been shown elsewhere[9,11] that $p' \simeq 0$ provided that (i) $\varepsilon' < 0$, and (ii) $\tau \ll t$, where τ is a relaxation time associated with the density fluctuations. Under these conditions equation (6) reduces to:

$$\frac{\mathrm{d}}{\mathrm{d}t}(\varepsilon' S^3) = (-q+r)S^3 < 0 \qquad (11)$$

Thus the non-radiative part of the internal energy of a co-expanding volume element diminishes monotonically with time. It follows that the quantity $\varepsilon_k/[\rho]$, which is proportional to the kinetic temperature of the distribution, increases monotonically with time, and that the amplitude α of the density fluctuations increases at least as fast as $S^{1/2}$. However, the temperature of the gas cannot have been appreciably smaller than 10^4 °K at any time during the period when $p' \simeq 0$, for at significantly lower temperatures the gas would have been so little ionized that radiative energy losses would have been negligible. The kinetic temperature would then have remained constant, and its present value would have been less than the lower limit given by equation (10). Considerations of the rate of energy loss by radiation suggest that the kinetic temperature of the gas would in fact have remained near 10^4 °K until galaxies began to separate out.

The preceding remarks may serve to indicate how certain properties of the present-day cosmic distribution of matter can be related to the kinetic temperature of the pre-galactic distribution. A deductive cosmogonical theory should relate this temperature in turn to the initial conditions of the universe. This problem has been briefly discussed elsewhere[11] and will be treated more fully in a forthcoming paper. The main point of the discussion is that the initial thermodynamic temperature of the universe must be assumed to be very low if the internal energy is ever to become negative. The simplest assumption is that the initial state of the universe is one of thermodynamic equilibrium at zero temperature. Such a universe would be matter-dominated throughout and would not possess a 'fossil' radiation field of the kind postulated by Dicke and Peebles.

The objections raised by Dicke and Peebles to the constant-temperature model seem to be based in part on their classical interpretation of equation (1). This interpretation encounters certain well-known conceptual difficulties. For example, the equation (but not the physical argument used to derive it) applies to a wholly collisionless gas. Again, the symmetry of the cosmic distribution would seem to ensure that one part of it cannot do work on another. It may perhaps be more satisfactory to interpret equation (1) as expressing non-conservation of energy resulting from the use of a non-inertial co-ordinate system. The quantities ε and p are defined in terms of particle velocities and momenta calculated with respect to the instantaneous locally inertial frames of reference of the individual particles (see equation (4)). With the help of Einstein's formula for the composition of velocities in special relativity, it can easily be shown[12] that the momentum of a test particle moving in a uniform and isotropic universe varies like S^{-1}. From this purely kinematic result it is possible to deduce the cosmological energy equation (1) for a uniform, isotropic, and collisionless distribution of gravitating particles, as well as the classical formula $\nu \propto S^{-1}$ for the frequency of a photon in a uniform and isotropic universe. The additional effects described by the cosmological energy equation when density fluctuations are present also admit a purely kinematic interpretation of an obvious kind.

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TANIFUJI–BUTLER MODEL FOR DEUTERON STRIPPING REACTIONS

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A MODEL which has been referred to as the 'snapshot' or 'sudden approximation' model has been recently proposed by Tanifuji[1] and Butler[2] for analysing (d,p) stripping reactions.

These authors emphasize that the deuteron is a large and weakly bound system. Such a system should stretch and dissociate as it encounters the surface of the target nucleus. To the extent that it does not properly take account of such stretching and dissociation, the conventional distorted wave Born approximation provides an unsatisfactory description of (d,p) stripping reactions.

The idea that the weak binding of the deuteron should be emphasized is not new. It was basic to the original Butler model[3]. The new feature in the 'snapshot' model is the use of the 'sudden approximation' to take account of this weak binding.

Although it may be desirable to develop a model of (d,p) stripping reactions which retains the emphasis of the original Butler model, we must point out that the use of the 'sudden approximation' is not justified.

We shall show in this article that the 'sudden approximation' cannot be used for the (d,p) stripping reaction, since it contains no mechanism by which the neutron can propagate into the bound final state. The exact 'sudden approximation' amplitude is zero. The non-zero amplitude of the 'snapshot model' results from a further 'approximation'. This further 'approximation' is artificial, and the stripping mechanism which results from it is unphysical.

Let us consider a (d,p) stripping reaction in the barycentric frame. We make the usual assumption that the residual nucleus contains a neutron bound to the unexcited target nucleus 'core'. We denote by H the total Hamiltonian, E the total energy of the system, and H_A the target Hamiltonian. We represent by V_n, V_p and V_{np} the neutron-target, proton-target, and neutron-proton interactions, respectively.

The exact amplitude for the (d,p) stripping reaction can be written[4]:

$$T_{fi} = \langle \psi_f \mid V_{np} \mid \Psi^{(+)} \rangle \qquad (1)$$

where $\Psi^{(+)}$ is the total wave function satisfying:

$$(H-E)\Psi^{(+)}=0 \tag{2}$$

The final state wave function $\psi_f^{(-)}$ which describes a proton scattered from the target nucleus 'core' satisfies:

$$\psi_f^{(-)}=0_f+\frac{1}{E-K_m-K_p-V_n-H_A-i\varepsilon}\,V_p\psi_f^{(-)} \tag{3}$$

where

$$0_f=\exp\,(i\,k_p'\cdot r_p)\,B(r_n,\xi) \tag{4}$$

where kp' is the momentum of the outgoing proton, and $B(r_n, \xi)$ the wave function of the residual nucleus. From equation (3), we see that the wave function $\psi^{(-)}$ is an eigenfunction of the Hamiltonian:

$$H_f=K_n+K_p+H_A+V_n+V_p \tag{5}$$

To obtain the 'sudden approximation' to the total wave function $\Psi^{(+)}$ we neglect the effects of the deuteron binding force once the deuteron interacts with the target nucleus. The 'sudden approximation' wave function $\Psi_d^{(+)}$ is then given by:

$$\Psi_d^{(+)}=\int dk_p G(k_p,k_d)\varphi_{np}^{(+)}(k_n,k_p,r_n,r_p,\xi) \tag{6}$$

where $\varphi_{np}^{(+)}$ satisfies:

$$\varphi_{np}^{(+)}=\left[1+\frac{1}{E_{np}-H_f-i\varepsilon}\,(V_n+V_p)\right]A_0(\xi)e^{ik_p\cdot r_p}e^{ik_n\cdot r_n} \tag{7}$$

with

$$E_{np}=\frac{\hbar^2}{2m}\,(k_p^2+k_m^2) \tag{8}$$

and we have chosen the energy of the ground state $A_0(\xi)$ of the target nucleus to be zero.

The wave function $\varphi_{np}^{(+)}$ in the expression (7) satisfies:

$$(H_f-E_{np})\varphi_{np}^{(+)}=0 \tag{9}$$

It describes two free nucleons scattering from the target nucleus. The 'sudden approximation' (6) for the total wave function contains only scattering states of the Hamiltonian H_f, and no states in which the neutron is bound to the target nucleus. It is therefore orthogonal to the final state $\psi_f^{(-)}$.

We follow the method of Butler[2] and write the exact amplitude (1):

$$T_{fi}=<\psi_f^{(-)}\mid E-H_f\mid\Psi^{(+)}> \tag{10}$$

We then replace $\Psi^{(+)}$ with the wave function (6) to obtain:

$$T_{fi}=\int dk_p G(k_p,k_d)<\psi_f^{(-)}\mid E-E_{np}\mid\varphi_{np}^{(+)}> \tag{11}$$

Because $\psi_f^{(-)}$, $\varphi_{np}^{(+)}$ are eigenfunctions of the same Hamiltonian, H_f corresponding to the different energies E, E_{np} respectively, the amplitude (11) is zero.

The 'snapshot' amplitude can be obtained from the amplitude (11) by introducing 'approximate' optical model wave functions. We replace $\psi_f^{(-)}$ with the wave function:

$$\psi_f{}^{A(-)}(r_n,r_p,\xi)=\chi_p^{(-)}(r_n,k_p')U(r_n)A_0(\xi) \tag{12}$$

where $A_0(\xi)$ is the ground state of the target nucleus, $U(r_n)$ the bound state of the captured neutron, and $\chi_p^{(-)}(r_p,k_p')$ an optical model distorted wave function which corresponds to the free proton momentum k_p. The wave function (12) satisfies:

$$[H_A+K_n+K_p+\mathcal{U}_n+\mathcal{V}_p]\psi_f{}^{A(-)}=E\psi_f{}^{A(-)} \tag{13}$$

where $\mathcal{U}_n$ is a real shell model neutron potential, $\mathcal{V}_p$ a complex optical model potential for the proton. We replace $\varphi_{np}^{(+)}$ with the wave function:

$$\varphi_{np}{}^{A(+)}=\chi_p^{(+)}(k_p,r_p)\chi_n^{(+)}(k_n,r_n)A_0(\xi) \tag{14}$$

where $\chi_p^{(+)}$, and $\chi_n^{(+)}$ represent optical model proton and neutron distorted wave functions. The wave function (14) satisfies:

$$[H_A+K_n+K_p+\mathcal{U}_n+\mathcal{V}_p]\varphi_{np}{}^{A(+)}=E_{np}\varphi_{np}{}^{A(+)} \tag{15}$$

where $\mathcal{V}_n$ is a complex optical model potential for the neutron.

The 'snapshot' model amplitude is then

$$T_{fi}{}^{S}=\int dk_p G(k_p,k_d)<\chi_p^{(-)}\mid\chi_p^{(-)}> \\ <U(r_n)\mid E-E_{np}\mid X_n^{(+)}(k_n,r_n)> \tag{16}$$

This amplitude is not zero because the Hamiltonians which appear on the left of equations (13) and (15) are no longer identical, so that the wave functions $U(r_n)$, $\chi_n^{(+)}(k_n,r_n)$ are not orthogonal.

If the 'snapshot' model has any validity, there must be some physical grounds for preferring the 'approximate' elastic scattering wave functions $\psi_f{}^{A(-)}, \varphi_{np}{}^{A(+)}$ to the 'exact' many-particle wave functions $\psi_f^{(-)}, \varphi_{np}^{(+)}$ in the 'sudden approximation' amplitude. These grounds have not been mentioned in the literature, although they must form the whole basis of the 'snapshot' model.

To discuss the physical content of the 'snapshot' amplitude we first consider a simple model reaction in which a deuteron impinges on a real nucleon potential. Intentively, we imagine that this model reaction contains the essential features of the real reaction. For this model reaction we see:

$$\psi_f^{(-)}=\psi_f{}^{A(-)}$$

$$\varphi_{np}^{(+)}=\varphi_{np}{}^{A(+)}$$

so that the amplitudes (16) and (11) are identical. The 'snapshot' result implies that the neutron is never captured when the deuteron is stripped.

Therefore we believe that the 'snapshot' model does not represent the physical stripping mechanism. The essential physical features have been lost in making the 'sudden approximation' which leads to the vanishing amplitude (11).

The main difficulties of the 'snapshot' model are illustrated in the neutron capture amplitude:

$$<U(r_n)\mid E-E_{np}\mid\chi_n^{(+)}(r_n,k_n)> \tag{17}$$

In order to be non-zero, this amplitude does not conserve energy. Thus, the mechanism for the neutron capture is somewhat artificial.

We have seen that the 'sudden approximation' replaces the total wave function with a packet of free neutron–proton pairs. In each pair the neutron and proton scatter from the target nucleus but do not interact with each other. Strictly the neutron cannot transfer energy to the proton and thus cannot lodge in a bound state without exciting the target nucleus 'core'.

The introduction of the optical model wave function in the capture amplitude (17) provides an artificial means for the incident neutron to shed its energy. We thus conclude that the 'snapshot' model does not represent the proper nature of the physical stripping mechanism. It has been shown elsewhere[5] that its predictions are in poor agreement with experiment.

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SOME ENGINEERING ASPECTS OF NON-NEWTONIAN FLOW

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THE subject of non-Newtonian flow is of acknowledged complexity. Treatment of it in engineering and other fields is frequently of an empirical nature representing a convenient means of correlating experimental data, probably for design and scaling procedures. Such treatment may not be of permanent value and subject to replacement by alternatives as the physical phenomena become better understood and further experimental data become available.

The following comments on (1) the measurement of normal stress in laminar viscoelastic flow, (2) dynamic similarity in non-Newtonian flow through a pipe, and (3) turbulent velocity profiles, are presented in the hope that they will assist in clarifying some of the confusing points to be found in the literature at the present time.

(1) Pitot Tube in Viscoelastic Flow

The simple theory treating the pitot tube technique for measuring velocity profiles is based on the Bernoulli equation, for incompressible flow:

$$\frac{p_1}{\rho} + \frac{u_1^2}{2} = \frac{p_2}{\rho} + \frac{u_2^2}{2} + E_L \qquad (1)$$

which is essentially a statement of the conservation of energy for a unit mass of material flowing from a point upstream of the pitot tube entrance in the undisturbed region (denoted by suffix 1) to the stagnation point at the tube entry (denoted by suffix 2). In equation (1) p is the fluid pressure, ρ is the (constant) density, u is the velocity and E_L is the energy transformed into heat between 1 and 2.

Neglecting the term E_L and noting that the velocity at the stagnation point is zero the well-known expression for the upstream velocity is obtained:

$$u_1 = \sqrt{\frac{2\,(p_2 - p_1)}{\rho}} \qquad (2)$$

It is satisfactory to neglect the E_L term at the axis of flow in laminar flow through a duct or more generally where the pathline to the stagnation point is a line connecting points of zero deformation rate. There is an error in using equation (2) for other pathlines, but a discussion of this would be out of context here.

Recently there has been a proposal that a pitot tube, used in the same way as for velocity profile measurement, is capable of measuring the axial stress known to be generated in laminar viscoelastic flow through a duct[7]. In contrast to the treatment for velocity profile measurement which is based on the conservation of energy, here the treatment is based on the conservation of momentum.

A suitable form for the steady state expression of conservation of linear momentum is, for an arbitrary fix surface in the flow (sometimes termed the control surface),

$$\int_A T^i \mathrm{d}\sigma = \tfrac{1}{2}\rho \int_A u^i u^j \nu_j \mathrm{d}\sigma \qquad (3)$$

where T^i is the surface force tensor per unit area, u^i (u^j) is the velocity vector, and ν_j is the unit normal vector at the surface area element $\mathrm{d}\sigma$. The density is assumed constant and the integrals are taken over the complete surface. The surface force tensor is related to the stress tensor by:

$$T^i = \tau^{ij}\,\nu_j \qquad (4)$$

Suppose a cylindrical control surface is chosen now which is the same radius as the 'impact' opening of the pitot tube (r') and extends upstream a distance z' into the region of flow where disturbances due to the introduction of the pitot tube are just undetectable. In the cylindrical co-ordinate system (r, θ, z) the velocity components at a point are (v, w, u). Assuming that with small error the w component of velocity may be neglected, then (3) may be rewritten as:

$$\int_0^{r'} \widehat{zz}_1(r) r\mathrm{d}r - \int_0^{r'} \widehat{zz}_2(r) r\mathrm{d}r + r' \int_0^{z'} \widehat{zr}(r',z)\mathrm{d}z = \tfrac{1}{2}\rho\left(r' \int_0^{z'} u(r',z)v(r',z)\,\mathrm{d}z - \int_0^{r'} u_1^2(r) r\mathrm{d}r\right) \qquad (5)$$

The quantity measured by the manometer attached to the impact opening is:

$$\int_0^{z'} \widehat{zz}_2(r) r\mathrm{d}r = \tfrac{1}{2}\,(r')^2\,\breve{\rho d}\; g(\Delta h) \qquad (6)$$

where $\breve{\rho d}$ is the density difference of the two fluids in the manometer limbs and Δh is the difference in fluid levels.

By putting $p_1 = \widehat{zz}_1$, $p_2 = \widehat{zz}_2$, neglecting the integrals $r' \int_0^{z'} \widehat{zr}\mathrm{d}z$, $\int_0^{z'} uv\mathrm{d}z$ (or assuming their equivalence) and assuming no variation of any of the quantities $\widehat{zz}_1$, $\widehat{zz}_2$ and u with r the equation (2) may be retrieved from (5). It has previously been mentioned that the energy approach to the derivation of (2) involves the neglect of viscous dissipation of energy so for consistency the neglect of $\int_0^{z'} uv\mathrm{d}z$ is implied in (5).

If the flow is viscoelastic and the integrals $\int_0^{z'} \widehat{zr}\mathrm{d}z$, $\int_0^{z'} uv\mathrm{d}z$ are similarly neglected, then there arises the possibility of sensing the upstream stress in the direction of flow by using (5) if u is known, as was discussed by Savins[9]. However, while the neglect of the shear stress $\widehat{zr}$ may be acceptable in the rapid flow of substances of small viscosity because:

$$r' \int_0^{z'} \widehat{zr}\mathrm{d}z \ll \rho \int_0^{r'} u_1^2 r\mathrm{d}r \qquad (7)$$

it is likely that the flow rate would have to be exceedingly rapid for the same assumption in the case of viscoelastic materials because they are often quite viscous.

As is the case for inelastic flow it is possible to consider the application of the conservation of energy to a particle moving from a point upstream to the stagnation point in front of the pitot tube opening, assuming that at this point it relaxes completely, then:

$$Ep_1 + E_{E1} + \tfrac{1}{2}\rho u_1^2 = \frac{p_2}{\rho} + E_L \qquad (8)$$

Here Ep_1 and E_{E1} are the pressure and elastic energy per unit mass respectively at a point upstream of the pitot opening. The other symbols are as previously defined for equation (1). There are considerable difficulties in using equation (8) to measure normal stress; not only would E_L in general be finite but the expression for Ep_1+E_{E1} as a function of $\widehat{zz}_1$ is unknown. From Savins[9] treatment:

$$\widehat{zz}_1 = Ep_1 + E_{E1} \qquad (9)$$

which may be true in isolated cases, but of course is not an acceptable general hypothesis.

There are other reasons why a pitot tube would be unsatisfactory for normal stress measurement such as the bending of the streamlines around the pitot tube entrance which could introduce aberrations into the stress measurement there[6].

In conclusion it may be stated therefore that the pitot tube is less likely to be as useful as other available methods for sensing the stress in the direction of flow[7].

(2) Dynamic Similarity

In Newtonian flow the familiar Reynolds number has proved an invaluable means for correlating experimental data and it is natural to search for a corresponding expression for non-Newtonian flow; however, it may be stated that although numerous attempts have been made, no generally acceptable similarity group has yet emerged. It may be expected that no single dynamic similarity group will be entirely adequate for non-Newtonian fluids since in turbulent flow the fluid elements are subjected to unsteady motion and it is doubtful whether any non-Newtonian fluid is purely viscous in its response to this type of motion. It is known for example that even suspensions of hard particles may show both in-phase and quadrature components of stress (and higher harmonics) in oscillatory experiments[8], which may arise not only from elastic effects but also from the effects of finite diffusion coefficients. None of the rheological formulae such as the 'power-law'*:

$$\tau = K\dot{\gamma}^n \qquad (10)$$

where τ is the shear stress, K is a constant coefficient, $\dot{\gamma}$ is the shear rate and n is a constant index, or the Bingham equation:

$$\tau - \tau_y = \mu_p\dot{\gamma} \qquad (11)$$

where τ_y is the yield stress, μ_p is the plastic viscosity, or the Casson equation:

$$\tau^{\frac{1}{2}} - \tau_y^{\frac{1}{2}} = c^{\frac{1}{2}} \qquad (12)$$

where c is a constant (to list only a few of those proposed), is satisfactory for unsteady motion and could scarcely serve as a basis for tubulent flow studies.

In Newtonian flow the Reynolds number for flow through a tube, diameter D, at a mean velocity of u_m is:

$$N_R = \frac{\rho u_m D}{\mu} \qquad (13)$$

or

$$N_R = \frac{\rho u_m D\,(8u_m/D)}{\tau_{\widetilde{WL}}} \qquad (14)$$

where $\tau_{\widetilde{WL}}$ is the wall shear stress if the flow was laminar (even though the flow may in fact be turbulent). It may be noted that the friction factor:

$$C_f \equiv \frac{\tau_w}{\frac{1}{2}\rho u_m^2} = \frac{16}{N_R} \qquad (15)$$

is automatically obtained from equation (14) in the laminar régime and a graph of log C_f against log N_R is a plot of log x against log $\frac{1}{x}$ which is bound to have a slope of -1. Thus there is no new information to be gained, as has previously been pointed out[2], and experimental data here cannot be taken as demonstrating the utility of a particular dynamic similarity group.

If a steady laminar shearing motion the experimental data can be fitted by equation (10) then for flow through a tube:

$$\tau_{WL} = K'\frac{(8u_m)^n}{D} \qquad (16)$$

where

$$K' = K\frac{(3n+1)^n}{4n} \qquad (17)$$

and by substituting equation (16) into equation (14) the Reynolds number is:

$$N_R = \frac{\rho u_m^{2-n} D^n}{8^n K'} \qquad (18)$$

as derived by Dodge and Metzner[3–5].

Now equation (10) cannot be expected to apply under the conditions prevailing in turbulent flow and thus the similarity group (18) may, at the most, apply only in steady laminar shearing motion.

If the experimental data cannot be fitted by equation (10) even in steady laminar motion it has been proposed to replace K' and n in equation (16) by their point values at the value of $8u_m/D$ (or $\tau_{\widetilde{WL}}$) under consideration. In other words, the tangent to the log $\tau_{\widetilde{WL}}$ against log $(8u_m/D)$ curve at a certain point is the curve of the equivalent 'power-law' fluid which would have the same Reynolds number (equation (18)), that is it would have dynamic similarity. But a dynamic similarity group based on a 'power-law' is of dubious value in turbulent flow and it is not surprising that other authors[2,3] have pointed out the failure of equation (18) to correlate experimental data.

For design and scaling procedures, it is satisfactory to have the relevant correlation of data for a particular fluid; it appears from the work of Bowen[2] that this is possible for a number of different types of non-Newtonian fluids and although a universal friction factor—Reynolds number curve is not achieved this is no serious disadvantage. The empirical correlating equation is:

$$D^x\,\tau_W = L u_m^y \qquad (19)$$

where x, y and L are constants.

(3) Turbulent Velocity Profile

Some time ago, by a dimensional analysis and by matching the velocity profile in the zone near to the pipe wall (where both viscosity and turbulent fluctuations are important with that in the fully turbulent core where viscosity is of minor importance), Millikan produced in an elegant manner the well-known logarithmic form for the Newtonian turbulent velocity profile.

An identical approach was used by Dodge[3] to formulate the logarithmic form for the non-Newtonian turbulent velocity profile utilizing the 'power-law' (equation (10)) which yields:

$$u^+ = A(n)\ln y^+ + B(n) \qquad (20)$$

where u^+ is the dimensionless (time-average) velocity, y^+ is the dimensionless distance from the boundary, A and B are constants for a given value of n. The profile (20) has since been published again[1] with a small correction factor which is a function of N_R.

It is now obvious that both the original and corrected forms are based on an unacceptable hypothesis (10) for turbulent flow.

It is possible, however, to derive an expression for the turbulent velocity profile based on Bowen's empirical

* There are also difficulties in the invariant form $P^{ij} = 2c(e^{ij}\,e_{ij})^{(n-1)/2}\,e^{ij}$, ($e_{ij}$($e^{ij}$) are the covariant (contravariant) components of the strain rate and P^{ij} is the (extra) stress tensor which has been proposed (ref. 10) In oscillatory motion the viscosity can also here become infinitely large and there will be both odd and even harmonics in the stress wave.

correlation and a diameter independence hypothesis such as is used in Newtonian flow to find the 1/7 power law velocity profile. It is hoped to publish this derivation in due course.

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CRYSTALLOGRAPHIC SHEAR AND DIFFUSION PATHS IN CERTAIN HIGHER OXIDES OF NIOBIUM, TUNGSTEN, MOLYBDENUM AND TITANIUM

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It is well known that the ease with which solids interact largely depends on the ready availability of suitable diffusion paths for the ions, and it is our purpose to show that the part played by a specific kind of defect, the crystallographic 'shear plane'[1], may be of vital significance. The existence of closely spaced families of phases is now well established in most transition metal solid-state systems. Niobium oxides of this kind are formed by substituting Ti^{4+}, Nb^{4+} or W^{6+} for Nb^{5+} (refs. 2 and 3), or alternatively by replacing O^{2-} with F^- in high-temperature niobium pentoxide[4]. Similar families of partly reduced tungsten or mixed tungsten–molybdenum oxides[5], and of reduced titanium oxides[6] represented as 'homologous series' with formulae W_nO_{3n-2}, $(Mo,W)_nO_{3n-1}$, and Ti_nO_{2n-1}, respectively, are also readily synthesized. Although the structural inter-relationships within each family have been investigated by crystallographic methods it is becoming increasingly important to understand the physico-chemical principles by which these groups of phases form. We therefore propose a model for a simple diffusion process involving the formation and the subsequent migration of a series of parallel shear planes within a crystal, which can occur rapidly and without drastic local changes of structure.

A shear is defined as a regular defect arising from the compression of a simple structure along certain planes, where the co-ordination polyhedra condense in a different way[1]. Fig. 1*a* represents the structure of NbO_2F (ReO_3-type), and any one octahedron has corners common to the six adjacent octahedra. Nb_3O_7F (Fig. 1*b* and *c*) is related to it, but contains parallel shear planes with every third octahedron in a particular row sharing edges with each of the two nearest octahedra and corners with the next five[7]. Nb_3O_7F, therefore, contains blocks similar to NbO_2F extending infinitely through the crystal in two dimensions, but with a finite width of three octahedra. The niobium, tungsten or titanium families of oxides each contain characteristic shear planes, and the members of one family differ only in the size of the block of the simple parent structure they contain. Their presence diminishes the oxygen-to-metal ratio of the parent phase.

NbO_2F rapidly decomposes at 750° C (ref. 8) according to the equation:

$$4NbO_2F \rightarrow Nb_3O_7F + NbOF_3\uparrow$$

The loss of the volatile phase $NbOF_3$ at the crystal boundaries is followed by the transport of equal numbers of Nb^{5+} and O^{2-} or F^- ions to empty spaces within the crystal. Migrating metal or non-metal ions move into the nearest interstitial position through a barrier of anions which, with their large amplitudes of thermal oscillation, need scarcely be pushed aside from their mean positions. In Fig. 1*b* and *c*, the arrows show the movement of the ions, proceeding by way of the longest O–O distance (3·07 Å) and thereby meeting least resistance.

Any one empty square in Figs. 1*a* and 2*a* bounded by four octahedra represents the projected view of a row in interstitial positions: the atoms migrating from the surface change it into a row of octahedra. In Fig. 2*a* and *b* is illustrated the second postulate of our model, the co-operative transformation in the same way of adjacent rows of empty sites by the movement of the Nb and O atoms in the octahedra labelled 1, 2, 3 and 4. The migration now continues as in Fig. 2*b* and *c* with the atoms 5, 6, 7 and 8, and then in Fig. 2*c* and *d* with 9, 10, 11 and 12. The atoms advance into the next row of interstitial positions of NbO_2F and leave this same structure behind, the net effect being the movement of a shear plane unidimensionally throughout the crystal. More planes form in the same way, and when the reaction is completed, they are frozen into the regular equilibrium positions of the phase Nb_3O_7F (Fig. 1*b* and *c*).

This mechanism is less disruptive than the proposal by Gadó[9] for the closely related WO_3–$W_{20}O_{58}$ reversible transformation. He postulated the extensive formation of vacant anion positions, but his detailed analysis of the diffraction data applies equally well to our model. The shear planes of $W_{20}O_{58}$ are also composed of octahedra sharing edges and corners, but differ from those in Nb_3O_7 by adopting another crystallographic orientation (Fig. 3*a* and *b*). These also move in unison through the crystal, the metals (plus oxygen) marked 2, 4, 6 and 8 in one direction, while the atoms 9 move the other way, and the whole structure takes up a regular periodicity at the conclusion of the reaction. There is no known tungsten oxide W_3O_8 the exact counterpart of Nb_3O_7F and the detailed structure of $W_{20}O_{58}$, which is formed instead, evidently reflects the loss of oxygen atoms alone, from the edges of the crystal. The oxidation of $W_{20}O_{58}$ to WO_3 could be initiated by the migration of the shear planes to the surfaces, where oxygen from the environment is captured, and the octahedral co-ordination for tungsten completed. The closely related members of the homologous series of compounds $(Mo,W)_nO_{3n-1}$ (ref. 5) may result from the loss of some of the more volatile molybdenum as oxide, the particular member of the series which forms thereby depending on the percentage of tungsten it contains.

The proposed diffusion mechanism is not confined solely to substances with the ReO_3 arrangement of atoms—these are comparatively rare in oxide systems. Compounds more commonly contain fragments of this particular structure present only as blocks of limited size, and the family of phases centred around Nb_2O_5 affords

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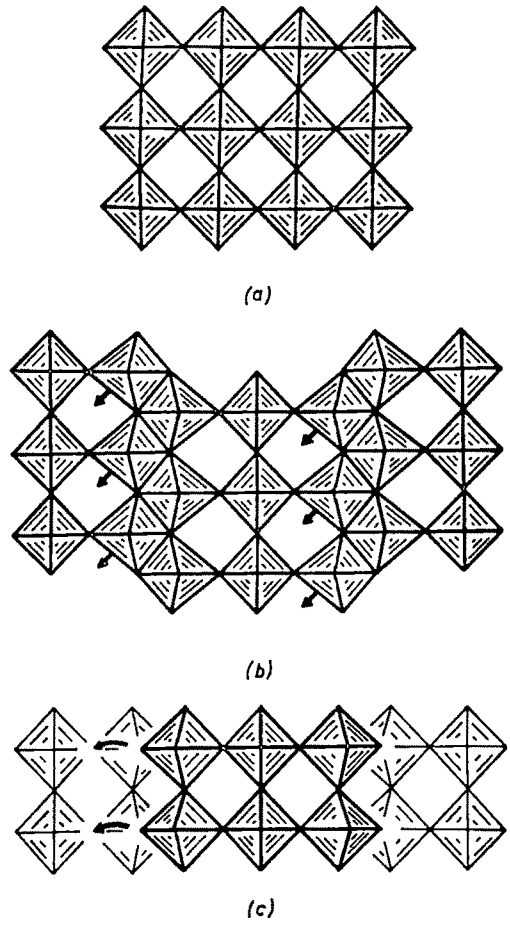


Fig. 1. *a*, Structure of NbO_2F in projection. Each hatched square represents an octahedron, with a niobium and an oxygen atom at the centre, and oxygens (or fluorine) at the corners. *b* and *c*, Two projected views of Nb_2O_5F (non-idealized) showing the relationship to NbO_2F. The shear planes are denoted by the octahedra sharing edges. The migrating niobium and oxygen (or fluorine) atoms move from the octahedra in the directions of the arrows to the interstitial positions (empty squares), which are consequently transformed into octahedra

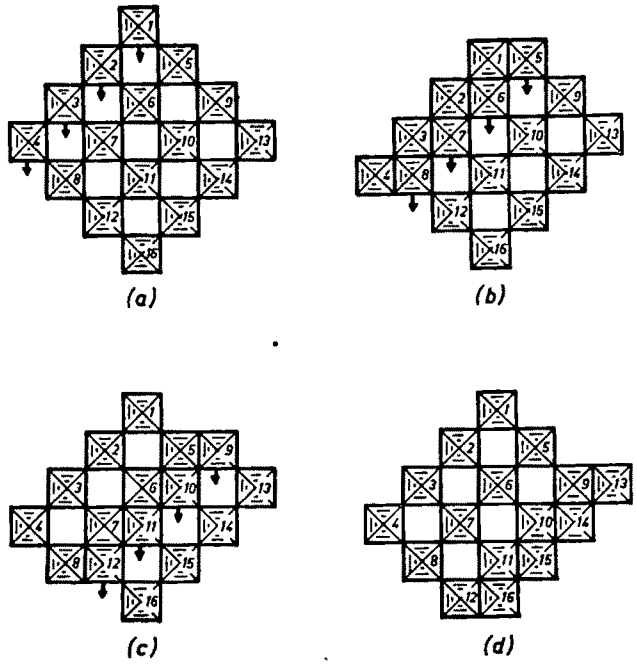


Fig. 2. *a–d*, The formation of a shear plane in NbO_2F and its subsequent migration. Note the resemblance to zone refinement of impure crystals

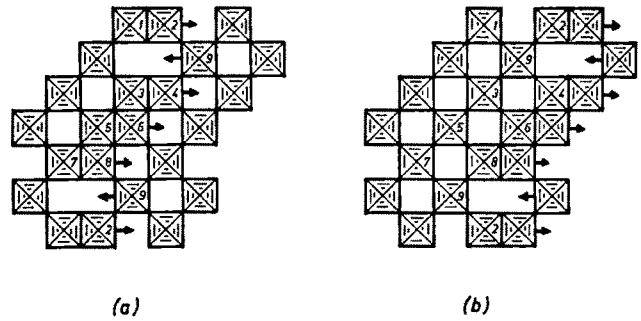


Fig. 3. *a* and *b*, The movement of shear planes in $W_{20}O_{58}$, formed by the reduction of WO_3

many examples of this type. Each oxide containing Nb^{5+} with a substituent metal of higher or lower valency, or with F^- replacing O^{2-}, is distinguished from the others in organization as well as in the size of the blocks[2,3]. Each is the ReO_3 structure containing two parallel but criss-crossing sets of shear planes forming the boundaries of the blocks, and tetrahedrally co-ordinated atoms are often present at their intersections. The tetrahedral atoms do not represent impenetrable barriers to the diffusion of metal or oxygen, as they can move unrestrained to any of four equally probable octahedral positions forming the immediate environment[10]. Thus, atoms are unaccompanied by migrating oxygens, and diffusion now involves the movement of one non-metal for every two metal ions.

Fig. 4*a* represents the structure of high-temperature Nb_2O_5 (ref. 10); and Fig. 4*b*, $TiNb_{24}O_{62}$ or $Nb_{25}O_{62}$ (ref. 2). The tetrahedral atom 7 in the former moves to an octahedral position in the latter, octahedral Nb 10 to a tetrahedral site, while the niobium and oxygen atoms 8 and 9 move to adjoining positions of the same kind as those they previously occupied. The movements of atoms 10, 11 and 12 in Fig. 4*b* to octahedral sites and of 13 to tetrahedral give the structure of $Nb_{11}O_{27}$ (ref. 10). The re-oxidation at low temperatures of $Nb_{11}O_{27}$ and $Nb_{25}O_{62}$ to new forms of Nb_2O_5 involves little change in the X-ray powder patterns[11]. Evidently the shear planes do not move, and the gross structure remains intact. But the tetrahedral atoms are free to migrate in the *b* directions of both phases (out of the plane of the paper) by way of the alternate filled and empty tetrahedral positions present in these structures[2,10], and form a film of new oxide on reaching the surface. When the temperature of oxidation is raised, the oxygen atoms, and therefore the shear planes, move and follow the reverse paths of Figs. 4*a* and *b*, giving high-temperature Nb_2O_5 as the end-product.

It is noteworthy that the many perovskite compounds ABO_3, where BO_3 is the ReO_3-type host structure containing *A* ions in the holes, do not form related shear structures. We have repeatedly, and without success, attempted to synthesize 'defect' perovskites of this kind, and evidently diffusion cannot occur when the holes are filled. Unless the structure is deficient in *A*, there is no way for the shear planes to form, and unless these ions are largely absent, there is no way for the planes to propagate, unless they too move and are lost, either as a volatile compound or as a new foreign solid phase. Authentic anion-deficient perovskites are unknown and alternative structures form instead[12–14].

Shear planes are also present in the Ti_nO_{2n-1} phases. Here the terminal octahedra of adjacent blocks of rutile (TiO_2) structure share faces[15], and not edges and corners (Fig. 5). The unreduced rutile structure contains interstitial octahedral positions, and in the reduced phases these are filled by some of the metal atoms belonging to the adjacent block[1,15]. A diffusion mechanism for the formation of the planes by the reduction of rutile involves the transport of planes of metal ions into the

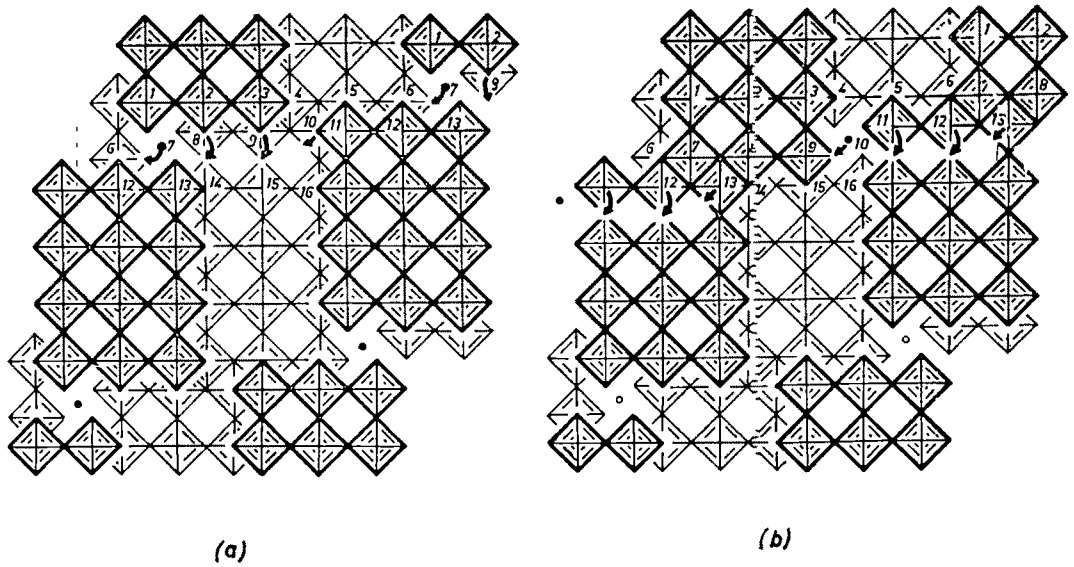


Fig. 4 *a* and *b*, Formation of $Nb_{22}O_{62}$ and $Nb_{12}O_{37}$ from Nb_2O_5 by the movement of a shear plane. *a*, Structure of high temperature Nb_2O_5. *b*, Structure of $Nb_{22}O_{62}$. The small black circles numbered 7 in *a* are tetrahedral metal ions which move (arrows) and form octahedra as in *b*. Niobium 10 moves from octahedral co-ordination in *a* to tetrahedral in *b*. The additional migrations indicated in *b* result in the structure of $Nb_{12}O_{37}$. Niobium 13 becomes tetrahedral in this latter structure and niobium 10 changes from tetrahedral to octahedral

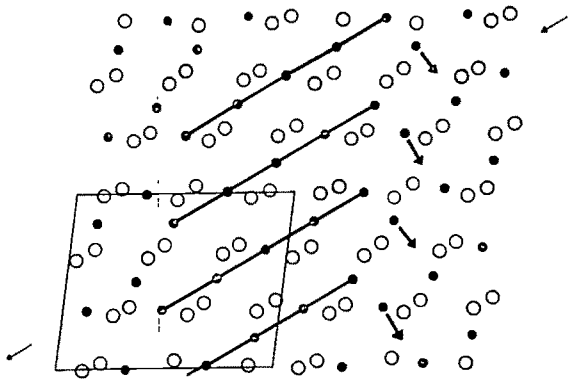


Fig. 5. Structure of Ti_5O_9. ●, Titanium; O, oxygen. The five titanium atoms joined by a thick line constitute a block of the rutile (TiO_2) structure, and the terminal octahedra share faces with those in the next block. The movement of the titanium atoms indicated by the arrows results in a block six titanium atoms long, which is characteristic of the next phase Ti_6O_{11} in the series Ti_nO_{2n-1}. The oxygens remain intact

next row of empty positions, each metal ion moving through a triangle of oxygens (an octahedral face) (Fig. 5) and no oxygen migration is possible. By close control of conditions, the oxidation of Ti_4O_7 ($n = 4$ of the series Ti_{2n-1}) to TiO_2 should result in the formation of each higher homologue ($n = 5$, 6, 7, etc.) in turn, and the inference that the shear planes have a high mobility in the solid is supported by the remarkable ciné films of reduced titanium dioxide, made by Eikum and Smallman in the electron microscope[16].

It is becoming increasingly evident that shear planes are a common feature in the higher oxides of the metals of Groups IV, V and VI. We believe that the properties of the phases in which they occur may well depend on their nature and mobility.

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Elachistosuchus, A TRIASSIC RHYNCHOCEPHALIAN FROM GERMANY

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Elachistosuchus huenei, a very small reptile with a skull some 26 mm long, from the late Keuper of Halberstadt near Magdeburg, was allocated to the Pseudosuchia by Janensch[1], on the grounds that it possesses a preorbital opening in a diapsid skull and thoracic vertebrae with "spectacle-shaped" rib articulations—this in spite of the acrodont dentition, large post-temporal fossae and lack of a mandibular fenestra. *Elachistosuchus* has been made the sole representative of a family and superfamily of the Pseudosuchia by Hoffstetter[2] and von Huene[3], the latter regarding it as a modified descendant of the Lower Triassic Proterosuchidae. It will be shown, however, that *Elachistosuchus* can be interpreted with greater probability as a rhynchocephalian, closely allied to the living *Sphenodon*.

Obviously the most important point to be established is the nature of the "preorbital opening". In *Elachistosuchus* (Fig. 1) a substantial part of the maxilla lies

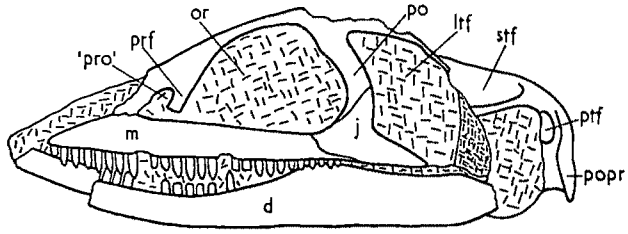


Fig. 1. *Elachistosuchus huenei* Janensch Left side of skull, × *c.* 2, after Janensch[1]. The posterior portion of the skull has been pushed downward and backward; the more heavily hatched area is a stain on the matrix representing the rear border of the lateral temporal fossa. Abbreviations for this and succeeding figures as in Romer[5], with the addition of *ltf:* lateral temporal fossa; *stf:* supratemporal fossa; *'pro':* preorbital opening of Janensch, herein identified as the lachrymal foramen

below the large orbit, and the bone ends posteriorly below the postorbital bar. This is quite unlike the situation in any pseudosuchian. Even in *Chasmatosaurus*[4], to which (among others) *Elachistosuchus* is compared by Janensch because of the acrodont dentition, the greater proportion of the maxilla lies below and in front of the preorbital fossa, this being the usual condition in the Pseudosuchia. In fact, the orbit of *Elachistosuchus* is rather like a pseudosuchian preorbital fossa, especially in the contribution made by the jugal, and I at first attempted a re-interpretation along these lines, but the arrangement of the more posterior openings in the skull makes such an explanation impossible.

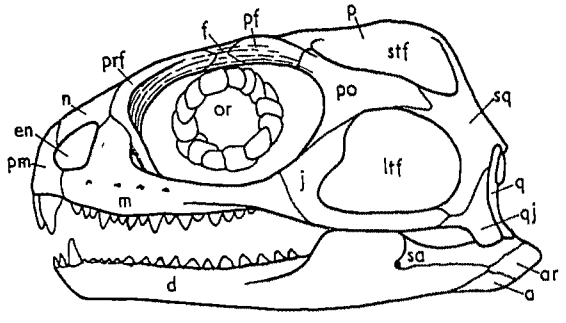


Fig. 2. *Sphenodon.* Left lateral view of skull, simplified from Romer[5], × *c.* 1

It is suggested that the key to the understanding of the skull of *Elachistosuchus* is supplied by the curious nature of the prefrontal in *Sphenodon*[5]. In this animal (Fig. 2) the lachrymal is missing and the prefrontal descends to the palate, passing medially within the anterior wall of the orbit and touching the palatine. In its lower course it encloses a large foramen ("canalis maxillare"[6] or lachrymal canal) between it and the ascending process of the maxilla. This arrangement is not homologous with the archosaurian preorbital fossa, the latter being bounded by the maxilla and lachrymal, with the jugal forming only a small part at the posterior inferior corner, and the prefrontal not entering at all. It is virtually certain that the so-called "preorbital fossa" of *Elachistosuchus* is really the lachrymal canal, since its relationships are practically identical with those in *Sphenodon*: a descending process from the skull roof at the position of the prefrontal forms its posterior border, and it is bounded in front by the ascending process of the maxilla (Fig. 3). Janensch's assumption that the right side shows this aperture more accurately is probably incorrect, since the left side of the skull is less distorted, the right having been pushed backward, according to that author. Thus it seems to have been a much smaller opening than is the case in most preorbital fossae, though nevertheless apparently larger than the lachrymal foramen in living *Sphenodon*. However, a posteroventral compression of the anterior part of the skull roof might account for the enlargement of the foramen and the difference in attitude of the prefrontal as compared with that of *Sphenodon*.

In *Sphenodon* again the jugal has a long anterior process lying in the floor of the orbit, concealed by the maxilla in side view, and reaching to within 2 mm of the prefrontal. Exactly such an arrangement occurs on the right side of the skull of *Elachistosuchus* (Fig. 3), but an anterior process of this type does not occur in Pseudosuchia, nor probably in any archosaur. The sutures between the jugal, maxilla and postorbital are, in fact, closely similar in *Sphenodon* and *Elachistosuchus*.

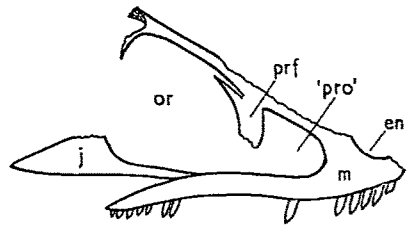


Fig 3. *Elachistosuchus.* Right side of facial part of skull, after Janensch, × *c.* 2

Other features which are anomalous for a pseudosuchian, but apt for a rhynchocephalian, may be dealt with briefly. These are: (*a*) the numerous acrodont, isodont teeth; (*b*) the very large post-temporal fossae and narrow supraoccipital (Fig. 4); (*c*) lack of a mandibular fossa (unusual in an Upper Triassic pseudosuchian); (*d*) low position of the paroccipital process, well below the upper temporal arch; (*e*) weak coronoid process (although stated by Janensch to be absent); (*f*) presence of a notch for the external naris at the anterior margin of the maxilla (Fig. 3)—in pseudosuchians the naris is either further forward or the maxilla curves smoothly upwards. Janensch (ref. 1, p. 227) also refers to a "bent down" fragment of the upper jaw at the anterior tip of the skull on the right side, which he suggests may represent the premaxilla. This would seem highly likely to be a rhynchocephalian "beak". The relatively short humerus with its strongly expanded extremities set almost at right angles to each other recalls the primitive "twist" retained in *Sphenodon*; the entepicondyle is very well developed. The lack of dermal ossifications is not conclusive, since they are not ubiquitous in the Pseudosuchia.

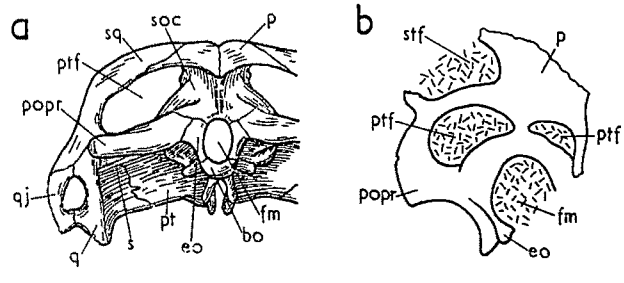


Fig. 4. Occipital views of *a Sphenodon*, after Romer, × *c.* 1; *b: Elachistosuchus*, after Janensch, × *c.* 2

There remain to be considered the vertebrae and their articulations with the ribs. Intercentra are present throughout the presacral series in *Sphenodon*, and though absent in the preserved vertebrae of *Elachistosuchus*,

this is not a bar to the affinities suggested here, since *Homoeosaurus*, a sphenodontid from the Upper Jurassic[2], lacks intercentra in the trunk region, and so probably does *Brachyrhinodon*[7] from the Upper Trias of Elgin, Scotland. The elongated, distinctly curved "cervical" ribs described by Janensch are more probably thoracic ribs, and his "block II" is thought more likely to succeed, rather than precede, "block III" because of the nature of the rib articulations; it does not necessarily follow that the vertebrae increase in size towards the sacrum, as they normally do in the Pseudosuchia, hence the preserved vertebrae probably include only the more posterior cervicals. The double rib-facets of *Elachistosuchus*, furthermore, are not strictly comparable to the "spectacle-shaped" articular areas seen in some pseudosuchians[8], or in *Ornithosuchus*[9], for when in the posterior thoracics the rib-facets become confluent (due to the gradual reduction in length of the diapophyses), they also come to lie almost on the same level, whereas in *Elachistosuchus* the parapophysis is distinctly below the diapophysis, although the two processes are virtually the same length. The most complete vertebra illustrated by Janensch (ref. 1, Figs. 6–8), which is probably the most anterior preserved, has, in fact, rib-facets which are very like those of the eighth cervical vertebra of *Sphenodon* (ref. 6, Fig. 19).

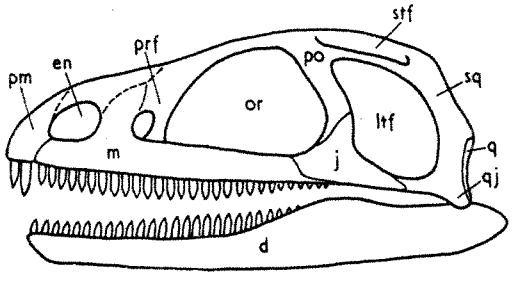


Fig. 5. Suggested restoration of the skull of *Elachistosuchus*, × *c.* 2. On the whole the restoration by Janensch[1] has been followed, but the quadrate and snout regions have been modified; no attempt has been made to correct for possible distortion of the prefrontal. The jaw has been moved back slightly on the ground that the coronoid process should pass up behind the lower temporal bar; this also gives a better fit with the premaxilla

Inasmuch as the lower temporal bar is not demonstrably complete in *Elachistosuchus*, due to the loss of the quadrato-jugal in the fossil, the possibility of lacertilian relationships cannot be excluded. In lizards also the prefrontal descends to the palatine, the lachrymal may be lost (although this is a relatively modern specialization; *Kuehneosaurus*[10], from the Upper Keuper, for example, has a large lachrymal), the post-temporal fossae are frequently large, and the jugal may have a long anterior process below the orbit (but visible in this case from the side), see *Iguana*, for example. The large number (twenty-eight) of small cylindrical teeth in *Elachistosuchus* is also rather lizard-like. On the other hand there is no reason to doubt that the lower arcade was originally complete, especially as the missing portion is extremely short. The acrodont implantation is suggestive, but not conclusive, of rhynchocephalian affinities. In particular the arrangement of the prefrontal, maxilla and jugal with respect to the orbit is extremely like that of *Sphenodon* and, in detail, unlike any lizard; the occiput is closer to that of *Sphenodon*, even the form of the foramen magnum (Fig. 4) being quite similar. When to these points is added the bicipital nature of the posterior cervical and/or anterior dorsal ribs, the form of the humerus and the possibility of a "beak", little doubt can remain of the rhynchocephalian nature of *Elachistosuchus*.

The superfamily Elachistosuchoidea may thus, with some satisfaction, be discarded, for *Elachistosuchus* may certainly be placed within the Sphenodontidae. It displays features of the skull and vertebrae so far undescribed or imperfectly revealed in the few Triassic genera which are available. These include the ventral extension of the prefrontal, the probable loss of the lachrymal, the relationships of the jugal with neighbouring bones, and the construction of the occiput. These details have survived with virtually no change since Upper Triassic times and add further emphasis to the already well known conservatism of the living *Sphenodon*.

Von Huene[3] divided the Sphenodontidae into the subfamilies Brachyrhinodontinae (Triassic), Monjurosuchinae (Jurassic) and Sphenodontinae (Jurassic to Recent), placing *Homoeosaurus*, however, as the type of a separate family. *Elachistosuchus* differs from *Sphenodon* and resembles *Homoeosaurus*[11], *Polysphenodon* from the Middle Keuper near Hannover[12], and *Brachyrhinodon* in that the parietals form a flat surface between the supratemporal fossae, their breadth here equalling or exceeding that between the orbits. It appears, therefore, that the narrow parietal crest of the living form is, as one might expect, a relatively modern specialization. The lachrymal had probably been lost in *Polysphenodon*, and perhaps also in *Brachyrhinodon*. My present work indicates that these two are extremely closely related, if not actually congeneric; von Huene[3] paradoxically reversed his previous opinion[2] and placed *Polysphenodon* in the Rhynchosauridae, in spite of its paired, terminal external nares and other differences. *Elachistosuchus* differs from these two genera in the more posterior position of the orbit, the less flattened and more lightly built skull, the more numerous teeth, and the lack of a marked coronoid process. Other Triassic genera are too imperfectly known at present for profitable comparison, the teeth of *Clevosaurus*[13], and *Palaeorodon*[14], evidently being very different. Although lacking intercentra in the thoracic region, *Elachistosuchus* is considered to be best placed in the subfamily Sphenodontinae, because of the close resemblance of the skull to that of *Sphenodon* itself. Janensch[1] regarded the dentition as being of an unspecialized type suitable for dealing with "small animals, arthropods, worms, etc." and also plant food. The relatively broad area between the supratemporal fossae, and the weak coronoid process, may reasonably be viewed as primitive features retained from a late Permian or early Triassic eosuchian ancestor in connexion with a likewise unspecialized jaw musculature. Hence *Elachistosuchus* presumably represents an extremely primitive rhynchocephalian stock, while the approximately contemporaneous Brachyrhinodontinae are a slightly more specialized side branch. Furthermore, if, as is possible, the premaxillary teeth of *Elachistosuchus* were similar to those of the maxilla (that is, it lacked the typical rhynchocephalian development) and, like *Polysphenodon*[15] and *Brachyrhinodon*, there were rows of teeth on the pterygoid as well as on the palatine, then we would have here a form structurally close to the common ancestry of Rhynchocephalia and Squamata.

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KINETICS AND MECHANISM OF ALBUMIN DENATURATION

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AND

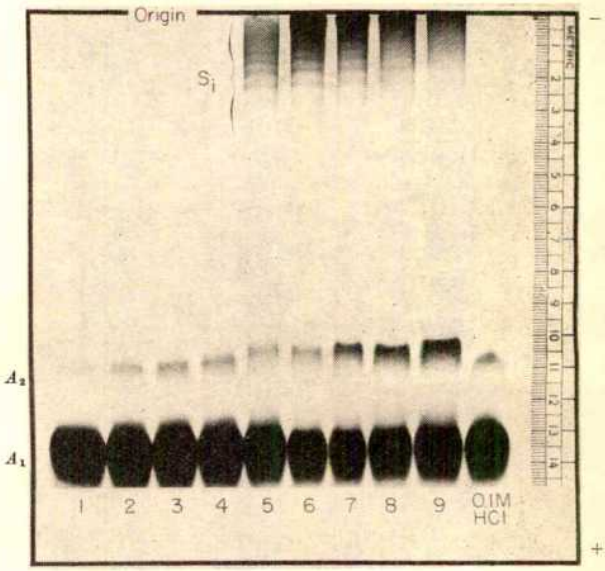
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THE ability of acrylamide gel zone electrophoresis to resolve protein sub-species which differ only in respect of charge, molecular weight and, possibly, conformation[1], coupled with the ability to quantitate the resultant patterns with a specially designed densitometer[2], prompted us to investigate the denaturation of bovine serum albumin (BSA) by urea. The objective was to investigate the kinetics of the denaturation process, and then to relate the various mechanisms to the structural determinants which define the geometry of the macromolecule in a given medium.

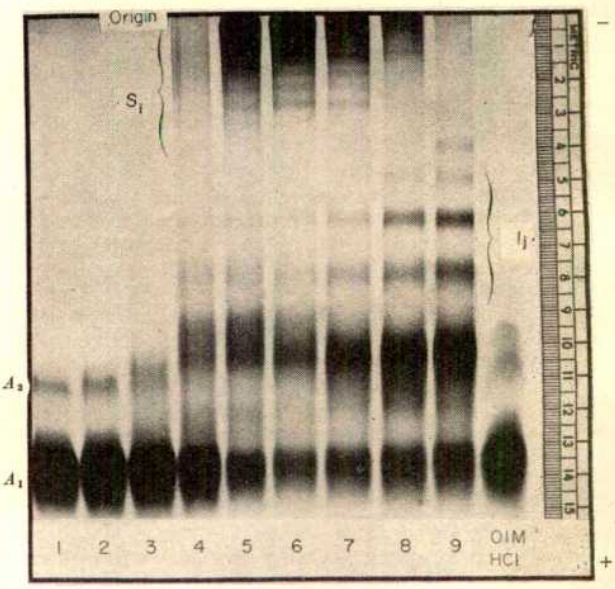
The denaturation procedure was conventional, using 10 mg of BSA (Armour and Co.) dissolved in 0·25 ml. of urea solution prepared from recrystallized urea (Mallinkrodt)[3]. Acrylamide gel zone electrophoresis was carried out on a vertical electrophoresis cell (Model 500, Arden Instruments, Inc., Rockville, Maryland) capable of the simultaneous analysis of thirty samples, using the gel slab technique. Sample volumes ranged from 10–50 μl. The supporting medium was 5 per cent 'Cyanogum 41' (American Cyanamid Co.) prepared with a *tris*-EDTA-boric acid buffer, *p*H 8·3, which also served as the electrolyte for electrophoresis[4]. Reversibility experiments involved the addition of 2 volumes of water to the denaturation system to stop the reaction. The urea was then removed by thin-film dialysis for 24 h against several changes of water. Evidence that the electrophoretic patterns produced by the denatured BSA were not artefacts was supported by the reproducibility of the results obtained using either the standard buffer or the buffer containing 3 M urea as the electrolyte for electrophoresis.

The effect of varying time and urea concentration is typified by the following systems in which BSA was exposed to 1–9 M urea at room temperature for 4 min and for 24 h before electrophoresis (see Figs. 1–4). A fast reaction occurs within 4 min at urea concentrations greater than or equal to 5 M, which produces a series of components, S_i, characterized by electrophoretic mobilities ranging from 0–30 per cent of that of the fastest component, A_1 (Fig. 1). The formation of the S_i fractions is associated with a concomitant decrease in the A_1 component (Fig. 3). With an increase in time and urea concentration there is a reduction in the S_1 fractions and the appearance of a new set of fractions, I_j, with mobilities intermediate to the A_1 and S_1 components (Figs. 2 and 4). The predominant species present in the system containing BSA exposed to 9 M urea for 24 h is a fraction with a mobility almost identical to A_2, a constituent found in the BSA before urea denaturation. Reversibility experiments using BSA exposed to 8 M urea for periods of time extending from 4 min – 24 h showed a linear relationship between the amount of water-insoluble protein produced with the length of urea contact time. During the course of dialysis the S_i fractions initially present disappeared with the appearance of the I_j fractions. The temperature dependence for denaturation was positive. The rate at 37° C was about ten-fold greater than that observed at 4° C. The *p*H rate dependence resembled a skewed



Urea concentration (molar)

Fig. 1. Electrophoretic patterns of 4 per cent BSA exposed to varying concentrations of urea in 0·05 M acetate buffer, *p*H 5·0 for 4 min. Electrophoresis was carried out using 5 per cent acrylamide gel, *p*H 8·3 buffer at 200 V for 4 h



Urea concentration (molar)

Fig. 2. Electrophoretic patterns of 4 per cent BSA exposed to varying concentrations of urea in 0·05 M acetate buffer, *p*H 5·0 for 24 h. Electrophoresis was carried out using 5 per cent acrylamide gel, *p*H 8·3 buffer at 200 V for 4 h

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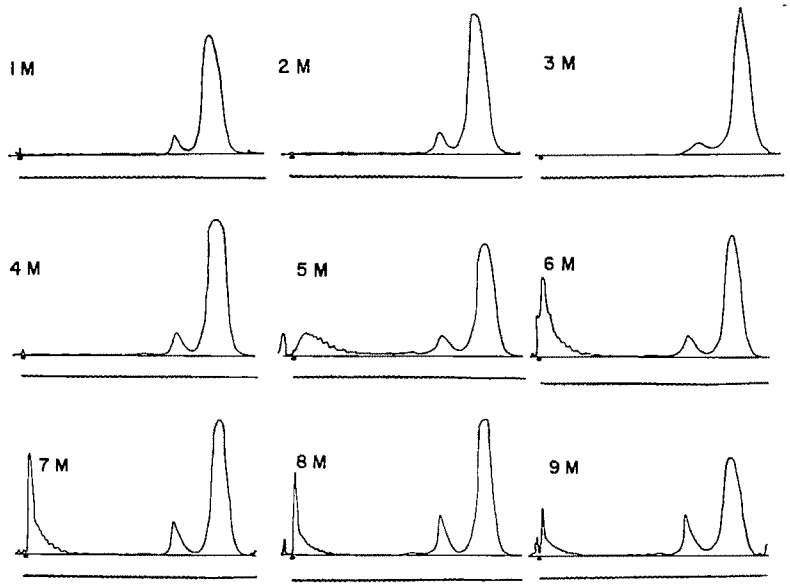


Fig. 3. Densitometer scans of the electrophoretic patterns shown in Fig. 1. The irregular line below each pattern is the integration pen tracing

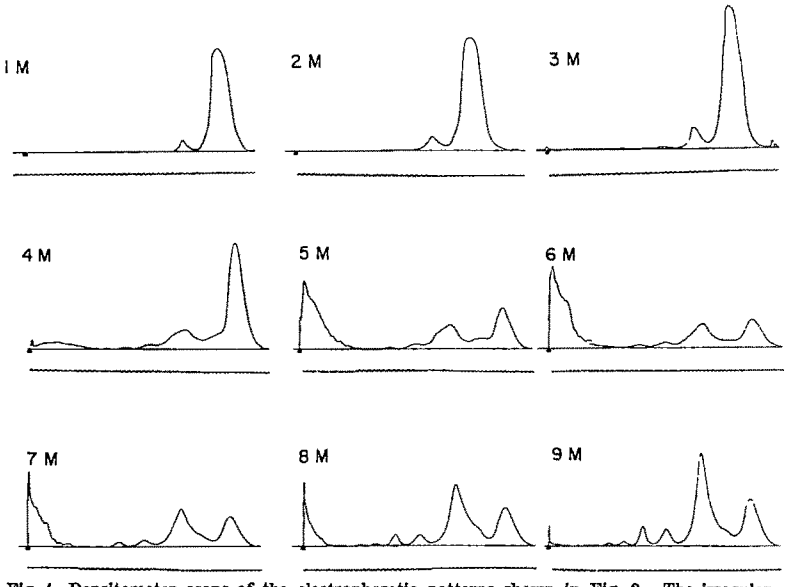


Fig. 4. Densitometer scans of the electrophoretic patterns shown in Fig. 2. The irregular line below each pattern is the integration pen tracing

geometric function with the maximal rate in the alkaline region; at *p*H 1·7 the rate was about five times as great as that found at neutrality, while at *p*H 10 it was greater by at least two orders of magnitude. The products produced by these systems were electrophoretically indistinguishable from each other. This was in contrast to the acid or alkali denatured BSA (exposed to 0·1 M reagents) the patterns of which differed in a characteristic way from each other and from the urea-denatured albumin.

A detailed discussion of the kinetics and reaction mechanisms will not be attempted, but a few of the salient features will be mentioned. Urea denaturation is initiated by a fast reaction involving conformational changes with the appearance of the S_f fractions. This is followed by a decrease in the S_f fractions which is due either to their conversion to components with zero mobility or to their transformation to the I_f fractions (Figs. 2 and 4). There is evidence that certain of the I_f fractions can be produced directly without passage through the intermediate or short-lived S_f state. On the basis of the distinctive electrophoretic patterns and reaction rates it is evident that the mechanisms for urea, acid, alkali and heat denaturation must differ substantially from each other.

The characteristic mobilities observed for the several species of denatured albumin can be attributed to any or a combination of the following factors. The values for the prototropic dissociation constants of the protein can be altered by the change in the macromolecular conformation or by the increase of the dielectric constant of the system on the addition of urea[6], thus producing a shift in the net charge on the protein and in its affinity for buffer ions. The transformation of albumin from the native globular form to a random configuration with an increased axial ratio results in a commensurate increase of frictional resistance to transport processes and in the subsequent reduction in electrophoretic mobility[6]. Another contributing factor is the slow time-dependent polymerization process that occurs during denaturation and produces substantial changes in both the molecular geometry and charge[7].

We hope to isolate, characterize, and establish the structural features that determine the electrophoretic

properties of the various fractions resolved by this technique.

We thank R. E. Easterling, K. J. Nelson and R. E. Budd for their invaluable technical assistance.

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IONIC COMPLEXES OF THIOUREA

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THIOUREA is one of the most versatile complexing agents yet discovered. It forms the well-known inclusion complexes with branched-chain hydrocarbons[1] and also has the ability to complex with various other organic substances[2] and with some salts of virtually every metal.

This latter property is largely a result of the formation of covalent sulphur-to-metal bonds[3]. However, thiourea has also been reported to form stable complexes with salts of elements which do not form covalent bonds to co-ordinated ligands. Another explanation must be sought for the existence of these complexes.

The prototype of these thiourea complexes is $NH_4Br.4TU$ (TU, thiourea), first described by Reynolds in 1888 [4]. The isostructural series includes the complexes formed with some halides of potassium, rubidium and caesium, with many ammonium and thallium (I) salts and with some lead (II) salts. These are all stoichiometric complexes and, depending on the oxidation number of both cation and anion, they have the general formulae:

$$MX.4\,TU$$
$$M_2X.8\,TU$$
$$MX_2.6\,TU$$

The nature of this family of complexes has now been established by a correlated chemical and crystallographic investigation. The preparations reported in the literature have been repeated and checked and some new complexes have been prepared. All the complexes have been examined by single-crystal or powder X-ray diffraction techniques and the unit-cell dimensions, space group and density (of single crystals only) determined. This has enabled some thirty-odd related complexes to be further subdivided into groups of isomorphous or nearly isomorphous structures. Representative complexes from each group were then chosen for structural analysis, thus enabling the relationship between the groups to be established. Some measure of disorder appears to be a feature of many of the structures at room temperature. While this has prevented determination of all the details of the structures, their main outlines have been settled. A broad survey and general description of the results obtained will be given in this article, to be followed by a series of more detailed papers now in preparation.

Classification of the Complexes

Morphologically the solid complexes are usually fibrous masses and only in some cases could single crystals suitable for crystallographic work be obtained. Well-defined X-ray powder patterns have, however, always been obtained.

The unit-cell dimensions and space groups show that the complexes fall into a number of well-defined groups. The largest of these includes the 1 : 4 complexes of alkali bromides and iodides, thallium (I) bromide, iodide and perchlorate. These crystals are all tetragonal with $a \sim 20$ Å, $c \sim 8{\cdot}3$ Å and space group $P4/mnc$. Closely related to these is a smaller group of complexes, including the thallium (I) nitrate, and sulphate (1 : 8) complexes, which have $a \sim 10$ Å, $c \sim 8{\cdot}3$ Å, space group $P4/mcc$. A Weissenberg photograph of the $TlNO_3.4$ TU complex at $-160°$ C showed that at this temperature this complex had $a \sim 20$ Å, $c \sim 8{\cdot}3$ Å and space group $P4/mnc$. Thus the smaller unit cell appears to be a partially disordered version of the larger ordered cell; this was established in detail by determining the structures of $TlClO_4.4$ TU and $TlNO_3.4$ TU at room temperature (the super-lattice reflexions of the latter are too weak even at $-160°$ C to allow reliable determination of the differences between the partially disordered and the ordered version of this structure). Two other ordered structures have been obtained. In one of these $a \sim b \sim 14$ Å, $c \sim 8{\cdot}3$ Å, while in the other $a \sim 2b \sim 21$ Å, $c \sim 8{\cdot}2$ Å. These structures, which have not been determined, would be expected to differ in detail only from that of $TlClO_4.4$ TU.

Another group is formed by the complexes of the alkali and thallium (I) chlorides, which have $a \sim 32$ Å, $b \sim 13$ Å, $c \sim 8{\cdot}5$ Å and space group $Bbam$ (this is the orientation most convenient for comparison with the other complexes). These complexes have only been obtained in polycrystalline (fibrous) form but one fluoride complex, $CsF.4$ TU. $2\,H_2O$ has cell dimensions ($a \sim 17{\cdot}0$ Å, $b \sim 12{\cdot}5$ Å, $c \sim 8{\cdot}45$ Å, space group $Ibam$, four formula units per unit cell) which are closely related to those of the chloride complexes. Single crystals of this fluoride complex have only been obtained recently and its structure has not yet been determined.

All the complexes discussed until now have been of thiourea with salts of monovalent metals, but two related complexes of divalent lead salts have also been reported: these are $Pb(NO_3)_2.6$ TU[5] and $Pb(ClO_4)_2.6$ TU[6]. Crystallographic investigations have shown that these two complexes also belong to this general family despite the difference in the ratio of cations to thiourea molecules. Both complexes have partially disordered structures (of different types) at room temperature, but X-ray structure analysis has revealed the general outlines of their structures and in particular has shown how a cation : thiourea ratio of 1 : 6 is possible.

The structural results will now be described in more detail. Some of the structures have been analysed by three-dimensional methods and some by two-dimensional methods; intensities were measured visually from Weissenberg photographs taken with copper $K\alpha$ radiation. The structures were generally solved by Patterson methods and refined by Fourier and least-square techniques. The R-factors are all below 12 per cent and there are no

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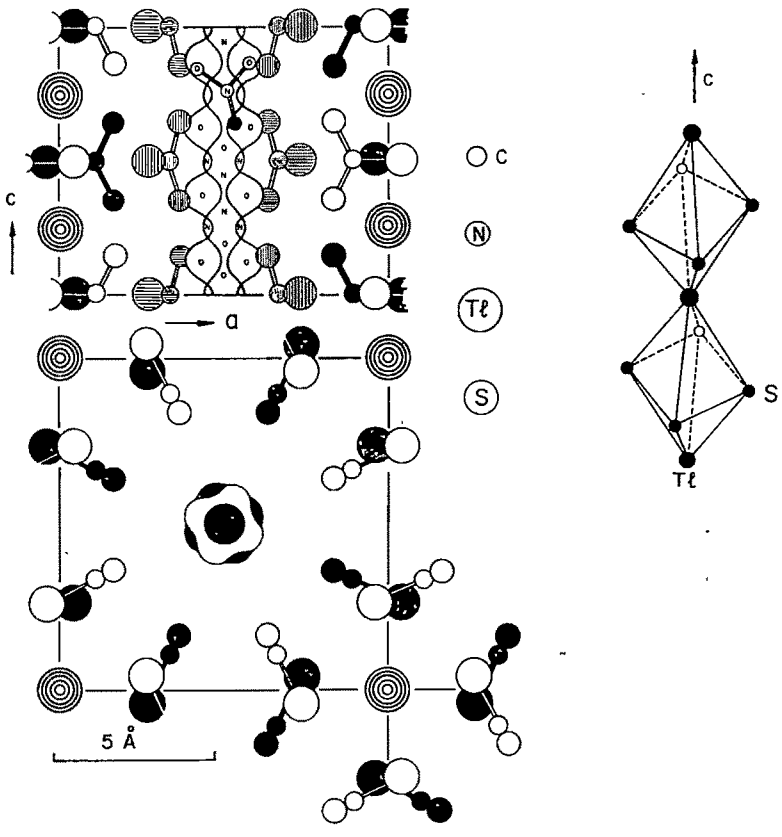


Fig 1 The crystal structure of the $TlNO_3$.4 TU complex shown in projection down [001] and [010]. On the right the arrangement of sulphur atoms and thallium ions in one of the chains along [001] is shown. In the projection down [001] the atoms represented by full circles lie in the plane of the paper while those represented by open circles are in the mirror plane lying $c/2$ above or below the plane of the paper. In the [010] projection the full-circle molecules lie about $a/4$ below the plane of the paper while those with open circles lie the same distance above it. The horizontally shaded molecules are about $a/2$ below the plane of the paper and the vertically shaded molecules the same distance above it

outstanding discrepancies between observed and calculated structure factors.

Structure of $TlNO_3$.4 TU

These crystals have $a = 10{\cdot}34$ Å, $c = 8{\cdot}29$ Å, space group $P4/mcc$, two formula units per unit cell. The results of the crystal-structure analysis are summarized in Fig. 1. The essential feature of this structure type, and the family as a whole, is the existence of separated cations and anions bridged by polar thiourea molecules. Each cation is surrounded by eight sulphur atoms at the corners of a distorted cube centring on the cation site (Fig. 1, right). The average thallium–sulphur distance is 3·43 Å, which is considerably longer than the separation between sulphur atoms of co-ordinated ligands and metal atoms. Indeed, the sum of the atomic radii of thallium and sulphur is about 2·7 Å whereas the sum of their ionic radii is about 3·3 Å. Thus it is clear that these are ionic complexes and that the major cohesive interactions in the crystals are ion-dipole interactions. The arrangement shown in Fig. 1 can be described as a linear chain of metal ions surrounded by neutral but polar thiourea molecules with the sulphur atoms of a set of thiourea molecules bridging successive cations. The separation between neighbouring cations in a chain is 0·5 c, or approximately 4·1 Å.

The thiourea molecules point away from the arrays of cations situated along the four [001] edges of the unit cell and in this way they enclose a cylindrical channel with its axis along $[\frac{1}{2}\frac{1}{2}z]$. The anions are in these channels and in an idealized structure would lie along the cylinder axis which is also a crystallographic four-fold axis. In the crystals that have been investigated the anions have lower symmetry and hence are disordered positionally. This type of structure is not directly comparable with the channel structures of the thiourea–hydrocarbon complexes since the thiourea molecules are not connected through S...H—N hydrogen bonds.

The structures of a number of crystals are known in which similar, approximately cubic arrangements of sulphur atoms about potassium and ammonium ions are found (for review see Wells[7]), while another close comparison can be made with the arrangement in thallosothallic sulphide, $Tl_2^{III}S_3.Tl_2^{I}S$ (refs. 8, 9, also ref. 7, pp. 900–901). The thallium (III) atoms in this structure are linked by sulphur atoms ($d(S\text{-}Tl^{III}) \sim 2{\cdot}6$ Å) to form chains of tetrahedra sharing opposite edges, while the thallium (I) atoms lie between the chains with eight sulphurs around them ($d(S\text{-}Tl^{I}) \sim 3{\cdot}3$ Å). The interactions between the sulphur and thallium (I) atoms appear to be ionic interactions somewhat similar to those found in the present family of complexes (however, ion-dipole forces are unlikely to be important in TlS).

Ordered Structure as found in $TlClO_4$.4 TU

These crystals have $a = 21{\cdot}07$ Å, $c = 8{\cdot}28$ Å, space group $P4/mnc$, eight formula units per unit cell. Their structure, which is characteristic of the majority of these complexes, differs from the $TlNO_3$.4 TU structure in that two neighbouring cation–thiourea chains are not crystallographically equivalent but are rotated to a small extent relative to one another and have slightly different ($\sim 0{\cdot}05$ Å) thallium positions; the doubling of the a axis is a consequence of these differences. The space group no longer requires the anions to lie on the axis of the cylindrical channel.

The structural details of the $TlClO_4$ and $TlNO_3$ complexes provide the key to most of the general crystallographic properties of the ionic thiourea complexes. In particular an explanation is provided for the fact that most of the complexes crystallize with a unit cell having $a \sim 20$ Å while the strongest reflexions define a sub-cell with $a \sim 10$ Å. If only the sub-cell is considered, both the cations and anions will be arranged in a linear chain with periodicity $c/2$. This is possible only if the distances between neighbouring cations and neighbouring anions are fortuitously the same. In the general case, therefore, the anionic arrangement is non-linear. This causes slight distortion of the cylindrical anionic channel of the sub-cell and the appearance of the superstructure. In the nitrate complex the deviation of the anionic chain from linearity is either masked by rotational disorder or else is so slight that it is beyond the limits of detection, but in the $TlClO_4$.4 TU structure the perchlorate tetrahedra are in an ordered array and displaced off the cylinder axis.

Complexes of Divalent Metal Salts

The complexes of this family formed by the salts of divalent metals (lead is the only known example) are essentially isostructural with the complexes of monovalent metal salts. The 1 : 6 ratio encountered in these complexes can arise in one of two ways, exemplified by the structures of the $Pb(NO_3)_2$ and $Pb(ClO_4)_2$ complexes.

Structure of the $Pb(NO_3)_2$.6 TU Complex

This complex caused some controversy in the past. It was described first by Rosenheim and Meyer[5], who assigned to it the formula $2Pb(NO_3)_2$.11 TU. The analytical work of Mahr and Ohle[6] supported the 2 : 11 ratio, but Brú *et al.*[10] concluded from crystallographic considerations that the most probable formula was 6 $Pb(NO_3)_2$.32 TU. However, the experimental crystal density of 2·03 g/cm³ is in good agreement with the 1 : 6 ratio deduced by Haworth and Mann[11] from their analytical results, but not with the densities calculated on the basis of the other proposed compositions. We have therefore used the 1 : 6 formulation. It should be emphasized that the crystal structure at room temperature is such that the $Pb(NO_3)_2$: thiourea ratio is not determined by space group considerations (in contrast, for example, to the 1 : 4 ratio in the $TlNO_3$ complex) and is not necessarily rational. The value used is an experimental value derived from chemical analyses and density measurements.

The complex has the space group *P4/mcc* characteristic of the $TlNO_3$.4 TU structure type. It has also $a = 10{\cdot}3$ Å, but $c = 24{\cdot}6$ Å, which is three times the *c*-axis of the other structures. There are four formula units per unit cell. Oscillation photographs about [001] show additional diffuse layer lines which indicate that there is considerable disorder in the structure. This has not been explained in detail. The electron-density projection down [001] is very similar to that obtained for $TlNO_3$.4 TU and this shows that the atomic arrangement must be essentially the same in both complexes. The suggestion of Brú[12] that this structure consists of an ordered framework of thiourea molecules which has tubular cavities in which lead nitrate molecules are contained is not correct.

Structure of the $Pb(ClO_4)_2$.6 TU Complexes

The $Pb(ClO_4)_2$.6 TU crystals have $a = 18{\cdot}6$ Å, $c = 7{\cdot}92$ Å, space group *P4/mcc*, four formula units per unit cell. The structure is based on that of the $TlClO_4$.4 TU complex, there being similar cation–thiourea chains along the cell edges and the central axis of the cell. The anion arrangements are also very similar. However, the cation sites along $[0\frac{1}{2}z]$ and $[\frac{1}{2}0z]$ are entirely vacant and these regions of the crystal contain half as many thiourea molecules as are in a cation–thiourea chain.

Water molecules can diffuse into these relatively open channels to give the hydrated complex $Pb(ClO_4)_2$.6 TU. 2 H_2O. The cell dimensions remain essentially unchanged when water is taken up in the structure, but the space group changes from *P4/mcc* to *I4/mcm*. A Fourier projection down [001] shows that the water and thiourea molecules in the regions about the empty channels are unresolved and it seems probable that what order there was in the positions of these thiourea molecules in the anhydrous crystals has become disrupted by the intruding water molecules.

Comparison of Structures of 1 : 4 and 1 : 6 Complexes

In the $TlNO_3$.4 TU complex there are two nitrate ions in each channel for each repeat distance of 8·2 Å along [001]; these ions do not completely fill the available space. In the $Pb(NO_3)_2$.6 TU complex there are eight nitrate ions in each channel for each repeat distance of 24·6 Å, or $2\frac{2}{3}$ per 8·2 Å. The available space in the channel is now completely filled. The requirements of stoichiometry result in there being four Pb^{++} ions to be distributed among the six cation sites of the one independent cation-molecule chain in each unit cell. Vacant cation sites result, the occurrence of diffuse scattering suggesting that these are not randomly arranged. The alternative of filling all cation sites in one set of chains and one-third of the cation sites in a second, non-equivalent, set is not used in this complex, presumably for both energetic and entropy reasons.

In the $Pb(ClO_4)_2$.6 TU complex quite a different means of compensating for the bivalent nature of the cations is used. The density of packing of perchlorate ions is much the same in the $TlClO_4$ and $Pb(ClO_4)_2$ complexes. In the $TlClO_4$.4 TU structure there are four Tl^+ ions for the two pairs of cation sites in the two crystallographically independent cation–molecule chains in the unit cell, but in a hypothetical $Pb(ClO_4)_2$ complex of the same structure there would be only two Pb^{++} ions for the four cation sites. Instead of half-filling all the sites in both chains, all the sites in one chain are filled and the other chain disappears. In its place one finds two thiourea molecules per unit cell (for the anhydrous complex) or two thiourea molecules and two water molecules (for the hydrated complex). These molecules do not appear to have any structural role in the crystals other than to fill space in the channels that exist in the loosely-bound framework formed by the cation–molecule chains and perchlorate ions. The differences in the two structures therefore stem from the different sizes of the nitrate and perchlorate ions.

Relationship of Other Complexes

The structures of a number of other complexes are known where a polar molecule forms a bridge between separated cations and anions. The greatest resemblance to the ionic thiourea complexes is shown by the ionic complexes $NaI.3(CH_3)_2NCHO$[12], $NaI.3(CH_3)_2CO$[13] and $NaI.3CH_3OH$[14]. These complexes all crystallize in hexagonal crystals and have similar structures. In the acetone complex, for example, there are chains of composition $(Na^+.3CH_3)_2CO$ running up the [00.1] axis. The iodide ions are in ordered positions in the channels between the hexagonally-arranged chains. Within a chain each Na^+ ion is surrounded by an octahedral arrangement of oxygen atoms of the carbonyl group while the methyl groups form the walls of the anion channel.

The approximately cubic and octahedral arrangements of sulphur and oxygen atoms about the cations of their respective complexes are in agreement with previous crystal-chemical results and can be explained in terms of the ratio of the radii of the ions involved (Pauling's[16] first rule). If comparable thiourea complexes of sodium salts can be prepared, then they will be expected to have an octahedral arrangement of sulphurs about sodium and thus will not belong to the present family of thiourea complexes. Similarly, complexes of oxygen-containing molecules with cations other than lithium and sodium will be expected to favour a cubic and not an octahedral arrangement of oxygen about the cations and thus differ from the complexes investigated by Piret *et al.*[15]. The fact that the crystals of the oxygen-containing molecules with sodium iodide favour hexagonal symmetry and those of the present thiourea complexes tetragonal symmetry follows from the respective octahedral and cubic arrangements in their cation–molecule chains.

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ELECTROPHORETIC MOBILITIES OF PEPTIDES ON PAPER AND THEIR USE IN THE DETERMINATION OF AMIDE GROUPS

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A SIMPLE model for the electrophoretic migration of molecules is of a sphere in an infinite medium impelled by electrostatic attraction and retarded by viscosity. It will then reach a terminal velocity, v, given by Stokes's law, and it can be shown (for example, ref. 1) that in this case, if the net electrostatic charge is ε, the radius of the molecule is r, the viscosity of the medium is η and the intensity of the electrostatic field is $-X$, then:

$$v = \frac{\varepsilon X}{6\pi r\eta} \quad (1)$$

The mobility, m, relative to some standard molecule run under identical conditions might then be given by a relation of the form:

$$m = k\varepsilon M^{-1/3} \quad (2)$$

where k is a constant and M is the mass of the molecule, the dimensional equivalent of r^3.

There are several difficulties in applying this model to practical situations. This is so for paper electrophoresis even after it has been noted that a correction is necessary due to the fact that the tortuous path taken by the molecule around the fibres of the paper makes the true potential gradient less than that which would be obtained by dividing the applied voltage by the straight line distance between the electrodes[2]. If, for example, the medium were not to be effectively infinite, a new treatment would be called for. In the limiting case, let it be supposed that the molecules were always very close to the fibres, or that retardation were to occur because the ionic atmosphere, being of opposite charge and solvated, causes a backward flow of solvent in the immediate vicinity of the migrating molecule. The retarding force could then be considered as the result of shear across a small element of liquid close to the migrating molecule. This would then be some function of the effective surface area of the molecule, in which case one might write:

$$m = k'\varepsilon M^{-2/3} \quad (3)$$

as area is dimensionally equivalent to $(\text{mass})^{2/3}$.

A number of important factors are neglected in both these models, in particular the possible contribution of the ionic atmosphere to the effective mass, and factors due to shape. They are simply mentioned as examples of the type of approach which might be adopted in order to rationalize actual experimental results without seeking a complete analysis of this very complex situation. Both types of model are examined with great mathematical rigour by Meuller[3]. Nevertheless, in an attempt to see if either of these crude models gave an answer near to the truth, relative mobilities were calculated for a large number of peptides. The experimental data came partly from records of preparative electrophoreses carried out in the course of sequential analysis of the coat protein of tobacco rattle virus (Offord, to be published), but also from similar and more extensive records obtained over some years by other workers in this laboratory (see final paragraph). These related to the determination of amino-acid sequences in chymotrypsin, elastase, cytochrome b_{551}, insulin from various animals, some Bence-Jones proteins, myosin, and glyceraldehyde phosphate dehydrogenase. The peptides contained only the usual L amino-acids except for the fact that cysteine and cystine had been converted to cysteic acid before running and that methionine was occasionally in the form $MetSO_2$.

The buffer system selected for investigation was pyridine–acetic acid–water 25 : 1 : 225 by volume (ionic strength about 0·07) and the paper, which hung vertically in an apparatus similar to that of Michl[4], was cooled by toluene–pyridine 23 : 2 (v/v). This arrangement corresponds to a pH of about 3·5 at which most groups that can be ionized—except the imidazoyl of histidine—would be expected either to be fully charged or fully discharged.

It was found that all records included reference to the migration of marker substances (usually the free amino-acids). An attempt was therefore made to correct for differences in mobility due to variations in ambient temperature and any other conditions by expressing all results relative to the mobility of free aspartic acid during the same run. Even if, as was occasionally the case, m had first to be expressed in terms of some other marker and then normalized, the values obtained have been shown to be repeatable to about ± 5 per cent over the range of temperatures (10°–40° C) experienced here. The position of the origin did not greatly matter, particularly as any disturbance in the mobility of a fast-moving spot on approaching the electrode troughs was usually matched to some extent by a similar effect on a marker. Endosmosis is quite considerable in this system (1–2 cm toward the cathode after 1 h at 3 kV) and the point of zero mobility was taken to be the mid-point of the free neutral amino-acid spot. Such a precaution is essential as peptides which are clearly neutral have sometimes been described as cationic, and anionic peptides as neutral (for example, ref. 5). The position of the leading edge of a migrating spot is the one best recorded; experiment shows that it is far less affected by overloading than that of the mid-point. Where possible, this was kept in mind when the relative mobilities discussed here were calculated.

Fig. 1 is a log–log plot of m versus molecular weight for all peptides of which details were available with the exception of peptides containing histidine and cysteic acid. The sign of m was ignored. It is seen that a series of straight lines is obtained with a slope of almost exactly $-2/3$. The values of ε assigned to the various lines on this plot are those which the peptides composing them were believed to have on other evidence before this graph was drawn. The slope of the lines and the approximately linear dependence of m on ε when M is held constant correspond to equation (3). It may therefore be that the crude model discussed in connexion with this equation has some elements of truth. Such a plot has a very practical application. The amino-acid compositions of peptides and proteins are obtained after acid hydrolysis sufficiently strong to convert asparagine to aspartic acid and glutamine to glutamic acid respectively. The distinction between these two groups of amino-acids is thus lost although their influence on the biological function of proteins must be very different. Electrophoretic mobility has often been

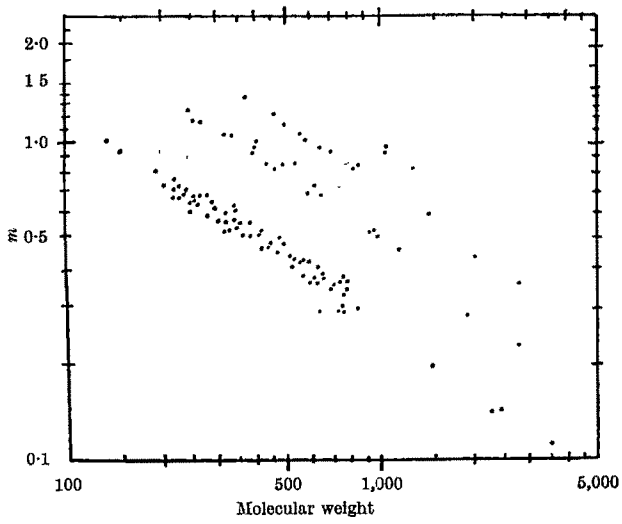


Fig. 1. A logarithmic plot of electrophoretic mobility (at *p*H 6·5, relative to aspartic acid) versus molecular weight for peptides without histidine or cysteic acid. The alignments which are observed correspond (in order of their distance from the molecular weight axis) to $\varepsilon = 1$, 2, 3 and 4

used qualitatively (for example, among many others[6,7]) to distinguish between the two possibilities, but it has been found useful to use Fig. 1 to extend the method to cases where it is necessary to distinguish between, say, a charge excess of one and two. Thus, if a peptide with a composition after hydrolysis of Asp_1Glu_2 has $m = 1{\cdot}05$, the net charge is clearly -2—presumably indicating a terminal carboxyl group, a terminal amino group, two side chain carboxyls free and one amidated. The scatter of the points is such that it may be expected that peptides with ambiguous mobilities may be found, but the lines, at least up to $\varepsilon = \pm 3$, are sufficiently far apart for it to be unlikely that a result could be obtained which would actually be misleading, especially if certain considerations, outlined later, are taken into account.

The plot can also be used either to predict the position of a peptide or in certain circumstances to estimate molecular weight. The spread of points is such as to recommend caution in these cases, especially the latter. While a peptide of composition Asp : Leu : : 1 : 1 and $m = 0{\cdot}65$ is clearly Asp_1Leu_1 and not Asp_2Leu_2 or $Asp_1Asn_1Leu_2$, there are cases where the results are ambiguous. The plot has, however, been found useful as a means of showing up errors in amino-acid analyses and other data.

The reservations referred to relate to the fact that ε is not always integral at *p*H 6·5. The range of *p*K for the imidazoyl side chains of histidine in peptides is about 5·8–7·5 and such peptides are thus unlikely to behave predictably at *p*H 6·5. None is included in Fig. 1, but some idea of the *p*K of this side chain in a particular peptide could presumably be obtained from its position between the lines on the plot.

Suspicions that ε may not be integral are not confined to the case of histidine peptides. The *p*K of the NH_2 group of some peptides may be quite close to 6·5 and it would have been preferable to have collected the data for a slightly lower *p*H, 6·0 for example. Many peptides run poorly at this *p*H as compared with 6·5, however, and the lower *p*H has never been adopted here for the routine preparative work that produced the mobility data. One class of peptides showing a low *p*K effect are certain of those in which basic residues (especially arginine), asparagine, serine and threonine are N-terminal. There appears in these cases to be a difference of at most 10 per cent in the mobility from that which would have been expected. These peptides are included in Fig. 1 and contribute a great deal of the scatter of the points. Anionic peptides of this class have their mobility enhanced and lie above the average line for their nominal net charge, while cationic peptides have their mobility reduced and lie below the line. Nominally neutral peptides of this type have a slight anionic mobility ($m = 0{\cdot}1$). This effect becomes serious at *p*H 7, but Fig. 1 shows that it is still within manageable proportions at *p*H 6·5 for peptides without cysteic acid.

The possession of cysteic acid is observed to cause the peptide to lie nearer the anode, even if this residue is not N-terminal (Fig. 2*a*). This effect is possibly due to the same reason as that already discussed and is seen in Fig. 2*b* to be particularly pronounced in cases where cysteic acid is N-terminal and where there are no other charged side chains. It is possible to devise explanations for the failure to observe such a strong enhancement by N-terminal cysteic acid in the presence of side chain carboxylic groups, but it would be preferable to wait for more data to be collected.

$MetSO_2$ may exert a similar effect to cysteic acid when it is N-terminal. The only peptide in the present collection of data in which this occurs also contains cysteic acid. It shows the enhanced anionic mobility normally charac-

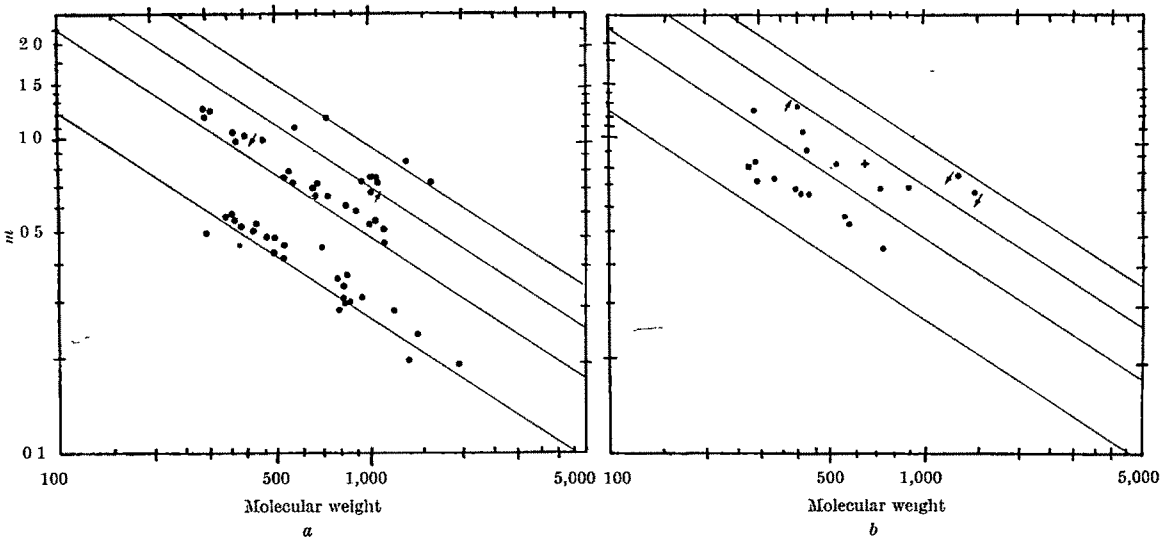


Fig. 2. *a*, A plot, similar to Fig. 1, showing the effect of cysteic acid in positions other than N-terminal (at *p*H 6·5). The diagonal lines indicate the positions of the lines of equal charge in Fig. 1. Certain points have the line on which they should lie indicated by an arrow. *b*, A similar plot showing the effect of cysteic acid in N-terminal positions (at *p*H 6·5). +, 2 cysteic acid residues, one N terminal; ×, 1 cysteic acid residue, $metSO_2$ N-terminal

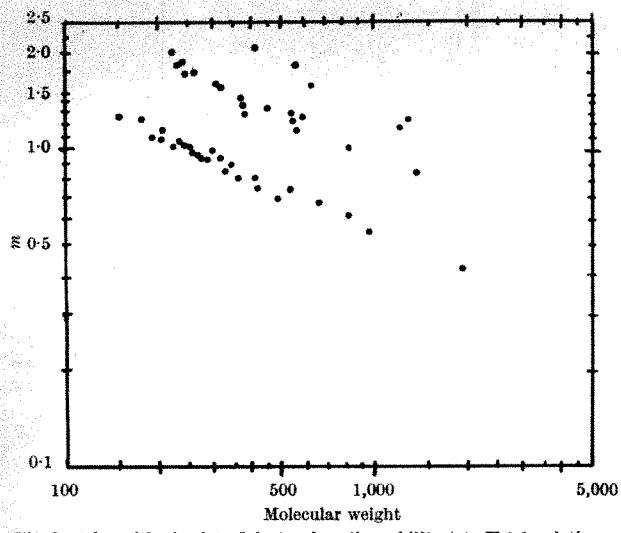


Fig. 3. A logarithmic plot of electrophoretic mobility (at *p*H 1·9, relative to serine) versus molecular weight for peptides without cysteic acid

teristic of N-terminal cysteic acid (Fig. 2*b*). There is some possibility that the sulphone group may suppress the ionization of the terminal carboxyl when $MetSO_2$ is at the carboxyl terminus, as such peptides, when nominally neutral, show in fact a slight cationic mobility at *p*H 6·5. A useful indication that one is dealing with a peptide of which the αNH_2 is suppressed may be obtained by staining on paper with the ninhydrin–cadmium reagent of Barrolier[8]. Such peptides are very frequently those which give a yellow, orange or brown colour as opposed to the normal red. The principal exceptions are peptides with N-terminal glycine which give a yellow colour but have normal *p*Ks, and peptides with basic residues N-terminal which give a red colour even when they have an abnormal *p*K.

A plot of the type described here has also been prepared for use at *p*H 1·9 (Fig. 3). The ionic strength of the 2 per cent formic–8 per cent acetic acid 'buffer' is about 0·01. It is, of course, not possible to distinguish between amidated and non-amidated side chains at this *p*H, but it has been of some use in looking for N-acetyl groups and as a general check on amino-acid analyses of peptides. *p*H 1·9 is dangerously close to the *p*K of some carboxyl groups and points may be found which lie well clear of the lines. One interesting point is that the slope of the lines in this case suggests a state intermediate between that expressed by equation (2) and that by equation (3). The ionic strength may be the determining factor.

Plots have also been made for 'dansyl' peptides[9] and for serine phosphate peptides[10]. It was hoped in the latter case that the plot would assist sequential investigations at the radioactive level, but the influence of the serine phosphate group on the *p*K of other charged groups considerably complicated matters. For such a plot to have been of any use beyond the stage of interpretation of preliminary results, considerably more information on mobilities of peptides in this class would have been required.

In conclusion, although the plot of electrophoretic mobility against molecular weight shown in Fig. 1 has been used extensively by workers in this laboratory, no clear indication of net charge given by it has yet been disproved. The use of this plot for any other purpose but this and the examination of different models of electrophoretic migration is to be viewed with some caution. The more complex circumstances applying to Figs. 2 and 3 also call for care in interpretation, as is the case for plots involving substituted peptide derivatives.

I thank R. F. Ambler, B. S. Hartley, C. Milstein, R. N. Perham, L. F. Smith and A. G. Weeds for supplying me with measurements and other observations on peptide mobility and for useful discussions on their interpretation, Dr. J. I. Harris and Dr. F. Sanger for their interest and encouragement, and the Medical Research Council for a scholarship.

[Enlarged copies of the figures can be obtained on request from the author.]

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ON SUDAN BLACK POSITIVE MATERIAL IN CONNECTIVE TISSUE

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Berenbaum[1,2] has described histological staining of bound substances, possibly lipids, by Sudan black *B* dissolved in acetone. This method sometimes stains reticulin[2,3] and material associated with collagen fibres[2,4]. Calcifying sites can be stained by Sudan black dissolved in 70 per cent alcohol, but only if the positive substance is unmasked before decalcification[5]. Fibroblasts are known to synthesize lipids[6]. These findings suggest that Sudan black positive material may be associated with the formation of hard and soft connective tissues. This possibility was tested in the investigation reported here.

Distal halves of growing tails of 9–11 day *A2G* albino mice were examined. The tails were frozen in solid carbon dioxide immediately after resection. Longitudinal sections of the unfixed, undecalcified tails were cut at about 8μ in a cryostat and thawed on clean slides at room temperature. Sections were post-fixed for 1 h in Baker's formol-calcium and stained by the following methods: in Sudan black *B* (Hopkin and Williams, Batch No. 41143) dissolved in 70 per cent alcohol, either at room temperature for 2 h or by Berenbaum's burnt method[1,2]; in Sudan black *B* dissolved in acetone for 6 h at 60° C[1,2]; in haematoxylin and eosin; by silver impregnation, using the methods of Gömöri for connective tissue fibres and von Kóssa for calcium deposits; and by the periodic acid–Schiff reaction. All sections were dehydrated, cleared in xylene and mounted in Canada balsam except those stained at room temperature in Sudan black *B* in 70 per cent alcohol, which were mounted in glycerin jelly. Although mouse tail contains a wide variety of tissues and is most useful for comparative studies, these observations are restricted to dermis, tendon, annulus fibrosus, endochondral cartilage and developing bone.

Tissues were regarded as being positively stained with Sudan black only if coloured jet black[7]. When stained by Sudan black/acetone (Fig. 1*a*), positively stained

intercellular material was sometimes seen in the 'basement membranes' of the skin, and in the dermis and tendons. By contrast, positive intercellular material was seen in most sections in the annulus fibrosus and in parts of the cartilage, namely the intercellular substance of the basal layers and areas continuous with tendons, and in calcified cartilage identified from sections processed by the von Kóssa method. Newly formed bone of the perichondral collar was also strongly stained in parts. The intercellular substance of maturing cartilage and the fibrous layer of the perichondrium and periosteum were rarely stained. The material coloured by Sudan black/acetone was not stained by either of the Sudan black/alcohol methods.

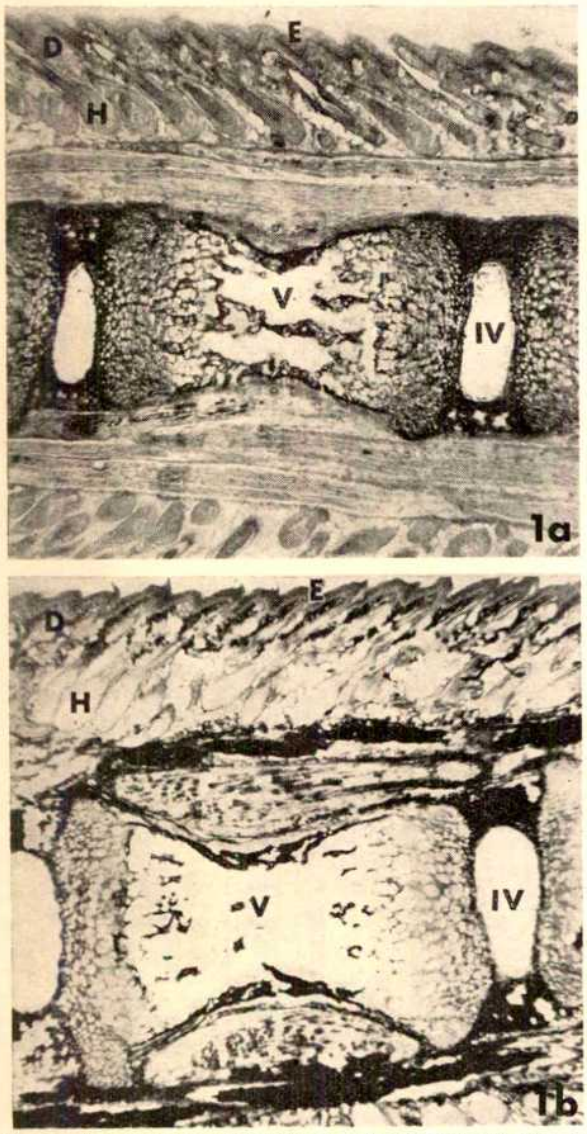


Fig. 1. Longitudinal sections through developing tail of mouse. *a*, Control; *b*, after extraction in chloroform : methanol : hydrochloric acid (2 : 1 : 1/300 v/v/v) at room temperature for 24 h. *E*, Epithelium; *D*, dermis; *H*, hair follicle; *V*, vertebra; *IV*, intervertebral disk. (Sudan black/acetone. ×40)

Collagen fibres were coloured black, red-black or red-brown, or golden by Gömöri's silver impregnation method. There seemed to be some correlation between the location of areas coloured a red-black hue by silver impregnation and black by Sudan black/acetone.

Unfixed cold microtome sections were extracted in a mixture of chloroform : methanol (2 : 1 v/v) or in absolute acetone, either at room temperature or boiling-point, for varying periods up to 48 h. Sections were post-fixed in formol-calcium, stained by the Sudan black methods and compared with non-extracted adjacent sections stained simultaneously. After extraction in these reagents at room temperature, sections stained in Sudan black/acetone showed reduction of "background staining" but increased sudanophilia in some areas, particularly in the annulus fibrosus and the intercellular substance of cartilage described above. These areas were not stained by the Sudan black/alcohol methods. Little or no material in dermis and tendon was stained by Sudan black/acetone after 24 h extraction in boiling chloroform : methanol or acetone, and the positive material in the annulus fibrosus was gradually removed. However, sudanophilic material in the intercellular substance of the basal attachment and calcified areas of the cartilage, but not usually of the bone of the perichondral collar, was increased. Forty-eight hours' extraction rarely removed all this stainable material from the calcified tissues, but frequently removed it from uncalcified cartilage.

It is known that some compound lipids can be isolated from neural tissue only by extraction in chloroform : methanol containing hydrochloric acid (see ref. 8). To determine whether further Sudan black positive material in connective tissue could so be disclosed, additional unfixed sections were extracted in chloroform : methanol : concentrated hydrochloric acid (2 :1 : 1/300 v/v/v). Room temperature extraction resulted in intense staining by Sudan black/acetone (Fig. 1*b*), but not by Sudan black/alcohol, of material associated with both hard and soft connective tissues. Strongly sudanophilic material was seen to be associated with the "basement membranes" of the skin, the connective tissue fibres of dermis, tendon, annulus fibrosus, perichondrium and periosteum, intercellular substance of basal and attachment areas of endochondral cartilage, calcifying cartilage and bone of endochondral collar, but only rarely with the intercellular substance of maturing cartilage. Staining of areas containing sudanophilic material in unextracted sections was intensified by extraction. Maximum staining was obtained after room temperature extraction for 1–48 h, the time varying between specimens. Thereafter, no noticeable increase in intensity occurred, and eventually there was gradual disappearance of stained material. Staining of calcified cartilage and bone of perichondral collar was more persistent but was prevented by extraction for 130 hours, which often caused hydrolysis of tissues. Extraction in boiling reagent removed all stainable material, sometimes within 24 h.

Some sections containing large quantities of material positively stained after extraction in chloroform : methanol : hydrochloric acid were demounted by soaking in xylene, and then rinsed in absolute alcohol. Subsequent transfer to chloroform : methanol (2 : 1 v/v) at room temperature removed the stain from soft connective tissue in 1 min or less, but stained material was still present in calcified cartilage and bone after 1 h. Attempts to restain the sections in Sudan black/acetone were largely unsuccessful. Periodic acid–Schiff positive material in sections extracted by the methods already described did not behave like the Sudan black-positive material.

In a further experiment, healthy skin and 8 mm punch wounds healing up to 25 days in the dorso-lateral skin of three *Macaca irus* monkeys aged about 5·5 years were examined by the methods already described. The monkeys had been fed a high carbohydrate-containing cariogenic diet for at least 4·5 years. Material, positively stained by Sudan black/acetone but not by the Sudan black/alcohol methods, and extractable by chloroform : methanol and by acetone, was found in healthy excised skin. It is not yet known whether this finding was in any way related to the animals' diet. This material seemed to be located in areas corresponding to those coloured red-black in adjacent sections impregnated by Gömöri's

silver method. Extraction at room temperature in chloroform : methanol : hydrochloric acid markedly increased the quantity of Sudan black/acetone stainable material associated with the collagen fibres.

Sudan black/acetone stained material was also found in relation to unextracted collagen fibres laid down in the healing wounds, again in areas apparently corresponding with those of a red-black hue in adjacent silver impregnated sections. The amount of material in the healing connective tissue stained by Sudan black/acetone after extraction in chloroform : methanol : hydrochloric acid at room temperature appeared to be related to the quantity of mature collagen fibres present in the wound. The intercellular substance of the connective tissue of the adjacent skin was always more heavily stained than the developing connective tissue of the wounds.

The material stained by Sudan black/acetone in unextracted sections in the foregoing experiments was never stained by either of the Sudan black/alcohol methods, but it could be extracted from soft connective tissues by acetone or chloroform : methanol. These findings suggest that it comprised bound lipids. These probably are not glycolipids, and their identification must await microchemical analysis.

Sudan black/acetone-stained material was seen in calcified cartilage, the sudanophilia of which has been established by Irving[5] using a different technique, and also in mineralized bone. However, the solubility of these substances is different from those in soft connective tissue, and this investigation has contributed little to their identification.

Bound lipids can be unmasked *in vitro* to histochemical reactions (see refs. 2 and 9). Some extracellular bound lipid-like substances in the connective tissues examined here have been unmasked and stained by Sudan black/acetone alone; others have been unmasked to Sudan black/acetone only after extraction, particularly by chloroform : methanol : hydrochloric acid. In this connexion, it is of interest that some protein-bound phospholipids can be extracted from brain partly by acetone and wholly by acidified chloroform : methanol[8]. Processes effecting the extraction of bound lipids appear to disrupt molecular attachments and relationships[8,10], thus possibly making the lipids accessible to staining reagents[11], although prolonged extraction removes them entirely. The ease with which sudanophilic material can be unmasked and extracted probably reflects its degree of binding in the tissues.

The use of extraction procedures indicated the presence of far greater quantities of sudanophilic material in these connective tissues than has hitherto been demonstrated. There was some evidence that in soft connective tissue the amount of material unmasked only by chloroform : methanol : hydrochloric acid increased with maturation. The sudanophilia of unextracted sections of soft connective tissues was variable, and it is suggested that this reflects "*in vivo* unmasking" of bound lipid-like substances which may represent a stage in their incorporation into developing connective tissue and perhaps in their dissociation from resorbing connective tissue. These possibilities are now being investigated.

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ISOLATION OF A CHONDROITIN SULPHATE PROTEIN COMPLEX FROM BOVINE INTERVERTEBRAL DISKS

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The intervertebral disk is a flattened cylindrical structure consisting of two distinct regions, an outer fibrocartilaginous ring or annulus and an inner gelatinous portion, the nucleus pulposus[1]. Several workers have shown the presence of acid mucopolysaccharides in both the nucleus and annulus in stained histological sections[2,3]; these have been isolated and identified as keratosulphate[4] and chondroitin-6-sulphate[5]. Marked changes in the proportions of these polysaccharides and their total amount in relation to the collagen content of the disk have been noted during ageing[6].

In most of these investigations the mucopolysaccharides were isolated by methods involving the destruction of tissue protein. However, Malawista and Schubert[7] showed that a more complex molecule containing mucopolysaccharide covalently bound to protein could be extracted from bovine nasal cartilage by homogenizing this tissue in water at 0° C. Further examination of the extracted molecule showed the presence of both chondroitin sulphate and keratosulphate bound to protein[8,9] and it has been suggested that both mucopolysaccharides are bound to the same protein[8].

Evidence for the presence of a similar mucopolysaccharide protein complex in bovine intervertebral disk is presented in this article.

Ninety grams of fresh bovine intervertebral disks were minced in a power mincer at 0° C and then homogenized in 1,500 ml. of distilled water as described by Malawista and Schubert[7]. The procedure was repeated until the nitrogen and hexosamine in the final extract were less than 5 per cent of that in the first extract. The residue was then further extracted with molar sodium chloride in a similar manner. The pooled extracts were dialysed and the content of acid mucopolysaccharide determined in a portion of the extracts by proteolytic digestion with papain and isolation of the mucopolysaccharides as described by Scott[10]. It was found that 87 per cent of the acid mucopolysaccharide in the tissue could be extracted under these conditions as determined by the hexosamine content of the quaternary ammonium precipitated material.

Treatment of the dialysed extracts from disks with the quaternary ammonium salt, cetyl trimethyl ammonium bromide (CTAB), resulted in the precipitation of a crude mucopolysaccharide protein complex. After washing

Table 1. ANALYSIS OF MUCOPOLYSACCHARIDE PROTEIN COMPLEXES ISOLATED FROM BOVINE INTERVERTEBRAL DISKS AND NASAL SEPTA

Percentage of composition in g/100 g dry weight

	Protein (a)*	Galactosamine (b)	Uronic acid (c)	Glucosamine (d)	Galactose (e)	Sulphate (f)	Ratio of KS/CSA (d/b)
Intervertebral disk complex	22·0	14·7	15·4	11·4	11·9	10·4	
Nasal cartilage complex	15·0	20·0	19·8	3·7	4·32	13·1	
		Molar ratio of components—calculated from total hexosamine as 1·0					
Intervertebral disk complex	—	0·56	0·62	0·44	0·51	1·08	0·79
Nasal cartilage complex		0·83	0·77	0·17	0·18	1·02	0·205

* Methods of analysis: (*a*) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951). (*b*) Cessi, C., and Seraphini-Cessi, F., *Biochem. J.*, **88**, 132 (1963). (*c*) Bitter, T., and Muir, H., *Anal. Biochem.*, **4**, 330 (1962). (*d*) Cessi, C., and Piliego, F., *Biochem. J.*, **77**, 508 (1960) (*e*) Trevelyan W. E., and Harrison, J. S., *Biochem. J.*, **50**, 298 (1952). (*f*) Dodgson, K. S., and Price, R. G., *Biochem. J.*, **84**, 106 (1962).

the precipitate with water, the quaternary ammonium ion was removed by shaking the precipitate with a saturated solution of potassium thiocyanate in ethyl alcohol at room temperature. This procedure was repeated three times and the product finally dissolved in distilled water. High-speed centrifugation for 2 h at 45,000 r.p.m. (Spinco model *L* No. 50 head) resulted in the separation of a small amount of gel similar to that found in preparations from bovine nasal cartilage[11].

Further purification of the dissolved mucopolysaccharide protein complex was achieved by passing the solution through a column of 'Ecteola' ion exchange resin in the chloride form. This treatment removed some protein, but 75 per cent of the mucopolysaccharide protein complex was present in the effluent. The eluate was dialysed and dried from the frozen state. The low capacity of the 'Ecteola' resin for the mucopolysaccharide protein complex makes this a useful method for separating the complex from free acid mucopolysaccharide. The capacity of the resin for mucopolysaccharide when isolated from the mucopolysaccharide protein complex is at least sixty times greater than that for the parent complex.

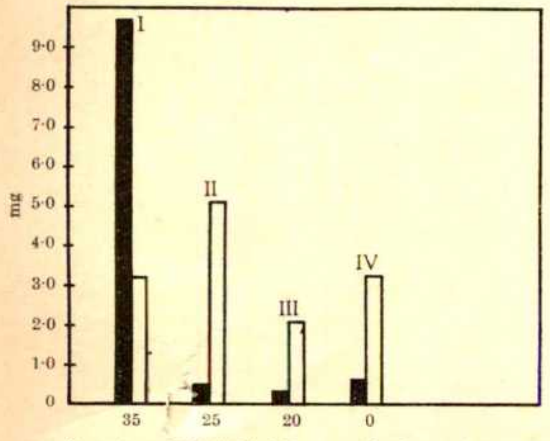


Fig. 1. Elution of keratosulphate (■) and chondroitin sulphate (□) from cellulose column with decreasing concentrations of ethanol

An analysis of the isolated mucopolysaccharide protein is shown in Table 1. The molar equivalence of galactosamine and uronic acid (1) and glucosamine and hexose (2) suggested the presence of both chondroitin sulphate and keratosulphate. This was confirmed by digestion with papain and the separation of the two mucopolysaccharides on a cellulose column (Fig. 1) as described by Gardell[12]. The protein content was similar to that found in the complex isolated from bovine nasal cartilage, but the ratio of keratosulphate to chondroitin sulphate was considerably higher (Table 1).

An examination of the sedimentation behaviour in the Spinco model *E* analytical ultracentrifuge showed that the preparation was apparently homogeneous (Fig. 2). A small quantity of a faster component was also present, but this could not account for more than 5 per cent of the dry weight of the material. Further purification did not remove it. The molecule remained at the origin when electrophoresis was carried out on starch or acrylamide gels, but moved as a single symmetrical peak in free boundary electrophoresis, at *p*H 5·0 in an acetate buffer, and at *p*H 6·4 in a phosphate buffer ($I = 0{\cdot}2$) (see Fig. 3).

A preliminary examination of the protein component showed it to contain little or no hydroxyproline, which

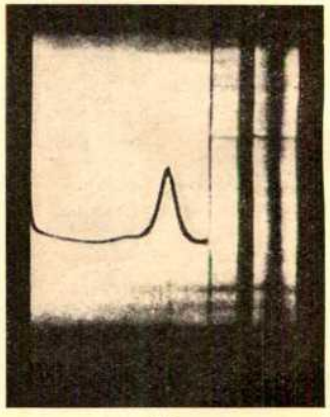


Fig. 2. Sedimentation velocity pattern of mucopolysaccharide protein complex. Sedimentation in 0·005 M phosphate buffer *p*H 7·8 containing 0·15 M potassium chloride. Mucopolysaccharide concentration 0·4 g/100 ml.

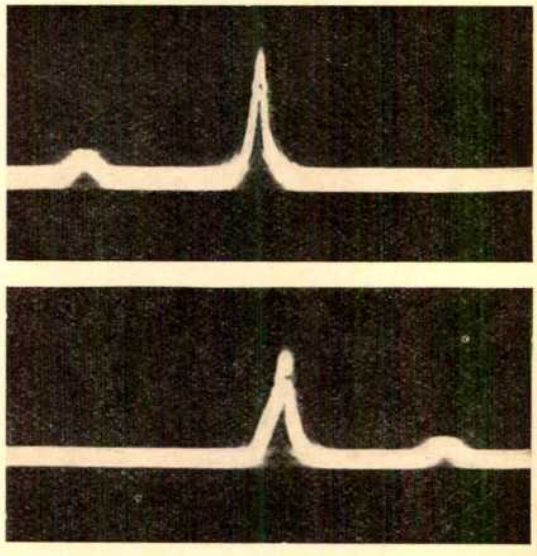


Fig. 3. Electrophoretic pattern of mucopolysaccharide protein complex *p*H 6·5 in phosphate buffer ($I = 0{\cdot}2$). Photograph taken after 60 min at a potential gradient of 6·1 V/cm². *A*, Ascending limb; *B*, descending limb

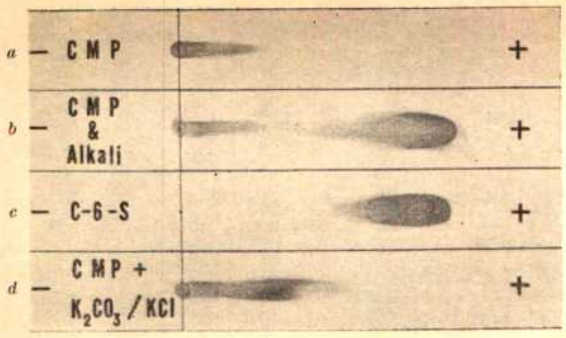


Fig. 4. Paper electrophoresis of the mucopolysaccharide protein complex and the derived mucopolysaccharides. 5 μl. spots containing 30–40 μg of preparation were applied to strips of Whatman 3*MM* paper (2·5 × 34 cm). Electrophoresis was carried out in an Eel apparatus in 0·1 M acetate buffer *p*H 5·0, containing 6 M urea, for 5 h at 4 V/cm. The strips were stained with aqueous toluidine blue. *a*, Untreated polysaccharide protein complex; *b*, alkali treated polysaccharide protein complex; *c*, papain digested polysaccharide protein complex; *d*, polysaccharide protein complex treated with potassium carbonate/potassium chloride mixtures. Mixtures of polysaccharide protein complex and polysaccharide can be completely resolved under these conditions

indicated that collagen was absent, and it did not give any precipitation lines with antibovine serum preparations when examined by the method devised by Ouchterlony[13]. Thus the protein does not seem to be either a serum component or collagen.

Muir[14] showed that the polysaccharide protein complex from pig tracheal cartilage was labile to alkali releasing free polysaccharide. Treatment of the intervertebral disk preparation with 0·5 N sodium hydroxide released material which on electrophoresis had the same mobility as chondroitin sulphate (Fig. 4). This alkali lability would account for the suggestion by Bernardi *et al.*[15] that material extracted from human nucleus pulposus with 1 per cent potassium carbonate containing 30 per cent potassium chloride (*p*H 10·5) contained a mixture of a mucoprotein and mucopolysaccharide. Treatment of purified mucopolysaccharide protein complex from bovine disks with the potassium carbonate/potassium chloride mixture for 60 h at room temperature resulted in a partial degradation as shown in Fig. 4.

We could not find any evidence for the presence of free mucopolysaccharide in our extracted material, thus 87 per cent of the total mucopolysaccharide in the tissue was extracted and shown to be present in a complex molecule containing chondroitin sulphate, keratosulphate and protein.

This investigation was supported by a grant from the Australian National Health and Medical Research Council. The free boundary electrophoresis was kindly performed by Dr. M. Gillespie of the C.S.I.R.O. Wool Research Division on a Tiselius apparatus, using Schlieren optics. The Ouchterlony diffusion test was undertaken by Dr. P. Ironside of the Department of Pathology, Monash University.

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DIMINUTION IN APPARENT ORGANIC PEROXIDE ("PRO-BLUE") IN MICE GIVEN AN ORAL CONTRACEPTIVE

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THE term "apparent organic peroxide" used here refers to what we have called "pro-blue"[1,2], an unknown substance or group of substances extractable by *n*-butanol from biological material and capable of oxidizing leuco-brilliant cresyl blue in the absence of air. We suspect, without proof, that pro-blue usually consists of hydroperoxides of unsaturated lipids formed by the chain reaction known as autoxidation. We have shown that pro-blue is increased not only by irradiation[2,3] but by alarm[4], and that some of the increase is in the fat surrounding sex organs[2] (ovaries in female, epididymis in male). The fact that deficiency of the natural antioxidant tocopherol causes sterility suggests that sterility may result from the autoxidation of ovarian or epididymal fat, even though tocopherol-deficient animals do not show an overall increase of pro-blue[5]. If this conjecture were true, the sterilizing effect of an unsuitable social environment in some animals could be a result of neurogenic autoxidation induced by frequent mild alarm. Neurogenic autoxidation could also explain the allegedly high incidence of arterial disease (including thrombosis) in business executives and others exposed to mental stress, as arterial disease is widely thought to be related in some unknown way to deficiency of unsaturated lipids, which could result (in addition to the production of toxic peroxides) from neurogenic autoxidation. Thus, neurogenic autoxidation may provide a link between neurogenic sterility and neurogenic arterial disease.

Pharmacogenic sterility has become of great social importance with the advent of progestin-like oral contraceptives. The suggestion that they may increase the incidence of thrombosis has been discussed[6], but evidence for it is weak or non-existent. Nevertheless it suggests, by analogy, the possible existence of pharmacogenic autoxidation as a common cause of sterility and thrombosis as in the partly hypothetical neurogenic case. A pharmacogenic rise in pro-blue is easily looked for in mice by our standard procedure, and we have done so.

The experiments were done with albino hairless mice (*hr hr*), individuals being selected at random and treated or not in random order. The contraceptives, in powder form (1·5 mg), were mixed with a paste of 'Horlicks Malted Milk' in water and given daily for 2 days to each experimental mouse. The control mice were given a similar amount of 'Horlicks' without the contraceptives. The precautions against alarm were as described in previous papers[1,3,4]. The peroxide estimation was as already described[2] with the following modifications.

Cadmium filings were made by filing a cadmium bar with a rough rasp. The filings were washed consecutively with dilute acetic acid, distilled water and acetone, and then dried in air. Copper powder was sieved and the

Table 1. MICROMOLES OF PEROXIDE ("PRO-BLUE") PER MOUSE

Control mice	Mice fed 'Enavid'
3·67	2·49
3·16	2·67
2·83	3·00
7·99	4·00
4·16	3·50
4·99	3·99
Mean 4·47	3·28

Control mice	Mice fed 'Ovulen'
5·16	5·00
6·33	3·17
4·00	2·83
7·66	4·67
2·00	3·33
4·00	4·17
Mean 4·86	3·86

Table 2

Control mice		Mice fed 'Ovulen'	
Micromoles of peroxide ("pro-blue") per mouse	Phase of oestrus	Micromoles of peroxide ("pro-blue") per mouse	Phase of oestrus
5·99	Met	7·83	Met
6·16	Di	4·83	Met
6·66	Met	4·66	Met
5·16	Met	4·17	Met
6·66	—	4·33	Di
6·33	Met	4·66	Met
5·00	Pro	4·33	Met
5·50	Oestrus	3·50	Met
6·83	Di	3·70	Met
6·00	Di	3·70	Met
5·83	Met	3·83	Met
4·64	Oestrus	4·49	Met
Mean 5·90		4·50	

The observed difference in means would occur by chance less than 1 in 30 times and is, therefore, significant.

fraction between 40 and 80 mesh per in. was used. The powder was washed consecutively with acetic acid, acetate buffer (*p*H 5), distilled water and acetone, and then dried in air. An 8×10^{-3} M solution of brilliant cresyl blue (molecular weight of hydrochloride, 245) was made in 0·5 M 2 : 6 lutidine plus 0·25 M acetic acid, all in *n*-butanol. This was diluted with 9 volumes of *n*-butanol for use. A 5×10^{-3} M solution of A.R. hydrated cupric chloride was made in 0·1 M lithium bromide plus 0·02 M lithium acetate, all in *n*-butanol.

In the first experiment, the results of which are shown in Table 1, two contraceptives were tested, 'Enavid' and 'Ovulen' (kindly supplied by G. D. Searle and Co., Ltd.). Six control and six experimental mice, 6 weeks old, were used with each.

A further experiment (Table 2) with 12 control and 12 experimental mice was done using 'Ovulen' only. The mice were 13 weeks old and therefore fully mature. 'Ovulen' in powder form (1·5 mg) was given daily for 5 days to each experimental mouse. A vaginal lave was taken from each mouse immediately after it was killed with nitrogen, to determine the phase of oestrus.

For the experiment in Table 3, mice 13 weeks old were used without contraceptives. After being killed with nitrogen the phase of oestrus of each mouse was determined. When six mice in any particular phase had been used, further mice found in that phase were rejected so that the work of pro-blue estimation could be concentrated where it was most needed.

Table 3. MICROMOLES OF PEROXIDE ("PRO-BLUE") PER MOUSE

	Pro-oestrus	Oestrus	Metoestrus	Means of rows
	2·16	4·33	6·33	4·27
	1·50	3·67	4·66	3·28
	8·33	8·16	6·50	7·66
	6·66	7·33	7·33	7·11
	6·83	7·16	7·99	7·33
	7·33	10·99	8·33	8·88
Means of columns	5·47	6·94	6·94	

The result in Table 2, which confirms the preliminary result in Table 1, has revealed a new phenomenon, the reverse of what was expected, in that the contraceptive 'Ovulen' has produced a statistically significant diminution in total pro-blue, and the preliminary results (Table 1) suggest that 'Enavid' may behave similarly. The observed differences in means shown in Table 1 would occur by chance about 1 in 6 and 1 in 4 times respectively and are therefore not significant. The pooled differences of means ('Ovulen' plus 'Enavid') are also not significant, but the pooled means of differences between treated and untreated mice examined at approximately the same time are significant ($P < 0{\cdot}02$), which suggests that a slow drift with time accounts for some of the large variance. The difference of means in Table 2 is significant with or without elimination of time.

We tried to explain the diminution in pro-blue by looking without success for an antioxidant action of 'Ovulen' *in vitro*. We also thought that the pro-blue level might vary with the oestrus cycle which might be blocked by 'Ovulen' in a low-peroxide phase. Actually, 'Ovulen' was found (Table 2) to block in the metoestrus phase (an unexpected and possibly new observation), but the mean pro-blue in metoestrus in control mice was no lower than in oestrus though a statistically insignificant fall ($P = 0{\cdot}33$) was observed in pro-oestrus (Table 3). In Table 3, the means of rows, which are in chronological order, show an obvious effect of time. This was found by analysis of variance to be significant ($P < 0{\cdot}01$); but even when it was allowed for, the pro-oestrus column was not significantly different from the other two.

The chief result of the present work is thus the discovery of an unexplained new phenomenon, low pro-blue in 'Ovulen'-treated mice. The explanation, when it comes, may throw some light on the means by which ovulation is regulated both naturally and artificially.

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5-HYDROXYTRYPTOPHAN UPTAKE BY DOG THYROID 'C' CELLS, AND ITS POSSIBLE SIGNIFICANCE IN POLYPEPTIDE HORMONE PRODUCTION

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THE *C* cells of the dog thyroid have been shown to be identical with the argyrophil parafollicular cells described in that animal by Nonidez[1,2]. As indicated in earlier papers[2,3], these cells are sensitive to physiological changes in serum calcium-levels and they respond to high calcium-levels, of short duration, by discharging their contents. It has been postulated that the secretion product of the *C* cells is calcitonin (thyrocalcitonin)—the calcium-regulating hormone in the serum[2,3].

Evidence was recently provided by Hargis *et al.*[4], who used an immunofluorescent technique on pig thyroid, that thyrocalcitonin "must be synthesized or stored in the same cells that elaborate thyroglobulin". These are indisputably the acinar ('*A*') cells. Notwithstanding,

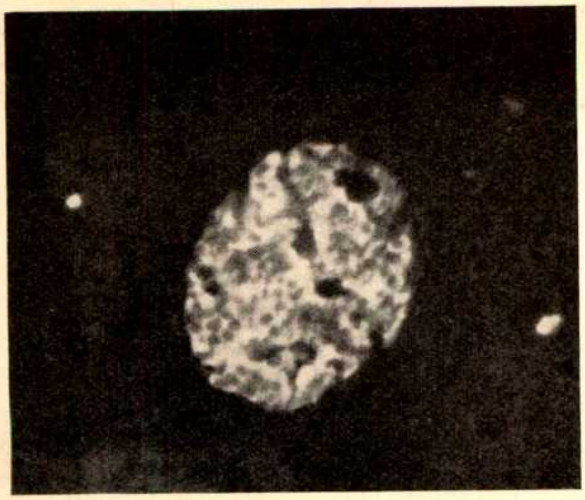


Fig. 1. Dog pancreas exhibiting 5-HT fluorescence in the β-cells after administration of 5-HTP. (× 420)

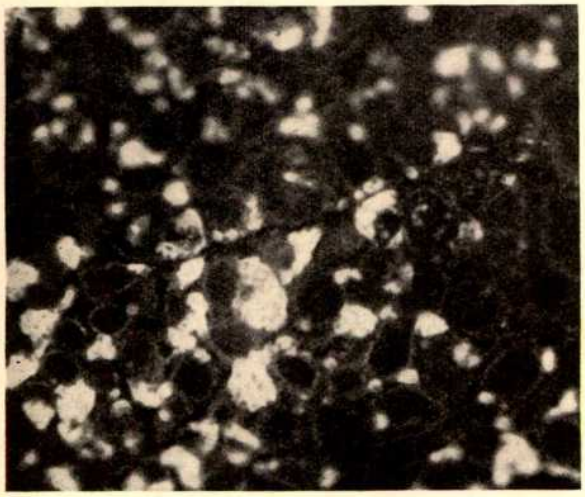


Fig. 2. Dog thyroid showing 5-HT fluorescence in the *C* cells after administration of 5-HTP. (× 180)

Bussolati and Pearse[5] have reported that when it is not subjected to the diffusing effect of "fixation" in solvent (acetone), calcitonin, in the pig thyroid, can be shown by immunofluorescence to be present in the *C* cells and possibly in the vascular pericytes. As yet it has not been proved that this indicates synthesis of calcitonin by the *C* cells as well as storage.

An interesting property of the *C* cells is their ability to concentrate injected 5-hydroxytryptophan (5-HTP) and, possibly, to convert this to 5-hydroxytryptamine (5-HT). Both can readily be shown by the fluorescent amine technique[6,7]. Puppies and young dogs were given 5-HTP (25 mg/kg) intraperitoneally and killed after 4–6 h. Control animals of similar weight and sex were also examined. Selected tissues were freeze-dried for 6–8 h at −40° C in a thermo-electric dryer[8]. They were then treated with formaldehyde vapour at 50° C for 1 h (28 per cent humidity). As indicated by Falck and Hellman[9] for the pancreas of the guinea-pig, specific 5-HT fluorescence was found in the β-cells of the islets. In the control dogs, however, it was only faintly visible and in a small proportion of the cells. After 5-HTP injection, the 5-HT fluorescence of the β-cells in the islets was greatly increased (Fig. 1).



Fig. 3. As Fig. 2. Characteristic autofluorescence in Golgi region of the *A* cells and induced 5-HT fluorescence of the *C* cells. (× 510)

A slight augmentation of 5-HT fluorescence was also observed in the anterior pituitary corticotrophs and in the melanotrophs of the pars intermedia. As shown by Pearse and MacGregor[10] for pig, rat and man, the normal corticotrophs have a moderately intense 5-HT fluorescence after freeze-drying and formaldehyde vapour treatment. The melanotrophs of pig, rat and dog were found to be positive; those of rabbit and guinea-pig were negative.

Of all the dog tissues examined, however, the strongest new 5-HT or 5-HTP fluorescence was shown by the parafollicular *C*-cells (Figs. 2 and 3). In all species so far examined (rat, rabbit, guinea-pig, dog, man), these cells have shown

Table 1. COMMON CYTOCHEMICAL PROPERTIES OF 5-HTP AND 5-HT CELLS

Organ and cell	Supposed origin	Product and mol. wt.	High 5-HTP uptake	Normal 5-HT content	High α-GPD* content	High esterase (E) or cholinesterase (ChE)
Adrenal medulla chromaffin	Neuroectoderm	Noradrenaline Adrenaline *c.* 180	Yes[11]	No	Moderate	ChE
Intestine, enterochromaffin	(1) Neuroectoderm (2) Entoderm	5-HT 175	Yes[11]	Yes[1]	Moderate	E
Mast cell	Mesoderm	Heparin Histamine	Yes[11]	Yes[1] (rat, mouse)	No	Chloracetyl E
Pituitary, corticotroph	Entoderm (Rathke)	ACTH *c.* 3,000	Yes	Yes[12]	Yes	E
Pituitary, melanotroph	Entoderm (Rathke)	MSH *c.* 3,000	Yes	Yes[13]	Moderate	E
Pancreas islet β-cell	Entoderm (Duct)	Insulin *c.* 6,000	Yes[11]	No (minimal)	Yes[14]	Low
Thyroid *C*-cell	Entoderm (possibly ultimobranchial)	Calcitonin *c.* 8,000	Yes	No	Yes	ChE

* Mitochondrial α-glycerophosphate dehydrogenase.

no specific 5-HT fluorescence in normal animals. The fine granular (orange) fluorescence seen in the acinar cells (Fig. 3) is invariably present in the normal dog thyroid. It is possibly associated with the formation of lysosomes in the Golgi region of the *A* cells. The colour of this fluorescence, and the fact that it does not fade, distinguishes it from that of 5-HT.

Uptake of 5-HTP by the parafollicular cells was first noted, in the mouse, by Ritzen *et al.*[11], who used an autoradiographic technique with ^{3}H-5-HTP. They found that the latter was initially accumulated "in the exocrine pancreas and other organs characterized by a rapid protein synthesis". After 4 h the pattern of storage closely approximated the "amine pattern" found after injection of ^{14}C-5-HT. Here storage was particularly in the adrenal medulla, the thyroid, the islets of Langerhans, bone marrow, spleen, lung, mast cells and enterochromaffin cells.

If one considers the principal cell types which are now seen to be involved in 5-HTP uptake and conversion (adrenal medullary cells, enterochromaffin cells, mast cells, pituitary corticotrophs and melanotrophs, pancreatic islet β-cells, thyroid *C* cells) it may be significant that in four out of seven cases the apparent principal product is a polypeptide hormone. It has already been suggested[2] that cells producing polypeptide hormones may have common cytochemical and electron microscopic properties. Cytochemical features common to the seven cell types are given in Table 1. No account is taken of species differences, which are often considerable, but most items refer to dog, rat, rabbit, or pig. The four cells of supposed entodermal origin fall into one group and the non-entodermal representatives into a second group. This division is not absolutely clear, however, as the enterochromaffin cells are now usually regarded as entodermal derivatives and it is possible that the thyroid *C* cells are non-entodermal (neuroectodermal). In some species these cells are not only found in the thyroid but also in other regions (parathyroid, thyroglossal duct), which suggests a common non-thyroid, non-parathyroid origin. Of two possibilities (ultimobranchial bodies, neural crest) the first is the more likely. A neural crest origin for the pituitary corticotroph and for the pancreatic islet β-cell also seems basically unlikely.

Where it is present, a high mitochondrial α-glycerophosphate dehydrogenase may indicate high phospholipid synthesis and high ChE (pseudocholinesterase) may have a similar implication. (In one animal, the rabbit, the *C*-cell enzyme reacts as a specific acetylcholinesterase.) The common denominator in the four polypeptide-producing cells may be the lipid-bound vesicles, averaging 2000 Å in diameter, which can be observed with an electron microscope. The specific product of the cells is apparently stored in the vesicles in a form available for immediate secretion. If in the latter process the vesicles are consumed, then high phospholipid synthesis will be required.

Whatever their precise origin, the details presented here suggest that pituitary corticotrophs, pituitary melanotrophs, pancreatic islet β-cells and thyroid *C*-cells develop from a common ancestral type. Their uptake of 5-HTP and its conversion to 5-HT may then be a demonstration of a no longer utilized ancestral function. It is not easy to postulate the use of 5-HTP as a tryptophan precursor for peptide synthesis. If this were the case the pancreatic islet α-cells (glucagon), with their high need for tryptophan would surely manifest high uptake. A possible function of 5-HT might be in connexion with capillary permeability and facilitation of the passage of the polypeptide product through the vessel wall.

Clearly, at the present time, no unifying concept is possible, but re-investigation of the developmental embryology of the four cell types, by cytochemical methods, is certainly indicated.

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EXTRUSION OF ATPase ACTIVITY FROM PINOCYTOTIC VESICLES OF ABUTTING ENDOTHELIUM AND SMOOTH MUSCLE TO THE INTERNAL ELASTIC MEMBRANE OF THE MAJOR ARTERIAL CIRCLE OF THE IRIS OF RABBITS

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IT is probable that Freidrick Arnold was the first to describe the circular venosus iridis in man[1]. Careful anatomical dissections later showed that this vascular structure was the larger of two arterial circuits formed by the anastomoses of the long posterior ciliary arteries and perforating branches of the anterior ciliary arteries. The long posterior and anterior ciliary arteries are branches of the ophthalmic artery. Light microscopy has shown that the major circuit is lined by a single row of flattened endothelial cells; the internal elastic lamina contains sparse numbers of fibrils, the media consists only of two rows of smooth muscle cells with meandering fibres between the contractile elements, the external elastic membrane is absent, and there are only a few fibroblasts and loose collagen fibres in the adventitia. The major arterial circuit of the iris, therefore, is structurally very similar to equally sized arteries of the brain. The iris, like the brain, is largely derived from ectoderm, for even the sphincter muscles, like the erector pili muscles of the skin, have that embryonic origin[2]. The iris, too, is a limiting organ to the flux of the humour of the anterior chamber of the eye and, in disorders of lipid metabolism, it is the site of a lesion known as arcus senilis. These considerations have suggested a high degree of metabolic activity in the iris.

Accordingly, a study of the cytochemical localization of ATPase activity in the major arterial circuit of the iris of albino rabbits was undertaken. The major arterial circuit is readily visualized in the white iris of albino

* John and Mary R. Markle Foundation Scholar in Academic Medicine.

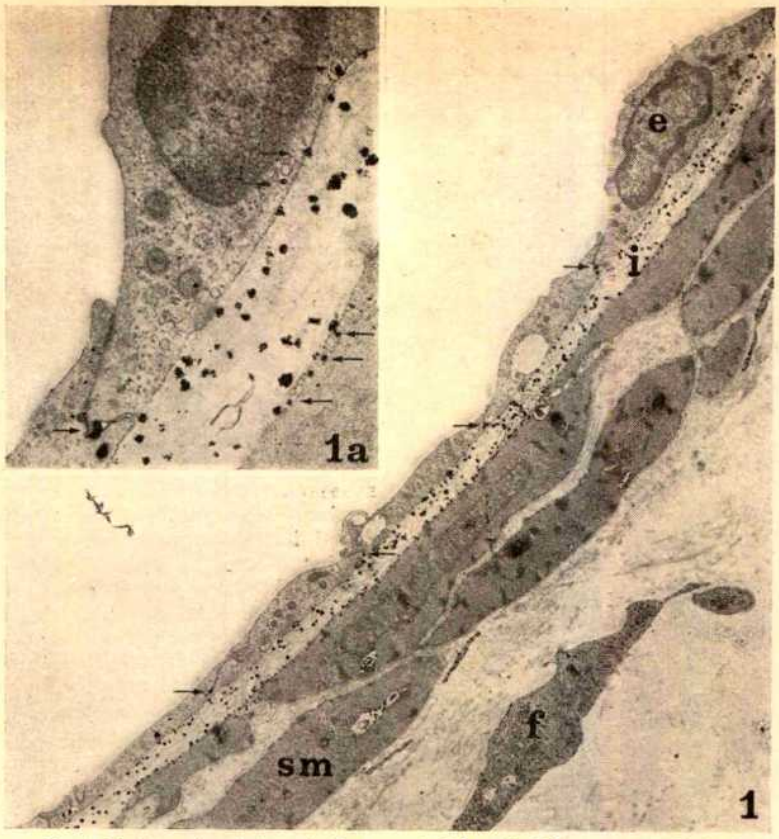


Fig. 1. Electron microphotograph of a segment of the wall of the major arterial circuit of the iris of a normal rabbit which was incubated in $1{\cdot}25 \times 10^{-3}$ M ATP for 0·5 h at room temperature (see text). Note the accumulation of reaction product (lead phosphate) at endothelial junctions (arrows) and the greater number in close proximity to the plasma membranes of the endothelium (*e*) and smooth muscle layer (*sm*) bordering the internal elastic lamina (*i*). Note that no reaction product is present in the luminal pinocytotic vesicles of the endothelium or in the pinocytotic vesicles of the smooth muscle that are not in close proximity to the internal elastic lamina. The cytoplasm of an adventitia fibroblast (*f*) contains minute granules of reaction product. (× *c.* 6,000)

Fig. 1*a*. Higher magnification of the upper right-hand corner of Fig. 1. Note reaction product deep within pinocytotic vesicles of the endothelial and smooth muscle membranes abutting the internal elastic lamina. In some instances reaction product partly extrudes into the interfibrillary spaces of the internal elastic lamina (arrows). Note the accumulation of reaction product beneath the desmosome at the endothelial junction in the lower left-hand corner of the photograph. (× *c.* 25,000)

rabbits. The irises were fixed by isobaric perfusion of each of the common carotid arteries of several rabbits with 300 ml. of cold 1·5 per cent steam-distilled glutaraldehyde buffered with 0·025 M *s*-collidine–hydrochloric acid at *p*H 7·4 for a period of 30 min. The fixative contained 0·015 M magnesium chloride and 0·015 M calcium chloride. Some of the cations were probably chelated by partially charged aldehydic groups and iso-osmolarity was achieved and controlled with complementary amounts of sucrose. The osmolar values of the fixative are given in Table 1. After perfusion, the irises were removed and narrow longitudinal blocks containing segments of the arterial circuit were washed three times for 4–6 h in the cold in iso-osmolar sucrose with 0·03 M magnesium chloride in 0·025 M *s*-collidine–hydrochloric acid buffer (*p*H 7·4). Cross-sections of the segments of the arterial circuit were then cut about 50μ thick by means of a McIlwain's tissue chopper[3] and placed in the test substrates for 30 min at room temperature. The incubating media were similar to those used by Marchesi and Barrnett[4] except that *s*-collidine–hydrochloric acid buffer was used throughout. The filtered substrate solutions were saturated with lead nitrate as the trapping agent and contained $1{\cdot}25 \times 10^{-3}$ M ATP (Nutritional Biochemical Corporation), 0·01 M magnesium chloride, and 0·08 M *s*-collidine–hydrochloric acid buffer at *p*H 7·0. Control studies included incubation of arterial segments in separate media each with equimolar concentrations of (*a*) adenylic acid (AMP) and (*b*) β-glycerophosphate as well as in (*c*) a solution without substrate. The tissues were then washed with buffered sucrose for 15 min and fixed for 0·5 h in the cold in 1 per cent osmic tetroxide in veronal buffer[4]. The tissues were then dehydrated in graded alcohols and embedded in 'Maraglas'[5]. The cross-sections of the arteries were properly oriented in the capsules by careful microdissection in partly polymerized plastic. Sections were made by means of a Porter–Blum microtome with a diamond knife, mounted on 200-mesh copper grids, and stained with lead hydroxide. Electron-microphotographs were taken with an R.C.A. *EMU 3G* electron microscope operating at 50 kV.

None of the irises incubated in control media showed any reaction product when examined under the electron microscope. The substructure of the components of the

Table 1. OSMOLAR VALUES OF FIXATIVE

	mosm/l. Theoretic	Actual
0·025 M *s*-collidine-hydrochloric acid buffer (*p*H 7·4)	50	
0·015 M magnesium chloride	45	
0·015 M calcium chloride	45	275†
1·5 per cent glutaraldehyde	163*	
0·035 M sucrose	35	35
	338	310

* This osmolarity was used in all preparations of fixative.
† This value was determined separately. About 28 mosm/l. were "lost" possibly by chelation of cations by partially charged aldehydic groups.

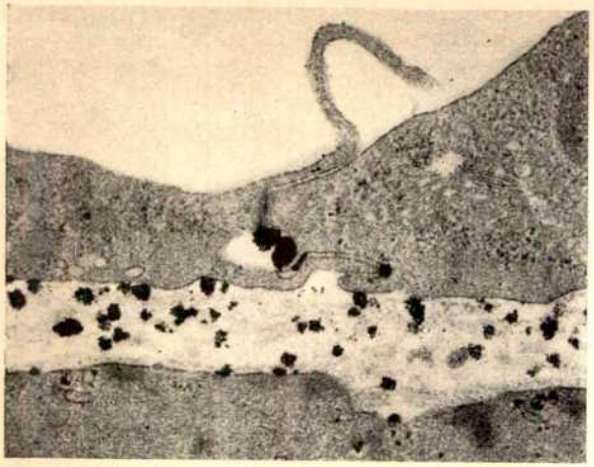


Fig. 2. Granules of electron-dense reaction product are present in cul-de-sac beneath a marginal fold and desmosome of two adjoining endothelial cells. Reaction product is also present in interstices of the internal elastic lamina and abutting pinocytotic vesicles. Tissue incubated in ATP media (see text). (× *c.* 31,500)

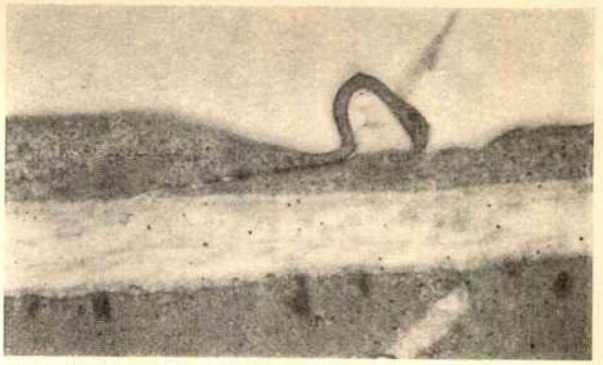


Fig. 3. Cul-de-sac beneath marginal fold and desmosome as well as the area of the internal elastic membrane and pinocytotic vesicles of abutting plasma membranes do not show reaction product in artery after incubation in media without ATP substrate. (× *c.* 24,900)

iris was well preserved. The arterial circuit was lined by sometimes overlapping endothelial cells that were interconnected by desmosomes. The endothelium was closely applied to the internal elastic lamina which was formed by a limited number of elastic fibres and contained numerous interfibrillary spaces. The subjacent media was formed by two rows of smooth muscle cells which were not closely applied to each other and elastic fibres were found coursing between these cells. No external elastic membrane was identified and the adventitia consisted of loose collagen fibres and scattered fibroblasts. Pinocytotic vesicles were numerous in the endothelium and smooth muscle. These vesicles seemed, in some instances, to be most numerous in the endothelial and smooth muscle membranes bordering the internal elastic lamina. Cytoplasmic filaments and glycogen-like granules were present in endothelial cells as well as in smooth muscle. Coarser osmophilic granules were rarely present in the endothelium and smooth muscle close to the nucleus, mitochondria, endoplasmic reticulum or the Golgi apparatus.

ATPase activity was demonstrated in the major arterial circuit of the iris. Reaction product (lead phosphate) was distributed in the internal elastic lamina and lined endothelium and smooth muscle (Figs. 1 and 1*a*). High magnification studies showed the accumulation of reaction product beneath desmosomes between endothelial cells (Figs. 2 and 3). At times, minute particles of reaction product were found deep within pinocytotic vesicles of abutting endothelium and smooth muscle; in other instances, the amount of reaction product was greater and partly extruded from these vesicles into the adjacent interfibrillar spaces of the internal elastic lamina. Many sections showed that there was a greater accumulation of granules of reaction product on either side of the internal elastic lamina immediately adjacent to the plasma membranes of the endothelial and smooth muscle cells than in the centre of the internal elastic lamina. No significant amount of reaction product was found in (*a*) luminal pinocytotic vesicles of the endothelial cells, or in (*b*) pinocytotic vesicles of the smooth muscle that were not in close proximity to the internal elastic membrane.

These findings suggest that both the endothelial and smooth muscle cells play a significant part in the maintenance of ATPase activity in the space subjacent to endothelial desomosomes and in the interfibrillary spaces of the internal elastic lamina of the major arterial circuit of the iris. ATPase activity seems to be highly selective in its localization in this arterial circuit. Activity of this enzyme appears to be greater just beneath desmosomes of the endothelium and in cytoplasmic pinocytotic vesicles of endothelial and smooth muscle cells abutting the internal elastic lamina. These observations suggest that ATPase is extruded into these extracellular spaces by way of selective pinocytotic activity of the endothelium and first layer of smooth muscle that border the internal elastic lamina. It is possible that a degree of commensality exists between these two different cellular elements with higher requirements for the release of high-energy phosphate bonds for transport of nutrients or other substances across the interface of the internal elastic lamina. In this respect, it is noteworthy that no ATPase activity was demonstrated in the luminal pinocytotic vesicles of the lining endothelium nor in the pinocytotic vesicles of smooth muscle which were not in close proximity to the internal elastic lamina.

I thank J. R. Guzmán-Rivero, C. Calloway and B. O. Spurlock for technical assistance in this work, which was supported by U.S. Public Health Service grants.

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A METHOD FOR ESTIMATING FREE ASPARAGINE AND GLUTAMINE IN BIOLOGICAL FLUIDS AS TRINITROPHENYL DERIVATIVES

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THIS article describes a method for the separate measurement of free asparagine and glutamine in small quantities of biological fluids containing 0·5–100 μg of each. It has proved equally suitable for extracts of animal tissues and for 0·5–1·0 ml. samples of blood or plasma. In simplicity and sensitivity the method has considerable advantages over others that are available at present[1–4].

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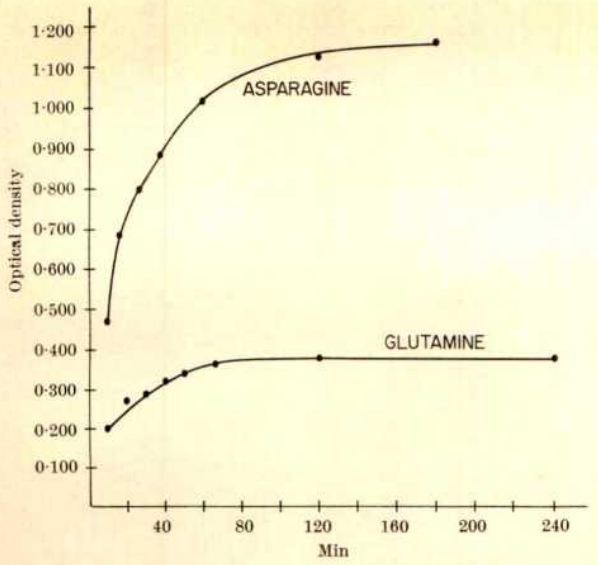


Fig. 1. Rates of trinitrophenylation of L-asparagine and L-glutamine. Incubation mixtures (39° C) consisted of chromatographically homogeneous L-asparagine 0·4 μmoles in 5 ml., 1·5 M phosphate buffer (*p*H 7·6) 0·2 ml., picrylsulphonic acid (Nutritional Biochemicals Corporation, Cleveland) 0·4 mg in 1 ml.; 0·5 ml. aliquots added at intervals shown to 0·5 ml. N hydrochloric acid to terminate the reaction (ref. 5). Water (5 ml.) added before making *O.D.* readings at 340 mμ. With L-glutamine (chromatographically homogeneous) a similar procedure was followed, but 0·2 ml. portions of the reaction mixture were used

In initial experiments, dinitrophenyl derivatives of asparagine and glutamine were prepared, but under conditions necessary for full derivatization large amounts of dinitrophenol accumulated which proved tedious to remove. To avoid this difficulty an alternative method was found in the preparation of 2,4,6-trinitrophenyl derivatives (TNP) using picryl sulphonic acid, according to a reaction recently described by Okuyama and Satake[5].

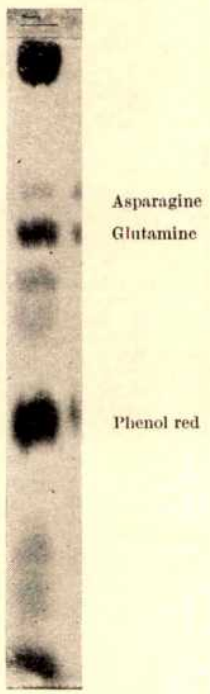


Fig. 2. Chromatography of amino-acid derivatives from tissue extract. Thymuses of 6 *C57/BL* mice (total weight 245 mg) homogenized in 13 ml. picric acid. A 5 ml. portion treated as described in text. Known samples of TNP derivatives of L-asparagine and L-glutamine run in parallel. (Whatman No. 4)

It is possible to separate out TNP derivatives of asparagine and glutamine by one-dimensional chromatography using tert-amyl alcohol saturated with phthalate buffer at *p*H 7·5, modifying the system of Blackburn and Lowther[6].

The stages of the procedure were as follows:

(1) *Protein precipitation with picric acid.* 0·5–1·0 ml. blood, plasma or serum was mixed with a 1 per cent solution of picric acid to a final volume of 6 ml., and the protein precipitate removed by centrifugation. Tissue samples weighing 50–800 mg were homogenized with 13 ml. picric acid solution in a Lourdes 'Omnimix' at 16,000 r.p.m. for 2 min. Filtration of the homogenate was preferable to centrifugation, owing to the tendency of fat-containing material to form a surface scum.

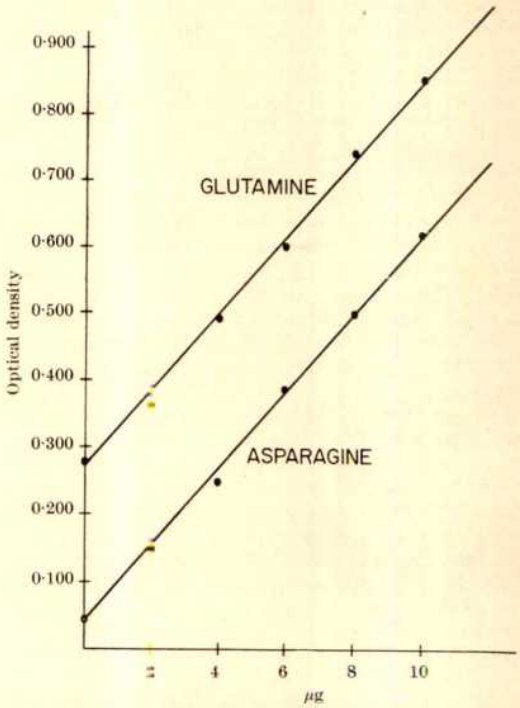


Fig. 3. Quantitation of TNP derivative formation from known amounts of L-asparagine monohydrate and L-glutamine added to samples of horse serum (1 ml.) and treated as described in the text. Eluates from chromatograms in 1 ml. water

(2) *Removal of excess picric acid.* 5 ml. quantities of protein-free supernatant were placed in stoppered test-tubes and acidified with one drop of 10 N hydrochloric acid. 5 ml. of ethyl acetate was then added and the layers gently mixed, avoiding the formation of an emulsion. After 2 washes picric acid was almost completely extracted from the aqueous layer. The remaining picric acid and dissolved ethyl acetate were removed by two extractions with 5–10 ml. ether, the residues of which were then removed at 37° C under reduced pressure.

(3) *Trinitrophenylation.* One drop of 0·17 per cent phenol red was added to the sample, followed by 0·2 ml. 1·0 M phosphate buffer and sufficient 1 N sodium hydroxide to make the colour of the indicator cherry-red, that is, *p*H 7·7. 1 ml. of 0·04 per cent picryl sulphonic acid was then added and the mixture incubated at 40° C for 3 h, which brought about complete trinitrophenylation (Fig. 1). As TNP derivatives of amino-acids are light-sensitive, this and further procedures were conducted as much as possible in the dark. It was found convenient to cover reaction tubes in aluminium foil.

(4) *Extraction of TNP derivatives of amino-acids.* After cooling the reaction mixture to room temperature

1 N hydrochloric acid was added until on testing with indicator paper the *p*H was reduced to 1·5–2·0. Amino-acid derivatives were then extracted in three thorough washes of 5 ml. ether, which were pooled in a 70 ml. centrifuge tube. The extract was dried at 35° C in a current of air.

(5) *Chromatography.* The dry extract of TNP amino-acids was dissolved in a few drops of moist peroxide-free ether. It was applied in a strip 0·5–1·5 in. long to chromatography paper (Whatman, Nos. 1, 4, or 3*MM*), thoroughly dried after saturation in 0·05 M phthalate buffer at *p*H 7·5. The chromatogram was developed by the descending technique using tert-amyl alcohol saturated with phthalate buffer; an over-night run was usually found adequate for the thinner papers. To obtain optimal results the quality of the tert-amyl alcohol was particularly important. Practical grades (boiling point 100°–103° C) provided better results than more highly purified preparations with which there was a tendency for streaking to occur. With such materials, however, the addition of 0·5 per cent pyridine greatly improved the resolution. The *p*H of the phthalate buffer was also critical. At normally used *p*H values (6·0–6·5)[6], asparagine and glutamine derivatives did not separate, but at *p*H 7·5, when phthalate had very little buffering activity for alkali, excellent separation was obtained.

(6) *Elution from chromatograms.* When the chromatograms had been dried, asparagine and glutamine spots were rapidly located and marked under long-wave ultraviolet light. They were eluted in 0·5–2·0 ml. distilled water. Corresponding areas behind the amino-acid spots were removed for blanks. Optical density measurements were made at 340 mμ in the Zeiss *PMQ* II spectrophotometer.

Fig. 2 shows the separation that can be obtained between glutamine and asparagine in an extract from mouse thymic tissue. R_F values of TNP derivatives are as follows: asparagine, 0·16; glutamine, 0·23; glutamic acid, 0·02; aspartic acid, 0·01; cystine, 0·20 and 0·56; glutathione (GSH), 0·12.

TNP-asparagine appears in a perfectly distinct spot and no other substance has been found to occupy its place. A cystine derivative, however, does not always clearly separate from glutamine. As it is formed in small amounts in the systems now used (giving about 5 per cent of glutamine values) it does not seriously interfere with results. However, to obtain most accurate values for both asparagine and glutamine concentrations, a blank should be prepared by incubating an aliquot of protein-free and picric acid-free filtrate (stage 2) with 0·2 ml. dialysed guinea-pig serum (L-asparaginase) and 0·1 ml. of glutaminase preparation[3] at *p*H 7·5 and 38° C for 1 h. The enzymes are then precipitated with an excess (100 mg) of picric acid, and the remaining procedures followed.

Fig. 3 shows the relationship between known amounts of glutamine and asparagine added to horse serum and the optical densities of eluates from the chromatograms. Recoveries are 101 ± 3 per cent of those that can be obtained from simple aqueous solutions of the amino-acids. The asparagine derivative prepared as now described gives an apparent molar extinction coefficient of $1{\cdot}173 \times 10^4$, the glutamine derivative $1{\cdot}018 \times 10^4$. Such values are similar to those obtained by Okuyama and Satake for other amino-acids[5].

The method described has proved valuable in investigating changes in amino-acid levels of animals treated with L-asparaginase—an enzyme causing powerful and apparently specific inhibition of a number of transplanted tumours[7–9].

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A FURTHER STUDY OF CATECHOLAMINE *O*-METHYLATION IN SCHIZOPHRENIA

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THE possibility that aberrant *O*-methylation in catecholamine metabolism is significant in the aetiology of schizophrenia was suggested by the pharmacological effects of a number of *para*-*O*-methylated catecholamines[1–3], and by the detection of an abnormal-*O*-methylated catecholamine metabolite in the urine of schizophrenics. Friedhoff and Van Winkle[4,5] were the first to report that 3,4-dimethoxyphenethylamine was excreted in the urine of a majority of schizophrenics but not in normal individuals. Later, they presented data derived from *in vivo* and *in vitro* studies to show that the di-*O*-methylated analogue is of endogenous origin[6]. Several investigators[7–9] confirmed the original observation of Friedhoff and Van Winkle on the unique occurrence of 3,4-dimethoxyphenethylamine in the urine of schizophrenics, but they reported significantly lower percentages of positive detections in the schizophrenic group. This discrepancy might arise from the varying percentages of acute and chronic schizophrenics in the several test groups. Others have reported contradictory data which suggest that the di-*O*-methylated derivative is not unique to schizophrenics and is probably of dietary origin[10–13]. That such derivatives are not unique to schizophrenia may also be inferred from a recent publication[14] describing the induction of akinesia in animals by di-*O*-methylated catecholamines, and associating these derivatives with the akinesia of Parkinson's disease. In this article and the succeeding one, data are also presented to suggest that abnormal *O*-methylation of catecholamines may not be a significant biochemical lesion in schizophrenia.

For the following *in vitro* investigations of catecholamine metabolism, autopsy specimens of liver and brain from normal and schizophrenic subjects were promptly frozen and stored in dry ice. Enzyme preparations were routinely made from a 10-g portion of liver or a 20-g portion of brain by homogenization at 4° C in 40 ml. of isotonic potassium chloride. After centrifugation for

10 min at 8,000*g*, the supernatant phase was decanted through coarse cheesecloth to remove the lipid layer.

For these metabolism investigations, a standard incubation procedure[15,16] was modified to the extent that each vessel contained 1·0 ml. of 0·5 M sodium phosphate buffer (*p*H 7·9), 0·5 ml. of 'Catron' (α-methylphenethylhydrazine, 5·6 mg/0·5 ml.), 0·2 ml. of *S*-adenosyl-L-methionine-methyl-^{14}C (10 μmoles, 10 μc./ml.), 0·2 ml. of dopamine (10 μmoles/ml.), 2·0 ml. of the filtrate, and 0·1 ml. of 0·5 M magnesium chloride. The *p*H of the incubation mixture was 7·6. Controls lacking dopamine were included in each series of determinations. The reaction vessels were flushed with nitrogen, stoppered, and incubated for 1·5 h at 37° C. The *p*H remained constant throughout the incubation.

At the end of incubation, 2 ml. of 0·5 M sodium borate buffer (*p*H 10) was added to the reaction mixture which was then adjusted to *p*H 10 with one drop of 10 N sodium hydroxide. The mixture was extracted[8] with three 4-ml. volumes of isoamyl alcohol–toluene (3 : 2). The organic phase was extracted with three 3-ml. volumes of 0·1 N sodium hydroxide to remove mono-*O*-methyl derivatives of dopamine, and then with three 1-ml. volumes of 0·05 N hydrochloric acid to remove di-*O*-methyl derivatives of dopamine. The acidic aqueous extract was reduced to dryness under a stream of nitrogen. The residue was dissolved in one drop of methanol and applied to a 20 cm × 20 cm thin layer of silica gel ('Silica gel *G*', E. Merck). The sample was flanked by 10 μg markers of the mono- and dimethoxy-phenethylamine analogues, and developed in butanol–ethyl acetate–15 N aqueous ammonia (3 : 1 : 1). The markers were sprayed with 0·2 per cent ninhydrin in pyridine–acetic acid–acetone (1 : 4 : 95), and the plate was heated at 100° C until the marker colours developed. The sample area was not sprayed because of the quenching effect of ninhydrin.

Fifteen consecutive 1 cm × 2·5 cm sections were marked in the sample area between the origin and the solvent front. The adsorbent in each section was scraped into a glass counting vial containing 20 ml. of a 4 per cent (w/v) 'Cab-O-Sil'—toluene scintillation solution consisting of 3·0 g of 2,5,diphenyloxazole and 100 mg of 1,4-*bis*(4-methyl-5-phenyloxazol-2-yl)-benzene per litre of toluene[17,18].

Radioactive counting of the contents of these vials for 10 min in a Packard model 3002 'Tri-Carb' liquid scintillation spectrometer gave a measure of enzymatic di-*O*-methylation. For a measure of catechol-*O*-methyltransferase (COMT) activity, a portion of the 0·1 N sodium hydroxide extract of the incubation mixture was counted in a toluene scintillation solution containing 30 per cent absolute ethanol.

Table 1. RESULTS OF *in vitro* STUDIES WITH HUMAN LIVER TISSUE

Specimen	Diagnosis	Age (yr)	Sex	Post-mortem autopsy interval	3-OCH$_3$-4-OH phenethylamine (c.p.m.)	3,4-Di-OCH$_3$ phenethylamine (c.p.m.)
1	Normal	2		5 h	1,057,820	844
2	Normal	26	F	4 h	504,167	498
3	Normal			3 h	957,956	81
1	Schizo				4,859	41
2	Schizo				3,269	0
3	Schizo	77	F	40 h	411,732	38
4	Schizo	78	F	8·5 h	50,273	36
5	Schizo	75	M	6 days	11,819	0
6	Schizo	89	F	3 days	262,232	21

Table 2. RESULTS OF *in vitro* STUDIES WITH HU

Specimen	Diagnosis	Age (yr)	Sex	Post-mortem autop
3	Schizo	34		

For the complete procedure, the efficiency of recovery of 2-(3,4-dimethoxyphenyl)ethylamine-1-^{14}C hydrochloride was established as 40 per cent and the limit of its detection as 50 ng by standard techniques.

The results of the *in vitro* investigations are summarized in Tables 1 and 2. Although only one set of data is reported for each specimen, most were tested on at least three occasions and gave similar data within experimental error. On the basis of these data, liver tissue preparations from six schizophrenic and three normal individuals and brain tissue preparations from four schizophrenic and one normal individual failed to convert dopamine to 3,4-dimethoxyphenethylamine. That such inactivity might be attributed to a loss of enzyme activity in necrotic tissue is readily conceded, but it is suggested that the relatively high COMT activity exhibited by these specimens is a valid indication of normal enzyme integrity.

At the conclusion of our *in vitro* investigations, we were informed of an independent investigation at Rockland State Hospital, Orangeburg, N.Y., in which 100 μc. of 3,4-dihydroxyphenylalanine-2-^{14}C was administered to two chronic schizophrenic patients. Though the courtesy of Dr. W. Sacks of that institution we were given a 24 h urine collection from each of these patients; equal portions of these specimens were subjected to extraction and two-dimensional thin-layer chromatography[8] to determine the levels of 3,4-dimethoxyphenethylamine and were examined for 3,4-dimethoxyphenylacetic acid[19]. Appropriate sections of adsorbents were scraped from the plates, eluted and counted as already described.

The *in vivo* data summarized in Table 3 suggest that 3,4-dimethoxyphenethylamine and 3,4-dimethoxyphenylacetic acid are not metabolites of 3,4-dihydroxyphenylalanine in the chronic schizophrenic.

Table 3. RESULTS OF HUMAN *in vivo* STUDIES

Patient	Diagnosis	3-OCH$_3$-4-OH phenethylamine (c.p.m.)	3,4-Di-OCH$_3$ phenethylamine (c.p.m.)	3-OCH$_3$-4-OH phenylacetic acid (c.p.m.)	3,4-Di-OCH$_3$ phenylacetic acid (c.p.m.)
A	Schizo	49,000	0	88,000	0
B	Schizo	332,000	18	71,000	40

We thank Dr. H. E. Himwich of Galesburg State Research Hospital, Dr. W. Strutton of Rockland State Hospital, and Dr F. Weinburg and Dr. S. Merlis of Central Islip State Hospital for the liver specimens. We also thank Dr. J. W. Auditore of Meharry Medical College for the brain specimens, and Dr. W. Sacks and Dr. P. Harper of Rockland State Hospital for the urine collections from two patients treated with 3,4-dihydroxyphenylalanine-2-^{14}C.

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OCCURRENCE OF 3,4-DIMETHOXYPHENYLACETIC ACID IN URINES OF NORMAL AND SCHIZOPHRENIC INDIVIDUALS

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DERANGEMENTS of amino-acid metabolism have been implicated in a variety of mental disorders[1]. In particular, recent investigations have directed our attention to a possible aberrant para-*O*-methylation of 3,4-dihydroxyphenethylamine (dopamine) in schizophrenia. Friedhoff and Van Winkle[2] reported that 3,4-dimethoxyphenethylamine was present in the urine of a majority of the schizophrenic patients studied, but was not excreted by normal subjects. Sen and McGeer[3], using a combined paper strip–gas chromatographic method, were able to detect the amine in urines of eleven of twenty-two schizophrenics, but no normal subjects were examined. In an extensive clinical study, Bourdillon *et al.*[4], using a slight modification of the Friedhoff technique, were able to detect a 3,4-dimethoxyphenethylamine-like component in the urine of many schizophrenics. On the other hand, some recent publications[5] record the failure to detect this amine in urines of schizophrenic patients. Friedhoff and Van Winkle[6] have reported that di-*O*-methylation of dopamine occurs *in vitro* when the substrate is incubated with a liver biopsy homogenate obtained from a schizophrenic patient known to excrete 3,4-dimethoxyphenethylamine, and that di-*O*-methylation of administered labelled dopamine can be demonstrated *in vivo*; however, others have been unable to confirm this observation[7]. Indeed, the identity of the urinary amine (the "pink spot", as it has been called[4]) has not been determined unequivocally, and the relationship of 3,4-dimethoxyphenethylamine to schizophrenia remains controversial.

We were unable satisfactorily to measure 3,4-dimethoxyphenethylamine in urines of schizophrenics by the Friedhoff paper strip technique, but using an ion exchange resin two-dimensional thin-layer chromatographic method[8] were able to demonstrate the presence of a compound indistinguishable from it in seven of twelve urine specimens from schizophrenic patients, whereas none was found in urines of ten normal subjects examined. The amounts measured, however, were low—3–10 μg/day, compared with the 20–50-μg value reported by Friedhoff.

The major metabolic end-products of the catecholamines are the corresponding acids resulting from deamination and oxidation—the vanilmandelic acid content of urine is normally considered to represent a major portion of metabolized epinephrine and norepinephrine. Evidence has been presented that administered 3,4-dimethoxyphenethylamine[6] follows the same metabolic pathway when administered to humans. This was also shown to be true with rats; animals treated with this ^{14}C-labelled amine excreted 3,4-dimethoxyphenylacetic acid[9] almost exclusively, but with a small portion of the parent amine unless a monoamine oxidase inhibitor was administered. Thus it is apparent that a met[illegible] to determine 3,4-dimethoxyphenylacetic [illegible] would permit an indirect estimation of [illegible] article describes such a method and [illegible] from investigations of urine speci[illegible] of schizophrenic and normal [illegible]

amount of 3,4-dimethoxyphenylacetic acid isolated.) When the chloroform layer had been washed with a small volume of water it was made alkaline by the addition of 2·5 ml. of 0·04 N sodium hydroxide and concentrated *in vacuo* to 5–10 ml. The aqueous layer was removed and combined with two additional aqueous extracts of the chloroform layer. The acids were separated from the aqueous solution by re-extraction into chloroform at *p*H 1·5–2·0. The residue obtained by evaporation of the chloroform extract under a nitrogen stream was redissolved in a small volume of that solvent and applied to a 20 × 20-cm silica gel *G* thin-layer plate. After development with the upper phase of a solvent mixture consisting of glacial acetic acid, benzene and water (3 : 2 : 1) in an atmosphere saturated with the lower phase, the 3,4-dimethoxyphenylacetic acid zone was located by visualization of guide spots of the acid (introduced initially adjacent to the urinary extract zones) with ultra-violet light, or by spraying with alkaline permanganate. The appropriate area was scraped into a small column containing a plug of glass wool and the 3,4-dimethoxyphenylacetic acid zone eluted with 6 ml. of chloroform containing acetic acid (5 per cent); the solvent was removed under a nitrogen stream.

The analysis of urinary aromatic acids (as their methyl esters) by gas chromatography is well established[10]. In order to increase confidence in the identification of methyl 3,4-dimethoxyphenylacetate two stationary phases of different chemical nature were used routinely—the polar polyester neopentylglycol adipate (NGA)[11] and the relatively non-polar two-component phase *F*-60-*Z* (ref. 12). (In the early stages of this investigation, when only one gas chromatography column was used and before the thin-layer chromatography conditions had been optimized, it was observed with several samples that, following acetylation, a major decrease in peak size (gas chromatogram) occurred, indicating that before acetylation an acylable compound was eluted at the same rate as methyl 3,4-dimethoxyphenylacetate. Under the final established set of experimental conditions this phenomenon was not encountered.) A second more polar polyester, ethyleneglycol succinate (EGS)[11], was used in selected instances. (Gas chromatographic conditions, NGA: 8 per cent NGA on 80–100-mesh 'Gas Chrom *P*' prepared according to the method of Horning *et al.*[13]; 6 ft. × 4 mm glass U-tube; 170° C; 13 lb./in.^{2}. Gas chromatographic conditions, *F*-60-*Z*: a mixture of 4·9 per cent *F*-60 (a methylpolysiloxane polymer containing a low percentage of *p*-chlorophenyl groups; Dow Corning Corp.) and 0·9 per cent EGSS-Z (a copolymer from ethylene glycol, succinic acid and a methylphenyl-siloxane monomer; Applied Science Labs., Inc.) on 80–100-mesh 'Gas Chrom *P*' prepared [illegible] × 4 mm glass U-tube; 150° C; 9 lb./in.^{2}. [illegible]ic conditions, EGS: 8 per cent ethyl[illegible]60–100-mesh 'Gas Chrom *P*' pre[illegible]m glass U-tube; 185° C; [illegible]ethyl 3,4-dimethoxy[illegible] min. *F*-60-*Z*:

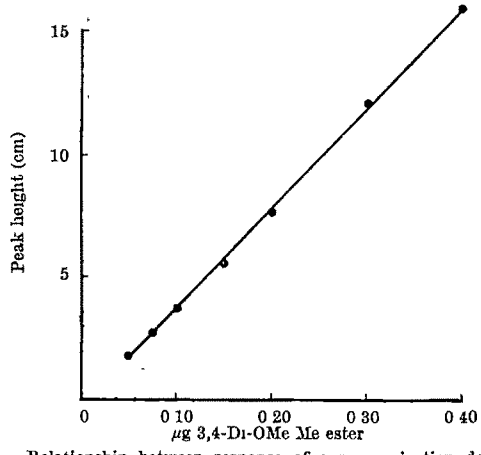


Fig. 1. Relationship between response of argon ionization detector (peak height in cm) and sample size (μg methyl 3,4-dimethoxyphenylacetate) with an *F*-60-*Z* column

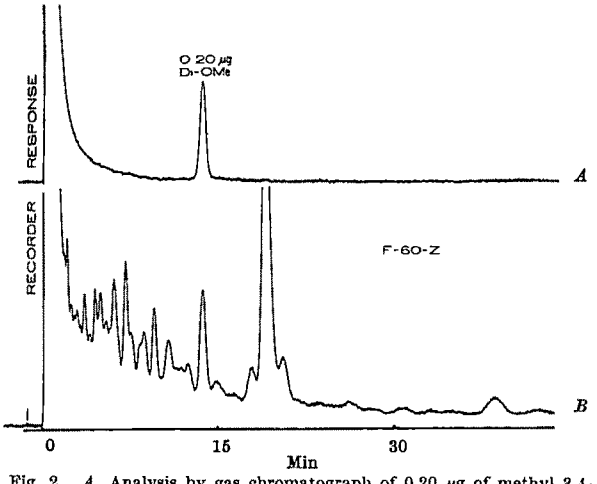


Fig. 2. *A*, Analysis by gas chromatograph of 0 20 μg of methyl 3,4-dimethoxyphenylacetate. *B*, Analysis by gas chromatograph (following esterification with diazomethane) of the 3,4-dimethoxyphenylacetic acid zone from the thin-layer chromatography of the urine of an acute schizophrenic individual This sample is equivalent to 5 ml. of urine, one two-hundred and fiftieth of a 24 h urinary output See text for column conditions

stream of nitrogen) some homovanillic acid, a normal constituent of urine, might be converted to methyl 3,4-dimethoxyphenylacetate was ruled out on the following grounds. Homovanillic acid was well separated from 3,4-dimethoxyphenylacetic acid by thin-layer chromatography, and in the few instances when methyl homovanillate was observed on the gas chromatograms (retention time relative to methyl 3,4-dimethoxyphenylacetate: *F*-60-*Z*, 0·92; NGA, 1·32) the quantities were too small to account for more than a minor fraction of the methyl 3,4-dimethoxyphenylacetate peak, since under the conditions of methylation less than 1 per cent of homovanillic acid could be converted to methyl 3,4-dimethoxyphenylacetate.

The linearity of response of the argon ionization detection system (800 V) to methyl 3,4-dimethoxyphenylacetate was determined by a dilution procedure. Fig. 1 shows a plot of peak height (cm) versus sample size (μg), and the relationship found for the system indicates that mass linearity existed at the level of sample size of interest for our work. In order to allow for slight day-to-day changes in the sensitivity of the detector and the operating conditions of the gas chromatograph, the detector response for known amounts of methyl 3,4-dimethoxyphenylacetate was determined during each series of sample analyses; the amount of the ester present in the urinary sample was then read from the plot. The reproducibility of detector response was excellent; for example, three consecutive injections of 0·15 μg of methyl 3,4-dimethoxyphenylacetate

Table 1. 3,4-DIMETHOXYPHENYLACETIC ACID CONTENT OF URINES FROM NORMAL AND THREE TYPES OF SCHIZOPHRENIC INDIVIDUALS (μg/g creatinine)

Normal	Acute	Sub-acute	Chronic
26	32	20	27
22	31	19	2*
19	23	13	2*
10	23*	12	
9*	12	12	
5*	2*	10*	
4*	Neg.	10	
Neg		10	
Neg.		9	
		6*	

* μg/l

gave an average peak height of 6·78 ± 0·05 cm. Reproducibility of data concerning the 3,4-dimethoxyphenylacetic acid content of urinary fractions was also satisfactory. After an interval of several days a sample which had exhibited a 3,4-dimethoxyphenylacetic acid level of 22 μg/g creatinine on EGS (19 on *F*-60-*Z*) was re-submitted for analysis on EGS on a double blind basis; the second value was 23 μg/g creatinine. (In general, values found with the different columns agreed to a satisfactory extent. One urinary sample, analysed on each of the three columns, gave values of 31 μg/g creatinine (*F*-60-*Z*), 31 μg/g creatinine (NGA) and 29 μg/g creatinine (EGS). The necessity for the use of multiple columns is well illustrated by the case of one urine which exhibited a high value (greater than 100 μg/g creatinine) on *F*-60-*Z*, but proved to be negative on NGA.)

The specificity and accuracy of the procedure were established by adding known quantities of 3,4-dimethoxyphenylacetic acid to distilled water or urine. Thus the addition of 3·0 μg of acid to 100 ml. of water gave a recovery of greater than 90 per cent, and the addition of 3 μg of acid to 100 ml. of a urine, previously shown to contain 3 μg acid/100 ml., led to a new value of 5·8 μg/100 ml.

Additional evidence for the identification of 3,4-dimethoxyphenylacetic acid was obtained by examining samples from the urine of a sub-acute schizophrenic and a normal individual on an *LKB* model 9000 gas chromatograph mass spectrometer using the molecular separator of Ryhage[14] developed at the Mass Spectrometry Laboratory of the Karolinska Institute, Stockholm. In both cases the peak in the gas chromatogram corresponding to methyl 3,4-dimethoxyphenylacetate was found to possess *m/e* values for the molecular ion (210) and for fragmentation products (for example, 151 and 121) in agreement with those of an authentic specimen of the compound.

As can be seen in Table 1, 3,4-dimethoxyphenylacetic acid was found to be present at levels of 10–30 μg/g creatinine equivalent in a large number of the urines examined. (It has been reported[10] that, under conditions allowing the detection of 300 μg/g creatinine, no 3,4-dimethoxyphenylacetic acid was found in normal urine.) No truly significant difference could be detected between the schizophrenic and normal urines investigated. In addition to the subjects detailed in Table 1, an additional fifty-six individuals were examined on a qualitative or semi-quantitative basis for their urinary excretion of 3,4-dimethoxyphenylacetic acid—here also no significant difference could be detected between the schizophrenic and normal group.

The identity of the urinary excretion product described in this article as 3,4-dimethoxyphenylacetic acid is suggested by chemical properties and thin-layer chromatography, strengthened by gas chromatography on a variety of columns and confirmed by mass spectrometry. In addition, the quantitative nature of the method is well established by accepted methods. (For a brief discussion of the need for reliable methodology in this field see ref. 15.) Therefore, consideration of these data requires some qualifications concerning the suggestion that 3,4-dimethoxyphenethylamine is a compound unique to the urines of schizophrenics. Although the presence of this amine in urine of schizophrenic patients has been indicated

by some investigations[2-4,6,8], including our own, the identity of the compound is based mainly on migration rates on paper or thin-layer plates, and thus cannot be considered to be rigorously established. It may well be noted here that a number of urine specimens examined in our present investigations for the acid metabolite were also studied for the presence of 3,4-dimethoxyphenethylamine by the method previously described[8], all with negative results. Thus our initial interpretation of the observation that the amine is present in urines of five of twelve acute and two of ten chronic schizophrenic patients must be altered in the direction of non-correlation of the occurrence of the amine with schizophrenia. It should be noted, however, that in the present investigations less excessively acute patients were available for study. The possibility that, under conditions of hyperexcitability, not necessarily related to schizophrenia, the di-*O*-methylated amine could be produced by normal COMT occurred to us as an explanation of the occurrence of the amine. Accordingly, we studied the soluble catechol-*O*-methyl transferase[16] of rat liver for the ability to di-*O*-methylate dopamine under a variety of conditions including large excesses of enzyme and *S*-adenosyl-methionine as well as varied amounts of the dopamine substrate. In no case was 3,4-dimethoxyphenethylamine detected although the normal mono-*O*-methyl derivatives of dopamine were formed in abundance based on the incorporation of label from *S*-adenosyl-methionine-methyl-^{14}C. The possibility that the recently described[17] microsomal catechol-*O*-methyl-transferase could account for the formation of 3,4-dimethoxyphenethylamine was similarly investigated, but again with negative results. The observation by Barbeau *et al.*[18] that urine of individuals suffering from Parkinson's disease contains large quantities of 3,4-dimethoxyphenethylamine is of interest and further questions the validity of the concept that the occurrence of 3,4-dimethoxyphenethylamine as a urinary metabolite is unique to schizophrenia. From this observation it can be speculated that the abnormal methylation in Parkinsonians causes a diminution of basic dopamine-levels and could explain the favourable response of such individuals to administration of dihydroxyphenylalanine. We are at present examining the urines of patients with Parkinson's disease for the presence of 3,4-dimethoxyphenylacetic acid.

The possibility that urine levels of 3,4-dimethoxyphenylacetic acid bear no relationship to amine-levels is remote, assuming no fault in monoamine oxidase activity in schizophrenics, as it has been established that in both human subjects and rats[9] administered 3,4-dimethoxyphenethylamine is largely excreted as the acid. Indeed, the endogenous nature of the amine has been questioned; some suggest it to be of exogenous origin[5]. We also feel that the endogenous nature of the metabolite is open to question, because our studies with urine samples from a chronic schizophrenia patient who had received labelled dihydroxyphenylalanine failed to indicate the presence of either ^{14}C-labelled 3,4-dimethoxyphenethylamine or the corresponding 3,4-dimethoxyphenylacetic acid, although gas chromatography of the eluted area on the thin-layer plate revealed the presence of 'normal' amounts of the unlabelled acid.

In summary, we have developed a sensitive method for determining 3,4-dimethoxyphenylacetic acid in urine. Because no significant differences could be detected in the amount of acid present in urines of schizophrenic or normal individuals, we must conclude either that the excretion of 3,4-dimethoxyphenethylamine is not characteristic of schizophrenia or that the amount of 3,4-dimethoxyphenylacetic acid present in urine is not a true reflexion of the amount of 3,4-dimethoxyphenethylamine present. On the basis of evidence at present available we prefer the former interpretation.

We thank Dr. S. Arthur Sugarman of New Jersey Neuropsychiatric Center; Dr. Hans Freymuth, Dr. Shaw Perry and Dr. Walter Hill of Trenton State Hospital; Dr. W. Sacks, Dr. Nathan Kline and Dr. A. Esser of Rockland State Hospital; and Dr. Arnold Friedhoff of New York University School of Medicine for urine specimens used in these studies. We also thank Prof. R. Paoletti and Mr. O. Holmström for making the *LKB* model 9000 gas chromatograph mass spectrometer available to us for the analysis of samples during the Second International Course on Methods for Lipid Research, Milan, Italy, in September 1965; Mr. S. Wikström for carrying out the analyses; and Drs. P. Capella, J. McCloskey and J. Sjövall for their help in interpreting the spectra.

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DIFFUSION-EXCHANGE RESPIROMETER USING THE CARBON DIOXIDE ELECTRODE

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TYPES of respirometers may be classed as either open or closed systems. The latter, such as the Warburg respirometer, generally measure the change with time of pressure, volume, or some other variable from which the rate of oxygen uptake (or carbon dioxide output) is deduced. The open-system respirometer, however, can be designed to indicate uptake or output rates directly by measuring the change in concentration of oxygen or carbon dioxide in air flowing through the respirometer at a known, constant rate. Nevertheless, the more extensive apparatus

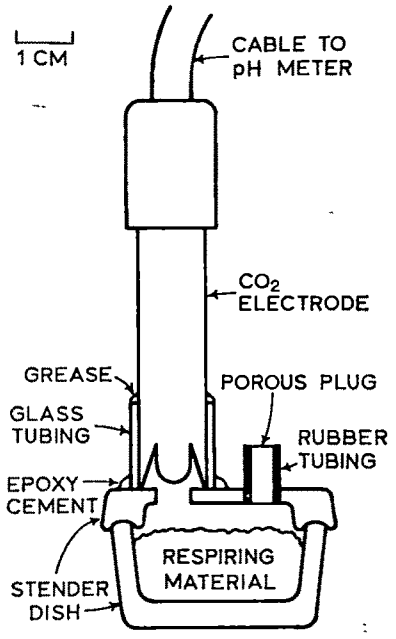


Fig. 1. A diffusion-exchange microrespirometer

required to produce, regulate and monitor the air stream has placed the open-system respirometer at a disadvantage compared with the simpler closed-system types.

The possibility of exchanging air in an open-system respirometer by the process of diffusion rather than mass flow has not apparently been considered previously, although Connelly *et al.*[1] measured the respiration of peripheral nerve with an oxygen cathode by a method based on the relationship of oxygen concentration, uptake and diffusion in the nerve trunk. Using a Severinghaus type of potentiometric carbon dioxide electrode[2] and diffusion exchange, we have devised a new type of open-system respirometer that measures carbon dioxide evolution and has the simplicity of closed-system types.

Essentially, the respirometer (Fig. 1) consists of a chamber containing the respiring material, an electrode to monitor the partial pressure of carbon dioxide within the chamber, and a porous plug in the chamber wall. Gas exchange with the external atmosphere occurs by diffusion through the porous plug in response to concentration differences. Additionally, the total pressure is kept atmospheric by equilibration through the porous plug. As will be shown, the partial pressure of carbon dioxide in the chamber under steady-state conditions is proportional to the rate of carbon dioxide evolution (respiration rate).

The rate of change of the quantity of gaseous carbon dioxide within the chamber at any instant is equal to the rate of carbon dioxide evolution by the respiring material minus the rate of loss by diffusion out through the porous plug, that is:

$$d\rho/dt = (rM/V - J)/v \quad (1)$$

where ρ is the density of carbon dioxide in the chamber in g/μl. of gas volume, t is time in min, r is the rate of carbon dioxide evolution in N.T.P. μl./min, M is the molar weight of carbon dioxide, V is the molar volume (22·41 l./mole), v is the gas volume of the chamber in μl. and J is the rate of diffusion of carbon dioxide out of the chamber in g/min.

If ρ does not change too rapidly:

$$J = h(\rho - \rho_0) \quad (2)$$

where ρ_0 is the density of carbon dioxide in the external atmosphere and h is the permeability coefficient in μl./min of the porous plug for carbon dioxide. Equations (1) and (2) combined are:

$$d\rho/dt = \frac{rM/V - h(\rho - \rho_0)}{v} \quad (3)$$

Equation (3) can be written in terms of the partial pressure p (in mm of mercury) of carbon dioxide by utilizing the ideal gas law:

$$p = \rho RT/M$$

where R is the gas constant (62·36 l. mm deg^{-1} mole^{-1}) and T is the temperature (°K). Equation (3) then is:

$$dp/dt = \frac{rRT/V - h(p - p_0)}{v} \quad (4)$$

where p_0 is the partial pressure of carbon dioxide in the external atmosphere.

Integration of equation (4), assuming that r is constant, yields:

$$p = (p_c + rRT/hV)(1 - e^{-ht/v}) + p_1 e^{-ht/v} \quad (5)$$

where $p = p_1$ at $t = 0$. The exponential terms become zero as the steady-state is approached and equation (5), solved for r, then is:

$$r = hV(p - p_0)/RT = k(p - p_0) \quad (6)$$

where $k = hV/RT$. Thus $p - p_0$ is proportional to r in the steady-state and may be used as a measure of r if h can be evaluated.

Determination of h may be readily done by flushing the empty chamber with carbon dioxide, then closing it and recording p as the carbon dioxide diffuses out through the porous plug. Equation (5) with $r = 0$ applies to this case; in logarithmic form it is:

$$\ln(p - p_0) = \ln(p_1 - p_0) - ht/v \quad (7)$$

Solving for h,

$$h = 2{\cdot}303v(\log[p_1 - p_0] - \log[p - p_0])/t \quad (8)$$

For air, $p_0 \sim 0{\cdot}2$ mm and is negligible in equation (8) if p and p_1 are large. The carbon dioxide electrode potential varies linearly with log p (for $p > 15$ mm); and, since h is constant, the potential should, according to equation (8), decrease linearly with time as the carbon dioxide diffuses out of the chamber. We verified this linearity experimentally as shown in Fig. 2. The slope of the line B is $-(\log[p_1 - p_0] - \log[p - p_0])/t$. By weighing the chamber before and after filling with water, v may be determined, and h and k then calculated.

For $(p - p_0) < 15$ mm, the electrode potential (curve A) in Fig. 2 departs from the linear relationship (B) of equation (8). This departure results partly from p_0 being significant at low p, but it is largely a characteristic of the electrode observed previously[3]. After the porous plug has been calibrated, it is better to make respiration measurements in this lower range of p to avoid any effects of higher carbon dioxide evels on the respiring material. Consequently, the electrode reading must be corrected and the curves in Fig. 2 may be used as a convenient nomogram for this purpose. The steady-state electrode potential from a respiration measurement is first located on the curve A. The point on the line B at the same abscissa then is that for $\log(p - p_0)$, and $p - p_0$ thus determined may be used in equation (6) to calculate r. Or, for a particular porous plug and temperature (k value), Fig. 2 could be scaled to read r directly.

In using Fig. 2 to obtain $p - p_0$, p_0 is assumed to be the same as when the curve was determined. Fluctuations of p_0, if not extreme, will introduce very small errors because p_0 itself is small for air. A porous plug with h too large (or a chamber with v too small) should not be used to obtain calibration curves as in Fig. 2, because the slope

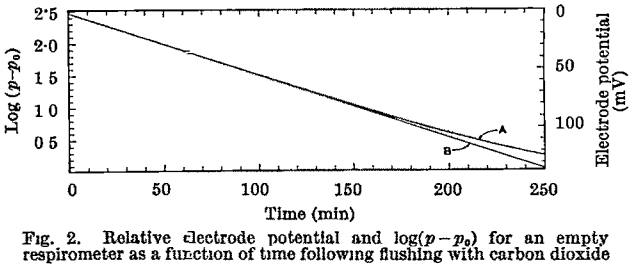


Fig. 2. Relative electrode potential and $\log(p - p_0)$ for an empty respirometer as a function of time following flushing with carbon dioxide

will then be great enough that a significant error from lag of the electrode response will be introduced.

A correction of the electrode potential for drift should be made occasionally by calibration with a gas of known carbon dioxide level (greater than or equal to 2 per cent to be in the region where A and B coincide in Fig. 2).

Fig. 1 shows a stender preparation dish converted to a respirometer which we have used for soil respiration measurements. Two holes were drilled in the lid, one for the porous plug and the other for the electrode. The rim of the dish was sealed with grease when a measurement was made. The soil was incubated in the same dish with an ordinary lid (ungreased) prior to measurement and was oven-dried and weighed in the same dish following measurement. The electrodes we used were temperature-compensated and kindly provided by Dr. G. Matsuyama, Beckman Instruments, Inc., Fullerton, California. Electrode potential was measured by a Beckman model 76 pH meter with an attached recorder. A special adaptor box between the electrode and pH meter contained temperature compensation circuitry.

Fig. 3 is a typical respiration measurement curve showing the approach to a steady-state carbon dioxide partial pressure in accordance with equation (5). The respiring material was Yolo silt loam with added sucrose. The time required to reach a steady state is 1–2 h. This long time is a disadvantage of the method. However, respiration rates that change slowly may be followed continuously with good accuracy once the steady state is approximated. Equation (5) indicates that the approach to a steady state may be hastened by increasing h and decreasing v. But increasing h reduces the steady-state value of p with a concomitant loss of sensitivity. Preferably, v should be reduced by filling the chamber as full as possible with the respiring material.

We made porous plugs as follows: 12 per cent by weight of solder glass (Code 7570, 325 mesh, from Corning Glass Works, Corning, New York) was mixed with 50μ-diameter glass beads ('Superbrite 660–5005', from Minnesota Mining and Manufacturing Co., Saint Paul, Minnesota) and poured into a length of 4-mm-inner-diameter glass tubing which served as a mould. On heating to ~600° C, the solder glass melted and formed capillary films at the contact points of the glass beads, thus cementing the beads into a porous solid when slowly cooled. The plugs were cut to length, removed from the glass tubing and inserted in tight rubber tubing so that only the ends were exposed. The dimensions of a plug for a given value of h could be approximately predetermined since h varies directly with the cross-sectional area and inversely with the length of the plug. The range of the respirometer can be prescribed by using a plug of appropriate h value (see equation (6)) and a chamber of such volume v as to give a suitable equilibration time according to equation (5).

The diffusion coefficient of carbon dioxide into air is nearly proportional to T^2 (ref. 4). Hence h should vary linearly with T^2 and k should vary linearly with T. For example, for a porous plug with $k = 0{\cdot}287$ μl./min/mm at 25° C we obtained a linear temperature coefficient from 14° to 33° C of 0·00228 μl./min/mm/°C. The departure of curve A from line B in Fig. 2 is also temperature dependent. For the electrodes we used, a single calibration curve could be used over a range of 5° without significant error.

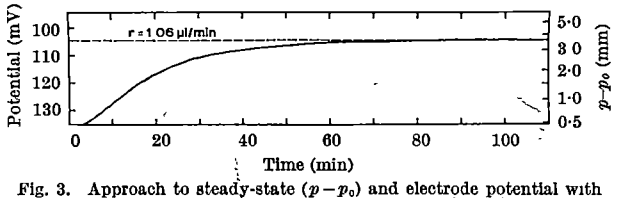


Fig. 3. Approach to steady-state ($p - p_0$) and electrode potential with time after closure of a respirometer containing respiring soil. $k = 0{\cdot}291$ μl./min/mm

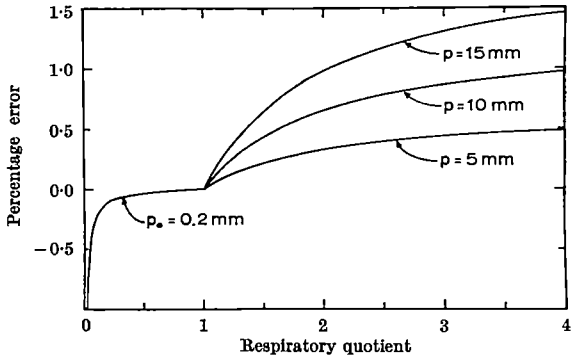


Fig. 4. Percentage error of r resulting from mass flow as a function of the respiratory quotient

Movement of the external air can also affect h by changing the thickness of the diffusive air layer at the outer end of the porous plug. For example, for a porous plug at 26·5° C, k was 4·5 per cent greater in a plant growth chamber with rapid air circulation than in a still room. Hence, average air movement during measurements should be similar to that during calibration of the plug.

If the respiratory quotient differs from unity, a total pressure change will develop within the chamber and produce a mass flow of air into or out of the chamber depending on whether the quotient is less than or greater than one. The mass flow of carbon dioxide may be approximately accounted for in the steady state ($dp/dt = 0$) by adding a term to equation (4):

$$0 = (rRT/V) - h(p - p_0) - \frac{760T(r-x)}{273P}p, \quad \mathrm{RQ} > 1 \qquad (9)$$

$$0 = (rRT/V) - h(p - p_0) - \frac{760T(r-x)}{273P}p_0, \quad \mathrm{RQ} < 1 \qquad (10)$$

where x is the rate of oxygen uptake in N.T.P. μl./min, P is the total (atmospheric) pressure in mm of mercury, and RQ ($= r/x$) is the respiratory quotient. If equations (9) and (10) are re-arranged and compared with equation (6), the error due to mass flow will be seen to be approximately:

$$760V(r-x)p/273RP \sim 0{\cdot}0013(r-x)p, \quad \mathrm{RQ} > 1$$
$$760V(r-x)p_0/273RP \sim 0{\cdot}0013(r-x)p_0, \quad \mathrm{RQ} < 1$$

The percentage error is obtained by multiplying by $100/r$.

$$\text{Percentage error} = 0{\cdot}13(1 - [x/r])p, \quad \mathrm{RQ} > 1 \qquad (11)$$
$$\text{Percentage error} = 0{\cdot}13(1 - [x/r])p_0, \quad \mathrm{RQ} < 1 \qquad (12)$$

Plots of equations (11) and (12) in Fig. 4 show that the mass flow error is less than 1 per cent for the usual range of RQ.

One advantage of the method is that v does not appear in equation (6). Therefore, once calibrated, using a chamber of known v, the porous plug may be used with other chambers of unknown volume. Also, no correction need be made for the volume of the respiring material; this is advantageous for material such as soil the volume of which cannot be measured directly. This fact, together with the fact that the interior of the chamber is at atmospheric pressure, makes convenient forms of the respirometer in which only an intact portion of an organism, for example, plant roots, can be sealed into the chamber and its carbon dioxide output measured.

Both h and $p - p_0$ can be determined to three significant figures with careful measurements, thus determinations of r to within 1 per cent should be possible for RQ values near unity.

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LETTERS TO THE EDITOR

PLANETARY SCIENCE

Sediment Transport by the Antarctic Bottom Current on the Bermuda Rise

THE basic pattern of bottom water circulation in the Atlantic Ocean was well described in the reports of the *Meteor* Expedition[1]. On the basis of dynamic topography, Wüst[2] calculated average current velocities of 2–15 cm/sec for the axis of the northward flowing Antarctic Bottom Current in the South Atlantic. These velocities, considered high by some workers, met with some scepticism[3], but recent direct current measurements in similar current systems indicate velocities of the same magnitude[4]. In the South Atlantic, a good correlation is observed[5] between the occurrence of ripple and scour marks and the location of the Antarctic Bottom Current as determined by Wüst. Recently, Heezen, Hollister and Ruddiman[6] observed strong scour and other effects of bottom currents[6] on the continental rise and Blake-Bahama Outer Ridge[7]. They inferred[6] that geostrophic 'contour' currents in the deep sea are not only effective in transporting sediments, but constitute one of the principal agents in the shaping of the great sediment bodies of the continental margins[8]. These conclusions were based on an investigation of oriented bottom photographs, sediment cores and echograms. We have extended this study to the Antarctic Bottom Current and its effects on the Bermuda Rise.

In November 1965, on a cruise of the Duke University R.V. *Eastward*, a series of bottom photographs were taken between Cape Hatteras and Bermuda (Table 1 and Fig. 1). One of the stations on the continental rise beneath the Western Boundary Undercurrent[9,10] showed identical scour to that reported by Heezen, Hollister and Ruddiman[6] and, as predicted by them, the direction of current features was south-west, parallel to the contours. A photograph station from the lower continental rise showed smoothing without recognizable texture and the water was noticeably murky.

Table 1. BOTTOM PHOTOGRAPH STATIONS

Station No.	Location	Depth (m)	No. of photographs	Current magnitude and direction	Description of bottom as shown in photographs
3479	33 06 N. 70 04 W.	5,354	21	Moderate (no compass)	Sea pens bent over; various size mounds with tracks and trails
3480	32 48 N. 68 04 W.	5,145	26	Weak N.E.	Numerous track trails and various size mounds. Few manganese nodules
3482	32 32 N. 66 29 W.	4,986	16	Moderate N.E.	Numerous manganese nodules; several large tracks and various size mounds; sea pens strongly bent down-current
3488	31 58 N. 64 49 W.	4,148	18	Tranquil	Numerous small mounds with burrows and tracks
3500	33 02 N. 69 20 W.	5,355	20	Weak N.E.	Generally smooth bottom with many small mounds and a few manganese nodules
3501	33 00 N. 70 32 W.	5,278	16	Moderate N.E.	Smooth bottom with sponge, sea pens and crinoids bent down-current. Tracks and trails and few small mounds. 'Coca-Cola' bottle with light coating of sediment
3502	32 59 N. 71 35 W.	5,272	15	Tranquil	Very smooth bottom; few various sized mounds, burrows and tracks
3503	33 00 N. 72 33 W.	5,261	9	Very weak	Very murky water, soft bottom with large mounds
3507	33 32 N. 74 33 W.	4,617	14	Moderate to strong S.W.	Large sediment streamers; very soft bottom; large mounds with few tracks and faeces; water murky

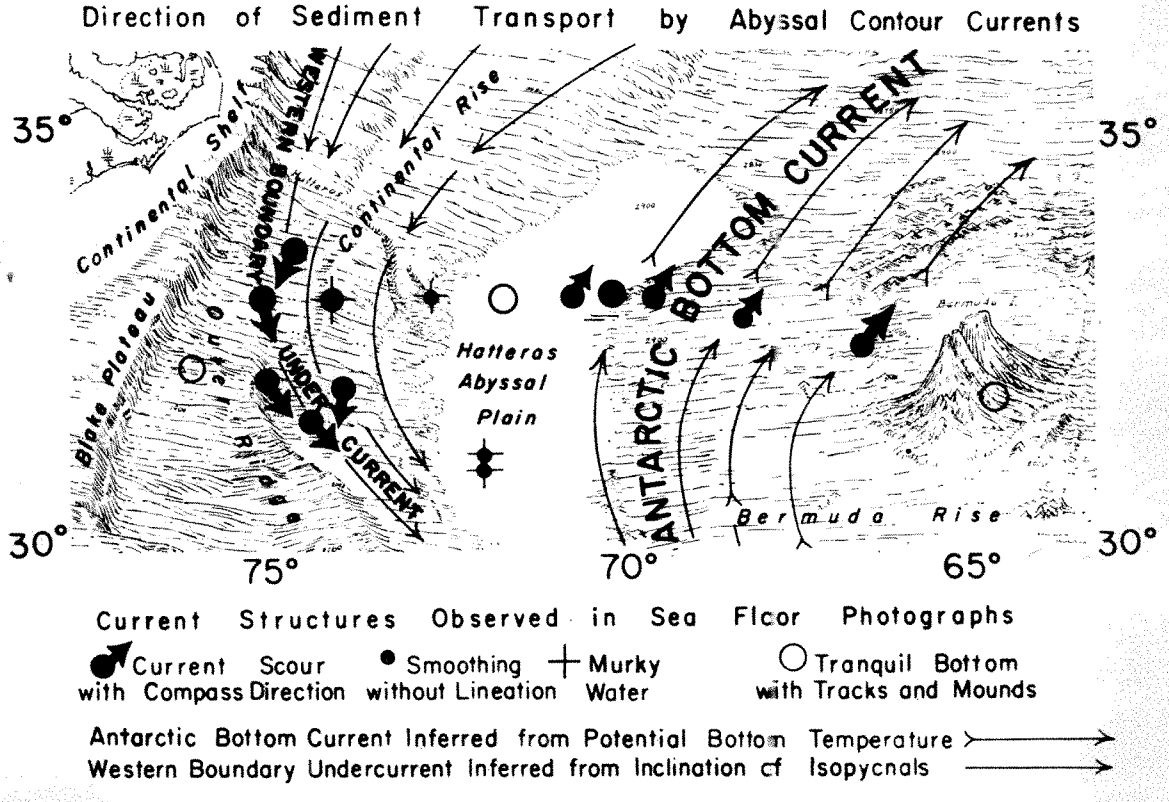


Fig. 1. Sediment transport in the western North Atlantic (ref. 6). Physiographic diagram (ref. 7)

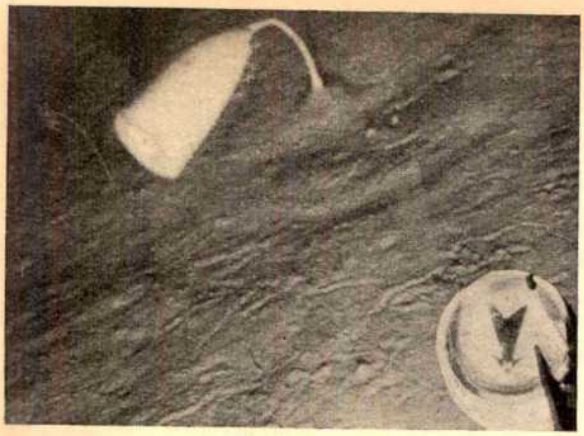


Fig. 2. Sponge and crinoid deflected to the north-east by the Antarctic Bottom Current. Compass 8 cm in diameter. Sponge has scribed a circular mark on the bottom from the north-west to the north-east suggesting that greater velocities sometimes bend the sponge to the sea floor and that the current varies in direction from N.W. to N.E. Station 3501

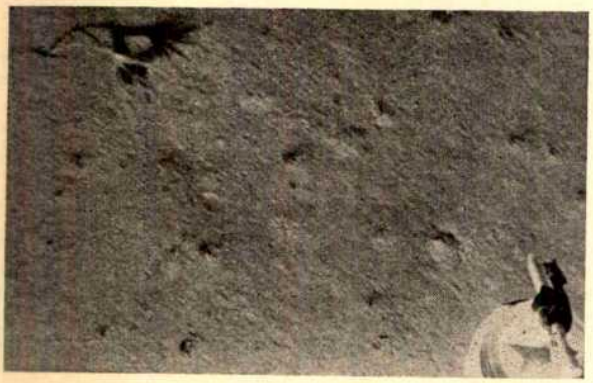


Fig. 3. The north-east fabric lineations result from the action of the Antarctic Bottom Current. Sea pen is strongly bent down-current. Note sediment streamers behind the dark-coloured manganese nodules. Station 3482

The Antarctic Bottom Current, as mapped by potential bottom temperature, is known to flow along the western flank of the Bermuda Rise[11,12]. The principal object of this series of photographic stations was to explore the effects of current on the bottom underneath this current. Five photograph stations on the bottom showed weaker current scour than was detected on the continental rise off Cape Hatteras, but the current was no less definite. The water appeared clearer and life was more abundant than observed under the muddy[13] Western Boundary Undercurrent. This latter fact led to the dramatic photographs of current-defected sponges and sea pens which were clearly bent with the current (Figs. 2 and 3). Manganese nodules were observed scattered over the bottom at several stations. Depositional tails of sediment on the lee side of these objects were often marked.

Evidence of the current was seen in the photographs taken on the eastern side of the Hatteras Abyssal Plain and on the Bermuda Rise. At all stations at which the pictures were compass-oriented, currents toward the north-east were indicated by the down-current deflexion of attached animals and the elongation of sediment streamers. These indicated a current parallel to the general contours of the area. The bottom photographs showed variations in the amount of scour and current evidence, but no quantitative estimates of current velocities could be made.

The sediments on the western Bermuda Rise lie near the boundary between red clay (more than 30 per cent carbonate) and globigerina ooze (less than 30 per cent carbonate)[14]. The actual range of carbonate contents is 25–35 per cent. The cores higher up on the Rise and those on the Bermuda Pedestal have a notably higher carbonate content. Most of the cores are thin-bedded. This is shown by variations of colours between manganese-rich and manganese-poor zones. Most of the dry cores split or break into beds along exceedingly thin laminae of very fine silt. The depth at which cold Pleistocene foraminifera give way to warm recent forms has been used to estimate abnormally high sedimentation rates for low carbonate pelagic lutites of the western Bermuda Rise. A post-glacial rate of approximately 3 cm/1,000 yr was inferred. As the penultimate interglacial was not recorded, late glacial rates could not be determined but were at least twice as high.

The Bermuda Rise is distinguished by smoothed low rolling hills. Seismic studies[16] indicate that its basement is covered by a relatively uniform mantle of nearly unstratified sediments 1–2 km thick.

The Antarctic Bottom Current may be a principal controlling factor determining the topography and sediment distribution of the Bermuda Rise. Its effects are less dramatic than the Western Boundary Undercurrent as it lacks a significant continental sediment source north of the equator. Its principal effects are in the redistribution of the slowly accumulating pelagic lutites. Since the Antarctic Bottom Current ceases to exist north of the Bermuda Rise, controlled deposition during its final dissipation may be responsible for the broad, thick, almost unstratified mantle of sediment which covers most of the Bermuda Rise.

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A Major East-north-east Dislocation Zone in Central Malawi

WORK by the Geological Survey of Zambia[1,2] has revealed the presence of a number of major E.N.E.-trending transcurrent dislocation zones in the Pre-Cambrian rocks of that country. Such zones are characterized by intense

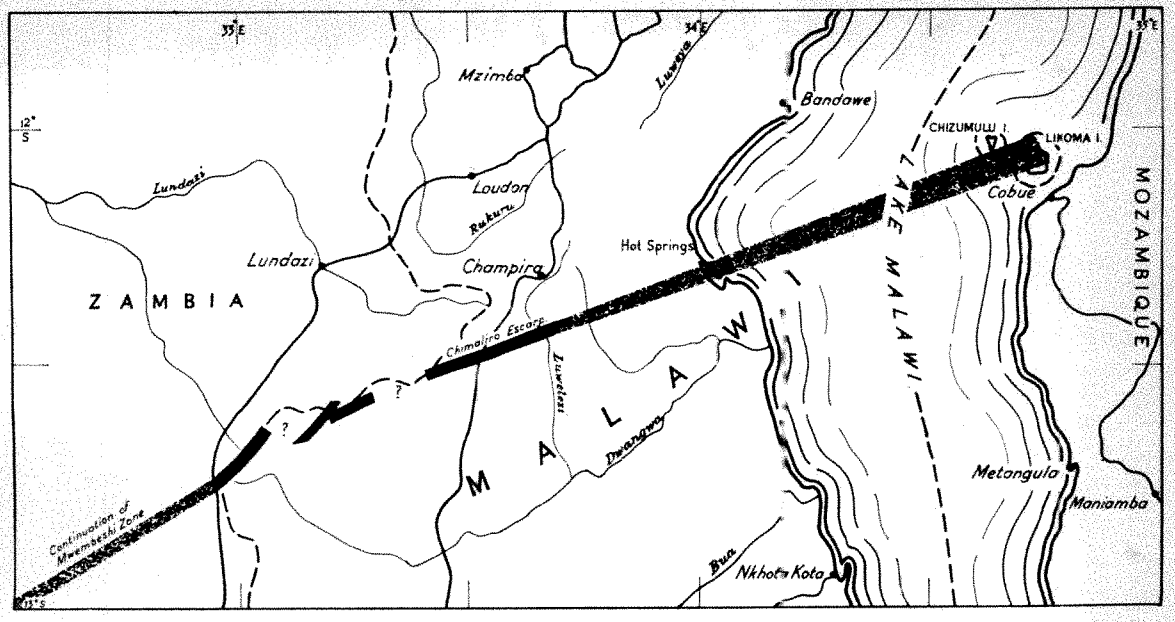


Fig. 1. The E.N.E. dislocation zone in central Malawi. The shaded bands represent probable extensions of the zone

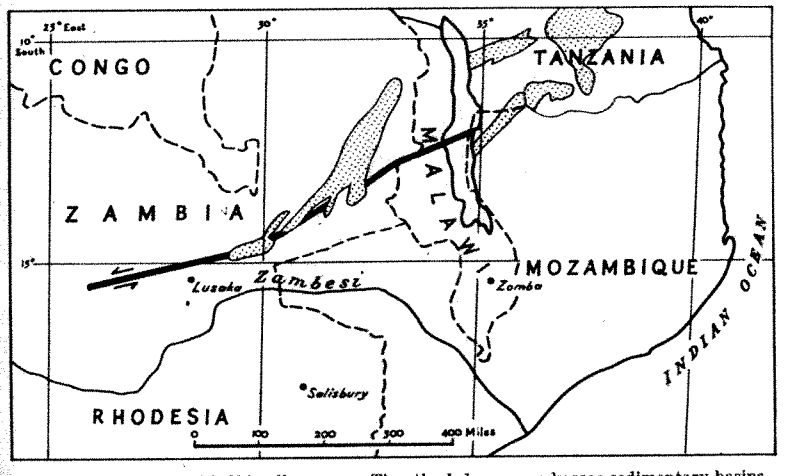


Fig. 2. The Mwembeshi–Chimaliro zone. The stippled areas are karroo sedimentary basins, partly fault-bounded

shearing, refoliation, blastomylonites and silicified gneisses. The best-defined is the Mwembeshi zone which can be traced discontinuously for some 500 miles eastwards from Mumbwa and is inferred[2] to enter Malawi at about the 12° 45′ S. parallel (Fig. 1).

Cooper's small-scale geological map of Malawi[3] shows a major fracture in the inferred position, separating rocks of Middle Pre-Cambrian age to the south from those of Upper Pre-Cambrian age to the north. Subsequent reconnaissance work has confirmed the existence of this fracture, and Chapusa[4] has traced a mile-wide band of mylonite for at least 20 miles along the crest of the Chimaliro escarpment (Fig. 1): the rocks to the north are high-grade psammitic gneisses containing garnet and sillimanite and, to the south, biotite-gneisses and phyllonites, contrasting lithologies but not of proved different ages. Peters[5] has mapped crushed quartzites, trending between N.E. and N.N.E., along the Zambia border between the 12° 45′ S. and 12° 30′ S. parallels and will have completed the mapping of the whole length of the fracture by the end of 1966.

Likoma and Chizumulu islands, which lie in Lake Malawi just off the Mozambique shore, have recently been shown to consist largely of graphic biotite–granite containing xenoliths of pelitic schist and (apparently) intruded by gabbros and microtonalites. The granites are strongly sheared along E.N.E. lines, a direction also followed by a number of major faults. The mafic rocks, although thermally altered, are not cataclastic and the relative age of the latest shearing movement is therefore apparent.

It is suggested that Likoma and Chizumulu lie on the eastern continuation of the Mwembeshi–Chimaliro dislocation zone, which thus trends E.N.E. for at least 600 miles from western Zambia to the eastern shores of Lake Malawi as shown in Fig. 2. Kazali hot springs on the western lake-shore also lie on this line.

Further to the east, in northern Mozambique and south-eastern Tanzania, no major dislocation is known, but the zone may be represented by the boundary faults of large karroo sedimentary basins in this area[6] and, still farther to the north-east, it may swing to follow the Tanzania 'fall-line'[7].

Although the Mwembeshi zone in Zambia follows an old line of weakness, its continuation in Malawi is represented, in part, by a mylonite band and a long escarpment and must be of comparatively recent age. Rejuvenation, possibly by normal faulting, seems likely. Further south, three other E.N.E. lines of weakness, apparently unrelated to those noted so far in Zambia, have been found to cross Malawi. One is followed by a line of infracrustal ring-complexes of Pre-Cambrian age[8] and the other two are associated with Lower Cretaceous plutonic activity[9,10].

The relationship of the Mwembeshi–Chimaliro zone to the rift faults bordering Lake Malawi merits some consideration. In Zambia the zone shows strong sinistral drag[1,2] but, if it is to be regarded as responsible for the apparent offset of the rift faults, movement in the opposite sense would be expected. An explanation of this may lie in the fact that the dislocation is caused by a transform fault as recently defined by Tuzo Wilson[11]. The parallel rift faults could have cut across an older E.N.E. line of weakness and the offset of the opposite sides is not a normal transcurrent displacement but due rather to a sinistral transform fracture as shown in Fig. 3. If this is so, it provides added support to Wilson's sugges-

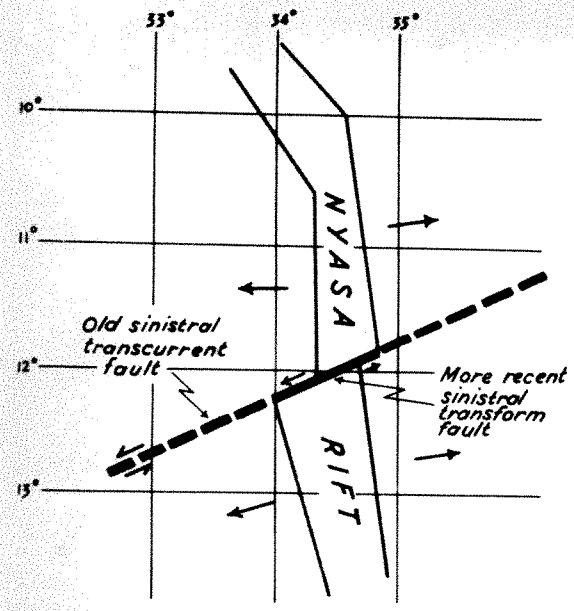


Fig. 3. Suggested relationship between the E.N.E. dislocation zone and the Nyasa rift

tion[11] that the African rift valleys are incipient mid-oceanic ridges.

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Modification of the Model for Internal Structures in Graded Beds to include a Dune Division

AN important recent advance in our understanding of sedimentation in flysch basins is the discovery that the internal structures of most graded sandstones and limestones follow a fixed sequence. Proceeding upwards in a graded bed, the model-sequence described by Bouma[1] consists of five structures: (*A*) graded division; (*B*) lower division of horizontal lamination; (*C*) division of current ripple lamination, often deformed into convolute lamination; (*D*) upper division of horizontal lamination; (*E*) pelite division (Fig. 1). This sequence, or partial sequences with base, middle, and/or top truncation of one or more of the divisions, characterizes graded sandstones and limestones in many flysch basins in North America and Europe.

Walker[2] correlates these internal structures with bed configurations observed in flume experiments using medium sand (Simons and Richardson[3]; Table 1). The sequence of internal structures in graded beds reflects bed configurations that progressively develop as a current slows down.

The configuration of the dune bed that characterizes the upper part of the lower flow régime in flume studies

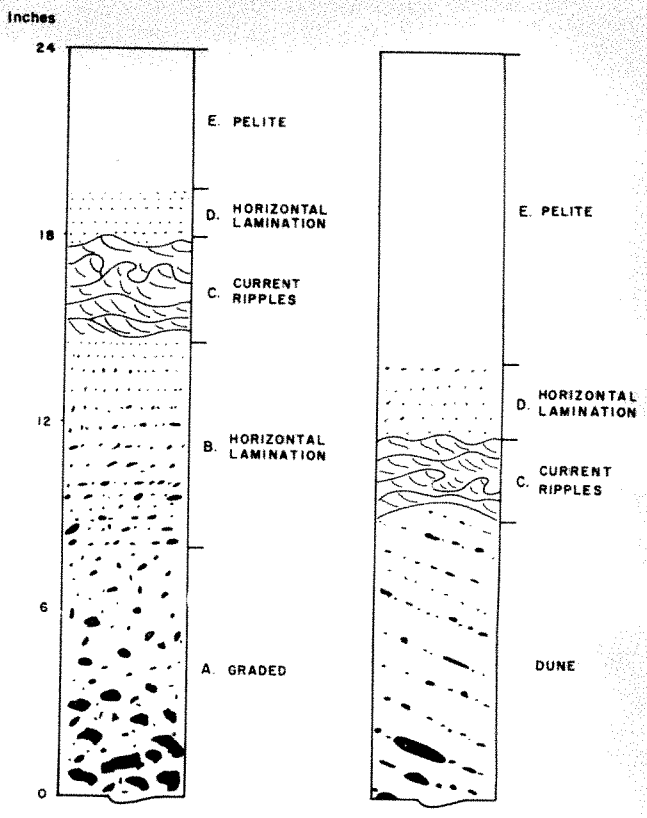


Fig. 1. Sequence of internal structures in graded sandstones and limestones. Sequence after Bouma (ref. 1) (left) and modification to include dune structure (right)

has not previously been reported for either graded sandstones or limestones. The dune structure does occur, however, for example in about 20 per cent of the graded limestones in a limestone flysch in the Upper Ordovician Whitehouse Formation in the Girvan District, southwest Ayrshire, Scotland. This limestone flysch section although faulted is at least 165 ft. thick, and consists of 65 per cent graded, sandy limestone beds, 8 per cent graded sandstones, 24 per cent silty micaceous green shale interbeds, and 3 per cent intraformational flat-pebble conglomerates (Table 2). The cross-bedded dunes in the limestones have wave-lengths of 15–20 ft. and amplitudes of 5–8 in. A *CDE* sequence of current ripples, horizontal lamination, and pelite overlies the dunes in each bed. The current ripples are occasionally deformed into convolute lamination whereas the dunes do not show deformed laminae. The *CDE* sequence is commonly slightly thicker than the basal dune structure.

The large wave forms in the Whitehouse graded limestones are dunes but not antidunes. In flume experiments[4],

Table 1. CORRELATION BETWEEN BED CONFIGURATIONS OBSERVED IN FLUMES AND INTERNAL STRUCTURES IN GRADED LIMESTONE AND SANDSTONE BEDS (AFTER WALKER) (REF. 2)

Bed configurations (Simons and Richardson (ref. 3))	Internal structures
Lower flow régime	
(1) Plane bed without movement	*E*. Pelite division. Mud settles out from current
	D. Upper division of horizontal lamination. Mud and silt settle out through laminar boundary layer
(2) Ripples	*C*. Division of current ripple lamination
(3) Dunes with ripples superimposed, and	Dunes
(4) Dunes	
Transition (bed roughness ranges from dunes to plane bed or standing waves)	
Upper flow régime	
(5) Plane bed with movement	*B*. Lower division of horizontal lamination
(6) Standing waves, and	*A*. Graded division. No specific bed configuration preserved
(7) Antidunes	

Table 2. WHITEHOUSE LIMESTONE FLYSCH SECTION, WHITEHOUSE FORMATION

Thickness and internal structure of 100 graded limestones and 28 graded sandstones, and thickness of green shale and intra-formational breccia interbeds

Thickness (in.)	Graded limestones (65 per cent)								Graded sandstones (8 per cent)							Green shale (24 per cent)	Intra-formational breccia (3 per cent)
	Dune-*CDE*	*ABCDE*	*BCDE*	*CDE*	*DE*	*ABC*	*C*	Total	*ABCDE*	*BCDE*	*CDE*	*BC*	*C*	*E*	Total		
0–0·5	—	—	—	—	1	—	3	4	—	—	1	—	7	—	8	8	—
0·5–1	—	—	—	6	3	—	—	9	—	—	2	—	3	—	5	13	1
1–2	—	—	—	12	4	—	—	16	—	—	1	2	1	1	5	27	1
2–4	1	4	2	8	—	—	—	15	—	—	3	1	—	—	4	30	—
4–8	5	4	1	2	—	2	—	14	—	—	1	—	—	—	1	11	1
8–16	8	7	3	—	—	—	—	18	1	1	—	—	—	—	2	7	—
16–32	7	7	2	—	—	1	—	17	1	—	1	—	—	—	2	2	—
32–64	—	4	2	—	—	—	—	6	1	—	—	—	—	—	1	—	1
64–128	—	1	—	—	—	—	—	1	—	—	—	—	—	—	—	1	—
Total	21	27	10	28	6	3	3	100	4	1	9	3	11	1	28	99	4

antidunes are structureless to crudely cross-laminated, with the cross-laminae inclined "upstream" at low (0°–10°) angles. The dunes in the Whitehouse limestones in contrast show distinct cross-lamination varying in dip from 5°–31°, averaging 17°. The cross-laminae are inclined uniformly down-current to the south-east in the same direction as the flutes on the limestone soles. Furthermore, the dunes in the limestones always occur directly below the current ripple division known to form in the lower flow régime.

The more complete sequences of internal structures tend to occur in the thicker graded limestones and sandstones of the Whitehouse Formation (Table 2). The dune-*CDE*, *ABCDE*, and *BCDE* sequences are common in beds that exceed 1 ft. in thickness. The graded division is commonly very thin, whereas the lower zone of horizontal lamination is thicker and simultaneously graded and laminated. In 1–2 in. beds, *C*, *CDE*, and *DE* sequences predominate. Flute casts occur sparingly on a few thicker and coarser limestones and sandstones implying, for each bed, that the same current both scoured the flutes and deposited the bed.

I have occasionally seen dune structures in graded sandstones in the Schlieren and Wäggital flysch sections of the External Prealps, Switzerland, and in the Lower Westphalian beds of North Devon. However, the reason for the common absence of a dune structure beneath the current ripple division in graded beds is not definitely established. Walker[2] suggests that either the water–sediment mixture lacks sufficient vertical thickness for dunes to form, or deposition was too rapid to allow time for dunes to develop. Perhaps a better lead for further investigation is that the dense, muddy current may inherently suppress development of dunes as suggested by flume experiments[5] in which, as more mud is added to sand, the dunes increase in wave-length and decrease in height.

The occurrence of a dune structure in the graded limestones of the Whitehouse Formation shows that such a structure should be incorporated below the current ripple division of the ideal model of a graded bed.

I thank Dr. E. K. Walton for directing my attention to the Girvan District. The University of Edinburgh provided office and laboratory facilities during my sabbatical leave (1964–65) from the University of Missouri. A grant from the University of Missouri Research Council paid for field expenses incurred. I also thank Dr. W. D. Keller for his help in preparing this communication.

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Glauconite in the Subathu Beds (Eocene) of the Simla Hills of India

AUDEN[1] described the geology of the Pre-Tertiary and Tertiary formations of the Simla Hills and mentioned in passing the occurrence of glauconite in the Subathu beds. No detailed study of the mineral has so far been made. A petrographical investigation of the Subathu rocks of the type area (lat. 30° 58′ N., long. 77° E.), which is still in progress, has revealed that there are several glauconitic horizons in the Subathu Series. In the type area, the Subathus have thicknesses of about 2,500 ft. and are lithologically comprised of limestones, including coquinites, calcareous sandstones and shales which are carbonaceous at the base. Glauconite has so far been found to occur in the limestones and calcareous sandstones. These are fine to medium grained rocks, greyish in colour and at places traversed by secondary veins of calcite. Under the microscope they show variable amounts of clastic constituents—quartz, feldspars, rock fragments (limestones and quartzite) and accessories like glauconite, rutile, zircon, tourmaline, pyrite, chlorite, micas and iron oxides embedded in a calcareous matrix. Among the organic constituents, microfossils (tests of foraminifera) are quite abundant.

From an investigation of thin sections, two modes of occurrence of glauconite in the limestones and calcareous sandstones have been recognized:

(1) *Fillings of chambers of foraminiferal tests.* The chambers of the foraminiferal tests are filled with glauconite, although the number of chambers filled varies from shell to shell. It is clearly a case of replacement of the original calcium carbonate of the chambers by glauconite. In some cases the replaced substance (calcite) is seen recrystallized, forming clusters within the shell (Fig. 1). It is interesting to note that glauconite occurs in the chambers of the broken tests only. On the other hand, the chambers of the intact tests are either colourless or are filled with a brownish isotropic substance which contains inclusions of calcite and quartz. It differs from the glauconite seen in the same slide both in colour and in its behaviour under crossed nicols. The exact nature of this substance could not be determined, but it seems similar to the "clay fillings of foraminifera" of Takahashi (in ref. 2). It might be the starting material in the formation of glauconite as postulated by Takahashi[2]. After the rupture of the tests the clayey material presumably altered into glauconite by adsorption of elements like potassium, magnesium and iron from the marine environment.

(2) *In the matrix.* The glauconite of the matrix has an irregular outline and varies in size from 0·06 to 0·15 mm. A few grains are oblong to rounded and correspond in shape and size to the chambers of the foraminiferal tests; evidently they have come from the broken tests. Isolated grains of glauconite can also be seen in the fragments of limestone embedded in the matrix, indicating that glauconitization has taken place after deposition. Inclusions of calcite in the glauconite and pseudomorphs of glauconite after the rhombohedra of calcite are occasion-

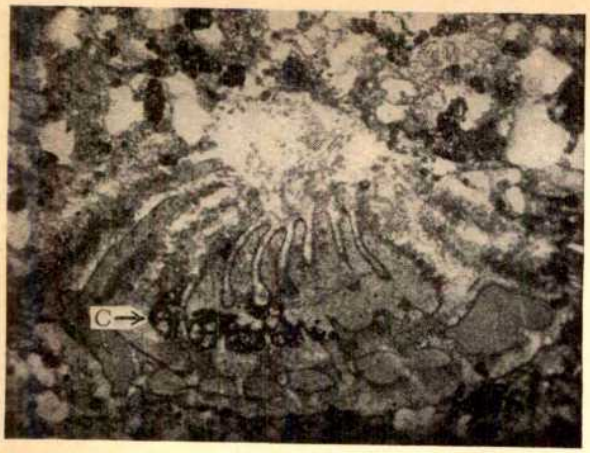


Fig. 1. Photomicrograph of calcareous sandstone showing chambers of a broken foraminiferal test filled with glauconite. The replaced carbonate has recrystallized as calcite (*C*), forming clusters within the test. Ordinary light. (×*c.* 50)

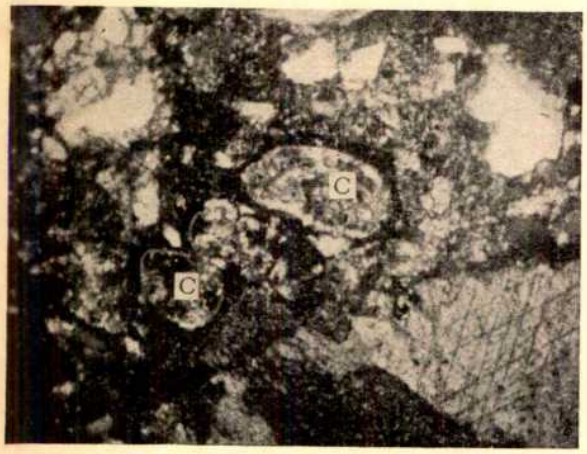


Fig. 2. Photomicrograph showing rings of glauconite round calcite (*C*) in the matrix of calcareous sandstone. Crossed nicols. (×*c.* 50)

ally seen. Continuous peripheral rings of glauconite around calcite are also observed (Fig. 2).

Glauconite forms less than 5 per cent by volume of the rocks as a whole. It has, therefore, not contributed significantly to the colour of the rocks.

As the mineral is soluble in hydrochloric acid, its separation from the matrix by treatment of the rock with hydrochloric acid was not undertaken. Optical investigations were made only in thin sections. Under the microscope the mineral has a yellow-green to light-green colour and is weakly pleochroic. A few grains show a tendency to limonitization. Cleavage is distinct in several grains. At times it is widely separated and in a few cases it is bent due to deformation. Extinction is nearly straight, but wavy extinction and aggregate polarization are frequently seen. Birefringence is low; grains are nearly isotropic. This may indicate a low content of iron in the mineral[3]. Detailed optical determinations are difficult due to the small size of the grains.

The following modes of origin have been ascribed to glauconite in sediments[4]:

Autochthonous glauconite: (*A*) of direct origin formed during sedimentation from colloidal matter, clays or alteration of biotite; (*B*) of replacement origin, involving matrix, microfossils, etc.

Allochthonous glauconite: of detrital origin.

The Subathu glauconite obviously originated by autochthonous replacement. A part of the glauconite of the matrix forming oblong to rounded grains may be regarded as allochthonous in the sense that it represents re-worked fillings of foraminifera. Evidence for replacement is found in the occurrence of inclusions of calcite in the glauconite grains, rings of glauconite around calcite and pseudomorphs of glauconite after calcite. Replacement has affected not only the foraminiferal chambers but also the matrix. In the case of the chambers, however, there is evidence that, in the first place, the original calcium carbonate was replaced by a brownish clayey material which subsequently altered into glauconite. The clayey material might be similar to the protoglauconite of Teodorovich (in ref. 5). Rhombohedra of calcite replaced by glauconite show that glauconite is younger than the diagenetic phase of early carbonate crystallization. Absence of glauconite from the veins of calcite indicates that the mineral was formed earlier than the veins.

Glauconite is an unusual mineral in sedimentary rocks and has gained much geological importance in recent years. The consensus of opinion is that a marine milieu is necessary for its formation in sedimentary rocks. This is borne out in the present case by the presence of glauconite in the Subathu beds of undoubted marine origin. Its occurrence in the calcareous sandstones and limestones and its association with foraminifera and pyrite lend support to the view that a slow rate of sedimentation and weakly oxidizing environmental conditions favour the formation of glauconite. It will be possible to throw light on the role of other factors like temperature, depth, salinity, etc., in its formation after the investigation of the Subathus is complete.

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Composition of the Earth

RECENTLY, Birch[1] reported data on the density and composition of the mantle and core. He wrote: "The resulting densities in the lower mantle are found to be in good agreement with shock-wave measurements on rocks having FeO contents in the range $10 \pm 2\%$ by weight . . . except for iron oxide, the chemical composition of the mantle is indeterminate. The density of the outer core is lower than that of iron by about 10%".

Of the two major groups of chondritic meteorites, the olivine-bronzite and olivine-hypersthene chondrites, the average composition of the bronzite chondrites[2] is close to Birch's initial requirement. Table 1 shows an FeO content of 9·7 per cent, equivalent to 12·6 per cent FeO in the silicate fraction (76·04 per cent of the meteorite matter). The average composition of the hypersthene chondrites is 14·70 per cent FeO, equivalent to 17·0 per cent FeO in the silicate fraction, which is much beyond Birch's requirement of 10 ± 2 per cent FeO. It therefore seems reasonable to use the average composition of the bronzite chondrites to derive a composition for the mantle (plus crust), the core, and for the Earth as a whole.

Birch provided two solutions for the density distribution within the Earth, which for the present purpose differ only slightly. Using his solution II, we have a mantle plus crust making up 67·6 per cent and a core 32·4 per cent of the mass of the Earth. The calculation of the composition (Table 2) is based on the following assumptions: (1) the composition of mantle plus crust is the same

Table 1. AVERAGE COMPOSITION IN WEIGHT PERCENTAGES OF OLIVINE-BRONZITE CHONDRITES (REF. 2)

A		*B*	
SiO_2	36 57	O	33·24
MgO	23·69	Fe	27 79
FeO	9·67	Si	17·10
Al_2O_3	2 30	Mg	14·29
CaO	1·77	S	1·93
Na_2O	0·86	Ni	1 64
Cr_2O_3	0 42	Ca	1 27
MnO	0 33	Al	1 22
P_2O_5	0·26	Na	0·64
K_2O	0·10	Cr	0 29
TiO_2	0 10	Mn	0 25
Fe	16 90	P	0 11
Ni	1·64	Co	0 09
Co	0 09	K	0·08
FeS	5 30	Ti	0 06
	100 00		100 00

A, Expressed as oxides, nickel-iron, and FeS; *B*, expressed as elements

as the oxidic material (silicates plus small amounts of phosphates and oxides) of the bronzite chondrites; (2) the 5·3 per cent of troilite in the bronzite chondrites is incorporated in the core, the remainder of which (27·1 per cent) is nickel-iron of the same composition as in the bronzite chondrites.

Table 2. CALCULATION OF THE BULK COMPOSITION OF THE EARTH

	Metal	Troilite	Silicate	Total
Fe	24 58	3 37	6 68	34 63
Ni	2·39			2·39
Co	0·13			0 13
S		1·93		1 93
O			29 53	29 53
Si			15·20	15·20
Mg			12 70	12 70
Ca			1·13	1 13
Al			1·09	1·09
Na			0·57	0 57
Cr			0·26	0·26
Mn			0·22	0 22
P			0·10	0 10
K			0 07	0 07
Ti			0·05	0 05
	27·10	5 30	67·60	100 00

Mass of core equals 32 4 per cent mass of Earth, mass of mantle plus crust equals 67·6 per cent mass of Earth (ref. 1); core assumed to consist of nickel-iron of composition of average chondrite plus 5·3 per cent troilite, mantle and crust assumed to have composition of silicate material of average chondrite.

These assumptions are certainly oversimplifications. For example, all nickel is assumed to be in the core, whereas terrestrial olivine always contains nickel. (That from peridotite inclusions in basalt usually has 0·2–0·3 per cent nickel.) Some nickel is certainly combined in mantle silicates. Again, the assumption that all the iron sulphide is incorporated in the core is clearly at best an approximation, because some sulphide is certainly present in the mantle and crust. However, the incorporation of iron sulphide into the core rather neatly meets Birch's second requirement, that the density of the outer core is less than that of iron by about 10 per cent. If 27·1 per cent of nickel-iron, density 7·90, is mixed with 5·3 per cent troilite, density 4·80, the resulting mixture will have a density at zero pressure of 7·15, thereby meeting this requirement quite closely. This obviates the necessity of supposing silicon to be in the core, as has been suggested by Ringwood[3] and MacDonald and Knopoff[4]. A nickel-iron core with free silicon is also incompatible with a mantle containing appreciable amounts of FeO, if mantle and core are in chemical equilibrium.

A comparison of the elemental composition of the mantle plus crust with that of the crust reveals some interesting fractionations. The mass of the crust (the solid material above the Mohorovičić discontinuity) is $0{\cdot}024 \times 10^{27}$ g, that of the mantle $4{\cdot}016 \times 10^{27}$ g, so the mass of the crust is only 0·6 per cent that of the mantle. Table 3 shows that the major elements oxygen and silicon and the minor elements manganese and phosphorus show no marked fractionation between mantle and crust. In the crust magnesium is much diminished, relative to the mantle; chromium is also strongly diminished, as the average in the crust is about 0·02 per cent; iron, on the other hand, is only slightly less concentrated in the crust than in the mantle. Aluminium and potassium are strongly enriched in the crust, sodium and calcium to a lesser extent—in terms of phase composition the differentiation of crust from mantle has largely been the accumulation of feldspar in the crust. One notable feature, if the figures in Table 3 are reliable, is the remarkable enrichment of titanium in the crust, almost as great as that shown by potassium. There is no apparent geochemical reason for this enrichment; in fact, titanium might be expected to remain in the mantle as $FeTiO_3$ or in substitution in the pyroxene structure.

Table 3. ELEMENTAL COMPOSITION, IN WEIGHT PER CENT, OF (*A*) CORE, (*B*) MANTLE PLUS CRUST, (*C*) CRUST (*A* AND *B* FROM TABLE 2, *C* FROM REF. 5)

	A	*B*	*C*
O		43·7	45 4
Si		22·5	25·8
Mg		18 8	3 1
Fe	86·3	9 88	6·5
Ca		1·67	6 0
Al		1·60	8·1
Na		0·84	2·2
Cr		0·38	
Mn		0 33	0·2
P		0·14	0 1
K		0·11	1 6
Ti		0 08	1·0
Ni	7 38		
Co	0·40		
S	5 96		

Green and Ringwood[6] recently discussed the composition of the mantle, and decided that it could probably best be represented by a dunite-basalt mixture, arguing that the partial melting of such a mixture would provide the basaltic magma so copiously injected into and through the Earth's crust, and leaving an upper mantle of dunitic composition. They decided the probable proportions of basalt and dunite were 1 : 3, and on this basis arrived at the composition of the mantle given in Table 4, where it is compared with the present estimate from chondritic composition.

Table 4. ESTIMATED COMPOSITION OF (*A*) MANTLE PLUS CRUST (THIS PAPER) AND (*B*) MANTLE (REF. 6)

	A	*B*
SiO_2	48 09	43·06
MgO	31 15	39·32
FeO	12·71	6·66
Fe_2O_3		1·66
Al_2O_3	3 02	3 99
CaO	2·32	2·65
Na_2O	1 13	0 61
Cr_2O_3	0 55	0 42
MnO	0 43	0·13
P_2O_5	0·34	0 08
K_2O	0·13	0·22
TiO_2	0·13	0·58
NiO		0 39
CoO		0 02
H_2O+		0·21
	100·00	100 00

When we consider that these two estimates have been made from completely different basic data, the general correspondence is quite remarkable. There are, however, numerous differences in detail. The calculated mineral composition is similar, with about 80 per cent olivine plus pyroxene and 13 per cent plagioclase, but the proportions of olivine and pyroxene differ in the two compositions, olivine greatly exceeding pyroxene in the Green–Ringwood composition as a result of their choosing dunite as a principal component. In their composition the total iron oxides correspond to 8·1 per cent FeO, just within the lower limit postulated by Birch, whereas the 12·71 per cent FeO in the present estimate slightly exceeds Birch's upper limit for FeO. The Green–Ringwood composition takes account of the nickel which is certainly present in the mantle, and also allows for a small amount of water, which is reasonable. With their figure for titania, the

titanium enrichment in the crust commented on previously becomes much less marked, although still significant. (It is possible that Poldervaart's estimate[5] of titanium in the Earth's crust is excessive.) The two estimates in Table 4 for the composition of the mantle show a marked degree of convergence, and suggest that the information now available does suffice to reach a fair approximation of the chemical composition of the inaccessible parts of our planet below the crust.

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Independent Fission-fields of Caesium-134

THE radionuclide caesium-134 has a half-life of 2·1 years and is reported to exist in the Earth's atmosphere and biosphere including man[1]. The caesium-134 activities measured in air, foods and man are in the range of 0·1–2 per cent of the caesium-134 activity[2–4].

The process responsible for the formation of caesium-134 has been a subject for speculation, but no definite explanation seems to have been offered. One obvious suggestion is the creation of caesium-134 in fission nuclear bombs, but no yield has been reported for the production of caesium-134 in the fission of uranium or plutonium. Other processes in connexion with nuclear bomb devices are neutron capture in inert caesium-133, and neutron-induced reactions such as (n,p), (n,α) and multiple neutron capture in fission products or elements in the environment.

In the present investigation an attempt has been made to ascertain the magnitude of the possible direct fission-yield of caesium-134 by thermal neutron fission of uranium-235. In a reactor, caesium-134 is also produced by neutron activation of the stable fission-product caesium-133. In order to interpret the formation of caesium-134 in the light of these two reaction mechanisms, enriched uranium samples were irradiated for different periods of time. Thus with a short period of irradiation the caesium-134 activity should originate mainly from the fission process. Conversely, the caesium-134 activity should be ascribed mainly to the (n,γ) activation process of caesium-133 when long exposure to irradiation is used.

The accurate determination of low activities of caesium-134 together with strong activities of caesium-137 has previously been connected with certain difficulties, owing to an insufficient resolution of the photo-peaks of these two nuclides with a sodium-iodine crystal. However, recently developed germanium semiconductor detectors revealing a high degree of resolution allow accurate analysis of this kind.

Enriched 5–10 mg samples of uranium (90 per cent uranium-235) were irradiated in the *R2* reactor, Studsvik, with a thermal neutron flux of $\sim 2 \times 10^{13}$ n/cm² sec (fast flux: $\sim 4 \times 10^{12}$ n/cm² sec) for periods of 2·5 h, 1 day, 3 days, 8·8 days and 28·1 days.

The samples were dissolved in nitric acid (1 : 1) containing a few drops of hydrofluoric acid. Caesium carrier was added as well as a few mg of citric acid. The solution was evaporated to dryness and the residue dissolved in diluted hydrochloric acid. The solution was neutralized, and the bulk of the fission products were then separated from the caesium-134 and caesium-137 activities through sorption on an anion exchanger in the form of the hydroxide[6] and also by precipitation of barium sulphate.

The semiconductor detector was a lithium-drifted germanium diode with 2 mm depleted depth and 2·8 cm² area. The gamma-ray spectrum of the separated caesium sample (irradiated for 28·1 days) obtained by this detector, which was connected to a 512-channel pulse height analyser (Nuclear Data), is shown in Fig. 1. Small remaining activities, probably due to zirconium-95–niobium-95, are observed between the caesium-137 : 0·667 MeV peak and the caesium-134 : 0·797 + 0·802 MeV peak.

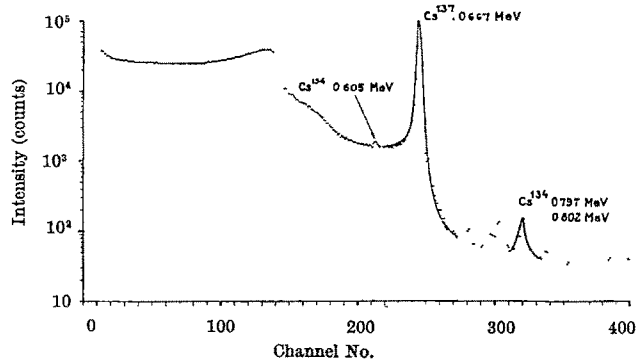


Fig. 1. Gamma-ray spectrum of caesium-134 and caesium-137 separated from the bulk of fission products, obtained by germanium semiconductor detector

Table 1 gives the measured caesium-134/caesium-137 disintegration rate ratio for the five samples investigated. Corrections have been made for differences in gamma-yields as well for the efficiencies of the full energy peaks corresponding to caesium-137 : 0·667 MeV and caesium-134 : 0·797 + 0·802 MeV. In the table are also given the caesium-134/caesium-137 ratios based on activation of casium-133, data from the compilation of Blomeke and Todd[6] being used in this connexion.

Table 1

Irradiation time	Caesium-134/caesium-137 disintegration rate ratio measured	Caesium-134/caesium-137 disintegration rate ratio calculated
2·5 h	$< 4 \times 10^{-4}$	$< 10^{-6}$
1 d	$< 4 \times 10^{-4}$	$< 10^{-5}$
3 d	$< 5 \times 10^{-4}$	$(8 \pm 2) \times 10^{-5}$
8·8 d	$(6 \pm 4) \times 10^{-4}$*	$(7 \pm 2) \times 10^{-4}$
28·1 d	$(35 \pm 9) \times 10^{-4}$*	$(50 \pm 10) \times 10^{-4}$

* Error is given as three times standard deviation.

In three samples irradiated for periods of 2·5 h, 1 day and 3 days respectively, caesium-134 could not be observed. Assuming the full energy peak of 0·797 + 0·802 MeV from this nuclide to be less than three times the standard deviation in the background in the peak region, an upper limit of 8×10^{-5} per cent is obtained for the thermal-fission yield of caesium-134.

For the samples irradiated for 8·8 and 28·1 days, respectively, the measured and calculated caesium-134/caesium-137 ratios were in reasonable agreement, as was expected.

The conclusion is that the direct formation of caesium-134 in fission is a process that may be ruled out as a possible explanation of the occurrence of caesium-134 in fallout. The caesium-134/caesium-137 disintegration rate ratio of 1·6 per cent measured in reindeer in the north of Sweden during March 1961[1] means that the yield of caesium-134 should have an order of magnitude of 10^{-2} per cent. This is a factor of more than one hundred times the upper limit determined in the present experiment.

This result is consistent with that reported by Baerg, Bartholomew and Betts[7], who found an upper limit of $1{\cdot}4 \times 10^{-5}$ per cent for the independent yield of caesium-134*m* in fission. The hypothesis of equal charge displacement indicates, moreover, that the yield of caesium-134 should be of the order of magnitude of 5×10^{-6} per cent[8]. This value is also consistent with the result obtained in the present experiment.

Other processes that might produce the caesium-134 found in fallout are now being investigated.

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THE SOLID STATE

Zinc Micas from Franklin Furnace, New Jersey

PLATY crystals of a dark mica occur in hancockite-bearing rocks from Franklin Furnace, New Jersey, and electron-probe analysis shows that the mica has high zinc (19·8 per cent) and manganese (12·5 per cent) oxide contents, corresponding to about 40 and 30 per cent respectively of the zinc and manganese end-members. So far as we are aware, no description of such a mineral has previously been published. This communication reports analytical, optical and X-ray data for two samples of the mica, one from the collection of the Department of Mineralogy and Petrology, Cambridge, the other from a hancockite–axinite rock presented by Prof. C. Frondel.

Table 1. ANALYTICAL DATA FOR TWO SAMPLES OF ZINC MICA

	1	2	3		
SiO_2	31 9	—	Si	5 44	8·00
			[Al]IV	2 56	
Al_2O_3	13 6	—	[Al]VI	0·17	
Fe_2O_3	4 9	5 0	Fe^{3+}	0 63	5·80
TiO_2	0 35	0 53	Ti	0 04	
MnO	12·5	14 9	Mn	1 80	
ZnO	19 8	16 9	Zn	2 47	
MgO	2 7	—	Mg	0 69	
Li_2O	0 04	0·04	Li	0 03	1·95
Na_2O	0·1	—	Na	0 03	
K_2O	8 6	—	K	1·87	
CaO	0 02	—	Ca	0 00	
BaO	0 3	—	Ba	0 02	
H_2O^+,F	3 95	—	(OH,F)	4 1	
H_2O^-	0 95	—			
Total	99 6				

1, Harvard mica, analysed by B. W. Evans; 2, Cambridge mica, analysed by R. G. J. Strens; 3, analysis of Harvard mica recast on basis of 24(O,OH,F). Total iron has been expressed as Fe_2O_3, total manganese as MnO.

The Cambridge sample (195037) contains large amounts of a yellow spandite garnet, with mica, franklinite, hancockite and axinite in moderate amounts, and some later chlorite. The Harvard sample was too small to section, but minerals identified in grain mounts and under the probe included major manganoan axinite and mica, with minor franklinite and hancockite.

Table 2. OPTICAL PROPERTIES OF ZINC MICA (HARVARD)

α	1 598	$(\gamma-\alpha)$	0·062
β	1 658	$a:z$	−6°
γ	1·660	$2V\alpha$	8°
Pleochroism	α	pale yellow	
	$\beta=\gamma$	light chestnut brown	
Absorption	(D)	$\gamma=\beta>\alpha$	
Dispersion		$r<v$, slight	

With the exception of lithium, which was determined by R. Allen using optical emission spectrography, and $(H_2O^+ + F)$, determined by heating a 75-mg sample of Harvard mica in air, all the constituents were determined on the electron probe, a systematic search being made for all elements with atomic numbers from 11 to 92. The following standards were used: biotite with average atomic number comparable with that of the zinc mica (magnesium, aluminium, silicon, iron), spessartine (manganese), sphalerite (zinc) and benitoite (titanium). Absorption, fluorescence and atomic number corrections were applied using procedures current in these laboratories. All iron has been stated as Fe_2O_3, in view of the presence of Mn_2O_3 in hancockite associated with the mica. A small fragment of the mica gave no red coloration when dissolved in a mixture of hydrofluoric and sulphuric acids, indicating that little if any Mn_2O_3 was present, and manganese has therefore been stated as MnO. The analysis in terms of oxides, and recast to 24(O,OH,F), is given in Table 1. The formula of a trioctahedral mica is readily apparent, though both alkalis and octahedral ions show a slight deficiency. Both samples have similar (MnO + ZnO) contents, but vary significantly in manganese : zinc ratio.

Table 3. UNIT CELL DIMENSIONS OF ZINC MICA (HARVARD)

a	5·34 Å	β	99 9°
b	9·312	V	505 5 Å^3
c	10 329	D_{calc}	3 30 g/cm^3

Polytype 1*M*

Single crystal photographs show the mica to be the 1 *M* polytype, in which respect it resembles manganophyllite rather than biotite. Powder photographs and diffractometer traces of the Harvard and Cambridge samples are identical, and the spacings and intensities are similar to those of manganophyllite.

The optical properties of the two samples are identical, presumably reflecting the similar (MnO + ZnO) contents, and indicating that manganese and zinc have similar effects on both optical properties (Table 2) and unit cell dimensions (Table 3).

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Apparatus for Continuous Dielectric-medium Separation of Mineral Grains

THE usual methods for concentration of minerals from rocks by means of magnetic separation, heavy liquids, etc., do not always give satisfactory results. In some of these cases it is known that a good separation can be obtained using differences in the dielectric properties of minerals[1,2]. Briefly, the separation is based on the principle that, in a non-homogeneous electric field, particles with a higher dielectric constant than that of the medium in which they are immersed tend to migrate from a zone of low field strength into a zone of high field strength. Likewise, particles of lower dielectric constant than the medium are repelled into a zone of low field strength.

An apparatus has been developed at this laboratory for the continuous separation of mineral grains with different dielectric constants by applying a homogeneous electric field. In this case, the separation is based on the fact that a substance with a higher dielectric constant tends to replace a substance with a lower dielectric constant between the plates of a capacitor held at constant potential. Essentially, the apparatus consists of two parallel flat electrodes at a distance of 3 mm, the lower electrode consisting in part of a 0·75 mm sieve (see Fig. 1). Both electrodes are immersed in a liquid intermediate in dielectric constant between the minerals to be separated. The electrodes are connected to a 50 c/s a.c. source which can be varied continuously from 0 to 2,000 V. The source consists of a transformer with a variable transformer in the primary circuit. A 100 m.amp fuse is placed in the primary circuit and a resistance of 1 MΩ in the secondary circuit. The mixture of minerals is introduced between the electrodes by means of a vibrating feeder, and the electrodes are vibrated by a second vibrator. The intensity of vibration of both vibrators is variable.

If a liquid with an appropriate dielectric constant is chosen, minerals with a dielectric constant higher than the liquid form "strings" between the electrodes, while those

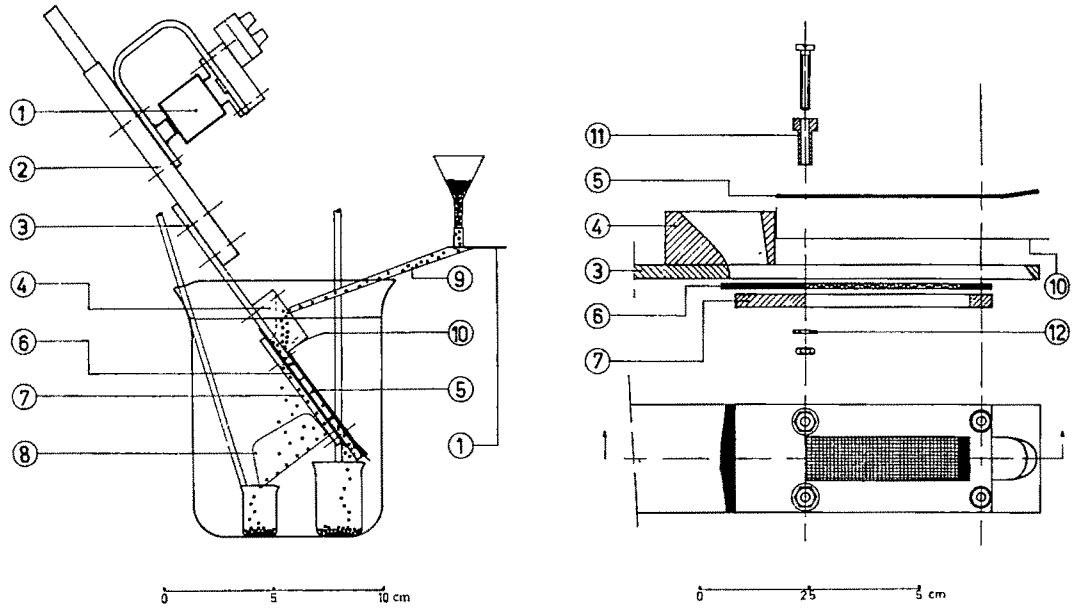


Fig. 1. Diagram of the separator. (1) Variable vibrator (the vibrator of the feeder is not shown), (2) vibrator-base (rigid polyvinyl chloride), (3) separator-body ('Perspex'), (4) separator-funnel ('Perspex'), (5) upper electrode (copper), (6) sieve-electrode (0·75 mm copper sieve partially soldered up), (7) clamping plate ('Perspex'), (8) chute ('Mylar'), (9) feeder-chute, (10) insulating-foil ('Mylar'), (11) bushing ('Teflon'), (12) ring ('Teflon')

with a lower dielectric constant fall through the sieve-electrode. Short-circuiting caused by conductive minerals is prevented by placing an insulating sheet of 'Mylar' underneath the upper electrode. The electrodes are given a forward slope so that the strings move towards the lower end of the electrodes, where they escape the electric field and consequently break down to loose particles.

The minerals falling through the sieve-electrode and those escaping the electric field at the lower end of the electrodes are collected in two small glass beakers placed in the liquid.

The apparatus was successfully applied to two mixtures of minerals difficult to separate by other methods: (1) nigerite, cassiterite and columbite-tantalite (from cassiterite-bearing albite pegmatites of Northern Portugal, supplied by Dr. C. Maijer).[3] and (2) biotite and astrophyllite (from riebeckite-gneisses in north-western Spain, kindly provided by P. Floor, Univ. Leyden), both containing minor amounts of other minerals. The former mixture contained roughly 65 per cent nigerite, 14 per cent cassiterite and 20 per cent columbite-tantalite with small amounts of tourmaline, zircon, chrysoberyl and gahnite. This mixture of minerals proved ideal for the development of the apparatus, because of their widely differing dielectric constants and the visual contrast between the red nigerite and the dark cassiterite and columbite-tantalite (the dielectric constant of nigerite seems to differ little from that of chloroform, as it is neither attracted nor repelled at 25° C and 50 c/s in a non-homogeneous electric field between two needle-shaped electrodes in this liquid. The dielectric constant of chloroform at 25° C and zero c/s is 4·7 according to Landolt-Börnstein. The dielectric constant of cassiterite at 10^{12} c/s is about 24 according to Landolt-Börnstein. According to Milner the dielectric constant of cassiterite is 50·0. Cassiterite is attracted in a non-homogeneous electric field at 25° C and 50 c/s in nitro-benzene with a dielectric constant of 34·67 at 25° C and zero c/s according to Landolt-Börnstein). Di-*n*-butyl-phthalate was chosen empirically as the dielectric medium (the dielectric constant of di-*n*-butyl-phthalate at 25° C and 10^7 c/s is 6·1 according to Landolt-Börnstein).

Nigerite, having a lower dielectric constant than the di-*n*-butyl-phthalate, fell through the sieve electrode; cassiterite, columbite-tantalite and intergrowths of nigerite with cassiterite and columbite-tantalite formed the strings. In this case two sieve fractions (+88–125μ and +125–250μ) yielded an almost pure nigerite concentrate after successive separations at 1,000 and 2,000 V. The first separation with the lower voltage gave a less pure concentrate but was required in order to prevent clogging of the space between the electrodes.

For the separation of a mixture of roughly 50 per cent biotite and 50 per cent astrophyllite with minor amounts of riebeckite, a mixture of butyl alcohol and kerosene was used in proportions of approximately 2:3, in order to create a suitable dielectric medium (the dielectric constant of butyl alcohol at 25° C and zero c/s is 17·7 according to Landolt-Börnstein. That of kerosene is 2·0 according to Milner). In this case, the astrophyllite and the riebeckite fell through the sieve-electrode, biotite forming the strings. Separation of this mixture of minerals proved to be less easy than that of the mixture of nigerite, cassiterite and columbite-tantalite, due to the apparent closeness of the dielectric constant of the minerals and to a gradual decrease of the dielectric constant of the liquid caused by evaporation of butyl alcohol. The index of refraction (1·4200 at 23° C) was used to control the composition. After repeated separations an almost pure biotite fraction was obtained. The astrophyllite fraction contained approximately 95 per cent astrophyllite.

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Paramagnetic Resonance Centres in Amethyst and Citrine Quartz

RECENT investigations[1,2] have shown that the colour and paramagnetic resonance (PMR) centres of violet amethyst quartz are related to one another in a rather complex way. This communication reports paramagnetic resonance lines in both amethyst and citrine (which is related to amethyst by heat treatment) not analysed previously, and the behaviour of these lines with heat treatment.

In all samples of natural citrine, a broad isotropic PMR line, about 100g in width, is observed (Fig. 1); its intensity is proportional to the depth of the yellow-brown colour. Both this line and the colour reach maximum intensity for annealing temperatures in the range 600°–700° C, and anneal out together at about 1,000° C. When natural amethyst is annealed at about 540° C, the violet areas turn yellow-brown; the appearance resembles that of citrine; and the broad isotropic PMR line appears. The colour of citrine has previously[3] been postulated as due to a yellow ferric compound (colloidal hydrous oxide); we suggest that the PMR line is due to Fe^{3+} in amorphous surroundings[4].

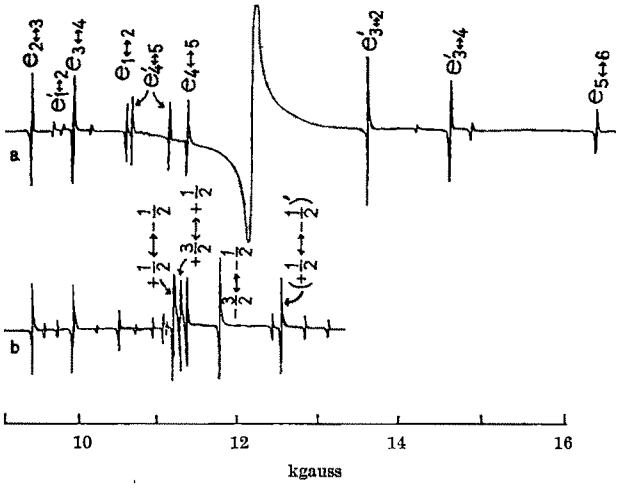


Fig. 1. 8 mm wave-length PMR spectra of (*a*) citrine, with $B\pi$ *a*-axis. The lines labelled *e* belong to the Fe^{3+} sites investigated by Hutton[1]; dashes denote sites with *x*-axes along the remaining *a*-axes. The energy levels have been numbered in order of increasing energy. (*b*) Amethyst; $B\pi$ *x*-axis of a site with $S = 3/2$

We have also observed that most samples of amethyst have three additional weakly populated sites for the Fe^{3+} centres previously analysed[1]. These sites have magnetic axes related to those of the strongly populated sites by a rotation of 60° about the *c*-axis. On heat-treatment to temperatures greater than the α- to β-phase transition temperature, the line strengths for the two sets of sites become nearly equal, due presumably to diffusion either of Fe^{3+} ions or the alkali precursors[1]. Typically, for one sample of amethyst, the ratio of the line intensities of the strong sites and the weak sites fell from 400 before annealing, to 100 after annealing at 500° C for 15 h, and to 1·3 after annealing at 700° C for 15 h.

We have also found two further PMR centres in amethyst, for which the magnetic axes are not related in any simple way to the crystallographic axes (Fig. 2). The observed three lines associated with these centres can be fitted with a spin-Hamiltonian

$$= g\beta\, B.S + 1/3\, D0_2^0 + E\, 0_2^2$$

where the 0_m^n are spin operators[5], $S = 3/2$, $g = 2{\cdot}004$, $D = 4{\cdot}24$ Gc/s, and $(E/D) = 0{\cdot}33$ (doublet zero-field splitting, 9·8 Gc/s). The *z*-axis for a site is defined as the axis making (E/D) a maximum. It was thought at first that these PMR lines might have been the stronger lines from a

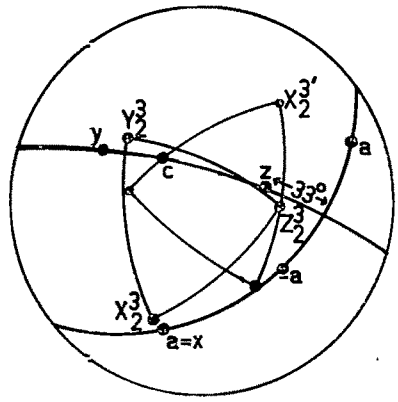


Fig. 2. Stereographic projection showing the relation of the magnetic axes of the Fe^{3+} centres (ref. 1) (*x*, *y*, *z*) and the centres with $S = 3/2$ (x'_2, etc.) to the crystallographic axes

centre with $S = 5'2$ (corresponding to Fe^{3+}); however, a careful search did not reveal the expected lines. No certain identification for this centre, which is not characteristic of all samples of amethyst, was possible.

Further work is planned on the correlations between changes in the optical absorption bands and changes in the PMR spectra produced by heat treatment.

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Effect of Frequency on Texture-induced Ultrasonic Wave Birefringence in Metals

AN ultrasonic shear (transverse) wave entering a stressed solid body splits into two components travelling at slightly different velocities depending on the relevant elastic 'constants' which are slightly different along the two principal stress axes normal to the wave propagation direction. Thus the velocity difference between the two orthogonally polarized shear waves propagated normal to, and polarized parallel and perpendicular to, a principal stress axis should be stress dependent because of this effect of 'acousto-elasticity' or 'sonoelasticity'. This shear wave birefringence has been used in recent years to detect internal stresses in polycrystalline metallic specimens[1,2], but the interpretation of the results was difficult because the preferred grain orientation (texture) had identical effects to the internal stress. In the work described here, attempts were made with some success to separate the effects of stress and grain orientation on the ultrasonic shear wave birefringence.

Measurements have been made of the effect of ultrasonic wave frequency on the shear wave birefringence in a stainless steel sample. The stainless steel (*FV*-535) bar selected for the purpose has a relatively large amount of preferred orientation parallel to the major axis of the bar. The ultrasonic shear wave velocity differences (birefringence) are small, but can be measured accurately by electronic techniques. The shear waves were generated by lead zirconate titanate (*PZT*-5) crystals mounted in a pressure-coupled transducer[3] and propagated across the centre of the stainless steel bar held in a hydraulic press.

The changes in the difference of the transit times of the shear waves propagating normal to the axis, with polarization parallel to and perpendicular to the major axis, were measured by noting the shift in the zero crossing of a selected cycle of a selected echo, as the transducer was rotated from the first (parallel) to the second (perpendicular) position.

Measurements of the change in the time of transit of the shear waves in the frequency range 2–5 Mc/s were made in a stress-free condition and with an applied stress of 10,000 lb./in.^{2}. The results of the measurement are shown in Fig. 1, where the differences in transit time are plotted as a function of frequency. It can be seen that, within the limits of measuring precision, there is a linear relationship between shear wave birefringence due to grain orientation and frequency, and stress has equal effect at each frequency. Thus the birefringence due to stress alone is independent of frequency. The latter result conforms with the observations of Rollins[2] on aluminium at two frequencies, 2 and 5 Mc/s.

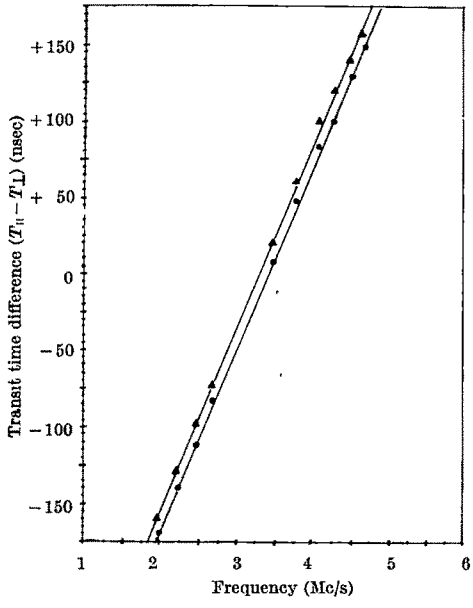


Fig. 1. Difference in time of transit plotted against frequency in stainless steel 1·25 in. thick. T_h[3], 19 μsec for "return trip". ●, Stress-free, ▲, stress of 10,000 lb./in.^{2}

The total fractional velocity difference $\left(\frac{\Delta V}{V}\right)_T$ could be represented by the expression

$$\left(\frac{\Delta V}{V}\right)_T = \left(\frac{\Delta V}{V}\right)_0 + \left(\frac{\Delta V}{V}\right)_S$$

where $\left(\frac{\Delta V}{V}\right)_0$ is the birefringence due to orientation alone, and $\left(\frac{\Delta V}{V}\right)_S$ is the birefringence due to stress alone. As calibration graphs could be plotted for the frequency dependence of $\left(\frac{\Delta V}{V}\right)_0$, as done in the present work, and stress dependence of $\left(\frac{\Delta V}{V}\right)_S$, already done by a number of workers, the above expression could be written as

$$\left(\frac{\Delta V}{V}\right)_T = \frac{\partial\left(\frac{\Delta V}{V}\right)_0}{\partial f} \cdot (f - f_0) + \frac{\partial\left(\frac{\Delta V}{V}\right)_S}{\partial S}(S - S_0)$$

where f_0 is the frequency at which the texture causes no birefringence, and the coefficient of $(f - f_0)$ is the constant slope of Fig. 1.

Thus:

$$S = \left[\left(\frac{\Delta V}{V}\right)_T - \frac{\partial\left(\frac{\Delta V}{V}\right)_0}{\partial f} \cdot (f - f_0)\right] \Big/ \frac{\partial\left(\frac{\Delta V}{V}\right)_S}{\partial S}$$

Effects similar to these were observed in samples of mild steel, aluminium and copper with different degrees of preferred orientation. A qualitative explanation of the results would be in terms of interaction of dislocations and ultrasonic vibrations. Future work will be done on samples having lesser degrees of texture and other types of steels and metals.

I thank Dr. D. I. Crecraft for suggesting this work, and Rolls-Royce, Ltd., for financing the project on residual stress analysis of which this work formed a part.

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Three-dimensional View of Phase Separation in Glass Ceramics

NUCLEATION and controlled crystallization of glass are now receiving considerable attention because of their importance in the manufacture of glass ceramics[1]. These materials are prepared by melting a glass batch containing a suitable nucleating agent and formed into a solid glass article by any conventional glass-making technique. The glass is later heat-treated in such a way that devitrification proceeds in a controlled manner and the article is converted into a glass-ceramic which is extremely fine-grained and nearly void-free.

As a result of extensive work[2,3] on this subject during the past decade, it is now believed that in many cases the first step in the formation of glass-ceramics is separation[4] of the melt into two metastable amorphous phases. Recently, Cahn and Charles[5] postulated that phase separation in glass can occur either by a nucleation and growth-mechanism or by spinodal decomposition.

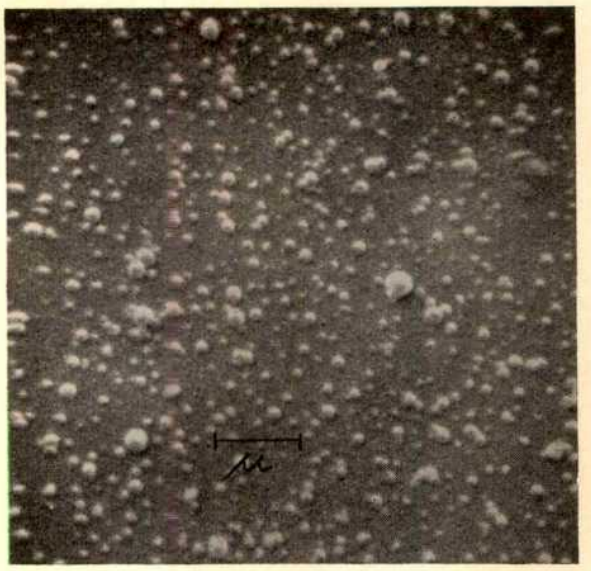
In the past, electron microscope transmission pictures, taken on very finely powdered samples or on ultra-thin (200–600 Å) sections or replicas of freshly fractured surfaces (often suitably etched), formed the main basis of discussion on phase separation and nucleus formation in heat-treated glasses. Owing to the small depth of focus of the transmission electron microscope, however, these photographs only showed the three-dimensional nature of phase separation to a very limited extent. Information regarding the geometry and the distribution of the separated particles is, of course, vital if the operative mechanism in phase separation is to be successfully explained.

It has already been demonstrated[6] that even with very fine grained solids such as set Portland cement, the scanning electron microscope (Stereoscan) offers certain advantages in this respect. It was, therefore, thought worth while to undertake some exploratory investigations of the nucleation processes in glass-ceramics with this instrument.

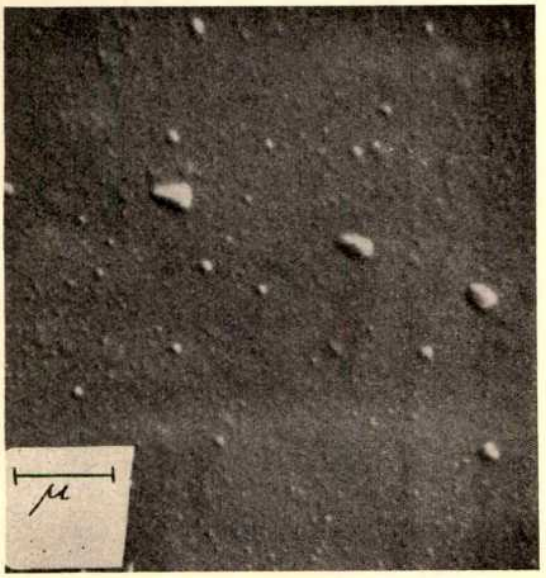
Two complex aluminosilicate glasses prepared from an industrial waste material were examined. Fig. 1*a* is a photomicrograph of the freshly fractured surface of the original glass. The surface appears to be uniformly homogeneous at the magnification used. The white lines in the photograph are ridges caused by conchoidal fracture. In the preparation of the glass, small amounts of nucleating agents, titanium dioxide and chromic sesquioxide, were added to the melt. Figs. 1*b–e* are photographs of the freshly fractured surface of the glass heat-treated at 725° C (it is believed that this is the optimum nucleating



a



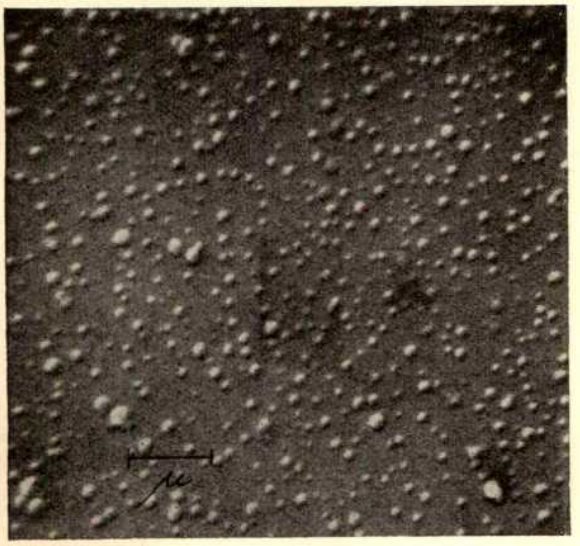
d



b



e



c

Fig. 1. Photomicrograph of a fractured surface of: *a*, the original glass; *b–e*, glass heat-treated at: 725° C for 15 min (*b*); 725° C for 30 min (*c*); 725° C for 1 h (*d*); 725° C for 2 h (*e*)

temperature) for varying periods of time. As can be seen in Fig. 1*b*, phase separation began after only 15 min heating, and even at this early stage the spherical particles generated show random distribution in size and position in the matrix, which suggests that nucleation and growth are operative mechanisms[6]. The progress of phase separation with time is clearly illustrated in Figs. 1*c–e*. The number of the spherical particles increased rapidly, and after heating for 1–2 h larger spheres are produced by coalescence of smaller ones. Thus the assumption that each small sphere acts as a nucleation centre for the formation of one crystal may not be valid. Another interesting feature of the advanced stage of phase separation appears to be the arrangement of small spheres into geometrical patterns which suggest some kind of dense packing before eventual fusion into larger spheres occurs.

An illustration of this dense packing can be seen in Fig. 2, which is a photomicrograph of the fracture surface of the second glass heat-treated at 725° C for 16 h. In this case, no external nucleating agent was added, but it is conceivable that minor components of the industrial waste material could have acted as such. The smallest

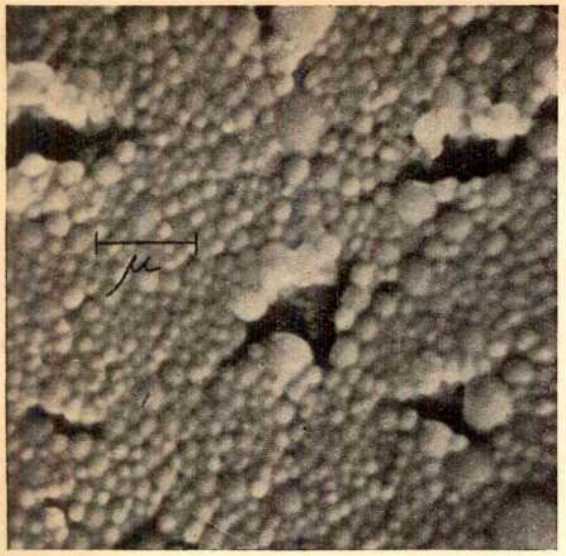


Fig. 2. Photomicrograph of a second glass heat-treated at 725° C for 16 h showing densely packed spherical particles resulting from phase separation

sphere discernible in Figs. 1 and 2 has an apparent diameter of about 600 Å. Without knowledge of the thickness of the metallic coating layer (in this instance gold–palladium alloy[6]), which is deposited on the specimen to facilitate thermal conduction and to prevent the specimen from becoming electrically charged, it is not possible to be more precise.

These preliminary results indicate that the scanning electron microscope may well provide a direct method of studying the nucleation mechanisms which operate in the production of glass-ceramics. Furthermore, it may now be possible to estimate the density of nuclei at different stages of heat-treatment with greater accuracy than heretofore and thereby to furnish useful data related to nucleation kinetics. Research into these aspects of glass devitrification is in progress.

The photographs were taken at the Research Laboratory of the Central Electricity Generating Board at Leatherhead, Surrey, and we thank Dr. C. Baker for his co-operation and assistance.

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PHYSICS

Liquid Drop Model and Asymmetric Nuclear Fission

RYCE has recently directed attention[1] to the resemblance between curves representing the division of electrically charged liquid drops and mass yield curves for nuclear fission. He points out that the set of curves obtained from liquid drop considerations are similar to the fission yield

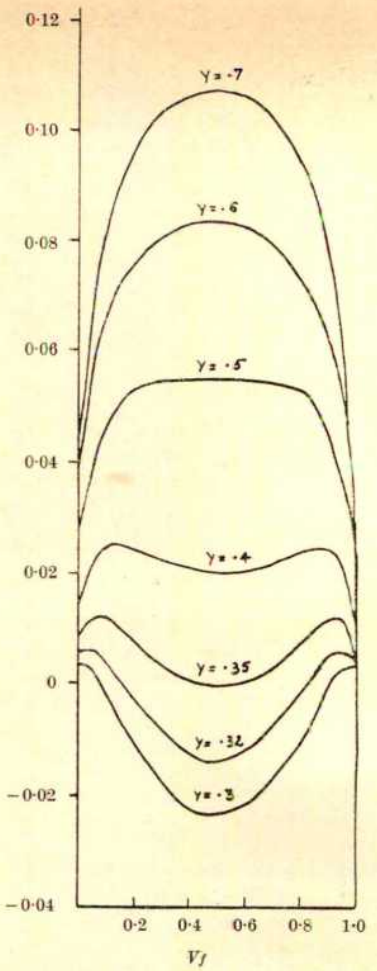


Fig. 1. Fractional energy change, E_f, plotted against the fractional volume change, V_f, for the division of charged drop where $Q \propto R^2$

curves (ref. 1, Fig. 1 left) obtained by Gibson[2] for fission of plutonium-239 induced by bombardment with deuterons of various energies. In the former, Ryce[1] has plotted the fractional energy change, E_f, versus the fractional volume change, V_f, for the division of a surface-charged drop. A V_f value of 0·5 corresponds to symmetric division. The parameter y is defined as:

$$y = \frac{Q^2}{16\pi R^3 T}$$

where Q is the charge on the drop, R the radius and T is the surface tension.

There is a superficial resemblance between the two sets of curves in a small range of values for y, and the relationship assumed seems to exist between the abscissae (drop volume and mass number) and the ordinates (excess energy and cross-section), although not necessarily linear in the entire energy range of the bombarding particle. We doubt whether the asymmetry of nuclear fission can be accounted for so simply by this model of surface-charged drop. Apart from the neglect of some vitally important variables which enter the mechanism of nuclear fission, we find that the similarity between the two sets of curves is misleading, as demonstrated in the following.

In the article by Ryce and Wyman[3] calculations were made for the volume-charged drops as well as for surface-charged drops. The corresponding curves for the volume-charged drop, an assumption more akin to a nucleus, bear no resemblance to Fig. 1 in Ryce's communication, in that they have no asymmetry whatsoever. It would be

incorrect to interpret this result giving much significance to the surface-charged drop model for nuclear fission, merely because of the similarity of the first two sets of curves in a very limited range. Calculations reveal that the form of the curves obtained does not depend in any way on how the charge density distribution is related to the radius. The only factor on which it does depend is how the total charge of a drop is related to the radius. The boundary conditions here considered are then $Q \propto R$ for the surface-charged drop and $Q \propto R^3$ for the volume-charged drop. For nuclei in the whole range of mass numbers, $Q \propto R^p$, where $2 \leqslant p \leqslant 3$. This conclusion is reached from a plot of Z and R for different nuclei (Z is the charge number and R the radius of the nucleus). In order to demonstrate that the similarity obtained with $Q \propto R$ is not truly significant, we obtained the corresponding set of curves for $Q \propto R^2$ shown in Fig. 1.

Our set of curves also corresponds to Ryce's Fig. 1, in a limited range of values of y between 0·32 and 0·60. Because a number of important variables have been ignored in this consideration, the match with nuclear fission yield cannot be expected to be better.

We believe that a liquid drop model with more simplifying assumptions and less stringent conditions than the Bohr and Wheeler[4] model is not likely to give results of much significance. Energy arising from deformation, activation energy, shell structure of nucleus, and nuclear binding energy should play a vital part in any calculation pertaining to nuclear fission.

We thank Frank Thomas, director of laboratories, for his help in the computational work involved.

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Anomalous Reflectivity of Shock Waves

Experimental and theoretical data on the reflexion of light from shock waves have given conflicting results. Hornig *et al.*[1-3] have proposed that, because the shock front separates two regions of different density, Fresnel-type reflexions should occur. Further, they have demonstrated that such reflexions can be observed from shock fronts propagating in dry atmospheres in shock tubes. Mallory[4] has observed a reflexion of a ruby laser beam from the vicinity of a shock front produced by an explosive detonation in air. He found the intensity of the reflected light was three or four orders of magnitude greater than that which could be explained on the basis of a Fresnel reflexion. It seemed that a different phenomenon was giving rise to such reflexions.

There are obvious limitations to the amount of information which can be obtained from the type of experiment carried out on explosions in the atmosphere. (1) It is not possible to control the atmospheric environment. (2) Because of limited accuracy, it is not possible to determine the exact location of the reflecting region. Therefore, it is possible that the reflexion could have originated before, at, or behind the front. (3) The resolution of the equipment used in the explosive experiments is not such that any conclusion can be reached as to whether the reflexions came from a plane or from an extended volume. (4) It is not possible to determine the effect of using a laser instead of a broad-band incoherent source.

It appears that the best method of clarifying previous data is to examine shock waves propagating in shock tubes for reflexions other than those of the Fresnel type.

An experimental configuration was used which was similar to that described by Cowan and Hornig[1]. Illumination was by means of a helium–neon gas laser and the shock tube was fitted with a means for controlling the humidity. No reflexions were observed when the shock tube was operated in a normal manner (the sensitivity was insufficient to enable us to observe Fresnel reflexions). In one experiment the driver section of the shock tube was evacuated rather than pressurized. When the diaphragm was punctured, an expansion wave was initially propagated down the shock tube. Scattering was then observed when, and only when, the propagation media contained moisture. Furthermore, as the shock wave and the expansion wave reverberated in the tube owing to reflexions from the closed ends, the moisture alternately condensed and re-evaporated.

These results led us to examine the pressure history of the shock waves under investigation. When the shock tube was operated in its normal mode, the pressure never dropped below its ambient value. However, the pressure dropped to less than the ambient when the shock tube was operated so that scattering was observed. In fact, the periodic appearance of scattering could be directly correlated with the fall of pressure below ambient.

These experiments suggest that the reflexions observed from the explosively generated shock waves may also have been scattering from water droplets which are condensed in the expansion phase which follows the shock front. Hydrodynamic equations were used to calculate the lowest pressure and temperature reached during this expansion phase. This temperature was below the dew point at the time the explosive experiments were made. Furthermore, the intensity of the observed reflexions was consistent with the assumption that the phenomenon was scattering from particles with radii of 0·1–5 μm.

It was recently brought to our attention that the formation of clouds observed in nuclear explosions had been explained by a similar mechanism[5]. The effect has probably not been previously observed with small explosions because the region in which the "cloud" forms is close to the explosion and the droplets are quickly evaporated by absorbing heat from the fireball. Scattering from the droplets is insufficient to make them observable in shadowgraphic photographs of explosions.

Anomalously high reflectivities have also been reported from regions of the atmosphere. It has been suggested that these reflexions come from regions of clear air turbulence. Munick[6] has shown that it is not possible to detect Fresnel reflexions from the interface between regions of different density. However, it seems possible that air turbulence can produce conditions where local regions of the atmosphere expand adiabatically and are cooled to below the dew point. The condensed water droplets (or ice particles) may be responsible for the observed phenomenon.

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A Simple Device for the Scintillation Counting of Aqueous Solutions of Calcium-45 and other β-Emitting Isotopes

THE methods at present available for counting calcium-45 by conventional liquid scintillation techniques, such as those of Carr and Parsons[1], Sarnat and Jeffay[2] and Humphreys[3], make it possible to count large quantities of low specific activity calcium at efficiencies of 50 per cent or more. However, these methods are laborious and time-consuming because of the lengthy chemical processing required to produce a calcium salt which will dissolve in the toluene-based scintillator mixture. Methods involving the use of hyamine for the direct solution of tissues or aqueous solutions in toluene[4–6] are much quicker, but are only capable of counting very small quantities of calcium at lower efficiency; they are also subject to quenching errors. The use of plastic scintillator fibres or beads and anthracene crystals by Steinberg[7–9] made it possible to count directly colourless aqueous solutions of calcium-45; however, the efficiency, except with anthracene, was low, and the sample and scintillator materials could only be recovered with difficulty. Plastic scintillator systems consisting of a vial[10] and a flow cell[11] are capable of counting calcium-45 in aqueous solutions without any corrections for quenching, but their capacity is 1 ml. or less and their efficiency only of the order of 6 per cent.

In many applications it would be useful to be able to count calcium-45 directly in larger volumes of aqueous solutions without quenching corrections, even if at low efficiency. An apparatus designed for this purpose which has proved useful in this laboratory is shown in Fig. 1. It consists of an array of sixty plastic scintillator filaments (*NE* 102*A*, Nuclear Enterprises, Edinburgh), diameter 1 mm, length 4 cm, spaced at intervals of 2 mm between centres in a 'Perspex' disk. The filaments pierce the disk and their ends are ground and polished flush with its base. The disk is fitted to the bottom of a 'Perspex' cylinder, the whole forming a container of volume 8 ml. The "brush" counter, as we have called it, is suitable for counting aqueous, dilute acid or dilute alkali solutions of β-emitting isotopes in a liquid scintillation counter with a single photo-multiplier. If the filaments are covered with a depth of liquid greater than the range of the β-particles being counted, it is not necessary to measure the volume of solution counted; the count-rate is proportional to the specific activity of the solution. The counter has the following efficiencies:

Isotope	Efficiency (per cent)
Phosphorus-32	40
Calcium-45	2
Carbon-14	0·7

The background count rate is about 1/sec.

The counter can easily be modified to count samples larger than 8 ml. The Ekco *N664B* liquid-scintillation counter used in this laboratory would accommodate a similar counter of maximum volume 20 ml. It can also be modified in design to make its construction simpler if only high-energy β-emitting isotopes are being counted. For example, only six filaments in a brush counter are needed to count phosphorus-32 with an efficiency of 8 per cent.

The brush counter can be compared in its mode of operation and usefulness with the *M6H* Geiger–Müller liquid counting tube. The *M6H* counts phosphorus-32 with an efficiency of about 7 per cent and a background of 0·25/sec, but has zero efficiency for both calcium-45 and carbon-14. Using the simple $\frac{(\text{count})^2}{\text{background}}$ test, the brush counter could achieve comparable counting statistics for phosphorus-32 in an eighth of the time, and it is also useful for the weak β-emitting isotopes. The brush counter is therefore more versatile than the *M6H* tube and, compared with liquid scintillation techniques, it has the advantages of great simplicity of sample preparation and it is not necessary to make corrections for any quenching effects.

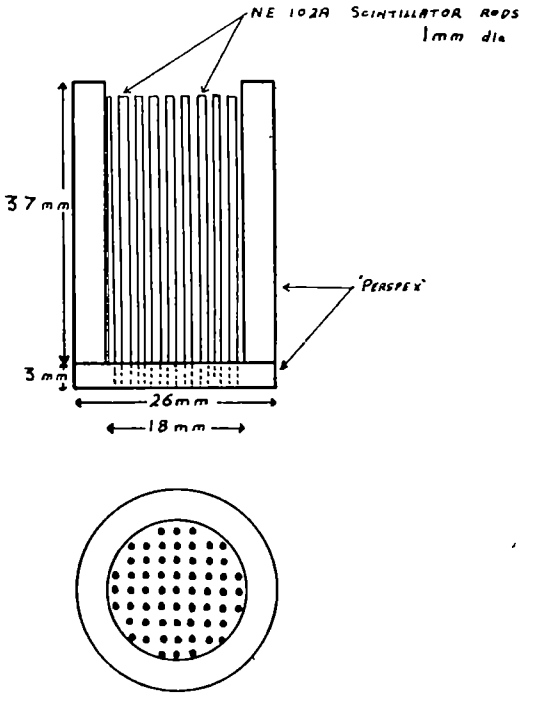


Fig. 1. Side elevation and plan of "brush" counter (×1)

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Changes in Loss Tangent of Polythene subjected to Partial Discharge

IN determining the resistance of materials to partial discharges, two distinct types of measurement tend to be used. In one, the time to breakdown is measured for film or sheet material mounted between rod/plane, needle/plane or hemisphere/plane electrodes. In the other, relatively large areas of sheet are subjected to discharge between plane/plane electrodes so that degradation after periods of exposure can be measured by following changes in conductivity, loss tangent, mechanical strength, etc. Because these properties change progressively with duration of exposure, the impression is being gained that the changes in the material which cause them are the same as those taking place in the breakdown process. This assumption leads to suggestions like that of Boeck[1] that impinging electrons become trapped in the material, forming a space charge and enhance the conductivity, the layer becoming ever deeper as fresh charge carriers are supplied.

We believe that, in general, the assumption is not justified, and that measurements on samples exposed to discharge between large plane electrodes can be misleading. Indeed, we suggest that the mechanisms leading to gross changes in measured conductivity are not the same as those leading to breakdown; the following experiment demonstrates this.

Films of low density polythene (MFI.7) 0·01 cm thick were subjected to discharges in a hemisphere/plane electrode system[2]. The gap between the hemisphere and upper film surface was 0·03 cm. Discharge was produced by applying 4 kV root mean square 500 c/s to the electrodes. On removing the film from the exposure rig after about 1 h and measuring the loss tangent at 500 c/s using plane/plane electrodes 1·75 cm in diameter, we found that the loss angle had indeed increased from its original value. The increase depended on the time of exposure, and at times just less than the time to breakdown (5–6 h) reached values in the range 0·04–0·5. During exposure to discharge an opaque white ring, 1·5 cm in diameter and 0·1 cm wide, was formed and by scanning the surface with a flat-ended rod electrode 0·3 cm in diameter, the high loss tangent was found to be confined to the material under the ring. Here the loss tangent reached 0·56; elsewhere, including the region immediately under the sphere, where breakdown invariably occurs, the loss tangent was less than 0·001 which was the limit of sensitivity. The ring was a surface deposit and could be wiped off with a dry cloth or better with ether, reducing the loss tangent to the original value. The deposit was not analysed, but oxalic acid and oxalates have been reported in similar deposits by other workers[3].

Interrupting the tests to wipe off the ring of deposit did not materially affect the time to breakdown. Breakdown frequently occurred soon after wiping when the loss tangent was low.

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CHEMISTRY

A Lower Transition Point for Polymethyl Methacrylate at 30°–32° C

IN view of the use of polymethyl methacrylate (PMMA) for the production of surgical and dental prostheses, its properties at body temperature and those encountered in the mouth are of particular importance. Furthermore, the necessity for the incorporation of metal inserts and porcelain teeth makes its thermal properties of unusual interest. The thermal expansion of both commercial PMMA ('Perspex') and samples prepared by dental techniques was examined by means of a specially constructed dilatometer. Increase in length was recorded by means of a dial gauge reading to 0·0005 mm, and temperatures were measured at three points along 70 mm specimens by means of a Leeds and Northrup potentiometer. Specimens were arranged in a silica vessel which in later experiments was filled with water to determine the effect of water absorption on the thermal properties of PMMA. Increase in length was plotted against rise in temperature between 20° and 40° C. The resulting graphs showed a change in upward slope between 30°–32° C. The thermal properties were studied daily for periods up to 35 days with the specimens immersed in water and for similar periods with the same specimen drying out. Graphs prepared as before showed no alteration in the position at which an upward change in slope occurred, although the thermal expansion was greater for saturated specimens than for dry ones. Several second-order phase transition points have been recorded[1] for PMMA, but so far as we can ascertain, no reference to one in the temperature range of 30°–32° C has previously been reported.

It is considered that this observation is important in relation to the testing of the material for compliance with the appropriate standards, and it is suggested that the temperatures at which testing is now done may require review.

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Molecular and Crystal Structure of a 5-Covalent Iron (III) Complex with Spin, $S=3/2$

THE magnetic properties of mononuclear compounds of tervalent iron (configuration $3d^5$) conform to the predictions of ligand field theory. Both high-spin $^6A_1$ (μ_{eff} = 5·9 Bohr magnetons (B.M.)) and low-spin $^2T_2$ (μ_{eff} = 2·3 B.M.) ground states are observed for complexes with octahedral microsymmetry. Quartet ground states (S = 3/2) are specifically excluded by the theory, so that the successful isolation of a new class of such compounds is of special interest.

We find that treatment with concentrated hydrohalic acids of a benzene solution of *tris*(dialkyldithiocarbamato)-iron (III) results in the immediate separation of crystalline compounds of the general type (Fe $(S_2C{\cdot}NR_2)_2X$), where X = Cl, Br, I and R = Me, Et, Pr^i, and Bu^{sec}. Typical of the series is the dark green crystalline compound, (Fe$(S_2C{\cdot}NEt_2)_2$Cl), which melts at 250°–255° C with decomposition and is monomeric in freezing nitrobenzene (at 1·5 × 10^{-2} M, molecular weight = 386 ± 10; calculated 388). The magnetic susceptibility of the powdered solid follows Curie's law between 130°–300° K with μ_{eff} = 3·98 B.M., close to the spin-only value of 3·88 B.M. for S = 3/2. In chloroform solution (3·1 × 10^{-2} M) at room temperature, the value 3·88 B.M. is obtained. The infra-red spectrum resembles that found for dithiocarbamates which are chelated to iron by way of two sulphur atoms. The iron-chlorine and iron-sulphur stretching modes occur at 309 and 353 cm^{-1}, and the high carbon–nitrogen stretching frequency in the region of 1,500 cm^{-1} suggests that delocalization of the π-cloud about the CS_2-group includes the adjoining nitrogen atom.

This evidence for a quartet ground state of an iron (III) compound is unambiguous, and to our knowledge is paralleled only by that for iron (III) phthalocyanine chloride as reported by Griffith[1]. A stereochemistry based on five-co-ordinated iron is suggested, but the data do not differentiate between the square pyramidal ($d_{x^2-y^2}\,sp^3$) and the trigonal bipyramidal ($d_{z^2}\,sp^3$) geometries. However, this ambiguity is resolved by X-ray methods using single crystals of the diethyl derivative.

The crystal data for the ethyl compound are as follows: $C_{10}H_{20}N_2S_4ClFe$, M = 387·8, monoclinic, a = 16·43, b = 9·42, c = 12·35 Å, β = 120·5°, U = 1713 Å^3, D_m = 1·52 (by flotation), Z = 4, D_c = 1·50 g cm^{-3}, space group $P2_1/c$, (C_{2h}^5; No. 14); co-radiation (unfiltered), single crystal oscillation and equi-inclination Weissenberg photographs, μ = 107 cm^{-1}.

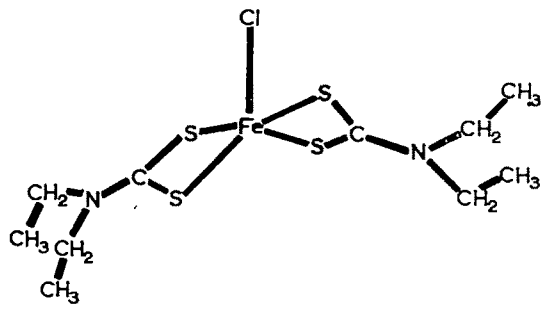


Fig. 1

The co-ordinates of the iron, sulphur, and chlorine atoms were obtained from a three-dimensional Patterson synthesis calculated using 1,440 independent reflexions. The carbon and nitrogen atoms were located in subsequent three-dimensional electron-density distributions. The structure is being refined by the method of least squares applying individual isotropic temperature factors. At present, the R-value, based on the observed reflexions only, is 0·16.

The structure consists of discrete molecules of composition $(Fe(S_2C{\cdot}NEt_3)_2Cl)$. Each molecule is separated from its neighbours at the normal van der Waals distances. The iron atoms are covalently bonded to four sulphur atoms and a chlorine atom at the corners of a somewhat distorted square pyramid (see Fig. 1). The Fe-Cl and the average Fe-S bond lengths of 2·27 Å and 2·32 Å, respectively, are normal. The four sulphur atoms are coplanar and the iron and chlorine atoms lie along a line which is almost exactly normal to this plane; the iron atom is, however, 0·63 Å above the plane. Apart from the terminal methyl groups, each of the ligand groups is planar and the iron atom is coplanar with both dithiocarbamate groups. The dihedral angle between the two planes through each of the groups is 50°.

Table 1

Compound	Distance of metal atom above basal plane	Ref.
$Co(S_2C{\cdot}NMe_2)_2NO$	0·54 Å	3
$Ni(5\text{-}Cl\text{-}SALen\text{-}NEt_2)_2$	0·36	4
$Co(OAsMePh_2)_4(ClO_4)_2$	0·32	5,6
$VO(acac)_2$	0·56	7
$Zn(disal)H_2O$	0·34	8
$Fe(S_2C{\cdot}NEt_2)_2Cl$	0·63	this work

There are no significant differences in the dimensions of the two independent ligand groups. The average carbon–nitrogen bond length of 1·36 Å is considerably shorter than the carbon–nitrogen single bond distance[2] of 1·472 Å. This together with the planarity of the ligand is consistent with the infra-red evidence indicating marked delocalization of the π-electrons in the co-ordinated dithiocarbamate group.

It is interesting to note that relatively few molecules with the tetragonal pyramidal stereochemistry for transition metal complexes have been established by X-ray methods. Nitroso(dimethyl-dithiocarbamato)cobalt provided the first example[3], and quite recently this novel stereochemistry has been reported for the N-β-diethylamineethyl-5-chlorosalicylaldimine derivatives of nickel (II) and cobalt (II)[4], as well as for monoperchloratotetrakis-(diphenylmethylarsine oxide) cobalt (II) perchlorate[5,6]. A dominant feature of these and related structures, based on a rectangular-based pyramid, is that the metal atom lies a considerable distance above the basal plane (see Table 1).

In addition to the mononuclear compounds listed in Table 1, many complexes of divalent copper have structures which involve dimeric or polymeric aggregates of approximately square pyramidal nature. Not infrequently the copper atom is found to lie above the basal plane.

It seems likely that this distortion from the regular geometry is a compromise between the condition of maximum σ-overlap with sulphur which requires the metal atom to lie in the planes of the chelating ligands and of steric expediency determined by the repulsive forces between the five ligands.

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Gas Chromatographic Determination of Non-linear Adsorption Isotherms at Low Concentrations

IN the course of an investigation of solid support effects in gas chromatography[1], it was necessary to determine the adsorption and distribution isotherms for a polar solute on an active support, both coated and uncoated. Because the concentrations of the sample in the gaseous phase were very low in this investigation, it was necessary to determine the isotherms for partial pressures in the range 0·5–10 mm.

There are three common methods for the gas chromatographic determination of adsorption isotherms. One involves the determination of the retention volume of a given concentration of sample by suddenly switching a mixture of sample and carrier gas into a clean column and measuring the retention volume of the sample front. This retention volume is equal to the quotient Q/C (ref. 2) Another method is the tracer pulse technique proposed by Helfferich[3]. However, the most common method is to calculate the isotherm from the shape of the diffuse rear boundary of an elution peak[4] or a desorption curve[5]. The mathematical basis for this technique was derived by Gluckauf[6].

Measurement of the Q/C values from sample fronts, or breakthrough curves, requires a separate experiment for each point on the isotherm, and one is restricted to rather large concentrations of solute. Rigid control of experimental conditions is required. For surface-active supports the most difficult experimental condition is the reproduction of the surface between runs.

The tracer pulse technique requires elaborate equipment for counting the radioactivity of the tagged sample and the method is restricted to samples that can be tagged with radioactive isotopes. In this case also, an experiment must be run for each point on the isotherm.

The saturation technique of Gluckauf has the advantages that the entire isotherm can be determined from a single experiment and it can be used at low concentrations. However, this method requires a knowledge of the area under the tailing portion of the chromatogram and thus cannot be used for small concentrations of polar solutes on an active support where the tailing portion of the curve approaches the baseline asymptotically.

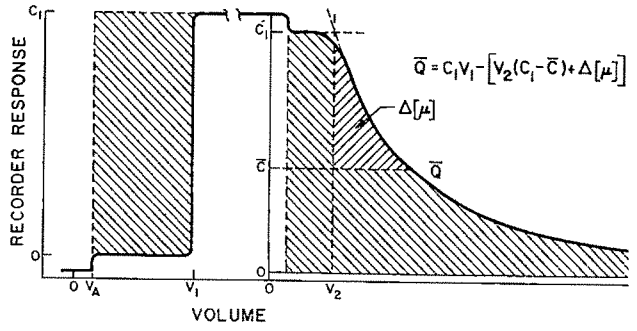


Fig. 1. Representative adsorption–desorption chromatogram

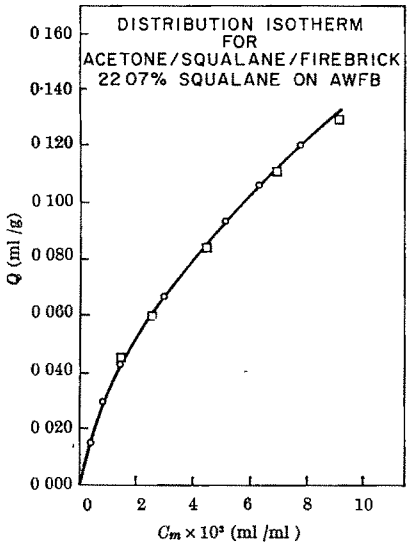


Fig. 2. Comparison of proposed isotherm calculation method with data obtained from break-through curves. ○, From $\bar{Q} = C_1V_1 - \{V_2(C_1 - \bar{C}) + \Delta[\mu]\}$; □, from $C_1V_1 = Q_1$

A combination of the sample front method and Gluckauf's method is the basis of the new technique. It has all the advantages of the Gluckauf method but does not require a measurement of the total area under the tailing portion of the chromatogram[7].

The small concentrations necessary for the sample in the carrier gas were obtained by bleeding a mixture of sample, nitrogen and carrier gas into the main carrier gas stream from a stainless steel tank with a capacity of about 1,000 in.³ (ref. 1). The nitrogen is used to give an air peak. This experimental arrangement is a distinct improvement over a liquid saturator or a constant-drive syringe. It gives better control at lower concentrations than either of these two arrangements and is much simpler.

The proposed method of determining the isotherm from an adsorption–desorption chromatogram is illustrated in Fig. 1. The retention volume of the sample front gives one point on the isotherm at the maximum concentration C_1. Mass balance requires that the area C_1V_1 be equal to the total area under the tailing portion of the desorption chromatogram. Measurement of C_1V_1 removes the necessity of measuring the area under the tail. Only the area designated as $\Delta[\mu]$ has to be measured, and this can be accomplished accurately down to the concentration at which the tail approaches the baseline.

From the initial saturation: $Q_1 = C_1V_1$ (1)

From the tailing portion[6]: $\bar{Q} = [\mu]_c + V_2\bar{C}$ (2)

By mass balance: $\bar{Q} = Q_1 - \Delta Q$ (3)

Where $\Delta Q = \Delta[\mu] + V_2\Delta C$ (4)

Substituting equations (1) and (4) into equation (3):

$$\bar{Q} = C_1V_1 - \{V_2(C_1 - \bar{C}) + \Delta[\mu]\} \quad (5)$$

Where C is the concentration of sample in the gas phase (ml./ml.), Q is the amount of sample adsorbed on the solid support (ml./g), V is the volume of carrier gas measured from the air peak (ml./g), $[\mu]_c$ is the area under the diffuse rear portion of the chromatogram (ml./g), and $\Delta[\mu]$ is the area under the curve as defined in Fig. 1 (ml./g).

Fig. 2 illustrates the distribution isotherm for acetone on a column of 20 per cent squalane coated on acid-washed firebrick. This isotherm was determined using equation (5). On the same graph a series of isotherm points determined using the sample front method alone are plotted to show the reliability of the new method.

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Primary Organic Radical formed in the Radiolysis of Aliphatic Alcohols

In view of contemporary interest in the transient species formed in the radiolysis of aliphatic alcohols, it may be of value to consider the evidence regarding the nature of the primary organic radical-precursor of vic-glycol and possibly aldehyde

Recent investigations by the pulse radiolysis method[1,2] have shown the existence of solvated electrons in irradiated ethanol, and other studies[3–5] indicate that these electrons and hydrogen atoms are the precursors of scavengeable hydrogen; the relative yield being $G_H/G_{e^-alc.} \approx 2$. The primary reactions can be denoted as follows:

$$ROH \longrightarrow ROH^+ + e^- \quad (1)$$

$$ROH \longrightarrow ROH^* \quad (2)$$

$$ROH^+ + e^- \longrightarrow ROH^{\pm} \quad (3)$$

$$\left.\begin{matrix} ROH^* \\ ROH^{\pm} \end{matrix}\right\} \begin{matrix} \nearrow R\cdot O\cdot + H \\ \searrow R'\cdot OH + H \end{matrix} \quad (4), (5)$$

It is the purpose of this communication to assess the relative importance of processes (4) and (5). Although reactions involving the parent ion or the solvated electron can also lead to the formation of these radicals[3], it is proposed here to consider only those reactions involving the decomposition of electronically excited molecules (whether formed directly or as the result of the neutralization reaction).

Possibly the most direct identification of the radical precursor of vic-glycol has been accomplished by high-speed spectrophotometry following pulse radiolysis[1,2]. The identification made with liquid ethanol was the hydroxy-ethyl radical, but as the formation curve for the radical was not observed it could not be established whether this was, in fact, the primary radical produced.

The hydroxy-ethyl radical has also been considered to be the primary species in work[6] concerned with the isotopic composition of hydrogen produced in the radiolyses

of ethanol and various deutero-ethanols. The results have been interpreted in terms of the following reactions:

$$C_2H_5OH \longrightarrow CH_3.CH.OH + H \quad (6)$$

$$H + C_2H_5OH \longrightarrow C_2H_5.O. + H_2 \quad (7)$$

However, it will be shown that these results are capable of another interpretation.

Finally, electron spin resonance spectra of irradiated alcohol glasses also favour the acceptance of the hydroxyalkyl radical as the primary organic species. Early determinations[7] assigned the resonance in irradiated ethanol to the radical ion $C_2H_4^+$, but in later work[8] the hydroxy-ethyl radical has been preferred. Nevertheless, some doubt must exist as to whether the primary and subsequent events are the same in glasses as they are in liquids or vapours. In glassy[9] and gaseous[10] methanol irradiated with ultra-violet light, for example, there would seem to be considerable differences in the reaction mechanism.

The evidence in favour of the primary production of the α-hydroxyalkyl radical ($R'.OH$) having been considered, let us now consider the case for primary production of the alkoxyl radical ($R.O.$) arising from reaction (4).

Photochemical evidence in favour of reaction (4). Early works by Phibbs and Darwent[11] on the mercury sensitized photolysis of CH_3OD reported that the C—H bond was broken, but later work by Porter and Noyes[10] was interpreted to show that the initial cleavage was of the O—D bond. Recently, the mercury sensitized photolysis of CD_3OH and $(CD_3)_2$ Hg at low pressures in a flow system has shown[12] that $CD_3O.$ is formed and not CD_2OH. As the result of this work it has been suggested that the early work reporting C—H cleavage was confused by the reactivity of the methoxyl radical. This radical has been shown to abstract hydrogen readily from suitable donors and it is thought[13] that the energy of activation for reaction (8) will be quite small:

$$CH_3O. + CH_3OH \rightarrow CH_3OH + CH_2OH \quad (8)$$
$$(R.O + R.OH \rightarrow R.OH + R'OH)$$

The result is that in systems where reaction (8) can occur the hydroxymethyl radical will be observed. This interpretation has been confirmed[14] in the mercury-sensitized photolysis of ethanol in the presence of NO in which ethyl nitrite was found as a product.

Radiation chemical evidence in favour of reaction (4). In a recent investigation[15], the abstraction of hydrogen from ethanol by hydrogen atoms has been studied in the radiolysis of aqueous ethanol solutions. These investigations conclusively show that, although a small amount of abstraction from the β-position occurs, it is from the α-carbon atom that the major part of the hydrogen is abstracted with very little contribution from the hydroxyl hydrogen. In the light of this evidence, Burr's results[6] may be re-examined and interpreted to show primary cleavage of the O—H bond. This work clearly shows that the hydrogen atom formed in the original rupture and that which is later abstracted originate from different sites—for example, radiolysis of CH_3CH_2OD leads to the predomination of HD in the total hydrogen yield. Taking the data on abstraction into account, this is consistent with a mechanism involving reaction (4) as the primary step followed by reaction (9):

$$H + C_2H_5OH \rightarrow CH_3CHOH + H_2 \quad (9)$$

Furthermore, recent evidence[4] has shown that, although reaction (8) readily occurs in irradiated ethyl alcohol, suitable scavengers can compete successfully leading to the formation of products characteristic of the ethoxyl radical. Such a scavenger is carbon monoxide which, in addition to reducing the yield of 2–3 butane-diol, leads to the production of ethyl formate which can be explained on the basis of the following reactions:

$$CH_3.CH_2.O + CO \rightarrow CH_3.CH_2.O.CO \quad (10)$$

$$CH_3.CH_2.O.CO + CH_3CH_2OH \rightarrow CH_3CH_2OCHO + CH_3CHOH \quad (11)$$

General. A mechanism involving reaction (4) as the primary step followed by reactions (8) and (9) is compatible with the bond energies involved[16]. Hydrogen atoms and ethoxyl radicals should abstract hydrogen preferentially from the α-carbon (C—H 90 kcal/mole) rather than from the hydroxyl group (O—H 101 kcal/mole). The possibility of abstraction from the stronger bond would exist in the case of steric hindrances, but the structure of ethanol suggests that these will not play a part. With the hydrogen atom abstracting, the further possibility exists that it might be formed with excess kinetic energy (that is, a "hot" atom) and thus easily undergo the more difficult abstraction. The fact, however, that hydrogen is readily scavenged[3–5] indicates that this is largely untrue and that the existence of the major proportion of hydrogen atoms in a "hot" condition cannot be accepted.

It is believed that these arguments strongly indicate that in the vapour and liquid phases the electronically excited states, produced by irradiation of the alcohols with ultra-violet light or gamma-rays, decompose to yield the alkoxyl radical. This conclusion does not necessarily contradict data from electron spin resonance, which may not be applicable to liquid or gas phase irradiations. Nor is it contradictory to the pulse radiolysis experiments which can, in fact, be interpreted as verifying the reactivity of the ethoxyl radical. Furthermore, it is not the purpose of this communication to gainsay the role of the hydroxyalkyl radical as the immediate precursor of vic-glycol and possibly of aldehyde. In fact, chemical evidence, in the case of ethanol, is strongly in favour of these products being formed by dimerization or disproportionation of this radical.

$$2\,C_2H_4OH \longrightarrow \begin{matrix} CH_3CHOH \\ | \\ CH_3CHOH \end{matrix} \quad (12)$$

$$2\,C_2H_4OH \searrow CH_3CHO + C_2H_5OH \quad (13)$$

This, of course, is not sufficient evidence in favour of C_2H_4OH being formed initially; the same end-result would be obtained if reaction (12) were preceded by reactions (4) and (8). It does seem strange, however, that products attributable to CH_3CH_2O (for example, di-ethyl-peroxide) are not formed in appreciable yields in irradiated liquid ethanol. The major reason for this probably lies in the reactivity of ethoxyl so that reaction (4) competes very favourably with inter-radical reactions. Furthermore, it has been shown[17] that even under favourable conditions the reaction:

$$2CH_3O \longrightarrow CH_3OOCH_3 \quad (14)$$

is very slow and that disproportionation is favoured. Unfortunately, this disproportionation reaction (15) cannot be distinguished from that of hydroxyethyl radicals (13) as both lead to the formation of acetaldehyde:

$$2CH_3CH_2O \rightarrow CH_3CHO + C_2H_5OH \quad (15)$$

The presence of ethoxyl is, however, clearly shown in the reaction with carbon monoxide (10 and 11) and by the

fact that, in the irradiation of the pure liquid, small amounts of $CH_3OC_2H_5$ and $C_2H_5OC_2H_5$ are formed[4,18].

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Adsorption of Caesium Tetraphenylborate from Aqueous Solutions on Glass, Polyethylene and Polytetrafluoroethylene

IT has previously been shown[1–4] that polyethylene (PE) and polytetrafluoroethylene (PTFE), unlike glass, do not act as cation exchangers. The adsorption of caesium from aqueous solutions on hydrophobic surfaces of PE and PTFE was negligible. This suggests a possible mechanism for such adsorption. PE and PTFE surfaces in aqueous solution acquire negative charge owing to adsorption of hydroxyl ions. These adsorbed ions may then attract caesium ions by electrostatic forces. In accordance with this hypothesis the adsorption values and the surface charge increase with *p*H. Comparing the adsorption values for caesium, thallium and silver ions on PE and PTFE[4] provides further evidence for a correlation between the adsorbability and the affinity of corresponding ions to hydroxyl groups.

In order to elucidate the mechanism of ion adsorption on hydrophobic non-ionogenic surfaces it was of interest to investigate the influence of other anions.

The present communication reports results of an investigation of the adsorption of caesium tetraphenylborate. It is known that the tetraphenylborate anion can selectively precipitate potassium, rubidium, ammonium, caesium, silver, tantalum and mercury ions in the presence of lithium, sodium and various di- and tri-valent ions[5]. Caesium can also be extracted selectively from aqueous solutions into organic solvents with high dielectric constants[6].

Let us now consider the data on the adsorption of caesium. The experiments were performed in 1 N sodium nitrate solution at varying concentrations of sodium tetraphenyl borate. The caesium concentration was approximately 1×10^{-9} M. The adsorption values were measured using caesium-137. The content of caesium-133 in the initial radioactive preparations was determined by the mass-spectrometric method of isotopic dilution[7]. Glass, PE and PTFE disks, 3 cm in diameter and 1 mm thick, were used to investigate the adsorption. The adsorption values were expressed as the ratio of caesium-137/cm^2 of adsorbing target to the activity of 1 ml. of solution under equilibrium conditions. The walls of glass vessels containing bathing solutions were first saturated with caesium ions until the radioactivity of the solution ceased to change. Adsorption on the targets scarcely affected the radioactivity of the solution, because the volumes of the bathing solutions were always much more than the surface capacity of all disks. The activity of the caesium-137 was measured by a scintillation spectrometer.

Table 1 shows that addition of sodium nitrate in the absence of sodium tetraphenyl borate strongly decreased the adsorption of caesium on all kinds of adsorbents. The adsorption coefficient (distribution coefficient) increases in the order: PTFE < PE < glass. The hydrophility of these adsorbents increases in the same direction[8]. As the wettability is determined primarily by the interaction of water hydroxyl ions with surface atomic groups, the caesium adsorption correlates with the wettability of the adsorbents. On glass, ion-exchange adsorption may also take place at low concentrations of sodium nitrate. The addition of sodium tetraphenylborate to the solution containing microquantities of caesium in 1 N sodium nitrate results in a strong increase of caesium adsorption and at the same time reverses the order of affinity of caesium ions to the surfaces investigated. The adsorption values are dependent on the concentration of tetraphenylborate and on adsorbability of this ion on surfaces of different natures.

Table 1. ADSORPTION OF CAESIUM FROM AQUEOUS SOLUTIONS OF SODIUM TETRAPHENYLBORATE ON GLASS, POLYETHYLENE AND POLYTETRAFLUOROETHYLENE SURFACES

Composition of the bathing solutions	Adsorption coefficients ($\times 10^4$) Glass	Polyethylene	Polytetrafluoroethylene
Water	22,400 ± 2,100	2,100 ± 200	2,000 ± 150
0 02 N sodium nitrate	610 ± 55	378 ± 25	187 ± 15
0 2 N sodium nitrate	60 ± 20	165 ± 30	73 ± 22
0 5 N sodium nitrate	20 ± 10	149 ± 25	25 ± 10
1·0 N sodium nitrate	—	53 ± 25	14 ± 8
1·0 N sodium nitrate + $2{\cdot}15 \times 10^{-3}$ N sodium tetraphenylborate	58 ± 10	240 ± 40	400 ± 55
+ $3{\cdot}40 \times 10^{-3}$ N sodium tetraphenylborate	258 ± 50	986 ± 124	1,570 ± 255
+ $4{\cdot}70 \times 10^{-3}$ N sodium tetraphenylborate	220 ± 35	3,300 ± 195	9,400 ± 750
+ $1{\cdot}1 \times 10^{-2}$ N sodium tetraphenylborate	630 ± 75	5,900 ± 450	25.400 ± 3,200
+ $2{\cdot}0 \times 10^{-2}$ N sodium tetraphenylborate	1,000 ± 160	9,000 ± 1,200	14,000 ± 2,500
+ $4{\cdot}8 \times 10^{-2}$ N sodium tetraphenylborate	490 ± 210	6,920 ± 1,100	27,500 ± 3,500
+ $1{\cdot}0 \times 10^{-1}$ N sodium tetraphenylborate	790 ± 145	32,800 ± 5,200	146,100 ± 21,000

At low concentrations of sodium tetraphenylborate, the major part of the caesium occurs in the ionic form[6]. The adsorption of caesium tetraphenylborate consists apparently of the following steps: (*a*) attraction of tetraphenylborate to surfaces by the van der Waals forces and/or hydrogen bonding; (*b*) selective attraction of the caesium ions by the adsorbed tetraphenylborate anions resulting in the formation of complexes on the given surface. With increasing concentrations of sodium tetraphenylborate considerable amounts of caesium can form the caesium tetraphenylborate complex directly in solution, with subsequent adsorption of this complex on the surfaces. As a result of adsorption, there are always non-charged complexes on the PE and PTFE surfaces because they have no ion-exchange groups in their structure[9]. Similar processes apparently occur on the glass in the case when the ion exchange adsorption is suppressed by high concentrations of sodium nitrate. In the range of caesium and sodium tetraphenylborate concentrations investigated, the solubility product of caesium tetraphenylborate was not reached[5]. Therefore the differences in adsorption values observed for glass, PE and PTFE are determined to a large extent by the molecular properties of the surfaces and adsorbate complexes. The ability of caesium tetraphenylborate

to selective adsorption was reported to occur at the oil/water interfaces[6].

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Nomenclature of Metallic Elements

SINCE the discovery of the transuranic elements, a consistent method of naming the newly discovered metallic elements has been adopted. All these new elements have been given names ending in "-ium". It seems pertinent now that systematic changes are evident both in scientific nomenclature and in measuring units to discuss some aspects of the naming of metallic elements.

Of all the known metals, the following do not have names ending in "-ium":

Antimony	Lanthanum	Platinum
Bismuth	Lead	Silver
Cobalt	Manganese	Tantalum
Copper	Mercury	Tin
Gold	Molybdenum	Tungsten
Iron	Nickel	Zinc

Some effort is needed to introduce a revision of the names of the metallic elements in terms of the suitably consistent ending "-ium". Several degrees of consistency (least, moderate, and total) are now suggested.

Least consistency. In Europe, and perhaps in the United States, there is now little doubt that the name "niobium" is preferred to "columbium". Similarly, the name "aluminium" is accepted everywhere except in the United States. The names of the following four elements might be spelled with the "-ium" ending, introducing only the minimal amount of novelty: lanthanium; molybdenium; platinium; tantalium. These changes would dispose of the only four "-um" endings. Why this latter ending was first introduced when these four elements were discovered is not understood.

Moderate consistency. Several elements have consistent Latin names from which the chemical symbols for these elements are derived, an example being gold—Au(rium). However, the names of some metals are so well established that it would be almost pointless to change them, unless one insisted on total consistency. Examples of these are copper, silver, gold, iron, mercury, and tin. Nevertheless, it would seem a simple process to learn to accept the modified Latin names for antimony and lead, namely, "stibium" and "plumbium". For moderate consistency these changes plus the changes suggested for least consistency are desirable.

Total consistency. The following new names would be required if total consistency were mandatory:

Argentium	Cuprium	Nickelium
Aurium	Ferrium	Stannium
Bismuthium	Hydrargyrium	Tungstenium
Cobaltium	Manganium	Zincium

The element helium, a gas at normal temperature, has, with respect to the present discussion, an inconsistent ending to its name. The name "helion" is suggested as a replacement for its present confusing name.

It seems surprising that something so basic as the naming of the elements proceeded for so long without complete systematic care. In this scientific age it is time that the English-speaking scientists reached international agreement on the systematic naming of at least the metallic elements and perhaps all the chemical elements. This communication was written with the intention of provoking some thought and discussion on the subject.

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BIOPHYSICS

Viscometry of Human Blood for Shear Rates of 0–100,000 sec^{-1}

Charm and Kurland[1] recently suggested that Casson's equation is applicable to the investigation of the viscosity of human blood for shear rates of 0–100,000 per sec.

I believe this statement to be only partially true. First, the physical interpretation given which supposes that the disaggregation of red cells proceeds in the range of rates of shear up to 100,000 per sec is quite wrong if the rates of shear refer to conditions in rotational viscometers. While some aggregates of red cells would be conceivable in axial flow in pipes (or vessels) even at very high rates of shear, the complete disaggregation[2] of red cells occurs already in the range of 10–60 sec^{-1} under conditions of homogeneous velocity gradient.

Any further decrease in the viscosity of blood, at progressively increasing rates of shear, is due not to the disaggregation of red cells, but to a gradual decrease in the internal viscosity of the red cell. Each red cell is in itself a complex rheological matter, both the interior and the membrane of the red cell forming a non-Newtonian system[3].

The viscosity of red cells, whether measured individually or as packed cells, will decrease progressively with increasing rates of shear. This phenomenon accounts for the experimental data and equations obtained by Charm and Kurland. In respect of rates of shear close to zero, from 0·001 to 10 per sec, the slope of the rheological curve (if correctly measured) is quite different from the one obtained at high rates of shear and is, indeed, due to the reversible aggregation of the red cells.

Although, by now, the statement of Charm and Kurland appears to be elaborated, other parameters unfortunately enter into the picture and make the final solution far from simple.

While the normal red cell (Hb AA), at its usual *p*H (about 7·4), will conform to the behaviour described, this is not necessarily true either for red cells containing abnormal haemoglobin or for red cells at abnormal (lower or higher) *p*H. A distinct possibility exists, although it has not yet been proved satisfactorily, that under some conditions the flowing blood might exhibit dilatant instead of thixotropic or shear-thinning properties.

In addition it is dangerous to deduce molecular or colloidal events from Casson's equation. The concept of critical yield stress might be especially misleading. It has been shown[4] that this concept has its place only as an arbitrary value. The critical yield stress is not a unique value of stress, but is a function of the time of application of this stress, and as such is represented by a curve if plotted as stress against time. A sensible (if arbitrary) concept of critical yield stress involves a state-

ment of time, for example, yield stress at 100 sec, or yield stress at 100 min.

Although it might be thought that in blood circulation the flow phenomena must be transient (one complete circulation of the red cell being of an order of 1 min), this is not so in pathological cases (various forms of stasis and intravascular clotting), and the time for one complete circulation might be quite appreciable in these cases.

Finally, I would like to suggest that rheological curves plotted on log–log scales have certain advantages in the micro-rheological[5] interpretation, as they make it relatively easy to account for the contributions of sub-phases or phases of blood to the blood viscosity.

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Fission of Virus and Protein under Electron Bombardment

IT has been reported[1] that, provided threshold bombarding energies and current densities are achieved, it is possible to transfer metallic films deposited on an anode to a cathode *in vacuo* by electron bombardment (a thin film thermionic emitter was used as a detector). The mechanism of release can be considered to arise from (*a*) an energy conversion yielding thermal evaporation over areas of atomic dimensions of the surface layer, or (*b*) an indirect one involving ionization *in situ* and the breaking of metallic bonds of a fraction of the surface atoms.

Recently, a gas-contaminated fine tungsten point was used to detect singly charged positive gas ions between a cathode and an anode[2]; about 400 impacts per sec were recorded at a pressure of 10^{-5} mm mercury. This work has now been extended to detect the fission into complex atom groups and single atoms as a result of bombarding with electrons, virus and protein material placed on the anode. *Pseudomonas* bacteriophage was used at a concentration of 7×10^7 virus particles per c.c. There is no *a priori* reason why the mechanism of detachment in an insulator should differ from that in a metal if the impact is a 'correct' one. To reduce impacts arising from any residual gas, the anode system consisted of a cylindrical box of copper–nickel 20 mm in diameter, 15 mm long and with a central 3-mm hole through which a tuft of four thoriated tungsten wires projected for a distance of 3 mm; these were insulated from the box, and faced a finely etched thoriated tungsten point detector, 1 cm away.

The method used in the experiment was to connect the box and tufts together so as to establish a 'threshold' voltage for gas ionization; if the anode was made 20 per cent negative to the tufts, it reduced the number of single impacts arising from any gas to very small value—something in the order of 20 per sec. An inspection of some 5,000 oscillograph traces showed the usual recovery time and form of the current variations—about 1 msec and 50 per cent, respectively; examples are shown in Figs. 1*a* and *b*. When the tufts were coated with the bacteriophage (about 100 virus particles), there was a time lag of 1–3 min before impacts were detected at the 'threshold', but a steady increase of single impacts up to 300 per sec soon became apparent. This effect is shown in Fig. 2*a* and indicates that the bonds in the virus and protein material on the tufts had been broken. In actual fact, the anode competes with the detector for the disintegration products and, when bombarded, gives a curve such as is

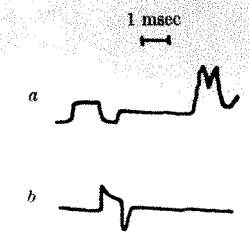


Fig. 1. Single impulses arising owing to the presence of gaseous ions *a*, Anode; *b*, tufts

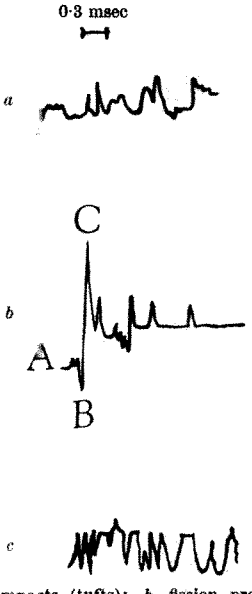


Fig. 2. *a*, Single impacts (tufts); *b*, fission products; *c*, successive impacts

shown in Fig. 2*b*, where the current variation is 500 per cent. The extremely small reaction time coupled with excessive variations in current are never recorded with gaseous ions, but it is probable that they arise from the fission of the virus and protein material. A possible interpretation of curve 1*b* is that the initial portion, *A*–*B*, arises through the cutting off of the beam as the particles proceed towards the detector; this region thus represents the transit time. Subsequent disturbance on the surface produces the 500 per cent current increase, *B*–*C*. On the basis that the average recovery time varies inversely as the square root of the mass, the portion *ABC* represents a fission product of about 400 Å diameter. Subsequent peaks indicate unusual complexes with masses of 40–140 times that of the oxygen atom. The striking succession of single ions which originate at a localized point, shown in Fig. 2*c*, nearly always occur in spasmodic bursts at some stage of the bombardment; this results in the great activity at the detector.

The layer on the tufts is polarized by the field, and may be some 1000 Å thick. This is easily penetrated by the 3–4-kV electrons; the secondary electrons should have sufficient energy to escape, but they are prevented from doing so because there are no fields in the right direction. Thus such electrons pass on their energy to the lattice, and help in breaking the bonds of some of the atoms penetrated. An approximate calculation shows that each primary electron releases $1/10^{24}$ of the atoms in the coating.

I thank Dr. Bell of the Department of Microbiology for supplying me with the solution of *Pseudomonas* bacteriophage for these experiments.

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PHYSIOLOGY

Effects of Cold Stress on Serum Protein Fractions of the Prairie Meadow Mouse

THE reaction of an animal to cold stress varies with the species and with the degree of stress imposed on the animal. Shields, Platner and Neubeiser[1] have shown that the albino laboratory rat reacts to a cold stress (4·0° ± 2° C for 20 days) with a marked decrease in serum albumin. They regarded catabolism of albumin as the prime factor responsible for its fall in serum.

The present investigation was undertaken to test the effects of cold stress on the distribution of serum protein fractions of a wild species of rodent, the prairie meadow mouse, *Microtus ochrogaster*, and compare its response with that of the rat. The meadow mouse, common throughout Missouri, was obtained from a stock raised in the laboratory. These animals do well in captivity and can be obtained in large numbers.

Twenty-four virgin females of the same age were divided into two equal groups. The control group was held at 22° C room temperature while the experimental group was placed in a cold room with a temperature of 5° C ± 1·5° C. Both rooms were on a timed 12-h light cycle. Each mouse was individually caged with water and feed *ad libitum*. The food was 'Purina' laboratory rat chow, identical to that used by Shields *et al.*[1] in their experiments on the rat.

After 20 days, the mice were bled by heart puncture. The blood was allowed to clot and the serum obtained was stripped on electrophoresis paper and migrated in the 'Beckman/Spinco Model *R*' cell with barbiturate (Veronal) buffer, *p*H 8·6, ionic strength 0·075. The paper strips were stained with bromophenol blue dye and scanned on the 'Beckman/Spinco Analytrol' equipped with the *B*-3 cam. Total protein was measured with a Goldberg refractometer (American Optical Co.).

Table 1. MEAN VALUES AS PERCENTAGE OF SERUM PROTEIN FRACTIONS OF MICE BEFORE AND AFTER EXPOSURE TO COLD

	Controls	Cold-exposed
Weight change (g)	+3·7 ± 0·78 (12)	+2·8 ± 0·69 (12)
Total protein (g)	5·2 ± 0·53 (7)	5·2 ± 0·18 (8)
Albumin	67·1 ± 1·50 (11)	70·7 ± 0·57* (10)
α-1 globulin	4·6 ± 0·47 (11)	4·9 ± 0·29 (10)
α-2 globulin	10·2 ± 0·30 (11)	9·0 ± 0·63 (10)
β globulin	15·0 ± 1·03 (11)	13·4 ± 0·57 (10)
γ globulin	2·7 ± 0·23 (11)	2·1 ± 0·16† (10)

* $P < 0.02$.
† $P < 0.05$.
Number of animals shown in parentheses.

The results are shown in Table 1. The gain in weight was not significantly different between the two groups. Nor was the total serum protein changed by the cold exposure. This observation agrees with previous reports[3]. The constituent serum proteins did, however, display a significant change by a rise in serum albumin and a drop in the serum gamma globulin fraction in the cold-exposed group. Such a rise in the albumin fraction of the prairie meadow mouse is not consistent with the findings reported for the laboratory rat. Apparently this species of mouse is able to meet its energy needs without resorting to catabolism of albumin. Its energy needs are apparently supplied adequately from non-protein sources. The significantly elevated albumin levels further imply an increased osmotic effect associated with serum proteins. A fall in serum γ-globulin is suggestive of an increased rate of antibody turnover and a decreased rate of antibody production by animals exposed to cold. Trapani[4] found similar results with rabbits exposed to cold. A comparison of control serum protein fractions of the meadow mouse shows that they are of the same order of magnitude as the data reported by Moore[2] for the rat, but they disagree with Shields's rat data. As total protein was not measured by the latter authors, the low control albumin values reported (31 per cent) raise a question regarding the general health of their rats.

Additional similar investigations should be performed on other wild species to ascertain their response to cold stress. It is possible that the laboratory rat is unique in using albumin as an energy source when exposed to cold.

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Decreased Lysosome Fragility in Response to Wounding in the Skin and Oral Mucosa of the Mouse

DURING a comparative histochemical investigation of repair in the skin and oral mucosa of the mouse, a marked change in the distribution of acid phosphatase activity in response to wounding was noted in the epithelial cells.

The Gomori lead nitrate technique, with all solutions made isotonic, was used to demonstrate enzyme activity in unfixed cold microtome sections. In the epithelium of normal skin and oral mucosa after incubation for 1·5 h, sites of enzyme activity were marked by particulate deposits of lead sulphide and by a diffuse cytoplasmic staining in the cells of the basal layers. Within 5 min of wounding (a single incision) the diffuse cytoplasmic staining was lost not only from the epithelial cells bordering the wound, but also from those some distance away from the wound margin (Fig. 1). The particulate deposits of lead sulphide persisted. Re-epithelialization occurred after 4 days, but the diffuse cytoplasmic staining did not re-appear until 7 days after wounding.

It is now generally agreed that sites of acid phosphatase activity in cells are confined to lysosomes or lysosome-like structures. Bitensky[1] has shown that the lysosome membrane has a given degree of fragility in the Gomori incubating medium, and that this varies not only for different tissues but also for the same tissue under differing experimental conditions. The diffuse and particulate deposition of lead sulphide obtained in this investigation indicates over-incubation and this was confirmed when a section of normal tissue was incubated for 1 h. In this case only a particulate deposition of lead sulphide marked sites of enzyme activity. However, as all the experimental



Fig. 1. Section through the skin surface of mouse lower lip 15 min after wounding. The wound margin is at the top left-hand corner of the photomicrograph. The epithelium adjoining the wound shows no diffuse deposition of lead sulphide. Gomori lead nitrate method. Incubation period 1·5 h

material was incubated for 1·5 h, the observed loss of diffuse cytoplasmic staining must represent a cellular response to wounding.

It was concluded that in both skin and mucosa there was a decreased lysosome fragility in the epithelial cells on both sides of the wound. This conclusion was tested by incubating an experimental section for 3 h and, as expected, there was a combined particulate and diffuse deposition of lead sulphide in the epithelium bordering the wound.

The functional significance of this decreased epithelial lysosome fragility in response to wounding is by no means clear, especially as Bitensky has demonstrated an increased fragility of lysosomes in damaged cells of other tissues. One can only speculate that the demonstrated decreased lysosome fragility indicates the cessation of some epithelial activity involving lysosomal enzymes, possibly keratinization, during the repair period.

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Resistance to Shearing of Heat-denatured Muscle in Relation to Shortening

IF an excised beef muscle is exposed to a temperature of 0°–10° C soon after death, a shortening of considerable magnitude occurs during the subsequent 1–5 h[1]. Both the extent and the rate of shortening are less with higher temperature in this range, and also with longer delay before exposure to the cold environment. By appropriate selection of temperature and delay, therefore, shortening values ranging from nothing to more than 60 per cent of the initial length can be obtained.

During an attempt to account for this cold-shortening phenomenon we observed an effect of possible relevance to investigations of muscle structure. Strips of sternomandibularis muscle from beef animals, obtained within a few minutes of slaughter, were made to become shorter by a variety of methods: (*a*) exposure to air at 2° C, after storage at 15° C for 1–24 h; (*b*) storage from 1 h to 48 h *post mortem* at several temperatures within the range 0°–15° C; (*c*) immersion in ice-water for 0–24 h before freezing in solid carbon dioxide–alcohol; and (*d*) thawing muscles at different rates after freezing in solid carbon dioxide–alcohol in a pre-rigor condition. The unfrozen or thawed strips were sealed in plastic film, after the completion of normal rigor or thaw rigor changes, and heated in a water-bath to an internal temperature of 80° C. The force (F, arbitrary units) required to shear the heat-denatured fibres with a blunt metal wedge was then determined[2].

Only relatively low forces (F_{min}) were needed to shear those samples which had shortened (before heat treatment) by less than about 20 per cent of the initial length. Beyond this point, however, the force requirement rapidly increased with further shortening, in some cases doubling with an additional change in length equal to only 4–5 per cent of the initial length. Maximum force values (F_{max}), which always occurred in those strips which had become shortened by about 35–40 per cent, were two to four times the initial value, and there is evidence to suggest that this ratio (F_{max}/F_{min}) would have been even higher if the muscle selected for study had had a lower connective tissue content, and thus a lower shearing force requirement in the unshortened state.

In those muscle strips which had shortened by more than about 40 per cent, the force required to shear the samples was found to decrease with further shortening,

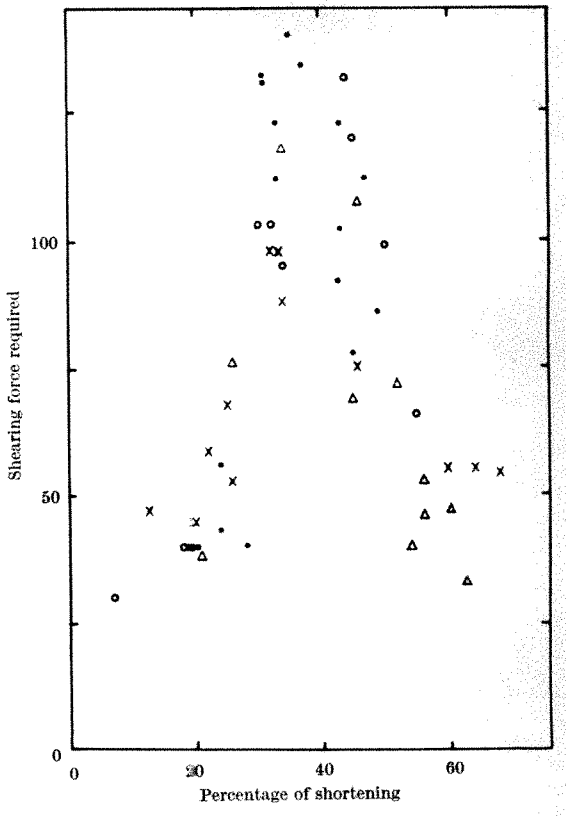


Fig. 1. The effect of degree of shortening on the force required to shear across the fibres of the heat-denatured muscle. Shortening produced by method (*a*) ○; (*b*) △; (*c*) ●; (*d*) ×

and at a considerable rate. The plot of force against shortening in the 40–60 per cent range, in fact, approximates to a mirror image of that in the 20–40 per cent range. The few results obtained from strips which had shortened by 60–65 per cent suggest that the force requirement remains low in this considerably shortened state, and about equal to that in the relatively unshortened condition.

The relationship between shortening and shearing force is illustrated in Fig. 1, which includes the results of one experiment for each of the four methods (*a*–*d*) already described to produce a range of shortening values. Our full results have revealed no exception in forty-two experiments which provided 530 points.

In this investigation, all shortening values were based on the initial length, that is, on the length of the muscle lying unrestrained for up to 1 h after excision, rather than on the true resting length of the muscle within the animal. If shortening had occurred as a result of excision and trimming[1], and before measurement of the initial length, then our critical shortening values of 20, 40 and 60 per cent would be too low. Despite this uncertainty which results from the absence of an absolute initial length, it is obvious from the magnitude of the changes in shearing force requirement that major structural changes have been detected at three distinct degrees of shortening.

The feature of this work most relevant to muscular contraction is the attainment of a peak force requirement at a certain shortening, followed by a reversal to low values with still further shortening. The effect would seem to be consistent with the sliding-filament theory of contraction[3]; the inter-digitating filaments present a resistance to perpendicular cleavage which is continuous along the structure (and therefore strong) at one degree of shortening, but regularly discontinuous (and thus more readily cleaved) on either side of it. It may also be significant

that the rapid decline in force requirement occurs at a shortening associated with the transition to the delta state[4], and when there is also a copious release of fluid indicative of membrane rupture[5].

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Response of Alpha Motoneurones to Direct Electrical Stimulation during Desynchronized Sleep

THE homonymous monosynaptic reflex, as well as the polysynaptic reflex, elicited by single-shock stimulation of the primary afferents of a muscular nerve is tonically depressed during desynchronized sleep in the cat[1]. The depression of these reflexes, however, is not complete. A phasic enhancement of inhibition frequently occurs in relation to the rapid eye movements during deep sleep[1]. An analysis was made of the mechanisms responsible for the tonic and phasic inhibitory influences impinging on the spinal cord during desynchronized sleep by taking electromyographical recordings of the recurrent discharge of the α-motoneurones in deafferented limbs of unanaesthetized, free-moving cats[2]. It was found that this response is depressed throughout desynchronized sleep, but that no further reduction of the recurrent discharge occurs during the bursts of rapid eye movements[3]. A more direct way of evaluating the excitability of the α-motoneurones is to record their response to direct electrical stimulation. The best method would be, of course, to inject the testing current through an intracellular microelectrode. Because this technique cannot be adopted in experiments on free-moving, unanaesthetized cats, an attempt was made in stimulate extracellularly the pools of motoneurones to the ventral horn.

The volley produced by direct electrical stimulation of the motoneuronal pool was recorded from the tibial (extensor) or the deep peroneal (flexor) nerve in the deafferented hindleg. Single shocks were applied unipolarly to the ventral horns through a stainless steel microelectrode (100,000–600,000 Ω). This electrode[4], as well as the electrodes for the electroencephalographic recordings, the electromyographic recordings of the posterior cervical muscles, and for recording the ocular movements, were implanted chronically under 'Nembutal' anaesthesia. The experiments started 1–2 days after the operation, that is, when the effects of the anaesthesia had disappeared. Rectangular negative pulses (0·05 msec at 1·5–2/sec) were used at a strength of 1·2–1·8 times the threshold for the peripheral response. Because the dorsal roots had been cut, the volley produced in the muscular nerve could only have arisen from impulses propagating orthodromically along efferent fibres. Measurements of conduction velocity indicated that the discharge recorded travelled along α-efferent fibres. The size of the nerve action potential was taken as a measure of the number of α-motoneurones excited by the stimulus.

The orthodromic response of the motor fibres to direct stimulation of the motoneuronal pool elicited in either the tibial or the deep peroneal nerve was not modified during transition from relaxed wakefulness to synchronized sleep. Furthermore, during synchronized sleep no significant difference was observed between spindle periods

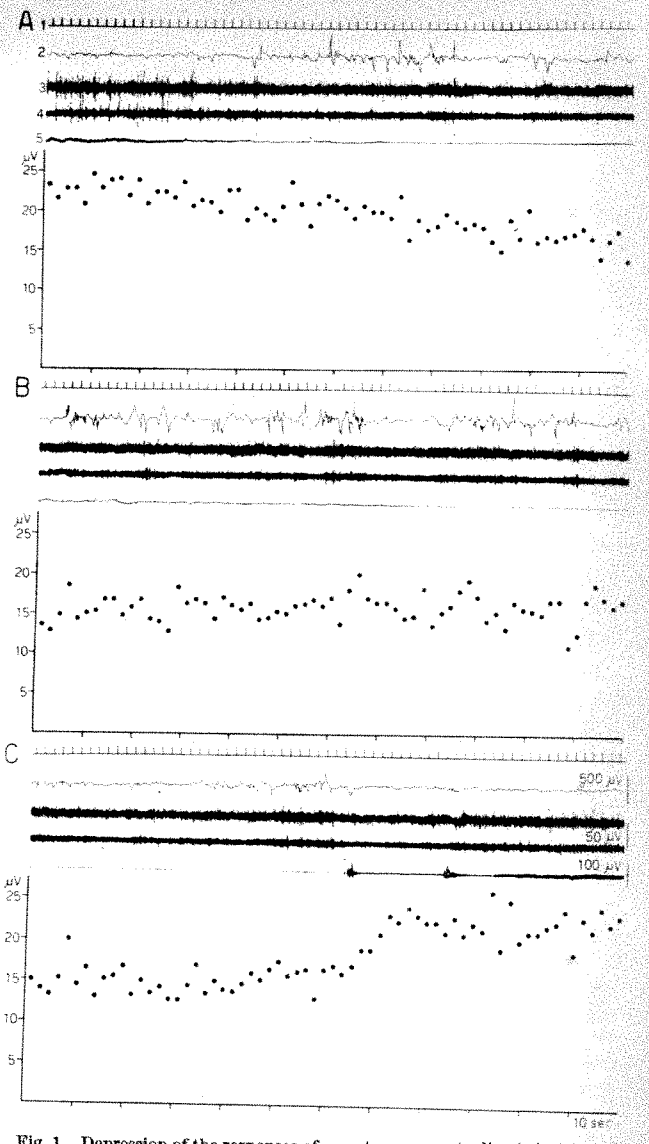


Fig. 1. Depression of the responses of α-motoneurones to direct electrical stimulation of the motoneuronal nucleus during sleep. Unrestrained, unanaesthetized cat. The experiment was carried out 2 days after chronic implantation of the electrode and complete deafferentiation of the left hindleg. A stainless steel microelectrode having a resistance of 100,000 Ω was chronically inserted into the motoneuronal nucleus supplying the deep peroneal nerve, while the orthodromic volley arising from direct stimulation of the α-motoneurones was recorded from the peroneal nerve with a bipolar electrode. (1) Stimulus artefact by stimulating the left ventral horn at the upper level of S_1 with rectangular pulses at one every 1·9 sec, 0·05 msec duration and 1·55 times the threshold for the orthodromic α-volley; (2) eye movements; (3) left parieto-occipital; (4) right parieto-occipital; (5) posterior cervical muscles. The amplitude of the volley arising from direct stimulation of the motoneuronal pool is represented by the position of the dots. *A*, Gradual depression of the responses of α-motoneurones during transition from synchronized to desynchronized sleep; *B*, soon after *A*, showing an episode of desynchronized sleep; *C*, recovery of the responses at the end of the episode of desynchronized sleep as monitored by the reappearance of the neck muscular activity

and interspindle lulls. The response of the α-motoneurones to direct stimulation was, however, tonically depressed during desynchronized sleep (Fig. 1). The decrease of the action potential was of the order of 20–30 per cent and lasted throughout the episode of desynchronized sleep. No phasic depression of motoneuronal excitability could be detected during the large bursts of rapid eye movements (Fig. 1).

These observations are in general agreement with those made on the recurrent discharge of α-motoneurones[3]. Both these methods of sampling the state of excitability of the α-motoneurones during physiological sleep and wakefulness lead to the conclusion that an increase in the

polarization of the motoneuronal membrane occurs during desynchronized sleep. This phenomenon, which is likely to arise from inhibitory postsynaptic potentials, would account for the tonic depression of the monosynaptic and polysynaptic reflexes which occur during desynchronized sleep[1].

Another mechanism must be postulated, therefore, to account for the striking phasic depression of the same reflexes that one sees in conjunction with the bursts of rapid eye movements[1]. The following evidence indicates that presynaptic inhibition, acting on the central endings of primary afferents, may cause the phasic depression of the spinal reflexes at the time of the rapid eye movements. No further depression of the motoneurones to antidromic[3] or direct stimulation (this report) was observed to parallel the bursts of rapid eye movements. In addition, during the bursts of ocular movements there is a phasic enhancement of the group I *a* antidromic volley[4], as tested in both flexor and extensor nerves following Wall's method[5].

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Central Effects of β-*N*-Oxalyl-α,β-diaminopropionic Acid and other *Lathyrus* Factors

ALTHOUGH an association between β-aminopropionitrile derivatives and osteolathyrism of experimental animals seems well established[1], there is less certainty concerning the nature of toxic substances present in *Lathyrus* species responsible for human neurolathyrism[2]. Acute neurological manifestations are produced in some laboratory animals by L-α,γ-diaminobutyric acid (present in numerous *Lathyrus* species, but not in *L. sativus*)[3,4], by β-cyano-L-alanine (present in *Vicia sativa* and *V. angustifolia*, possible contaminants of *L. sativus*)[5] and by β-*N*-oxalyl-L-α,β-diaminopropionic acid (recently isolated from *L. sativus*)[6]. The degree of susceptibility to these toxic agents varies between different animal species, and it has yet to be demonstrated whether these substances do, in fact, produce human lathyrism. Nevertheless, in view of the potent effects of many relatively simple amino-acids on mammalian central neurones[7], these lathyrus factors are of considerable interest. Consequently an investigation has been made of the action of these and related compounds on single nerve cells of cats anaesthetized with pentobarbitone.

Action potentials were recorded extracellularly from spinal interneurones and Betz cells by means of the centre barrel of five-barrel glass micropipettes of overall tip diameter 4–5μ, while the compounds under investigation were administered electrophoretically into the immediate extracellular environment of single neurones from the other barrels[7]. Previously this technique has been used to demonstrate that certain acidic amino-acids related to glutamic acid are excitants of nerve cells, whereas structurally similar neutral amino-acids of the γ-aminobutyric acid group are depressants[7].

β-Cyano-L-alanine, β-cyano-D-alanine, α-cyanoglycine and γ-cyano-L-α-aminobutyric acid neither excited nor depressed spinal interneurones when administered as cations from aqueous solutions of *p*H 2·8–3·4. When similarly administered, L-α,γ-diaminobutyric acid depressed the synaptic responses of these cells, but had a much weaker action than γ-aminobutyric acid or β-alanine[7]. In marked contrast, however, β-*N*-oxalyl-L-α,β-diaminopropionic acid, administered as an anion from a *p*H 7·5 solution of the sodium salt, was a powerful excitant of spinal interneurones and Betz cells. This substance was more potent than D,L-homocysteic acid, and was only slightly less active than *N*-methyl-D-aspartic acid, the strongest amino-acid excitant of feline nerve cells yet reported[7]. When injected intraventricularly into 4-week-old mice[8], β-*N*-oxalyl-L-α,β-diaminopropionic acid caused flexor convulsions and hyperactivity in doses of 25–50 μg. Related amino-acids, γ-*N*-oxalyl-α,γ-diaminobutyric acid, *N*-oxalylglycine and *N*-oxalyl-β-alanine were without effect on spinal interneurones.

β-*N*-Oxalyl-L-α,β-diaminopropionic acid is thus the most powerful excitant L-amino-acid yet tested. It is tempting to speculate that a direct action on central neurones accounts for the effects which follow intraperitoneal administration to immature chickens[6], and that the lack of action on mice and rats[6] is associated with the failure of the amino-acid to penetrate the blood-brain barrier. If this barrier is relatively impermeable in adult animals, then difficulties may arise in explaining the relationship between the oxalyl derivative and human lathyrism, unless of course the diffusional barrier is altered by severe nutritional deficiencies. Presumably the toxic effects produced by β-cyano-L-alanine[5] and L-α,γ-diaminobutyric acid[3] are not produced by a direct action on nerve cells, and may be associated with disturbances of the metabolism of L-glutamic acid, γ-aminobutyric acid and related substances.

We thank Dr. C. Ressler for samples of cyano-amino acids, Prof. P. S. Sarma for a sample of natural β-*N*-oxalyl-L-α,β-diaminopropionic acid and some synthetic oxalyl derivatives of amino-acids, and Dr. D. R. Rao for bringing the recent work of Prof. Sarma and his colleagues to our attention.

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Fate of Intracisternally Injected 5-Hydroxytryptamine in the Rat Brain

THE uptake of 5-hydroxytryptamine (5-HT) by brain tissue *in vitro* and *in vivo* has been investigated by many workers[1–4]. The present experiments investigate the fate of 5-HT in the brain tissue after administration into cerebrospinal fluid.

Albino male rats weighing 110–130 g were used. The animals were anaesthetized with ether and received an intracisternal (cisterna magna) injection[5] of 5-HT solution. 5-Hydroxytryptamine creatinine sulphate (Light) was dissolved in an isotonic solution of sodium chloride and administered in a dose of 0·1 mg/100 g body-weight.

To obtain bloodless brain, the animals were bled with simultaneous intravenous infusion of saline until the effluent was almost colourless. After decapitation, the cerebral ventricles were opened, the arachnoid was cut with a razor blade and the whole brain was washed with running saline to remove cerebrospinal fluid. In this way, all 5-OH indoles outside the brain tissue are probably removed. The animals were killed in this way at various intervals after injection. The whole brains were homogenized with 0·1 N hydrochloric acid. After precipitating the proteins with 10 per cent zinc sulphate and 10 per cent sodium hydroxide, portions were taken for spectrofluorimetric determination of total 5-OH indoles[6], 5-hydroxyindoleacetic acid (5-HIAA)[6] and 5-HT[7].

The results are shown in Fig. 1. It is clear that 5-HT penetrates into the brain very easily. The maximal level is achieved 10 min after injection. There is a rapid decline in 5-HT content from 20 to 40 min. A progressive but less rapid fall in 5-HT is manifested from 40 to 80 min.

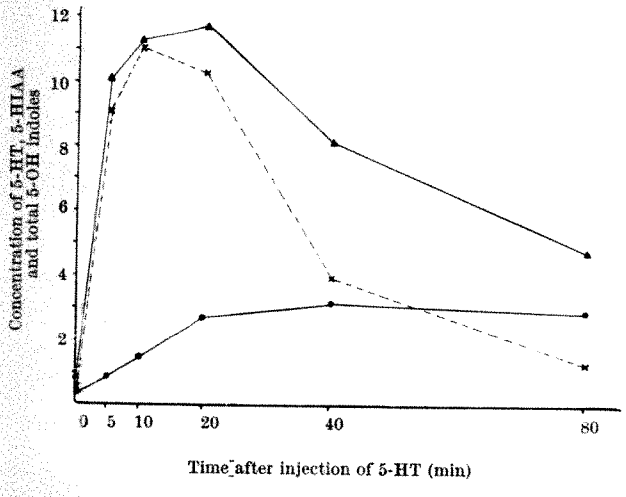


Fig. 1. Amount of 5-HT, 5-HIAA and total 5-OH indoles in the rat brain (μg/g) at various intervals after intracisternal (cisterna magna) injection of 5-HT (0·1 mg/100 g body-weight). Each point represents mean of five experiments. × — — — ×, 5-HT; ●——●, 5-HIAA; ▲——▲, total 5-OH indoles

The content of 5-HIAA, main metabolite of 5-HT, increases continuously up to 40 min. Thereafter it remains almost unchanged.

The amount of total 5-OH indoles oscillates, depending on the levels of 5-HT and 5-HIAA.

Surprisingly, the rapid decrease of 5-HT from 20 to 40 min is not followed by an adequate increase of 5-HIAA. This discrepancy could be explained in many ways: 5-HIAA rapidly leaves the brain; 5-HT is not completely metabolized via 5-HIAA; 5-HT leaves the brain partly unchanged.

Comparing the results from our previous work[8] with those described, it is evident that 5-HT penetrates from cerebrospinal fluid into the brain in a far greater amount than from blood. There are many possible reasons for this phenomenon. (1) 5-HT may be rapidly taken up by blood platelets. (2) 5-HT may be carried by blood in the different tissues and metabolized. (3) 5-HT may be very diluted when given intravenously, the volume of the blood being greater than that of the cerebrospinal fluid.

Summarizing the results of our experiment, it can be concluded that intracisternally administered 5-HT penetrates into the brain easily. Its metabolism is very rapid in the brain and it is almost complete after 80 min.

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PHARMACOLOGY

Effect of Methylhydrazine Derivatives on the Survival of Second-set Skin Homografts

METHYLHYDRAZINE derivatives were originally introduced as a new class of anti-tumour agents[1]. Since then, as with other cytotoxic drugs, immunosuppressive effects have also been demonstrated[2,3]. In an investigation of the pharmacological susceptibility of the primary skin homograft reaction, it became increasingly clear that the methylhydrazine derivatives were among the few agents which provided a definite prolongation of graft survival in mice[4]. The present experiments were carried out to determine the effect of these compounds on the survival of second-set skin homografts.

Inbred strains of mice were used. Skin grafting was done by the method of Billingham and Medawar[5]. Grafts were taken from the general body skin of donors. The dressings were removed on the sixth day and the grafts were inspected daily thereafter. The survival times were taken as end points, that is, until complete graft breakdown was evident. 'White grafts', when present, were lost shortly after the removal of the dressing and were then classified as surviving 6 days.

First-set homografts had been applied to the contralateral side of the backs of the mice 3–5 weeks before the second transplant. The donor and recipient animals were both of the same sex.

Two methylhydrazine derivatives were used for treatment: *Ro* 4–6467/1 (= 1-methyl-2-*p*-(isopropylcarbamoyl) benzylhydrazine hydrochloride) and *Ro* 4–6824 (= 1-methyl-2-*p*-allophanoylbenzyl = hydrazine hydrobromide). In addition, *cyclo*phosphamide and azathioprine were included in the strain combination *C57BR←CBA*. The drugs were applied daily subcutaneously, beginning on the day of transplantation or 12 days beforehand. Two doses were used for each compound. According to preliminary trials, they were of approximately equal toxicity during administration over a period of several weeks.

Table 1 shows the prolongation of the survival of second-set skin homograft, provided by *Ro* 4–6467/1 and *Ro* 4–6824 in mice. Definite prolongations in comparison with the untreated control groups were obtained in two donor/recipient combinations. In the first, transplants from F_1 hybrids between two strains with 'strong' histocompatibility differences were placed on a parent strain (*C57BL←(CBA* × *C57BL)*F_1). Fig. 1 represents the respective individual graft survival times. The second combination (*C57BR←CBA*), as characterized by its first-set skin homograft survival time of 9·6 days[4] behaved like one with 'strong' antigenic differences and *H*-2 locus disparity. In these two combinations, with an effective drug regimen, the prolongation of the survival of the second-set graft was of the order of 50–70 per cent.

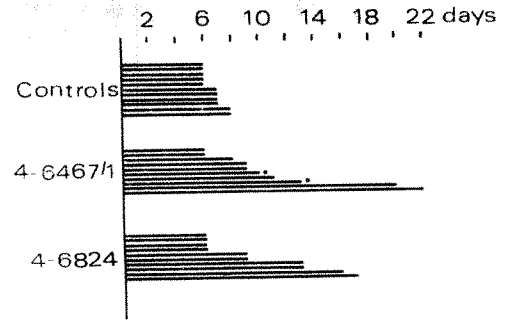


Fig. 1. Prolongation of $(CBA \times C57BL)F_1$ second-set skin homograft survival in *C57BL* mice given *Ro* 4–6467/1 and *Ro* 4–6824 daily (150 mg/kg, from day −12). See also Table 1. ○, Mice died before the rejection of the graft was completed

In contrast, *cyclo*phosphamide and azathioprine did not significantly retard the rejection of *CBA* grafts on *C57BR* hosts. Small but statistically significant extension of graft survival was caused by the methylhydrazines in the combinations *C3H←C-* and *CBA←A*. As twenty-four of twenty-five control grafts were already rejected at the first evaluation, inspection on day 5 rather than on day 6 might have led to somewhat more pronounced results.

When the two methylhydrazine derivatives are compared, it is clear that although they have practically equal immunosuppressive activity, *Ro* 4–6824 is less toxic in mice. Treatment of the hosts before grafting was more effective than treatment from the day of transplantation onwards. These two observations confirm earlier findings made with first-set skin homografts[3,4].

The second-set homograft reaction is considered highly resistant towards modification by radiation or drugs. To our knowledge, no drugs have been successful when applied to this purpose. In temporarily successful organ transplantation, as with kidneys, a constant antigenic stimulation is likely to occur. Therefore, immune states might develop which resemble the situation in second-set transplants. Drugs which affect the second-set model might consequently, besides facilitating the acceptance of primary transplants, also be indicated to control such phenomena in clinical organ transplantation as the late 'acute rejection crises', which are considered as attempts of the host to destroy primarily accepted organ homografts.

The biological mechanism by which the methylhydrazine derivatives exert their action is not yet fully understood. The marked and clinically predominant effect of *Ro* 4–6467/1 in malignant lymphoma[6,7] seems to indicate a specific vulnerability of lymphoid tissue. The intensively proliferating lymphoid cells which characterize the secondary immune response to homografts are likely to be an equally sensitive target for these drugs.

We have also found a relatively pronounced effect of the methylhydrazine derivatives on transplantation immunity in experiments now in progress using skin homografts in rabbits. The use of these agents in clinical organ transplantation, particularly with adequate pretreatment, should be more thoroughly assessed.

I thank Miss Maryan Richner for skilful technical assistance.

Table 1. SUMMARY OF SECOND-SET SKIN HOMOGRAFT SURVIVAL TIMES

No. of mice	Treatment	Dose (mg/kg)	Start (graft day 0)	Median survival time (days)	Range of survival (days)	Change against controls (%)	Mortality of mice before completed rejection
			C57BR←CBA				
34	Controls*			6·3	6–7		
8	*Ro* 4–6824†	150	−12	10·6‖	7–17	+69	0/8
7	*Ro* 4–6824	150	0	6·1	6–7	−3	0/7
17	*Ro* 4–6467/1†	150	−12	9·4‖	6–15	+49	6/17
7	*Ro* 4–6467/1	150	0	7·9‖	7–9	+25	0/7
15	*Ro* 4–6467/1	300§	0	10·1‖	6–22	+61	3/15
12	*Cyclo*phosphamide	12	−12	7·0	7–10	+11	0/11
6	*Cyclo*phosphamide	20	−12	7·5	6–10	+19	0/6
5	*Cyclo*phosphamide	20	0	6·0	6	−5	0/5
11	*Cyclo*phosphamide	30	0	6·6	6–8	+5	0/11
10	Azathioprine‡	4	−12	6·4	6–7	+2	0/10
5	Azathioprine	4	0	6·7	6–7	+6	0/5
6	Azathioprine	6	0	6·2	6–7	−1	0/6
			C57BL←(CBA × C57BL)F$_1$				
11	Controls			6·7	6–8		
10	*Ro* 4–6824	150	−12	10·1‖	6–17	+51	0/10
10	*Ro* 4–6467/1	150	−12	11·4‖	6–22	+70	2/10
			C3H←C-				
15	Controls			6·2	6–9		
20	*Ro* 4–6824	150	−12	7·0‖	6–13	+13	0/20
18	*Ro* 4–6467/1	150	−12	8·3‖	6–12	+34	1/18
			CBA←A				
10	Controls			6·0	6		
7	*Ro* 4–6824	150	−12	7·0‖	6–9	+17	0/7
7	*Ro* 4–6824	300	0	6·6	6–7	+10	0/7
9	*Ro* 4–6467/1	150	−12	6·8¶	6–9	+13	0/9
7	*Ro* 4–6467/1	300	0	6·6	6–7	+10	0/7

* 0·1 ml. saline/10 g/day subcutaneously.
† By courtesy of Dr. W. Bollag (F. Hoffmann-LaRoche and Co. *AG*).
‡ By courtesy of Dr. R. S. F. Hennessey (Wellcome Research Laboratories).
§ 100 mg/kg from day +15 onwards.
‖ $P < 0{\cdot}05$.
¶ $P = 0{\cdot}05$.

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BIOCHEMISTRY

Direct Effect of Vitamin A on Boar Testis

ABOUT 40 years ago the effect of vitamin A deficiency on male sexual organs was investigated in rats by Wolbach and Howe[1] and Gross[2], and in guinea-pigs by Wolbach and Howe[3]. Later the influence of this vitamin on reproduction was investigated in various species of domestic animals, for example, in bulls by Hodgson *et al.*[4] and Madsen *et al.*[5,6], and in rams by Lindley *et al.*[7], but until I reported my investigations[8], it would seem that boars had not been examined. Termination—or a marked reduction—of the spermatogenesis as a consequence of the A-avitaminosis characterized all findings in the species of animal examined. It was demonstrated in these experiments that the administration of vitamin A to an A-avitaminotic boar resulted in complete normalization of the spermatogenesis within 3 months.

Investigations were continued with the view of elucidating whether vitamin A acted directly on the spermatogenetic epithelium or whether a hormonal factor was involved.

As with previous experiments, Danish Landrace boars were used, and from the age of 6 weeks were fed a diet deficient in vitamin A. The vitamin A status was controlled by analysis of liver biopsies which, together with the markedly increased cerebrospinal pressure, formed the basis by which it was checked that the animals (at the age of 4–5 months) were depleted of vitamin A. At this stage, daily doses of vitamin A acid were supplied to some of the boars, the clinical condition of which remained normal but in which a vitamin A deficiency was manifest in the spermatogenetic tissue. This feature was investigated earlier in boars (unpublished data), and is in accordance with findings of Thompson *et al.*[9] in rats. About a month later, vitamin A alcohol or vitamin A aldehyde in peanut oil containing small quantities of indian ink was injected into one of the testicles. The doses of vitamin A alcohol injected varied between 6,000 and 8,000 I.U., and vitamin A aldehyde between 0·6 and

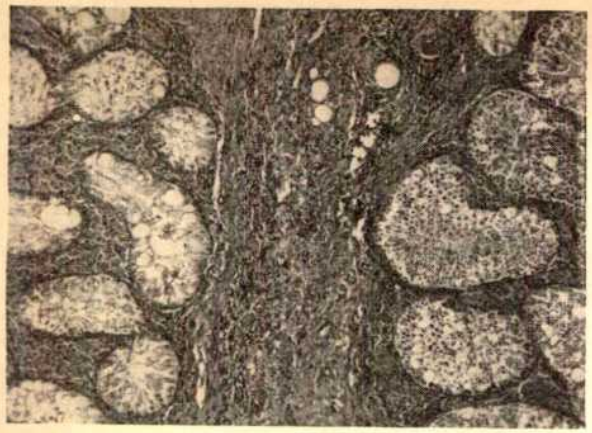


Fig. 1. Testis from vitamin A-deficient boar. The septula testis, which contains vacuoles and grains of indian ink, separates normal spermatogenetic tubules (right) from degenerating tubules (left). (Stained with haematoxylin-eosin. × 60)

1·6 mg. In a few boars, both preparations were applied to the same testicle, one preparation being injected at a distal, the other at a proximal site. At intervals of 3–4 weeks this treatment was repeated on four or five occasions after which the animals were killed.

The weight of the testicles was found to be much lower in all experimental boars than in their normal littermates; members of the former group were about a third or a half the normal weight. There was no characteristic difference in weight between treated and non-treated testicles. Histological examination of the testes, however, revealed a distinct difference between the testicular areas into which the vitamin had been injected and the surrounding tissue and the non-treated testicle. In some boars spermatogenesis was found to be normal at the site of application (Fig. 1), yet at the same time there was total degeneration of the spermatogenetic epithelium in the surrounding tissue and the non-treated testis as the tubules were found to contain only Sertoli's cells and a few inactive spermatogonia. Generally the areas treated with vitamin A were well defined, and radial septa of testicular connective tissue separated the vitamin A-rich from the vitamin A-deficient areas (Fig. 1). Besides the normal spermatogenesis in the former areas, grains of indian ink could be seen embedded in the interstitial tissue which also contained some circular or oval vacuoles of somewhat variable sizes. These vacuoles represented the 'negative picture' of the vitamin A-containing peanut oil used for the injection, and fat-staining of frozen sections of the testicles with Sudan III gave a positive result. Vitamin A alcohol and vitamin A aldehyde thus seem to have similar direct effect on the spermatogenetic tissue, with the exception that vitamin A aldehyde seems to result in a more well-defined demarcation between the active, vitamin-treated and the vitamin-deficient testicular tissues.

As already mentioned, the way in which the vitamin A deficiency provokes the characteristic testicular changes remains unknown. An explanation which immediately suggests itself is that it might be a matter of hormonal interference, and for this reason interest has been focused primarily on the gonadotropic hormones of the pituitary. The results obtained in investigations using experimental as well as domestic animals have been most contradictory. Whereas Mason and Wolfe[10] found that the content of gonadotropins in the pituitary increases in vitamin A-deficient rats, Van Os[11] and Mayer and Goddard[12] considered that supplies of gonadotropic hormones would counteract the degenerative influence on the sexual organs otherwise involved in such vitamin deficiency. Hodgson *et al.*[4] and Gunn *et al.*[13] (the former group used A-avitaminotic bulls, while the latter group used rams) found that the spermatogenesis was accelerated after administration of pregnant mare serum; however, Lindley *et al.*[7] in more comprehensive experiments with rams showed that supplies of pregnant mare serum failed to prevent the development of A-avitaminotic, testicular changes.

As early as in 1952 Fell and Mellanby[14] demonstrated in tissue cultures that high dosages of vitamin A might be responsible for a cellular A-hypervitaminosis and that consequently they might be considered to act directly on cells *in vitro*. Numerous experiments carried out by Fell[15] substantiated this finding, not merely in A-hypervitaminosis but also in A-hypovitaminosis.

Thus our findings show that the degenerative changes of the spermatogenetic tissue caused by A-avitaminosis are not indirectly provoked by changes—arising from the avitaminosis—in the hormonal regulation, but instead that they are of a direct nature. That is to say, they can be attributed to a disturbed function of the spermatogenetic tissue brought about by the vitamin A deficiency, and thus can be counteracted by direct application of vitamin A alcohol or vitamin A aldehyde to the tissue, but not by application of vitamin A acid.

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Separation of Connective Tissue Mucopolysaccharide–Protein Complexes from Unrelated Proteins

IT is now generally accepted that most, if not all, of the mucopolysaccharides (MPS) in connective tissues are bound to small amounts of protein or peptide. Recent estimates indicate that this protein content may be as little as 2 per cent for hyaluronic acid and as much as 15–20 per cent for cartilage protein–polysaccharide complexes[1]. Crude extracts of connective tissue always contain large amounts of protein not associated with the MPS. Therefore, in almost all procedures for isolating the MPS components, deproteinization is an essential first step before purification. It is usually assumed that the variety of proteolytic enzymes used, such as pronase, papain or trypsin, specifically degrade the "unrelated" protein but do not attack that complexed to the MPS. Although this is probably true there is, nevertheless, little direct experimental evidence to support this assumption. Other methods commonly used to deproteinize crude tissue extracts are adsorption on kaolin, Lloyd's reagent, shaking with chloroform-isoamyl alcohol (Sevag's procedure) and dialysis against a cationic resin[2]. Besides the possibility of degradation, these procedures generally result in great losses of material.

It seemed desirable to find milder methods for the deproteinization of crude extracts, especially those

derived from tissues with only small amounts of MPS. The present communication describes a procedure in which the ratio of protein and uronic acid can be reduced from 70/1 to as little as 5/1 by passing crude tissue extracts through columns of *CM*- or *SE*-'Sephadex'. Besides the rapidity and simplicity of this procedure, it has the additional advantage of causing little loss of MPS. These strongly acidic substances, being repelled by the fixed negative charges of the gel, pass quantitatively through the column while most of the proteins are retained on the gel. At this stage, when only relatively little protein remains, most of it can be removed by the addition of 55 per cent ammonium sulphate.

The *CM*- and *SE*-'Sephadex', *A*-50 Medium (Pharmacia) were washed first with 0·5 N hydrochloric acid and afterwards with buffer; the latter step is a very critical one since it was found that maximum adsorption of protein could be achieved only under carefully controlled conditions. Whereas relatively little protein is retained on the column when phosphate buffer, *p*H 6·5–7·0, is used, maximum adsorption can be attained with citrate-phosphate buffer at *p*H 5·2–5·5. The ionic strength of the starting buffer is not critical; buffers ranging from 0·005 M to 0·02 M were found to be equally effective. Although the *CM*-'Sephadex' requires a considerable number of washings before equilibrium is reached[3], *SE*-'Sephadex' was found to equilibrate very rapidly. Both are equally effective in adsorbing proteins. Columns 2–3 cm in diameter and 30–50 cm in height were used in all the experiments. Tissue extracts were dialysed against two changes of starting buffer. Any residue which formed was removed by centrifugation and the dialysed supernatant solutions were then applied directly to the column.

The results of two typical experiments are summarized in Table 1. The initial ratio of protein/uronic acid in an extract of bovine cornea was 42/1; after two passages through *CM*-'Sephadex' this ratio had dropped to 6·2/1. A similar fall in the ratio of protein/uronic acid was observed in preparations obtained from cattle retina[4]. The largest amount of protein which can be applied to a column of this size is about 250 mg. Samples containing quantities greater than this are best chromatographed in batches.

Table 1. PURIFICATION OF CONNECTIVE TISSUE EXTRACTS

Tissue	Protein* (mg)	Uronic acid† (mg)	Protein/uronic acid
Corneal extract			
Initial preparation	176·0	4·22	42·0
First *CM*-'Sephadex'	46·1	3·86	12·0
Second *CM*-'Sephadex'	21·4	3·44	6·2
After ammonium sulphate	4·3	3·30	1·3
Retinal extract			
Initial preparation	192·0	2·76	69·0
First *CM*-'Sephadex'	47·5	2·60	18·3
Second *CM*-'Sephadex'	13·0	2·40	5·4
After ammonium sulphate	1·92	2·31	0·8

* Assayed according to the method of Lowry *et al.* (ref. 5).

† Assayed according to a modification (ref. 6) of the carbazole method of Dische (ref. 7).

It should be noted that passage through *CM*- or *SE*-'Sephadex' did not completely remove all the protein initially present. Moreover, additional chromatography did not cause any further drop in the ratio of protein/uronic acid. However, it was afterwards found that at this stage the relatively small amount of protein remaining could be removed almost completely by precipitation with 55 per cent ammonium sulphate; almost no loss of uronic acid-containing material resulted. Ammonium sulphate is apparently only effective at relatively low protein concentrations. When added to the crude extracts, considerable loss of MPS resulted, due undoubtedly to co-precipitation of mucopolysaccharide–protein complexes with the protein.

To sum up, the overall procedure outlined here results in the removal of about 98 per cent of the original protein in these preparations, while substances containing uronic acid are recovered in yields of 80 per cent or more. It may be assumed that a substantial portion of the protein remaining is probably bound to the MPS. The mild conditions used undoubtedly ensure the preservation of these complexes in their native state, and thus provide suitable starting material for ultimate fractionation and purification. Because of the high recovery of uronic acid-containing substances, this procedure is especially suitable for tissues with low MPS content.

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Anomalous Protein Binding of Iodine-131 in the Presence of Iodate

As a preliminary step to some work which we wish to undertake with insulin labelled with iodine-131 and other proteins, we have recently compared some of the methods available for the separation of protein-bound iodine and free iodine from mixtures of the two.

In the course of this work we have studied the behaviour of two mixtures, containing human serum and iodine-131 in the form of sodium iodide and with the addition of either inactive sodium iodide or iodate. In the presence of sodium iodide alone, it has proved possible to effect virtually complete separation of the iodide from the serum (as determined by distribution of radioactivity) by (i) trichloroacetic acid precipitation, (ii) zinc hydroxide precipitation, (iii) separation on ion exchange resin, and (iv) gel filtration. In the presence of iodate, however—though the latter three methods gave results comparable with those found with iodide—precipitation with trichloroacetic acid gave completely anomalous results and 74 per cent of the activity was found in the precipitate. This is demonstrated quite clearly by the summary of results given in Table 1.

The mechanism for this precipitation of the activity is not clear. The fact that the molecular sieving achieved by the gel filtration indicates the activity to be associated

Table 1

Method of separation	Percentage of total radioactivity associated with protein in the presence of Sodium iodide	Sodium iodate
(i) Trichloroacetic acid	1·0	74·0
(ii) Zinc hydroxide	2·0	3·0
(iii) Gel filtration	1·0	2·0
(iv) Ion exchange	1·0	4·0

Notes: (*a*) The mixtures consisted of 10 ml. of pooled human serum to which had been added approximately 2 μc. of iodine-131 and 5 mg of either sodium iodide or iodate. 0·5 ml. portions of these were used for all determinations. (*b*) Trichloroacetic acid precipitations were carried out with 5 per cent trichloroacetic acid. The precipitates were centrifuged and washed three times with 5 ml trichloroacetic acid. (*c*) The procedure adopted for the zinc hydroxide precipitations was that developed by Somogyi (ref. 3). (*d*) Gel filtration was performed on 20 cm × 1 cm columns of 'Sephadex *G*-25', using normal saline as the eluent. (*e*) Columns containing about 1·5 g of 'Amberlite' resin *IRA* 400 Cl were used for the ion exchange separations and normal saline was again used as the eluent.

with small molecules would seem to preclude true protein binding of the iodide. Moreover, the objections which have been raised in the past with regard to the use of trichloroacetic acid as a precipitating agent for protein-bound iodines[1,2] (on the grounds that inorganic iodine is occluded in the precipitate) would appear to be only partially valid; in *in vitro* conditions at least, the phenomenon only occurs in the presence of iodate.

Though doubts have been cast on the accuracy of the method, the precipitation of protein-bound iodine by trichloroacetic acid in thyroid function tests is well established. The technique has also been used as a criterion of the degree of protein labelling achieved in methods in which iodinating mixtures containing iodide and iodate are used. Our findings suggest that results obtained by this method should be examined critically, as very different results can be obtained when other methods of separation are used.

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Actomyosin-like Protein from Crayfish Nerve: a Possible Molecular Explanation of Permeability Changes during Excitation

THE purpose of this communication is to show that a protein which resembles the contractile actomyosin of muscle (also an ATPase) is present in nerve membranes.

A detailed hypothesis has been presented elsewhere[1], together with supporting evidence, suggesting that the complex permeability of the membrane of excitable cells is controlled by an adenosine triphosphatase (ATPase) enzyme system, which is able to change its molecular configuration as a function of the supply of energy-rich substrate. Changes in effective pore diameter and/or position of charged groups would produce a modification of selective cation-permeability. Such a "mechanoenzyme" system[2] would correspond with that present in the mitochondrial membrane[2,3] and would account for the changes in axon length[4] and in light scattering properties of the axon membrane[5] which follow electrical stimulation. Its enzymatic activity would also contribute to the observed changes in inorganic phosphate liberation which follow nerve activity[6].

Crayfish (*Astacus pallipes*) nerve cords were homogenized and suspended in a solution of 0·25 M sucrose and 1 mM ethylenediamine tetraacetic acid (EDTA), buffered with 30 mM *tris* at *p*H 7·3. The homogenate was centrifuged at 0° C and the nuclear and mitochondrial fractions discarded. The resulting supernatant was diluted with an appropriate solution of salts (AnalaR grade) in *tris* buffer and examined in a Hilger–Watts recording spectrophotometer. Changes in absorbancy at 19° C were measured at a single wave-length 410 mμ[3]. Each cuvette contained approximately the supernatant from three nerve cords; this was equivalent to a final dilution of 0·8–1·2 mg protein/ml. Determinations of enzyme activity were made by incubation with ATP (*tris*-salt, Sigma) and the inorganic phosphorus (P_i) liberated was measured by the method of Fiske and Subbarow[7].

The supernatant was found to possess ATPase activity, with both glycoside-sensitive and glycoside-insensitive fractions, as found in the membrane fragments of crab nerves[8].

When the preparation was placed in the spectrophotometer its absorbance was in the range 0·17–0·25, when compared with a distilled water blank. However, the absorbance and turbidity of the preparation proved to be very sensitive to the effect of the addition of ATP. At *p*H 7·3 and with an ionic concentration of Na^+ = 80 mM, K^+ = 120 mM, Mg^{2+} = 4 mM, the addition of ATP to a final concentration of 2 mM produces an increase in absorbance (approximately 50 per cent), but higher concentrations of ATP (4–6 mM) cause large absorbancy changes. The action of ATP is clearly diphasic. On the addition of ATP there is a large initial increase in absorbance, followed by a slow, steady increase, the rate of which declines over the following 45 min. Small variations in the value of the absolute turbidity change were found, but typical results are illustrated in Table 1, experiments 1–4. Essentially the same overall pattern of changes in absorbance was obtained when either the ATP was added at one time or in successive smaller quantities, although successive application produced a higher overall change (Table 1). The large initial changes at 4 and 6 mM ATP were clearly evident by eye.

These changes in the absorbancy of the supernatant are very sensitive to ionic concentration and *p*H and to their interacting effects. For example, reducing the ionic concentration to Na^+ = 15 mM, K^+ = 15 mM, Mg^{2+} = 6 mM, *tris* 30 mM increased the magnitude of the absorbancy change produced when ATP was added. This effect was accentuated still further by the removal of all cations except Mg^{2+} (6 mM). When the *p*H of the medium was reduced to 5·5 or 6·5, in the presence of 6 mM Mg^{2+} only, low concentrations of ATP (0·5–1·0 mM) were sufficient to produce very large increases in turbidity. On the other hand, the preparation was very much more stable in the presence of ATP when the *p*H was raised to 7·4 and only small changes in absorbancy were recorded.

These results concerning the superprecipitation produced by ATP, and the effects of ionic concentration and *p*H, are characteristic of actomyosin-like proteins[9] and provide evidence for the presence of a similar contractile system in the nerve membrane. Two fractions of ATPase activity are known to be present in the excitable mem-

Table 1. ATP-INDUCED CHANGES IN TURBIDITY OF SUPERNATANT, AS MEASURED BY ABSORBANCY, *A*, AT 410 mμ

Expt. No.	Ouabain 10^{-4} g/ml.		Initial reading	ATP concentration 1·5 mM 10″	1·5 mM 30′	2·0 mM 10″	2·0 mM 30′	2·5 mM 10″	2·5 mM 30′	3·0 mM 10″	3·0 mM 30′	3·5 mM 10″	3·5 mM 30′	4·0 mM 10″	4·0 mM 30′	6·0 mM 10″	6·0 mM 30′
(1)	–	*A*	0·184			0·204	0·272							0·522	1·051	1·130	1·537
		per cent				11	48							184	471	514	735
	+	*A*	0·178			0·200	0·295							0·524	1·074	1·183	1·545
		per cent				12	66							194	503	564	768
(2)	–	*A*	0·254	0·271	0·271					0·420	0·593						
		per cent		7	7					65	133						
	+	*A*	0·255	0·286	0·286												
		per cent		12	12												
	–	*A*	0·254					0·316	0·387								
		per cent						24	52								
(3)	+	*A*	0·190													0·740	1·180
		per cent														289	521
(4)	–	*A*	0·226	0·242	0·244			0·289	0·607			0·714	1·038				
		per cent		7	8			28	169			216	359				
	+	*A*	0·288			0·254	0·410			0·510	0·960						
		per cent				11	79			123	317						

At each successive addition of ATP, the change in turbidity is also expressed as a percentage of the initial absorbancy.
Spectrophotometer readings show 'immediate' response at 10 sec (10″) and after 30 min (30′). *p*H = 7·3, 19° C. Ions, Mg^{2+} = 4 mM, K^+ = 120 mM, Na^+ = 80 mM, *tris* = 30 mM.

brane, one glycoside-sensitive and the other glycoside-insensitive. The former is associated with the active cation pump[8,10], and it is suggested[1] that the latter controls the passive permeability of the membrane. Addition of the glycoside ouabain does not significantly alter the superprecipitation effects produced by ATP (experiments 1 and 2, Table 1). However, the process of superprecipitation followed an approximately exponential time course over the 30 min period after addition of ATP, whereas parallel enzyme investigations showed that the rate of ATP hydrolysis at 19° C was low and followed a linear time-course. Furthermore, at the higher ATP concentrations the enzymatic activity of ATPases is inhibited[8,10], while in our experiments increased superprecipitation is observed at these substrate concentrations. The observation that the addition of ouabain has little effect on the process of superprecipitation does not, therefore, necessarily exclude the possibility that the cation pump is also associated with the activity of a contractile protein. The action of low *p*H in accelerating superprecipitation confirms the view that this phenomenon is not directly related to enzyme activity. Either one or, more probably, both of the ATPase components could be a protein able to change its molecular configuration on the addition of ATP. The results correspond with the actomyosin-like protein which has been identified[11] in and isolated[12] from the mitochondrial membrane, and the results of experiments concerning the permeability of erythrocytes[1,13] also strongly suggest that a comparable system that controls permeability is present in the erythrocyte membrane.

The concentrations of ions and ATP used in these experiments are either identical with[14] or correspond very closely with[8,10] those used in many of the classical studies concerning the ATPase enzyme activity of nerve and erythrocyte membrane. The superprecipitation produced at 4 and 6 mM ATP might explain the substrate inhibition observed in other experiments at concentrations of 2·0–2·5 mM and greater[8,10].

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New Assay of the Ribonuclease-reactivating Enzyme based on Disulphide Exchange

RECENTLY, Straub and I described experiments which suggested that the ribonuclease-reactivating enzyme isolated from pig pancreas probably acts through a rearrangement of disulphide bridges of the substrate by means of disulphide–sulphydryl interchange[1,2]. Further evidence in favour of this hypothesis has been reported by Givol *et al.*[3]. However, the action of this enzyme was followed in both laboratories by indirect methods, that is, by measuring the rate of appearance or disappearance of the activity of another enzyme (ribonuclease or chymotrypsin). This communication describes a convenient assay method which eliminates many uncertainties of the indirect reactivation–inactivation tests and further supports the proposed interchange mechanism.

The substrate used in this assay was a randomly mixed disulphide, formed from radioactive cysteine and reduced ribonuclease. Reduced ribonuclease[4] (1 mg/ml.) and sulphur-35-labelled cysteine (20 μg/ml., 2×10^6 c.p.m./ml.) were allowed to oxidize spontaneously at *p*H 8·5 in 8 M urea for 120 h. When the reaction was complete, the mixture was filtered through 'Sephadex *G*-25' gel equilibrated and eluted with 0·1 N acetic acid. The macromolecular fraction which contained 9 per cent of the originally added radioactivity was lyophilized and dissolved in a small volume of 0·1 N acetic acid. When this

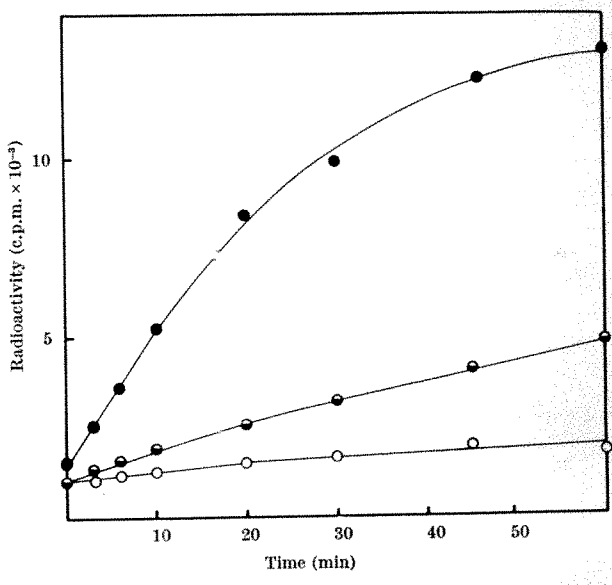


Fig. 1. Time curve of the release of soluble radioactivity. Incubation mixture: 0·2 M *p*H 7·5 *tris*-hydrochloric acid; 10^{-5} M labelled substrate (3×10^4 c.p.m.) in 1 ml. volume. ○, No addition; ◒, 5×10^{-5} M 2-mercaptoethanol added; ●, 5×10^{-5} M 2-mercaptoethanol added with 180 μg enzyme. The measured radioactivities were calculated to the total incubation mixture and plotted against time

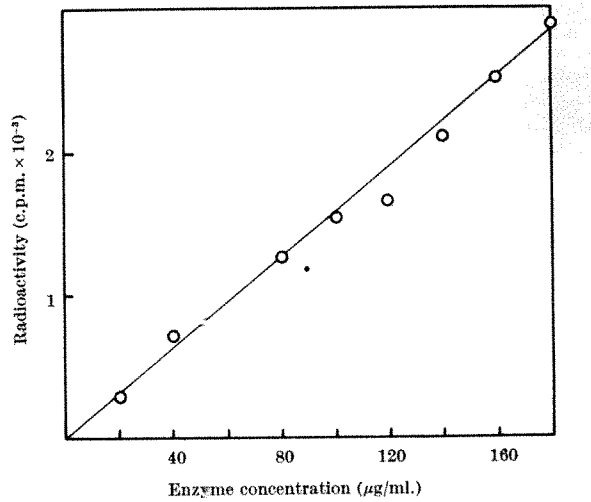


Fig. 2. Enzyme concentration curve. Incubation mixture: 0·2 M *p*H 7·5 *tris*-hydrochloric acid; 10^{-5} M labelled substrate (3×10^4 c.p.m.); 5×10^{-5} M 2-mercaptoethanol; plus varying amounts of the enzyme. Volume: 1 ml. Incubation time: 10 min. The control value (radioactivity released in the absence of enzyme) was subtracted from the measured radioactivities

substrate was incubated with the partially purified RNase-reactivating enzyme[5], radioactivity was released from the protein. The assay was carried out as follows. The labelled substrate was incubated at 30° C in *p*H 7·5 *tris* buffer in the presence of the enzyme and 2-mercaptoethanol. At various intervals, 0·1 ml. portions of this mixture were pipetted into 2 ml. cold acid–acetone (acetone : 1 N hydrochloric acid, 39 : 1). 0·05 ml. of 2 per cent serum albumin solution was added as carrier and the precipitated protein was removed by centrifugation. Equal portions of the supernatant solutions were dried on alumina planchets and their radioactivities determined by means of a windowless gas-flow counter.

Fig. 1 shows the increase of soluble radioactivity in the presence or absence of the enzyme. Fig. 2 demonstrates that during the initial stage of reaction the radioactivity released is proportional to the amount of enzyme present. It should be emphasized, however, that the concentrations of mercaptoethanol and the enzyme in these experiments were lower than those used in our earlier assay method based on the measurement of ribonuclease activity[5]. Under the present conditions, the reactivation of RNase in the first 10 min of incubation was scarcely measurable. On the other hand, by elevating the mercaptoethanol and enzyme concentrations to levels where considerable reactivation occurs, the release of soluble radioactivity was practically complete within the first few minutes of incubation.

It would seem that the assay method described here is much more sensitive than the reactivation test, and its sensitivity can be further increased by decreasing the concentration of mercaptoethanol, elevating the *p*H (the optimum lies between 8·2–8·4), or extending the time of incubation. I believe, moreover, that it offers better possibilities for investigating the detailed mechanism of action, specificity, and other enzymological properties of the enzyme.

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Hormone–Enzyme Complex : Insulin–Hexokinase

CORTISONE acetate dissolved in β-lipoprotein (isolated from blood plasma of normal animals) has been shown to inhibit the activity of yeast hexokinase, while on addition of insulin the inhibition is reversed, and the activity of the enzyme restored[1]. It has also been found that blocking of yeast hexokinase sulphydryl groups with parachlormercuribenzoate[2] or *N*-ethylmaleimide[3] failed to affect the activity of the enzyme. Consequently, it was obvious that sulphydryl groups of hexokinase played no part in catalysis of the glucokinase reaction[4].

The hexokinase inhibiting effect of cortisone may also be displayed when free sulphydryl groups of the enzyme are blocked, while insulin (preventing or reversing the inhibition) fails to have any effect after the sulphydryl groups become bound (Table 1).

Table 1. EFFECTS OF CORTISONE AND INSULIN ON YEAST HEXOKINASE ACTIVITY AFTER BINDING FREE SH-GROUPS OF THE ENZYME

Conditions of incubation	Hexokinase activity: Without inhibition		In the presence of *N*-ethylmaleimide (1×10^{-3} M)	
	μg P-ATP	Inhibition (per cent)	μg P-ATP	Inhibition (per cent)
No addition (control)	40	0	40	0
Added cortisone	22	45	22	45
Added insulin, followed by cortisone	40	0	—	—
Added cortisone, followed by insulin	40	0	22	45
Same + added cysteine excess	—	—	40	0

Table 2. HEXOKINASE ACTIVITY IN ELUATES FROM SPOTS ON STRIPS II AND III (FIG. 1)

Eluates from spots:	Hexokinase activity: μg P-ATP	Percentage
Strip II Hexokinase	230	100
Strip III		
(1) Hexokinase	75	32·6
(2) Insulin	0	0
(3) Third component	145	63·0
Sum of 1 + 3	220	95·6

Fig. 1 shows electrophoretograms (with corresponding 'densitograms') of crystalline insulin, crystalline hexokinase and of a mixture of them, incubated for 15 min at 37° C.

It can be seen in strip III that, in addition to the insulin and hexokinase spots, a third spot (as well as a third peak on the densitogram) has been revealed, representing a component, which exhibits reduced mobility in the electric field, as compared with hexokinase, but greater mobility than insulin.

Table 2 gives the results of an assay of hexokinase activity in eluates from the spots on strips II and III (see Fig. 1).

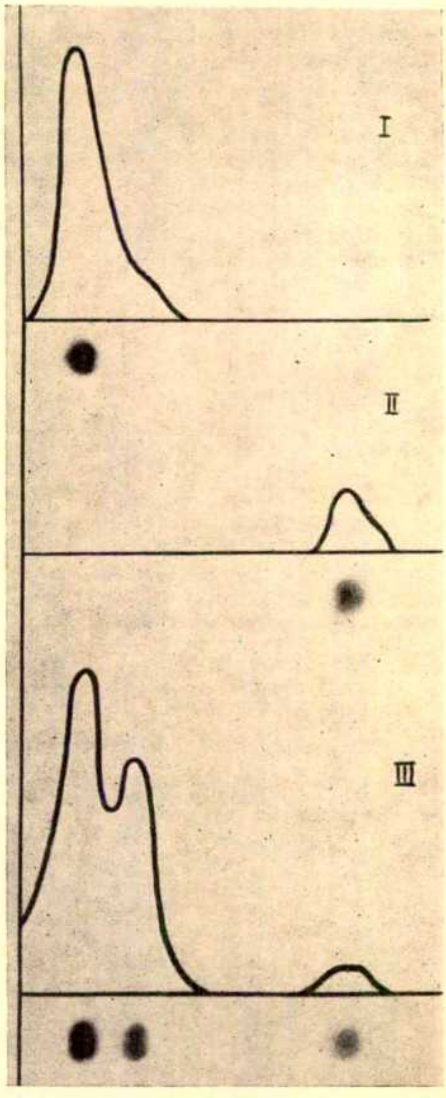
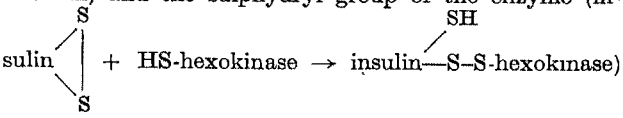


Fig. 1. Electrophoretogram (with corresponding 'densitogram'). Electrophoresis was carried out on 1·5 per cent agar gel with 0·05 M veronal buffer (*p*H 8·6). (I) 200 μg insulin; (II) 1 mg hexokinase; (III) 200 μg insulin + 1 mg hexokinase, preincubated for 15 min at 37° C

Enzymatic activity, which is absent from the hexokinase spot, has been quantitatively recovered from the spot formed by a new, third component, resulting from incubation of insulin with hexokinase (Fig. 1).

The results of this experiment demonstrate the formation of a new complex during incubation of insulin and hexokinase—a hormone–enzyme complex: insulin–hexokinase. The total hexokinase activity remained unchanged within the system. The appearance of such a complex would account for the capacity of insulin to prevent, or reverse, the inhibitory effect of cortisone on yeast hexokinase activity (Table 1).

We suggest two alternative interpretations for the mechanism of interaction between insulin and yeast hexokinase: (1) Formation of the hormone–enzyme complex insulin–hexokinase, through a disulphide bond between the –S–S– group of the hormone (probably its *A*-chain) and the sulphydryl group of the enzyme (in-



can be attributed to the action of an allosteric effector (insulin), which results in a change in the conformation of the enzymatic protein and thus its inability to interact with cortisone. (2) Fixation of insulin on the hexokinase molecule determines the capacity of the former to interact with glucocorticoid, which results in prevention of inhibition, or in liberation of the "active centre" of the enzyme when inhibition of the enzyme by the glucocorticoid occurs.

The second interpretation would seem to be similar to G. Weber's[5] views on the interaction between insulin and glucocorticoid—as revealed by its influence on hepatic glucose-6-phosphatase synthesis: repression, in the case of insulin, and reversal of the repression by glucocorticoid.

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Preparation, Properties and Metabolism of Iodinated Bromosulphthalein

BROMOSULPHTHALEIN (phenoltetrabromophthalein disulphonate sodium) is metabolized by organisms and forms a conjugate with glutathione[1–4]. Rose bengal (tetrachlortetraiodofluorescein), which resembles bromosulphthalein in structure, does not form similar conjugates but undergoes partial deiodination in the organism[5–7].

While studying derivatives similar to bromosulphthalein and their metabolism we became interested in the effects of iodinating bromosulphthalein. We assumed that iodine would link to the phenolic hydroxyl in the *ortho*-position as in rose bengal.

In iodinated bromosulphthalein we expected two metabolic processes: conjugation with the sulphydryl (SH) group and deiodination.

Tubis *et al.*[8] have described the preparation and properties of iodinated bromosulphthalein. They quote an iodine content of 5·3 and 7·9 per cent in the final preparation, but this does not correspond with the required 23·2 per cent for a disubstituent. They worked with a preparation which was not precisely defined and did not investigate its metabolism. We therefore set ourselves the task of elaborating a method for the preparation of defined iodinated bromosulphthalein and of studying its metabolism.

As the initial product for the preparation of iodinated bromosulphthalein we used bromosulphthalein as prepared by Merck, *VEB*, Berlin, and also some which we prepared ourselves. 100 mg of bromosulphthalein was dissolved in 10 ml. of methanol, and 0·05 ml. of iodine monochloride was added; the mixture was heated on a boiling water bath and later 10 ml. of distilled water was added. This solution was repeatedly extracted with 25 ml. of petrol ether in order to remove the excess iodine monochloride. The aqueous solution was then evaporated to dryness on a water bath. The preparation was repeatedly recrystallized from methanol and dried for 48 h over phosphorus pentoxide in a vacuum (15 mm) and at the temperature of boiling chloroform.

The iodine content was estimated by the method developed by Schöniger[9]; it proved to be 22·5 per cent, which is in keeping with the requirement of a disubstituted derivative such as $C_{20}H_6O_{10}Br_4I_2S_2Na_2$—23·2 per cent. The spectral absorption maximum of the preparation occurred at 600 mμ and the molar extinction coefficient was 52,400. Readings were taken on a Unicam '*SP* 500' apparatus.

In a system of propanol, acetic acid and water[10] and in McMillion's system[11] the preparation was found to be chromatographically homogeneous—the R_F in the propanol system was somewhat higher than the R_F of bromosulphthalein. When we attempted a similar preparation of the dibrominated derivative of bromosulphthalein, we obtained a chromatographically heterogeneous preparation with similar properties to iodinated bromosulphthalein. It is probable that partial desulphonation had occurred as a result of the action of bromine (this is known to occur on *o*-phenol sulphonic acids[12]).

When studying the metabolism of bromosulphthalein, 5–5·5 mg/100 g body-weight of our preparation (dissolved in water) was injected intrajugularly into 24 h fasted adult Wistar rats under urethane anaesthesia. The animals were killed after 6 h by decapitation, and the small intestine was removed and divided into three equal parts, which were then rinsed in 5 ml. distilled water. The dye was only ever present in the proximal third part.

For chromatography of the content of the proximal part, a system of propanol, acetic acid and water was used, and 0·2–0·8 ml. solutions were applied to the start. The chromatographic analysis revealed that apart from free iodinated bromosulphthalein two or three other metabolites were also present. The first metabolite was blue in colour (R_F, 0·63) and occurred in the greatest concentration; the second was also blue (R_F, 0·47) but in much lower concentration than the first; and the third violet metabolite (R_F, 0·42) was present only in trace amounts which could only be detected when very large quantities of the solution were applied at the start. The third metabolite had the same R_F as one of the bromosulphthalein metabolites. Free iodinated bromosulphthalein had an R_F of 0·78 and bromosulphthalein an R_F of 0·70.

The iodinated bromosulphthalein prepared in our laboratory corresponded, from its iodine content, to a disubstituent. Although evidence is not available to say for certain whether iodination occurred in the *ortho*-position in relation to the phenol groups, we assume by analogy with rose bengal that this is so. The bromination of tetrabromophenolphthalein also occurred in the *ortho*-position to the OH phenolic groups[13]. An iodine bound in the *meta*-position would not be in line with the normal halogen substitution of the phenol nucleus which is encountered in organic chemistry[14]. The substitution of bromine for iodine in the nucleus of phthalic acid is also unlikely as the iodine content would not correspond to 23·2 per cent.

The formation of coloured metabolites from iodinated bromosulphthalein as with bromosulphthalein itself would suggest that conjugates with glutathione are also formed

similarly as with bromosulphthalein. The finding of a violet derivative, though only in minute amounts, suggests that deiodination also occurs, as the loss of iodine in the iodinated bromosulphthalein molecule would mean that there is a shift of the blue colour back to violet.

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Paradoxical Influence of Calcium Ions on the Permeability of the Cell Membranes of the Isolated Rat Heart

IN the course of an investigation of the specificity of acetoacetate which causes electromechanical dissociation[1] we found that pyruvate also inhibited contractility of the rat heart and dog heart, leaving electrical activity intact[2]. Later we found that perfusion of the heart with sodium citrate caused essentially the same phenomenon. Although the inhibition of cardiac contraction by potassium citrate has already been demonstrated by Melrose *et al.*[3], it is essential to use sodium citrate in order to demonstrate electromechanical dissociation, as high potassium-levels *per se* inhibit electrical activity.

The phenomenon of electromechanical dissociation by calcium-free solutes is well established[4]; we decided to investigate whether acetoacetate, pyruvate and citrate act by lowering the available calcium of the heart cell. This possibility was strongly supported by the finding that inhibition of contractile activity by acetoacetate, pyruvate or citrate could be abolished by raising the level of calcium ions in the perfusion medium (unpublished observations).

It is well known that calcium plays an essential part in the ATP-dependent contraction-relaxation cycle of the actomyosin system; however, its influence on cell structures, such as the cell membrane, is not yet fully understood.

In the course of our investigations, we found that alterations in the calcium concentration in the perfusate give rise to large changes of membrane permeability.

This communication reports the details of our investigation.

White rats, weighing between 200 and 250 g, were anaesthetized by an intraperitoneal injection of sodium pentobarbitone. After the rats had been heparinized, their hearts were removed and perfused at 37° C using Langendorff's technique[5]. A total heart block was introduced by cutting through the bundle of His, and the hearts were electrically stimulated with a fixed frequency of 4·5 beats per sec. The procedure for simultaneous recording of the isotonic contractions and electrocardiogram has been described by Meijler *et al.*[6]. All the hearts were perfused for 30 min with the normal solution before the experiments proper were started. The effluent fluid from the hearts was collected at 1 min intervals, using a refrigerated 'Buchler'-fraction collector. The absorbancy of the effluents at 260 mμ was continuously registered.

The perfusion medium used was a modified Tyrode's solution[7]. It contained in 1 l.: 7·5 g sodium chloride; 0·35 g potassium chloride; 0·15 g calcium chloride; 1·7 g sodium bicarbonate; 0·05 g sodium dihydrogen phosphate; 0·1 g magnesium chloride; 2·0 g glucose. After gassing with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide, the *p*H of the solution at 37° C was found to be 7·4; the final osmolarity was 325 mOsm. The activity of creatine phosphokinase in samples of the effluent fluid was measured spectrophotometrically at 340 mμ in cuvettes of 1 cm light path at 25° ± 2° C. In addition to 0·02 ml. of the sample, they contained 250 mM glycine buffer (*p*H 9·0), 4·2 mM magnesium chloride, 1·7 mM adenosine triphosphate (ATP), 0·13 mM phosphoenolpyruvate, 0·10 mM reduced nicotinamide-adenine dinucleotide (NADH) (except in the control), 4 μg lactate dehydrogenase, 8 μg pyruvate kinase and finally 25 mM creatine after the NADH oxidation had been registered for 10 min, so that the creatine dependent NADH oxidation could be calculated. The final volume was 1·2 ml. The activity of lactate dehydrogenase was also determined spectrophotometrically at 25° ± 2° C in the effluent fluid at 340 mμ in cuvettes containing in 1 ml.: 100 mM *tris*-hydrochloric acid (*tris*-HCl) buffer *p*H 7·6, 0·12 mM NADH (except in the control) and a sample of the heart effluent. The reaction was initiated by the addition of 5 mM pyruvate.

The enzyme activities were calculated on the basis of the initial rates of the reactions and expressed in mU (each mU denoting 1 mμmole of NADH oxidized/min). Potassium and calcium were measured by means of a flame-photometer. Adenosine diphosphate plus adenosine triphosphate were measured in some fractions by the method described by Slater[8]. Myoglobin in the effluent fluid was identified by its column chromatographical behaviour on 'DEAE-Sephadex'[9] after dialysis against 0·01 M *tris*-HCl-buffer (*p*H 8·6). Cytochrome *c* was eluted from such a column with 0·01 M *tris*-HCl, whereas the myoglobin and haemoglobin were retained at the top of the column. Myoglobin, in contrast to haemoglobin, was eluted by 0·05 M *tris*-HCl *p*H 8·6.

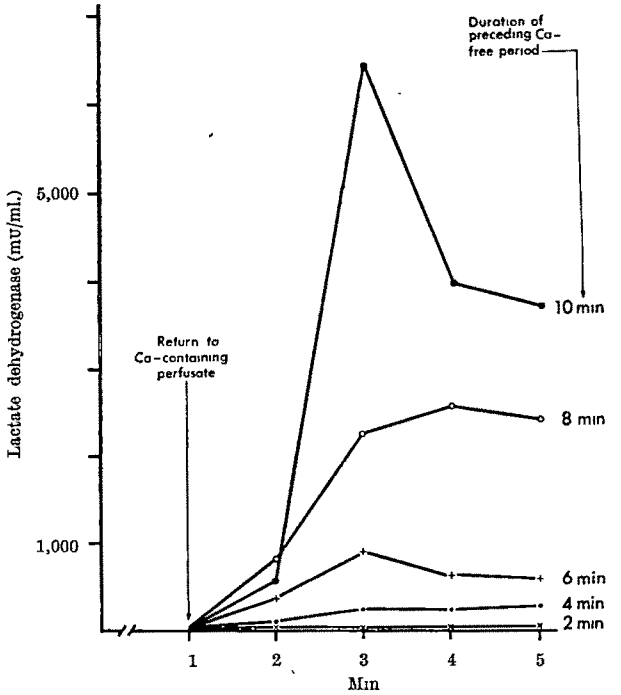


Fig. 1. Lactate dehydrogenase activity in the effluent fluid from isolated rat hearts after calcium-free perfusion for various periods of time

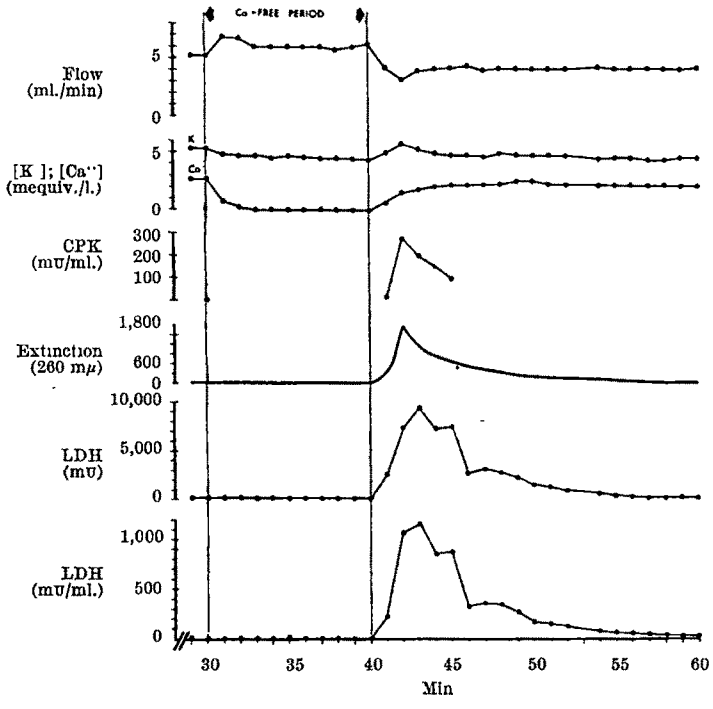


Fig. 2. Analysis of potassium- and calcium-ions, creatine phosphokinase (CPK), lactate dehydrogenase (LDH) and 260 mμ-absorbing material in the effluent of an isolated rat heart during cardiac arrest by perfusion with a calcium-free medium and the subsequent period of perfusion with a medium containing 2·6 mequiv. calcium ions. The coronary flow was calculated from the effluent fractions obtained at 1 min intervals. Total LDH activity was calculated by multiplication of the flow in ml. by the LDH activity per ml. (lower two curves)

During perfusion with a calcium-free solution it was found that contractility was abolished, but electrical activity was maintained. There was a definite increase in coronary flow. Returning to a normal perfusate after more than 2 min resulted in the disappearance of the electrocardiogram, and, after the heart had contracted a few times, these contractions passed off completely and contractile activity was not restored. The heart lost its colour and acquired a pale and mottled appearance. This phenomenon, whereby the heart maintained its red colour and electrical activity when the perfusion medium was changed from normal to calcium-free, but lost these properties on return to normal, we called the "calcium paradox". We found that the extent of these changes strongly depended on the duration of the calcium-free perfusion.

The bleaching of the hearts was accompanied by the loss of a brownish substance from the heart, which coloured the effluent yellowish-brown. A loss of myoglobin was considered to be the most likely explanation for this. This hypothesis was confirmed by measuring the absorption spectrum of the effluent. Clear peaks at 540 and 419 mμ were obtained, suggesting the loss of a haemoprotein. A spectral shift occurred after addition of dithionite. Further proof for the presence of myoglobin was obtained by chromatographic identification of the product as described earlier. No cytochrome *c* could be detected in the effluent by this method. Because myoglobin (a protein of low molecular weight) was found in the effluent, it was necessarily concluded that the calcium shift gave rise to an increase in the permeability of the cell membrane, leading to a loss of this cytoplasmic constituent. It therefore seemed of interest to investigate whether, on changing the calcium-free perfusate back to a calcium-containing perfusate, other intracellular proteins of a higher molecular weight also passed through the membrane structures. Because lactate dehydrogenase and creatine phosphokinase activities are known to be high in heart muscle, we undertook to determine whether these proteins were also present in the perfusate. It can be seen from Fig. 1 that lactate dehydrogenase indeed leaves the heart muscle after the heart has been perfused by a calcium-free solution on changing back to the calcium-containing medium. It can also be seen that the magnitude of the loss of lactate dehydrogenase is strongly dependent on the time during which the heart was perfused by the calcium-free medium. It is of interest to note that in this series of five rats, only the heart that had been perfused for 2 min with the calcium-free solution resumed its regular contractile activity after changing back to the normal perfusate. Hearts perfused for longer periods with a calcium-free solution were found to be irreversibly damaged, although a few initial contractions were observed.

Fig. 2 shows an experiment with a heart that had been perfused for 10 min with a calcium-free medium after it had been equilibrated for 30 min with normal medium. On returning to the normal perfusion medium, an almost immediate loss of lactate dehydrogenase, creatine phosphokinase, 260 mμ-absorbing material and potassium ions from the heart ensued. The correlation of these events is very striking. From the finding that the absorption at 280 mμ was about one-third of that at 260 mμ, it appeared likely that the contribution of protein to the material lost from the heart cells was relatively small. Indeed, estimation of energy-rich adenosine phosphates in the fractions with maximum 260 mμ absorption showed that at least one-third of the 260 mμ absorbing material resulted from the presence of these high-energy phosphate compounds.

We conclude from these results that, after calcium-charge, the membrane of the cell is no longer capable of retaining intracellular constituents.

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BIOLOGY

Distribution of Labelled Indolyl-3-acetic Acid in Intact Cucumber Plants

INDOLYL-3-ACETIC acid (IAA) applied to intact plants may induce specific morphogenetic changes, by affecting the differentiation of certain organs in those parts of the plants to which it is transported. The causal relationship between endogenous and applied growth substances and the sex expression of cucumber plants was extensively studied in this laboratory[1]. The use of radioactive IAA makes possible an accurate determination of the dis-

Table 1. DISTRIBUTION OF IAA-2-^{14}C IN VARIOUS PLANT ORGANS OF MONOECIOUS CUCUMBER PLANTS*

Plant organ	Time interval between injection and harvest: 4 h, Experiment 1 Dry weight (g)	4 h, Experiment 1 C.p.m./plant organ	4 h, Experiment 2 Dry weight (g)	4 h, Experiment 2 C.p.m./plant organ	50 h, Experiment 1 Dry weight (g)	50 h, Experiment 1 C.p.m./plant organ	50 h, Experiment 2 Dry weight (g)	50 h, Experiment 2 C.p.m./plant organ
Apex	0·017	6,610	0 015	9,165	0·020	23	0·020	34
Third leaf	0·061	2,313	0·058	2,654	0 243	136	0·222	127
Second leaf	0·392	1,002	0 356	205	0·638	88	0 719	126
Second petiole (place of application)	0·022	53,490	0 020	48,869	0 047	2,673	0·046	2,810
First leaf	0·462	65	0·450	30	0·552	13	0 600	18
First petiole	0·034	222	0 033	165	0 045	24	0 053	65
First internode	0 053	4,139	0·062	3,041	0 135	206	0·125	64
Cotyledons	0·353	63	0 339	23	0·400	26	0·378	37
Hypocotyl	0·145	226	0 120	144	0·178	56	0·181	33
Roots	0 055	171	0·064	93	0·102	17	0·102	36
Total	1·594	68,301	1·517	64,389	2·360	3,262	2·446	3,350

* IAA-2-^{14}C was injected into the petiole of the second leaf and harvested 4 or 50 h after application. Results are expressed in c.p.m. obtained in purified ether extract.

tribution and *in vivo* degradation of applied IAA. The distribution of IAA-2-^{14}C in monoecious cucumber plants was studied in order to provide data for the correlation of this distribution, with the morphogenetic effects caused by this growth substance.

A considerable amount of information exists regarding the adsorption, translocation and destruction of IAA in plant sections, but little is known about these processes in intact plants. In the present investigation, radioactive IAA was applied by several means; for example, by droplets to leaf blades, by lanoline—or polyethylene glycol ('Carbowax')—pastes applied to petioles or on stem tips, and by injection. The subsequent distribution of label was detected by autoradiographic and extraction techniques. Such tests were repeated under various environmental conditions and the auxin was applied to seedlings at different stages of development. The most uniform quantitative results were obtained when application was by means of injection. Results of a typical experiment are presented in Table 1. One μl. of solution containing 1 μg IAA-2-^{14}C (specific activity 9·2 mc./mmole) was injected into the second petiole of plants, which had developed two leaves. Ten plants were used in each treatment. After injection, the plants were left for either 4 or 50 h in the greenhouse and then appropriately sectioned. The sections were immediately killed by treatment with liquid air, freeze-dried in a Virtis 'Lyophilizator' and the dried, pulverized material was extracted three times for 1 h at 0°–1° C with a total volume of 30 ml. peroxide-free diethyl-ether. The combined ether was reduced *in vacuo* to 10 ml. and washed with 15 ml. of 0·5 M sodium bicarbonate. The sodium bicarbonate solution was acidified to *p*H 3·0–3·5 with 0·1 N hydrochloric acid and re-extracted with ether to yield the acid-neutral fraction. Radioactivity was measured by a liquid scintillation spectrometer and results were expressed in counts per minute (c.p.m.) for each plant organ.

It can be seen from Table 1 that a relatively small amount of the injected IAA was translocated away from the site of application. This would indicate that a strong limitation of IAA transport occurs in intact plants, as has been also confirmed in the coleoptiles of *Avena*[2].

The greatest accumulation of radioactivity was found in the plant apex 4 h after IAA injection. Although the stem tip is the smallest organ on a weight basis, the highest absolute amount of label was translocated to it. A comparatively minute amount of radioactivity was found in the leaves. Only about 5 per cent of the total radioactive dose injected was detected in all the plant organs 50 h after injection. This disappearance is most probably due to *in vivo* destruction. Only about half the activity detected in the purified plant extract could be identified by paper and thin-layer chromatography in the R_F region of IAA.

This rapid disappearance of IAA from intact plants is in contrast with results obtained with carbon-14-labelled 2,4-dichlorophenoxyacetic acid and other synthetic growth regulators used as herbicides[3]. It is assumed that the lack of successful recovery of IAA arises from enzymatic breakdown or conversion to other substances, most probably when growth occurs in light. It seems that endogenous IAA in the plant is much more resistant to degradation. It may be that combination with protein, with inhibitors or with other compounds provides this protection. The question as to how the application of IAA causes a marked effect on sex expression in cucumber plants[4] despite its apparent rapid degradation, however, remained unsolved.

Studies are at present being carried out, using synthetic auxin also, to gain a better insight into the processes by which auxin accumulation induces morphogenetic changes in plants.

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A Self-fertile Polyhaploid Plant in an Autotetraploid Variety of Sugar Beet

A POLYHAPLOID seedling with eighteen chromosomes has been found among the offspring of cytologically controlled tetraploid plants of sugar beet. The plant survived the winter months and developed a number of seed stalks in the following spring. At flowering time the plant was isolated in a greenhouse and, in addition, one branch was bagged. Despite the fact that the original diploid variety was classified as self-sterile, the new diploid (polyhaploid) plant set a number of seeds and proved to be partially fertile[1].

Polyhaploids in autotetraploid beets were reported by Fischer[2] and Kruse[3] during investigations of polyembryony in *Beta vulgaris* L. The frequency of polyhaploids in the tetraploid beets investigated here was about one plant per thousand. If a plant such as that described here grows undetected within a tetraploid variety the consequences are obvious. While a self-sterile diploid plant in a tetraploid variety only causes the production of a certain number of triploid seeds, the self-fertile plant besides provoking aneuploidy also reproduces itself in chromosome number. Within a few generations the tetraploid variety would be invaded by plants from different chromosome levels. This emphasizes the necessity of repeated

cytological controls in autotetraploid varieties. It also indicates that polyploid material is hampered not only by chromosomal instability but also by changes at the genome level connected with unexpected genic changes.

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Production of Pectic Enzymes by *Phytophthora infestans*

PECTIC enzymes have been shown to be produced *in vitro* by many plant pathogenic fungi, and evidence has been presented which suggests that they may play a part in the mechanisms of infection of the host plant[1]. Most work has been concerned with relatively non-specialized facultative parasites. Little is known about obligate parasites, for which the only record is of polygalacturonase production by germinating uredospores of *Puccinia graminis tritici*[2], and the difficulty of obtaining uredospore suspensions in axenic culture makes this work somewhat suspect. *Phytophthora infestans* shares certain features in common with obligate parasites, showing a high degree of host specificity[3], has much more exacting nutritional requirements *in vitro* than most facultative parasites[4,5], and forms haustoria[6]. The processes of germination *in vitro* appear similar to that of many obligate parasites, as zoospores readily germinate on a wide range of natural and synthetic media but do not readily continue growth to form actively growing colonies[7]. Thus the following observations on the ability of *Phytophthora infestans* to produce pectic enzymes may be of some interest.

An isolate of race 4 was grown in a range of natural and synthetic media. The basal medium contained in g/l.: potassium dihydrogen phosphate, 0·5; $MgSO_4.7H_2O$, 0·25; asparagine, 1·0; yeast extract, 5·0; thiamine, 0·001, and additions were made as shown in Table 1. The *p*H was adjusted to 5·5 before autoclaving. After 14 days' growth, the culture filtrates were tested for pectin methyl esterase (PME), exo- and endo-polymethylgalacturonase, exo- and endo-polygalacturonase[8,9], and tissue macerating activity using potato tuber disks[10]. The results are given in Table 1. A further seven isolates (two of race 4, one of race 1 and four of unknown race type) were grown in medium *F* and the culture filtrates tested for PME and macerating activity as before, and for polygalacturonase activity by the cup-plate technique[11]. Again PME was the only enzyme activity detected.

Table 1. PECTIC ENZYMES PRODUCED BY AN ISOLATE OF RACE 4

Medium	Pectic enzymes: PME	Polygalacturonase complex	Macerating activity
A 'Birds Eye' french beans	+	−	−
B Potato stems	+	−	−
C Potato tubers	+	−	−
Synthetic media (basal medium with additions as shown in g/l.)			
D Glucose 25 g	+	−	−
E Apple pectin 10 g	+	−	−
F Glucose 25 g + apple pectin 10 g	+	−	−

+, Enzyme activity demonstrated. −, No enzyme activity demonstrated.

The failure to detect polygalacturonase activity of any kind conflicts with the results of Grossmann[12], but it is possible that these are inducible enzymes only produced under conditions which were not provided in these experiments. The absence of macerating activity was not unexpected as the host's tissue is not macerated *in vivo*. Tissue disks infiltrated with culture filtrates did not go brown, and were not killed because the cells could still be plasmolysed and deplasmolysed, and so the toxicity usually associated with macerating activity was also not present[13,14]. The only enzyme activity which could be demonstrated in any filtrate was PME. This was produced in media both with and without pectin, and so behaved as a constitutive enzyme. This would suggest that it may also be produced *in vivo* and play some part in the mechanisms of infection.

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Mechanism of Fluid Exudation from Isolated Maize Roots

IT is commonly believed that salts are secreted into the xylem vessels of isolated roots by some mechanism involving metabolic energy and that the concomitant water movement is driven by the osmotic pressure gradient between xylem sap and the external medium. In particular, House and Findlay[1] have found quantitative support for this process in maize roots. These workers considered that the exudation from the basal ends of isolated roots was described by:

$$J_w = L_p RT[C_s^x - C_s^0] + \varphi_w^0 \qquad (1)$$

where J_w (cm³ exuded per cm² of root surface area per sec) is the fluid exudation rate, $L_p RT(C_s^x - C_s^0)$ is the net osmotic water flux from the external medium into the root and φ_w^0 is net water flux independent of any osmotic gradient. This latter water flow might possibly be analogous to that found in many animal epithelial preparations bathed in identical salines. In equation (1), L_p (cm/sec atm.) is the osmotic permeability or, more correctly, the hydraulic conductivity of the roots to transverse water flow, R the gas constant, T the absolute temperature, C_s^x (mole/cm³) the osmolarity of salt (for example, potassium chloride) in the exudate and C_s^0 the osmolarity of salt in the external medium. The observed difference in salt concentration ($C_s^x - C_s^0$) is likely to depend not only on the net salt flux into some compartment, like the xylem, but also on the rate of water entry into this region.

Several investigators[2-4] have proposed essentially similar descriptions of water flow to equation (1), but apparently there has been no quantitative support for this relation under steady-state conditions apart from our investigations[1]. The purpose of the experiments reported here was to test equation (1) by examining the relationship between J_w and L_p for isolated maize roots.

Grains of maize (*Zea mays*, white horse tooth) were soaked and germinated as previously described[1]. Primary roots were excised from 4- or 5-day-old seedlings which had been pretreated with a solution, π_1, containing 0·1 mM calcium chloride and 1 mM/l. potassium chloride, for 24 h before the experiment. The basal end of each root

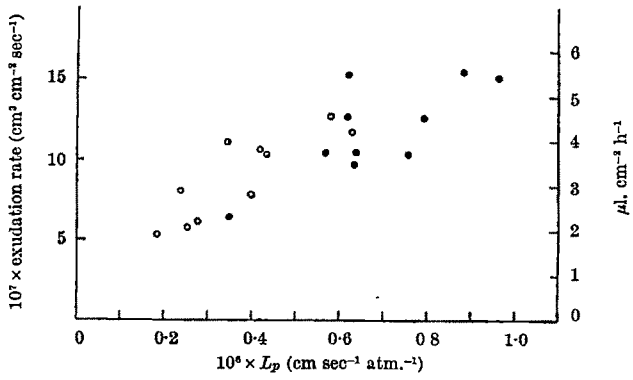


Fig. 1. The relation between the exudation rate and the estimated value of the hydraulic conductivity for isolated maize roots. Each point represents the results of an experiment on a single root. ● and ○ denote roots 5–7 cm and 9–16 cm long, respectively. The ordinate axis on the right of the figure expresses the exudation rate in conventional units

was inserted into an enlarged orifice in the end of an L-shaped glass capillary tube (internal diameter 0·05 cm) so that each root was immersed vertically in the medium. The fluid exudation rate was measured by observing the horizontal motion of the capillary meniscus with a Vernier microscope. The excised roots were 5–16 cm long and about 0·1 cm in diameter at the basal ends; the roots had no visible lateral roots or root hairs. All experiments were performed at 25° ± 1° C and under conditions of continuous aeration. For the purpose of expressing the fluid exudation rate in μl./cm² h (or cm³/cm² sec), the root surface area was assumed to be cylindrical.

After an equilibration period of at least 1 h in the basic medium, π_1, the exudation rate, $J_w{}^{\pi}{}_1$, was measured over six 5 min intervals. In every experiment, $J_w{}^{\pi}{}_1$ was constant over this period and the external medium was later replaced by a solution, π_2, containing 0·1 mM calcium chloride and 50 mM/l. potassium chloride. A rapid decrease in the exudation rate was observed and within about 150 sec the exudation had reached a minimum rate, $J_w{}^{\pi}{}_2$. Assuming that $\varphi_w{}^0$ and $C_s{}^x$ remain constant during this experiment, it is possible to show that, in these circumstances:

$$L_p = 1{\cdot}02 \times 10^4 \, (J_w{}^{\pi}{}_1 - J_w{}^{\pi}{}_2)/RT \text{ (cm/sec atm.)}$$

where RT is $2{\cdot}5 \times 10^4$ cm³ atm./mole and $J_w{}^{\pi}{}_1$ and $J_w{}^{\pi}{}_2$ are expressed in cm³/cm² sec.

Fig. 1 shows the results of these experiments on twenty roots. The mean ± *S.E.* value of L_p for roots 5–7 cm long (● in Fig. 1) was $(0{\cdot}69 \pm 0{\cdot}06) \times 10^{-6}$ cm/sec atm., while the corresponding value for roots 9–16 cm long (○ in Fig. 1) was $(0{\cdot}38 \pm 0{\cdot}05) \times 10^{-6}$ cm/sec atm. These values for L_p agree with other estimations[1] of this parameter for maize roots. Moreover, our estimates of L_p are in general agreement with those values cited by Bennet-Clark[5] for bean roots (calculated from Brouwer[6]) and by other investigators[7,8] for various plant cells and tissues. Nevertheless, a detailed discussion[9] of the experiments reported here suggests that this method of estimating L_p yields an underestimate of its actual magnitude.

Although our experiments indicate that the water permeability of the isolated maize root is not a uniform property along its length, an examination of the data in Fig. 1 showed that the relation between J_w and L_p was linear and homogeneous for the two groups of roots. Equation (1) may be considered, therefore, an adequate quantitative description of the fluid exudation from isolated roots as Fig. 1 indicates that $\varphi_w{}^0$ is non-zero; however, a satisfactory direct demonstration of the existence of $\varphi_w{}^0$ needs further investigation since there are alternative explanations[1,9] of our data.

It is concluded that the water movement in isolated maize roots is produced by an osmotic mechanism with the additional possibility that a small component of the exudation is non-osmotic in origin.

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GENETICS

Does *Vicia faba* have Multi-stranded Chromosomes ?

THE broad bean, *Vicia faba*, has been extensively used for cytological investigations because, among others, it has the advantage of unusually large chromosomes. Several different kinds of investigation using this species have given results which suggest that its chromosomes are multi-stranded. These include autoradiographic investigations of chromosome replication[1], investigations of meiosis and mitosis following X-irradiation[2] and, most recently, direct observation of the unravelling of chromosomes following enzymatic digestion[3].

In the hemipteran tribe Pentatomini, the occurrence of species which have a tetraploid number of autosomes but DNA values like diploids[4] suggests that the latter have twice as many strands per chromosome as the former. This example illustrates the value of studying species related to those which are thought to have multi-stranded chromosomes.

We have investigated the karyotypes of *Vicia faba* and of five closely-related species of the section Euvicia of the genus *Vicia*[5]. We have modified the quantitative method for comparing karyotypes described by Martin and Hayman[6] in two ways. First, we have measured the percentage of the area of chromosome arms, rather than the percentage of their lengths, because this quantity seemed more appropriate for chromosomes which were small and, following colchicine treatment, highly contracted. Secondly, we have measured relative DNA values of mitotic root-tip nuclei isolated by the method of McLeish[7].

Vicia faba has been assigned a DNA value of 100 arbitrary units. A group of two species had 16–19 per cent of this value and a second group of three species had 50–59 per cent. Fig. 1 shows these DNA values and derived karyotypes. The percentage of area is printed against each arm, but modified (except in *V. faba*) to allow for relative DNA values. Thus in all species the areas are in the same units and are directly comparable.

The two species with low DNA values, *V. sativa* and *V. amphicarpa*, would seem to have four pairs of chromosomes in common, that is those chromosomes connected by arrows have arm areas which do not differ at the level of probability $P = 0{\cdot}05$. In the second group, the three species do not seem to share any chromosomes; however, it is not possible to distinguish with certainty four pairs of chromosomes in *V. hybrida* so that these must be grouped for statistical tests. Five similar pairs in *V. lutea* must also be grouped. When comparisons are made between these two groups, the short arms do not differ ($0{\cdot}90 > P > 0{\cdot}80$) but the long arms do ($0{\cdot}02 > P > 0{\cdot}01$), but real similarities may be obscured by the artificial grouping.

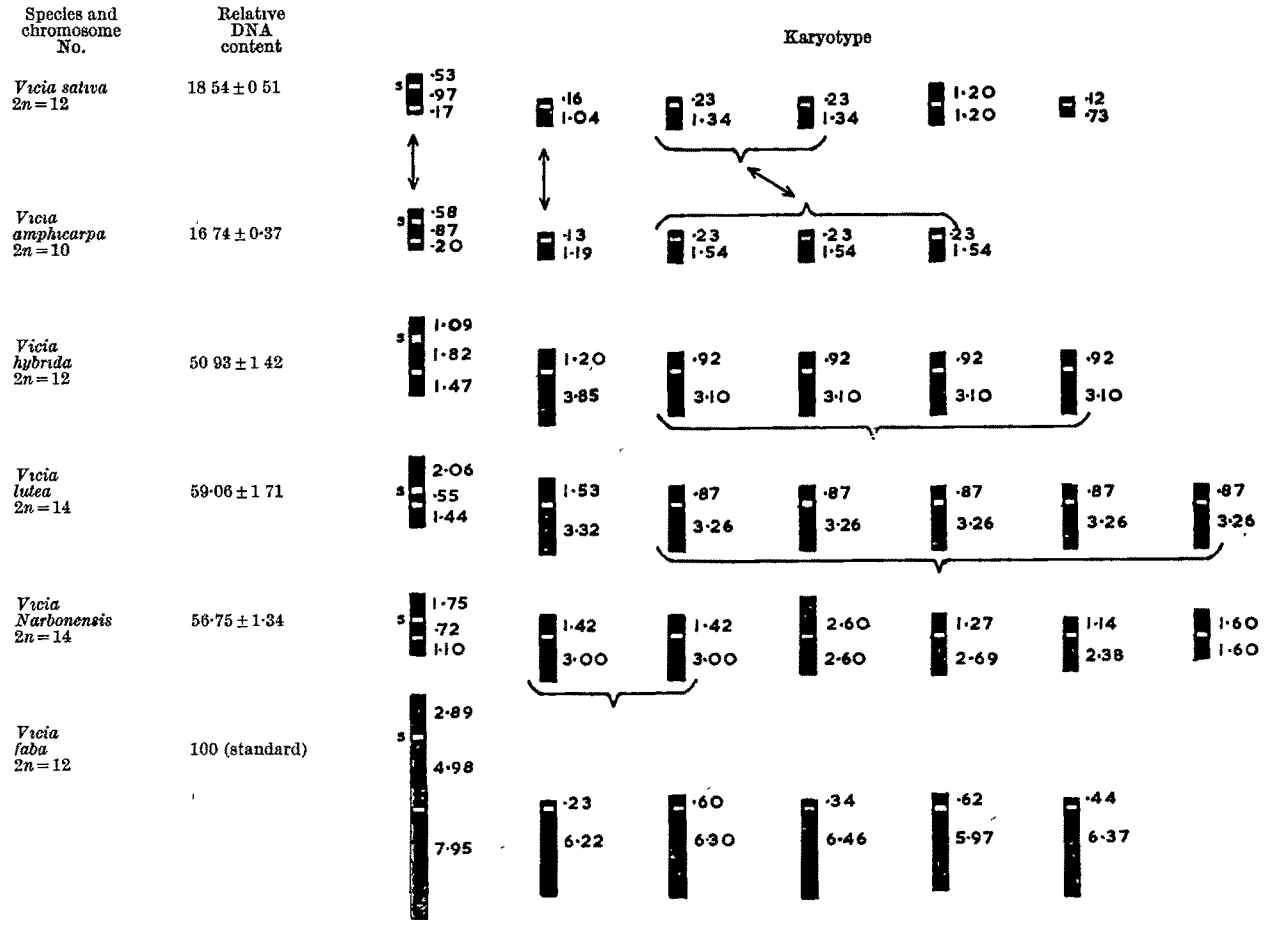


Fig. 1. Relative DNA contents and karyotypes of six species of *Vicia*. *Notes* (1) DNA values were derived from fifty cells (root-tip prophase) of both the species named and of the standard species. (2) The values alongside chromosome arms are the mean percentage of areas from photographs of twelve different cells at metaphase. Percentages are based on the diploid chromosome complement. The areas have been modified to take account of relative DNA values so that the values in different species are directly comparable. (3) Interspecific comparisons which are not significantly different are indicated by arrows. (4) *s*, Secondary constriction. (5) Chromosomes which cannot be distinguished with certainty are bracketed

The differences between the three groups of species are great and many chromosome re-arrangements are evidently involved. Unless duplications associated with re-arrangements alone can account for the large inter-group differences, it is necessary to consider the possibility that they differ in degree of polynemy. Differential polynemy has been discussed by Darlington[8] and recently reviewed by Lewis and John[9].

It has been suggested[10] that the great interspecific variation in DNA content of angiosperm nuclei is related to different adaptive values of different cell sizes. When DNA content changes by polyploidy, aneuploidy, or duplication in association with re-arrangements, advantages of changed cell size may be counteracted by disadvantages arising from reduced fertility or, except in the case of polyploidy, from genic imbalance. If changed cell size can also arise from changes in degree of polynemy, then genic imbalance should not occur; but if no disadvantages are to arise from reduced fertility, then meiosis must be able to proceed regularly. If *V. faba* does indeed have multi-stranded chromosomes, then it is able to accomplish meiosis without changing the degree of polynemy. We have found that DNA values in meiotic prophase are twice those of the preceding interphase, four times those in newly-formed pollen grains and equal to those in mitotic prophase in root-tips.

The disadvantages of multi-stranded chromosomes would be associated with hindering adaptive changes normally effected by mutation and selection. It is possibly significant that Rowlands[11] failed to detect any response to selection in *V. faba*. If it can be shown that multi-stranded chromosomes do occur in the genus *Vicia*, this may account for the comparative rareness of polyploidy in the genus[12,13]. Here the advantage of chromosome duplication would have been gained through polynemy.

The evidence discussed here suggests that different degrees of polynemy exist within the genus *Vicia*. One direct test of this would be to perform unravelling experiments similar to those of Trosko and Wolff[3]. These should reveal different numbers of strands in species from the three groups described.

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Differential Response of Some Tomato Genes to Base-specific Mutagens

A NUMBER of chemicals are now known which affect specific bases of the DNA molecule[1]. These include the base analogues (such as fluorodeoxyuridine) and others like hydroxylamine and hydrazine which react mainly with cytosine and thymine respectively under particular conditions of treatment. The action of many of these chemicals on chromosomes and genes in higher organisms has not been tested extensively, but some reports are available which show that they induce localized damage in the nucleus[2–4]. We have observed a differential reaction of tomato chromosomes when pollen mother cells are treated with hydroxylamine[5]. The main finding was that a few of the chromosomes, particularly chromosome 2, were much less damaged than other members of the complement. These experiments with hydroxylamine and a number of other chemicals and X-rays have now been extended to compare the spectrum of mutations induced by them; the present communication relates to some of the results obtained.

The different treatments, details of which are presented in Table 1, were given to seeds (500 in each case) presoaked in water for various durations. The *p*H's of the freshly-prepared solutions were adjusted in a manner described elsewhere[5]. The observations on induced mutations were recorded mainly on seedlings which are easier to handle in large numbers. Also, a number of gene mutations affecting seedling characters are known to give such characteristic phenotypes that the induced variation can easily be scored for them[6].

These observations make it clear that hydrazine and hydroxylamine induce mutations at a number of loci with very different frequencies. These loci, moreover, are not randomly distributed in the genome. A number of them (*d*, *aw*, *wo*) are known to be located on one particular chromosome (chromosome 2). It should be emphasized that although hydroxylamine failed to induce mutations at these loci, the overall mutation rate with this chemical was higher than that with hydrazine, as shown by the frequency of lethal seedlings and other variation not included here because it could not easily be classified in relation to any of the known genes. Also, for comparable concentrations, hydroxylamine is found to induce significantly greater chromosomal damage and reduce germination percentage to a much greater extent (see Table 1). Ethylmethanesulphonate, which is known to react with several bases[7], differs from both in inducing chlorophyll deficiencies of various kinds, but apart from this, it appears to correspond in its action more to hydroxylamine than to hydrazine.

The observations, and the cytological analysis reported earlier, suggest that a number of loci, particularly on chromosome 2, may be characterized by an inhomogeneity in the distribution of adenine-thymine and guanine-cytosine base pairs in their DNA. Differences in base composition of this nature, if confirmed, may prove to be of considerable interest, for they may be of significance in relation to what Rick[8] has described as the asymmetrical distribution of genes on tomato chromosomes, with chromosome 2 having nearly 25 per cent of all the mapped genes located on it. No satisfactory explanation for this asymmetry has so far been available. Further analysis using techniques based on autoradiography and ultracentrifugation is in progress.

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CYTOLOGY

Cytoplasmic Deoxyribonucleic Acid-containing Bodies in Amoebae

EVIDENCE has been presented, from investigations involving techniques of nuclear transfer and injections of cytoplasm, that certain cellular properties of *Amoeba proteus* and *Amoeba discoides*, such as nuclear diameter and sensitivity to streptomycin, are influenced by self-replicating cytoplasmic hereditary determinants[1–6]. The finding of DNA- and RNA-containing bodies in the cytoplasm of *Amoeba proteus*[7–9], followed by the demonstration that these bodies are self-replicating within the cell[8,9] and that they show a certain degree of interaction with the nucleus of amoeba[8], has stimulated interest in the possibility that they contain the physical basis of this cytoplasmic inheritance[6].

Cells of the strain of *Amoeba proteus*, $_{Tl}P$, and the strain of *Amoeba discoides*, $_{Tl}D$, used in the nuclear transfer

Table 1

Treatment	M_1 seed germination (per cent)	Frequency of different mutant seedlings (Percentage of M_1 seedlings) Dwarfs (*d*)	Without anthocyanin (*aw*)	*d* + *aw**	Woolly (*wo*)	Chlorophyll deficient	Lethals†
Hydrazine (*p*H 8 5)							
(i) 0·1 M, presoaked for 16 h, treated for 20 h	40·8	1·96	0 98	0 49	0 98	–	0 98
(ii) 0 1 M, presoaked for 16 h, treated for 30 h	40·0	0·50	–	1·00	0·50	–	1·50
(iii) 0·05 M, presoaked for 20 h, treated for 12 h	63·0	0·95	0 95	0 63	0·63	–	–
(iv) 0·00 M, presoaked for 16 h, treated for 30 h (Control)	64·8	–	–	–	–	–	–
Hydroxylamine (*p*H 7 0)							
(i) 0·05 M, presoaked for 16 h, treated for 20 h	17·4	–	–	–	–	–	5 74
(ii) 0·025 M, presoaked for 20 h, treated for 12 h	46 4	–	–	–	–	–	3·87
(iii) 0·012 M, presoaked for 20 h, treated for 30 h	45·9	–	–	–	–	–	3·26
(iv) 0 00 M, presoaked for 16 h, treated for 20 h (Control)	76 6	–	–	–	–	–	–
EMS (*p*H 7·0)							
(i) 0·03 M, presoaked for 16 h, treated for 20 h	47·6	–	–	–	0·42	0 84	5 45
(ii) 0 03 M, presoaked for 16 h, treated for 30 h	45 4	0 44	–	–	–	1 32	7 92
(iii) 0 00 M, presoaked for 16 h, treated for 30 h (Control)	80·8	–	–		–	–	–
X-ray (dry seeds)							
(i) 20,000 r.	64·2	–	–	–	–	–	0 21
(ii) 30,000 r.	53·8	–	–	–	0 28	–	0·82
Control	86·2	–	–	–	–	–	–

See Rick and Butler (ref. 6) for a description of the mutant phenotypes. Control mutant stocks for comparison were made available by Dr L. Butler, whose generous gifts of seeds are gratefully acknowledged.

* The simultaneous induction of these double mutants of linked genes is obviously of considerable significance.

† These variously deformed seedlings do not survive beyond the cotyledonary or 1–2 true leaf stage. A statistical analysis shows that hydroxylamine and hydrazine induce them with significantly different (1 per cent level) frequencies.

experiments[1-6] were kindly provided by Dr. S. E. Hawkins. The cells were grown in a dilute salt solution[10] at 18° C and fed daily on *Tetrahymena geleii*. Cells of both species were treated and examined in conjunction. Living cells were fixed either directly or after centrifugation at 10,000*g* for 20 min, digested with ribonuclease and stained with acridine orange[7]. Light microscope autoradiographs[7] and electron microscope autoradiographs[9] were made of cells fixed directly or after centrifugation, which had been incubated for 36 h in amoeba medium supplemented with 10 μc./ml. of tritiated thymidine (8,600 μc./μmole) (exposure was for 71 days). Normal and centrifuged cells were also fixed and stained with basic fuchsin after treatment for 7 min at 4° C with 6 per cent perchloric acid. The specificity of dyes, and of label due to incorporated tritiated thymidine, was controlled by deoxyribonuclease digestions (0·3 mg/ml. distilled water containing 10^{-3} M magnesium sulphate, adjusted to *p*H 7·0 with sodium monohydrogen phosphate, 4 h, 38° C), ribonuclease digestions (0·3 mg/ml. distilled water, adjusted to *p*H 7·0 with sodium monohydrogen phosphate, 4 h, 38° C) and treatment with 10 per cent trichloroacetic acid (TCA) at 90° C for 20 min. Controls of the digestions were prepared by incubating in similar media but with the enzymes omitted, and of the hot TCA by treatment with 10 per cent TCA at 4° C for 20 min or distilled water, 90° C for 20 min.

Examination in the fluorescence microscope of amoebae digested in ribonuclease and stained with acridine orange revealed the presence of 0·5μ–0·8μ yellowish-green bodies in the cytoplasm in the case of *A. discoides* but not *A. proteus*. In centrifuged cells of *A. discoides* the bodies were found in the region of the nucleus as described previously[7,9]. In cells stained with basic fuchsin, cytoplasmic bodies were again seen only in *A. discoides* and corresponded in size and, in centrifuged cells, in position, to those seen under the fluorescence microscope. The yellowish-green fluorescence is sensitive to DNase and removed by hot TCA, and no bodies could be found if digestion with RNase or treatment with hot TCA preceded basic fuchsin staining. In light microscope autoradiographs, about 30 per cent of the *A. proteus* cells and about 60 per cent of the *A. discoides* cells examined showed nuclear label; but stratifiable, DNase-sensitive, acid-insoluble, cytoplasmic label was found only over cells of *A. discoides*. In the electron microscope cytoplasmic bodies similar to those described previously[9] were found only in *A. discoides*, and in electron microscope autoradiographs these bodies were found to be the only entity that was significantly labelled.

In order to explain all the results of the nuclear transfer and cytoplasm transfer experiments[1-6], hereditary factors must be present in the cytoplasm of cells of both the strain of *A. discoides* and the strain of *A. proteus* used in these experiments. The present results rule out the possibility that at least in the cells of *A. proteus* the physical basis of the cytoplasmically determined differences in cell characteristics already noted is contained in the DNA- and RNA-containing bodies described by Rabinovitch, Wolstenholme and Plaut, or in similar bodies.

Two other strains of *A. proteus* (*PROT I*, and *K*) which were examined by the ribonuclease-acridine orange technique, and by basic fuchsin staining, were found to be free from the cytoplasmic bodies. The finding of strains of *A. proteus* free from cytoplasmic bodies offers a possible explanation for the single band observed in a caesium chloride gradient analysis of DNA extracted from starved amoebae[11]. As the results indicate that the cytoplasmic bodies are not an essential entity of the cytoplasm of amoeba, their suggested origin from the nucleus of amoeba[9] seems unlikely. These results tend to support the alternative hypothesis that the bodies are endosymbionts[9]. Evidence for nuclear control of the cytoplasmic bodies has already been presented[8] and, although the present results oppose the suggestion that the bodies are responsible for the cytoplasmically determined cell characterstics so far investigated, it is most probable that when present they do influence hereditary processes in the cell. As Lederberg has pointed out[12], and as has been found wherever such relationships have been thoroughly investigated, the contained organisms "comprise part of the genetic determination of the organic whole".

I thank Dr. S. E. Hawkins for cells of *Amoeba proteus* and *Amoeba discoides*, Dr. J. Griffin for *Amoeba proteus* (*PROT I*) and *Tetrahymena geleii*, and Dr. K. Holter for *Amoeba proteus* (*K* in the text). I also thank Dr. E. F. J. van Bruggen for the use of his electron microscope facilities and Prof. W. Beermann and Prof. J. A. Beardmore for constructive criticism of the text.

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HAEMATOLOGY

Binding of Normal Serum Globulins to Tanned Red Blood Cells

ACCORDING to Bregman and Kirsner[1] normal human serum globulins are not usually fixed to tanned red blood cells and this is in contrast to some pathological serum globulins which have a binding capacity for these cells. These conclusions were drawn from the observation that tanned red blood cells coated with normal sera were not usually agglutinated by antiglobulin serum, whereas their coating with some pathological sera resulted in a positive Coombs's test. The difference between normal and pathological sera was even more evident when an anti-non-γ-globulin serum was applied instead of antiglobulin serum. In the anti-non-γ-globulin the anti-γ-constituent of the Coombs's serum is blocked by previous addition of γ-globulin, so that it reacts predominantly with the component of the non-γ-globulin serum[2]. In the present investigation, in which the same method was applied, evidence was obtained for the existence of an inhibitor in normal concentrated sera which interferes with the binding of the non-γ-globulin component to tanned red blood cells.

Tanning of red blood cells was effected according to the technique developed by Stavitsky[3] and a 1 : 40,000 dilution of tannic acid (Merck) in normal saline was used. 0·5 ml. of a 2·5 per cent suspension of tanned red blood cells and 1·5 ml. of the test serum serially diluted in normal saline were incubated at room temperature for 2 h. The tubes were centrifuged, and the sensitized tanned red blood cells were washed twice with normal saline and then resuspended in 0·5 ml. of normal saline.

Three millilitres of antiglobulin serum (Sevac, Prague) were repeatedly absorbed with 0·5 ml. of packed, tanned red blood cells at room temperature for 30 min, until the spontaneous agglutination with antiglobulin serum had disappeared. Anti-non-γ-globulin serum was prepared by mixing equal parts of absorbed antiglobulin serum and an 8 per cent solution of human γ-globulin (Norge).

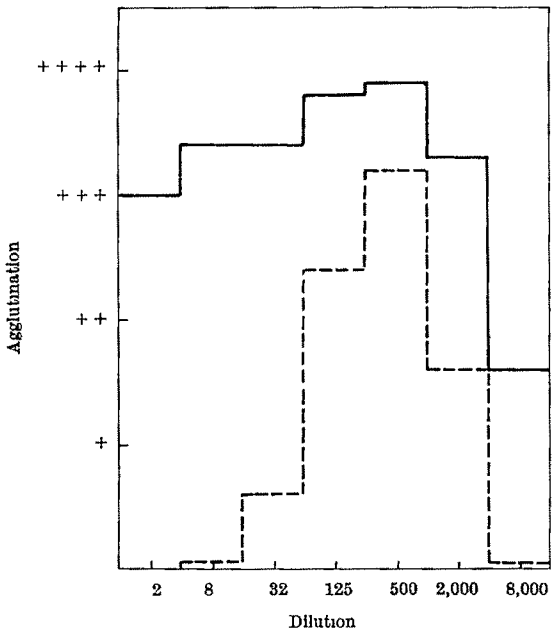


Fig. 1. Agglutination by anti-human globulin (AG) (———) and anti-non-γ-globulin (- - - -) serum of tanned human red cells coated with human normal serum (average of 16 experiments). With the anti-non-γ-globulin serum, in contrast to the antiglobulin serum, the agglutination appears only in diluted sera and is then proportional to the dilution with a maximum at 1 : 500. At all dilutions the agglutination is significantly lower with the anti-non-γ-globulin than with the antiglobulin serum ($P < 0.001$; at 1 · 500, $P = 0.01$–0.02)

Mixtures of 0·05 ml. of antiglobulin serum or anti-non-γ-globulin serum and of 0·05 ml. of the suspension of sensitized tanned red blood cells were incubated for 60 min at 37° C. The agglutination was read microscopically; its degree ranged from one to four plus. These experiments were carried out on sixteen sera.

Control experiments include: (1) tanned but uncoated red blood cells, treated with antiglobulin serum and anti-non-γ-globulin serum; (2) tanned red blood cells coated with the serum to be tested, but only washed in two changes of normal saline. In the absorption experiments carried out on five sera, 3 ml. of serum and 0·5 ml. of packed tanned red blood cells were incubated at 20° C for 30 min. After centrifugation the supernatant was separated. This procedure was repeated up to ten times.

The results are shown in Fig. 1. Tanned red blood cells sensitized with normal sera agglutinate on addition of antiglobulin serum. The degree of agglutination is higher with diluted than with concentrated sera tested. Absorption of the sera in advance by tanned red blood cells did not affect the agglutination (Table 1). Except for occasional agglutination with tanned red blood cells sensitized with concentrated sera and washed with normal saline, no agglutination was observed in other control experiments.

In contrast, the addition of anti-non-γ-globulin serum to sensitized tanned red blood cells resulted in an agglutination only with diluted sera. In concentrated sera, however, it occurred when these had been previously absorbed with tanned red blood cells (Table 1).

These phenomena would justify the assumption that both normal serum globulins and their non-γ-constituents have a binding capacity for tanned red blood cells. The binding of the non-γ-globulins, however, seems to be hindered by an inhibitor present in concentrated sera. Dilution of these sera, or their absorption with tanned red blood cells, would remove this inhibitor, the exact nature of which is as yet unknown. According to preliminary work in our laboratory it seems, however, that serum albumin might be involved in this inhibitory phenomenon.

Table 1. EFFECT OF PRIOR ABSORPTION BY TANNED RED CELLS OF A TEST SERUM ON THE DEGREE OF AGGLUTINATION

Serum dilutions		1 : 2	1 · 8	1 32	1 : 125	1 . 500
AG serum	Unabsorbed test serum	+++	++++	++++	++++	++++
	Absorbed test serum	+++	++++	++++	++++	++++
ANGG serum	Unabsorbed test serum	–	–	++	++	+++
	Absorbed test serum	+++	++	++++	+++	++

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IMMUNOLOGY

Antibodies to Synthetic Corticotrophin

ADRENOCORTICOTROPHIC hormone (ACTH) is a polypeptide of particular interest as its structure is known, it has been synthesized, and the molecule has both biological and antigenic activities. In earlier publications[1,2] it was shown that antibodies to highly purified pig ACTH induced in the guinea-pig form a soluble complex with ACTH labelled with iodine-131, and that this binding is reversible.

As synthetic ACTH and its biologically active or inactive fragments have become available, it was thought that it could be used as a model for the investigation of structural features on immunogenicity and antigen–antibody interactions, and also on the relationship between biological and antigenic properties of the molecule.

The synthetic preparations of ACTH-peptides used in this investigation were: (*a*) β 1–39 ACTH, which corresponds to the natural pig full chain ACTH and has full biological activity[3]; (*b*) β 1–24, a biologically active fragment[4]; (*c*) β 1–10; and (*d*) β 11–24, fragments derived from the preparation of β 1–24 corticotrophin and without biological activity. We would like to thank Dr. R. Schwyzer, of the Department of Molecular Biology, Zurich, for the gift of these preparations.

Four groups of six to eight guinea-pigs were injected with different preparations of ACTH, as described elsewhere[2]. The guinea-pigs received five doses of 1 mg of either natural A_1 ACTH (a gift from Prof. Tausk, Organon, Oss, Holland), or synthetic β 1–39 or β 1–24 ACTH. Controls were injected with Freund's adjuvant alone.

The strengths of the antisera produced were compared by their ability to bind β 1–39-^{131}I in the radioimmunological system described previously[2]. The results shown in Fig. 1 indicate that β 1–39 was as potent in eliciting antibody formation as was the preparation of natural origin, but β 1–24 was scarcely antigenic. No binding of β 1–24-^{131}I by the sera from the guinea-pigs injected with β 1–24 could be detected, thus eliminating the possibility that such sera may contain specific antisera for β 1–24, which would not bind β 1–39-^{131}I.

Unlabelled ACTH and labelled ACTH compete for binding to antibodies against ACTH[1]. This is the basis for a radioimmunoassay. The specificity of the antibodies for ACTH can thus be examined by comparing the binding of the labelled species in the presence or absence of different unlabelled species when the antibody is at

Table 1. DISPLACEMENT OF ACTH-^{131}I FROM ANTIGEN–ANTIBODY COMPLEX BY 100 NG/ML. OF DIFFERENT PREPARATIONS OF ACTH OR FRAGMENTS OF ACTH

Preparation	Percentage of displacement
A_1 ACTH (natural, Organon)	100
β 1–39 (synthetic)	200
β 1–24	15–33 (occ. 100)
β 1–10	0
β 11–24	0·5–2

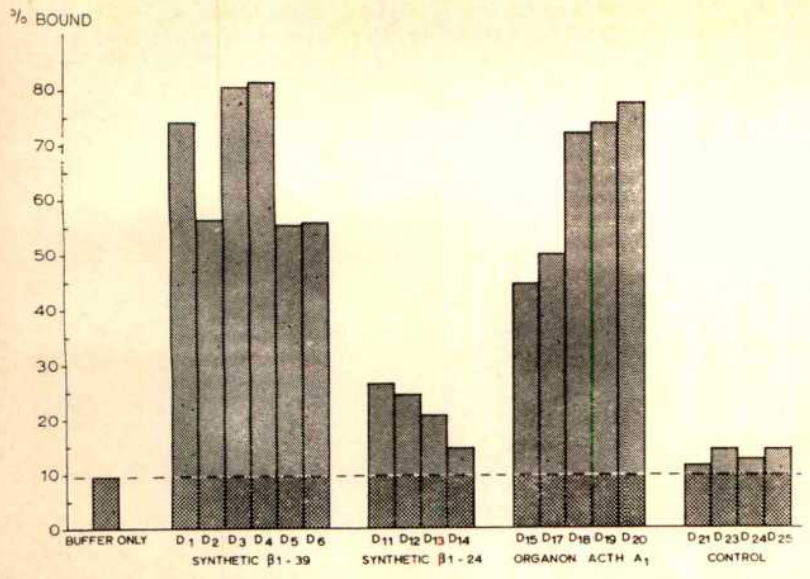


Fig. 1. Relative strengths of antisera produced by guinea-pigs against various ACTH preparations. The strengths of the antisera are expressed as the percentage binding of a fixed quantity of β 1-39-^{131}I by the same dilution (1/500) of antisera produced in a series of guinea-pigs by the injection of the preparations of ACTH shown in the figure. The control animals were injected with saline in place of hormone

such a limiting concentration that competition occurs. The results of such experiments are given in Table 1. It can be seen that β 1–24 binds to the antibodies to β 1–39, although it produces less displacement than the full chain ACTH. Smaller fragments β 1–10 and β 11–24 do not compete in any appreciable degree for the antibody sites.

The best known and best studied of the biological effects of ACTH are the adrenotropic and peripheral lipolytic activities. Lebovitz and Engel[5] have shown that the same minimum structure is necessary for both activities. In order to verify that the binding of ACTH to its antibody corresponds to a loss of the biological activity of the hormone, it was decided to investigate the effect of ACTH antiserum on the *in vitro* lipolytic activity of β 1–39 and β 1–24 synthetic corticotrophin. The method used was essentially that of Lebovitz and Engel[6], with the exception that glycerol was measured rather than FFA (free fatty acid), as glycerol is not recycled. After 1 h preincubation and 3 h incubation in 3 ml. Krebs–Ringer bicarbonate buffer containing 2 per cent albumin and the substances the effects of which on lipolysis are to be tested, the extent of lipolysis was determined by measuring the medium glycerol concentration in each flask by the method of Wieland[7]. The results (Table 2), which are expressed as micromoles glycerol/g tissue/3 h incubation, indicate the dependency of the glycerol production of epididymal fat pads on the ACTH concentrations.

The effects of antibodies on the biological activity of both the 1–39 and 1–24 polypeptides are shown in Table 3. While non-specific effects of antisera were found at low dilution, this was not the case at the dilutions used in these experiments. It is interesting to see that the antibodies obtained from guinea-pigs injected with the 1–39 synthetic peptide block the activity not only of the complete polypeptide but also of the 1–24 fragment. This finding establishes a relationship between activity inhibition and radioimmunological binding.

To sum up, these experiments show that the synthetic full chain β 1–39 ACTH is antigenic, whereas the 1–24 fragment has only little, if any, immunogenic activity. It behaves as a hapten and binds to antibodies produced against the complete ACTH chain. Binding to antibodies is followed by the loss of *in vitro* lipolytic activity of the full chain or the 1–24 fragment.

This work was supported by a grant from the Fonds National Suisse de la Recherche Scientifique.

Table 2. LIPOLYTIC EFFECT OF SYNTHETIC β 1–39

Additions to medium	μmoles glycerol/g tissue/3 h incubation
None	5·04 ± 1·61 (6)
25 ng/ml. β 1–39	8·16 ± 2·28 (6)
100 ng/ml. β 1–39	10·53 ± 2·08 (6)
250 ng/ml. β 1–39	12·83 ± 3·45 (6)

Tissues were incubated for 3 h at 37° C in Krebs-Ringer bicarbonate medium containing 2 per cent beef serum albumin in an atmosphere of 95 per cent oxygen : 5 per cent carbon dioxide. Results are expressed as the medium glycerol concentration after incubation per g tissue per 3 h of incubation and are given as mean ± standard deviation. The figures in parentheses are number of observations.

Table 3. EFFECT OF INCUBATION WITH ANTISERUM ON LIPOLYTIC ACTIVITY OF β 1–39 AND β 1–24

Additions to medium	μmoles glycerol/g tissue/3 h incubation
(*a*) β 1–39	
None	4·40 ± 0·71 (6)
100 ng/ml. β 1–39	14·20 ± 6·71 (6)
100 ng/ml. β 1–39 + antiserum 1/3,600	8·12 ± 0·90 (8)
Antiserum 1/3,600	4·59 ± 1·25 (6)
(*b*) β 1–24	
None	3·04 ± 0·57 (5)
100 ng/ml. β 1–24	8·20 ± 0·94 (8)
100 ng/ml. β 1–24 + antiserum 1/3,600	6·09 ± 0·69 (5)
Antiserum 1/3,600	3·31 ± 0·14 (4)

All media were allowed to stand at 4° C for 1 h before adding tissue. The tissue had been pre-incubated for 1 h. Incubation in the media was for 3 h. Results are expressed as the medium glycerol concentration after incubation per g tissue per 3 h incubation, and are given as mean ± standard deviation. The figures in parentheses are numbers of observations.

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HISTOLOGY

Cells, Collagen Fibrils and Vessels in Dinosaur Bone

COLLAGEN has been found in mummified human remains several thousand years old, and in mastodon bones preserved under permafrost conditions. It has been stated that bone fragments after glacier preservation had all the appearances of a fresh bone[1]. Studies on Pleistocene bones have demonstrated that it is also possible to observe collagen and other organic materials in a fossil bone[2,3]. Electron micrographs of fossil bones of a deer-like animal from the Miocene (25 million years old) revealed collagen fibrils and the intact walls of canaliculi[4]. There have also been reports of striated muscle fibres, epithelial cells and melanophores being found in tissues of specimens from the Eocene (40 million years old) deposits of Geiseltal (eastern Germany)[5]. Furthermore, electron microscope investigations of patches of almost completely demineralized bone of a dinosaur from the Upper Trias

(200 million years old), and the dermal armour of Devonian ostracoderms, have revealed a fibrillar structure, while a further examination of the same material showed a mixture of mineral and collagen patterns[6]. Analysis of fishes from the Middle Devonian (380 million years old) has indicated that degradation products of collagen can still be recognized. More recently[7,8], it has been demonstrated that fossil dentine and bone-like tissues in Devonian ostracoderms can be decalcified and sectioned on a microtome. The presence in these tissues of polysaccharides as well as collagen has been indicated.

The specimen analysed and described in this communication was the phalange bone of a dinosaur from the Upper Cretaceous (80 million years old) of the Gobi Desert, Southern Mongolia. It was collected during the Polish–Mongolian Palaeontological Expedition in 1965[9].

Ground sections of the bone revealed the outlines of osteocytes, the lacunae of which were connected by numerously branched canaliculi. The lacunae were typical in shape of those present in normal bone, but varied according to the plane of cutting. By carefully decalcifying this bone material a suspension was obtained which after decantation showed elements akin to isolated osteocytes. In certain parts osteocytes were disposed longitudinally around the vessel just as in fresh bone tissue.

The same sediment was washed in Sörensen's buffer and water, and embedded in methacrylates. Sections 1-μ thick revealed the contents of the bone lacunae as well as the canaliculi together with their processes (Fig. 1).

Electron microscope examinations of the material, using the microreplica technique, revealed the presence of collagenous material in the wall of the vessel (Fig. 2). At the point indicated by the arrow, cross-banding can be seen on the surface of the fibrils with a repetition estimated to be about one every 600 Å. The electron micrograph also reveals the plaited texture of the fibrils. The specific pattern of collagen fibrils could only be determined in the wall of the vessel. This texture could not be distinguished in the fossilized element of the bone lamina, and for this reason it is assumed that the preservation of the collagen fibre may be a consequence of 'specific mummification' arising from a separation of the vessel area by a border barrier—an occurrence well known to histologists concerned with bone tissue. A similar border barrier was probably also responsible for the preservation of the structural material of the osteocytes and their processes after decalcification.



Fig. 1. Thin section (thickness *c.* 1μ) of an isolated osteocyte

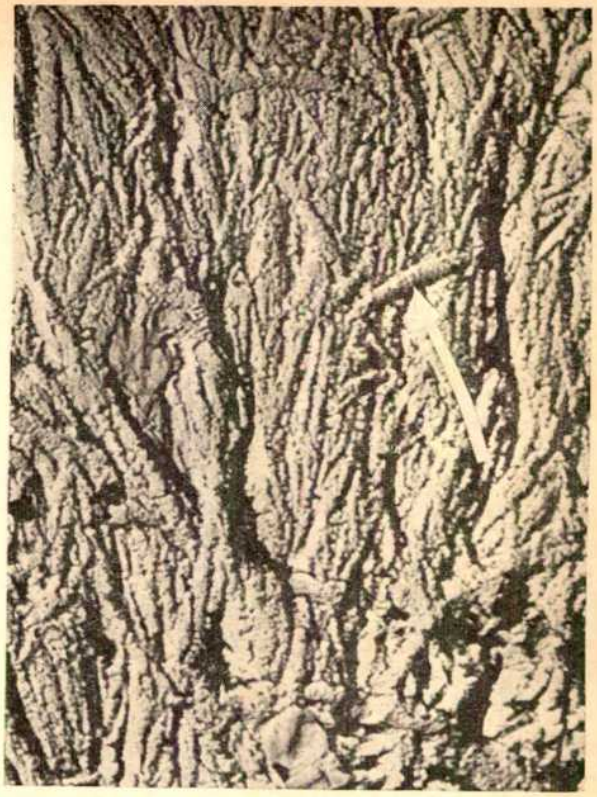


Fig. 2. Electron micrograph of the texture of collagen fibrils; the arrow shows the cross-banding. Two-step replica, evaporated with carbon and platinum. Zeiss D_3 electron microscope. (×22,600)

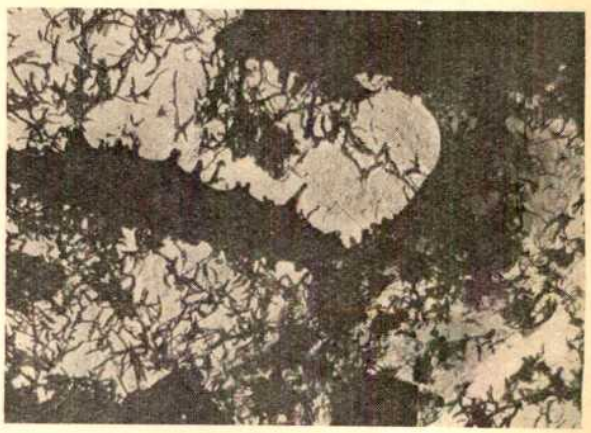


Fig. 3. Fragment of the loop of an isolated vessel with a profile of an osteocyte. Extraction replica of the preparation. Zeiss D_3 electron microscope. (×2,000)

Fig. 3 shows a layer macerated in nitric acid and reveals a curved loop of a vessel and the profile of an osteocyte with numerous bone canaliculi penetrating into the vessel.

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MICROBIOLOGY

Enhanced Susceptibility of Thymectomized and Irradiated Mice to Infection with *Mycobacterium leprae*

Since 1960 it has been established beyond doubt that a limited infection with the human leprosy bacillus, *Mycobacterium leprae*, can be produced in the mouse foot-pad[1,2]. Quantitative analyses have shown that the multiplication of *Myco. leprae* is limited, is dependent on the size of the inoculum and is confined to the site of inoculation. For example, inocula of 10^4 *Myco. leprae* yield 10^6 in 6–8 months, and although smaller inocula may eventually give the same yield, larger ones give no higher yields, and inocula of 10^6 or more fail to multiply significantly. Once the bacterial population in the foot-pad has reached approximately 10^6, it remains steady for many months although there is a gradual increase in the proportion of dead bacilli[2]. An identical type of limited infection has been obtained in the hamster ear and foot-pad[3,4]. Therefore, although the long-awaited successful transmission of *Myco. leprae* to animals offers for the first time an opportunity for examining human leprosy experimentally, progress in this field is now being restricted by the limited nature of the infection. One of the most likely limiting factors is an immunological one resulting from the infection. This suggestion is strengthened by the observations of Shepard[5] that prior vaccination with BCG given either locally in the foot-pad or at other sites in the body, more or less completely inhibits the multiplication of *Myco. leprae* inoculated later into the mouse foot-pad. Cortisone[6] and suramin[7,8], compounds which have been shown to enhance significantly infection with other species of mycobacteria, have no effect on infections with *Myco. leprae* in the mouse foot-pad[2,9]. On the assumption that the limiting factor has an immunological basis, an attempt to enhance the infection by prior exposure of the mice to thymectomy plus total-body irradiation was tried because of the known powerful immunological depressive effect of these combined procedures in the homograft reaction and antibody production.

Two preliminary experiments have been undertaken using twenty-four male *CBA* mice, twelve of which were thymectomized and later X-irradiated and twelve kept as untreated controls. Thymectomy was undertaken when the mice were approximately 2 months old, and 16 days later they were exposed to 900 r. and immediately afterwards transfused with bone marrow from *CBA* mice in the ratio of one donor to three recipients. Four weeks later the right hind foot-pad of each mouse was inoculated with 10^4 *Myco. leprae* (counted as acid-fast bacilli). Three groups (normal *CBA*, thymectomized-irradiated *CBA* and normal *P* strain mice) of six mice each were used for each of two strains of *Myco. leprae*. The *P* strain mice were included because our previous studies with *Myco. leprae* have been undertaken with this strain of mice. In experiment *A* the mice were inoculated with a strain of *Myco. leprae* direct from man and in experiment *B* with a first passage strain from a mouse foot-pad. One mouse from each group in the two experiments was killed at the fifth month, and thereafter at about 2-monthly intervals, and the foot-pads were homogenized to determine the total yield of acid-fast bacilli. (The methods for inoculating and collecting material from the foot-pads have already been described[2].) The proportion of degenerate (dead) *Myco. leprae* in the foot-pads at the time of harvest was determined by the method of Rees and Valentine[10]. The results of the two experiments are shown in Table 1 and Fig. 1. Comparable yields of bacilli were obtained from both strains of *Myco. leprae* in the *P* and normal *CBA* mice. In both experiments the infection was enhanced in the thymectomized-irradiated *CBA* mice resulting in significantly higher yields of bacilli which continued to increase for a longer period with a lower proportion of degenerate bacilli than in the normal *CBA* mice. In experiments *A* and *B* there were, respectively, about ten and a hundred times greater yields of bacilli in the thymec-

Table 1. Yield and Proportion of Degenerate Bacteria in the Foot-pads of Thymectomized-irradiated *CBA* and Normal *CBA* and *P* Strain Mice Inoculated with 10^4 new (*A*) or Passage Strain (*B*) of *Myco. leprae*

Experiment	Harvest time (months)	Mouse strain and treatment: Normal *P** Yield *AFB*† (×10⁶)	Normal *P** Degenerate *AFB* (per cent)	Normal *CBA* Yield *AFB* (×10⁶)	Normal *CBA* Degenerate *AFB* (per cent)	Thymectomized-irradiated *CBA* Yield *AFB* (×10⁶)	Thymectomized-irradiated *CBA* Degenerate *AFB* (per cent)
A	5	0·8	(0/—)	2·1	(1/18)	2·0	36
	7·5	5·1	2—	6·0	56	16·7	17
	8					*H*†	
	9·5	2·5	7—	3·8	57	33·1	15
	10	0·9	(5/—)				
	11·5	1·8	7—	2·6	73	33·3*I*†	28
	12·5			1·2	(6/10)		
	13					35·7	41
	14·5			5·5	61		
B	5	0·8	(0/—)	2·7*I*	(0/23)	1·8	(0/15)
	7	0·5	(1/—)	6·4	52	11·7*I*	5
	9	1·3	7—	0·7	(9/16)	108·0*I*	23
	11	5·8	4—	2·6	(19/22)	261·7*I*	30
	12			2·9	58		
	12·5	1·8	6—			583·0*I*	48
	14·5	8·8	4—	5·7	54	304·6	32

* One died at 3 months.

† AFB, Acid-fast bacilli. *H*, Sick mouse killed for histology. *I*, Inoculated into the foot-pads of further mice.

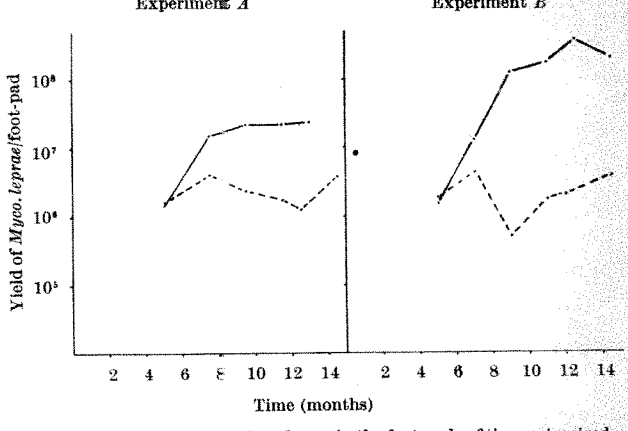


Fig. 1. Growth curves of *Myco. leprae* in the foot-pads of thymectomized-irradiated and normal *CBA* mice inoculated with 10^4 new (*A*) or passage (*B*) strain of bacteria. - - - -, Normal *CBA*; · ———, thymectomized-irradiated *CBA*

tomized-irradiated than in the normal *CBA* mice. On the other hand, there was no evidence of systemic spread of the infection in the thymectomized-irradiated mice as no acid-fast bacilli were seen in Ziehl-Neelsen stained impression smears from the livers and spleens, and only one inguinal lymph node showed a few bacilli. Bacilli recovered from thymectomized-irradiated mice (designated "*I*" in Table 1), when passaged to normal *P* or *CBA* strains of mice, reverted to infections yielding not more than 10^6 bacilli/foot-pad. Finally, while histological examination of the foot-pad of one thymectomized-irradiated mouse killed at 8 months revealed a large number of bacilli, the organisms were for the most found within striated muscle fibres, which is the characteristic distribution observed in normal mice[11].

It is reasonable to conclude from these preliminary investigations that at least one factor limiting multiplication of *Myco. leprae* in the mouse foot-pad is the development of immunity during the course of infection. This conclusion is strengthened by the reports that bacterial[12] and viral[13] infections are enhanced by thymectomy in rats and mice, respectively. Furthermore, it has been demonstrated that the post-neonatal thymectomy wasting syndrome is almost certainly based on an infectious aetiology since the syndrome does not occur in thymectomized germ-free animals[14]. These studies have particular interest because they represent the first deliberate attempt to use thymectomy plus irradiation for increasing the susceptibility of animals to chronic infections. However, the impaired immune response following thymectomy plus irradiation still resulted in only limited multiplication of *Myco. leprae*, albeit at a ten to a hundred times higher level than obtained in normal mice, and there was no spread of infection beyond the foot-pad. Failure to obtain under these conditions a completely progressive infection with systemic spread may be comparable with the similar significant, but only partial, depression of the ability of thymectomized-irradiated mice to reject the more foreign skin grafts[15]. In order to demonstrate beyond doubt an immunological basis for the enhanced infection following thymectomy plus irradiation, it will be necessary to show that the enhancement can be neutralized by lymphocyte replacement. Because only a partial enhancement of infection has been obtained it is not possible to exclude other limiting factors.

Thus thymectomy plus irradiation already provides a means for significantly increasing the yield of *Myco. leprae* in the mouse foot-pad infection. Further experiments must be undertaken to determine whether similar treatment will sufficiently diminish the resistance of animals to allow larger inocula (not less than 10^6 bacilli/foot-pad) to multiply or to allow intravenous inocula to multiply systemically.

I thank Dr. R. B. Taylor for performing the thymectomies and for his advice and guidance.

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Antagonism between Vegetative Cells and *L*-Forms of *Bacillus licheniformis* Strain 6346

Landman and Halle[1] showed that lysozyme or penicillin in suitable circumstances could cause mass conversion of vegetative cells of *Bacillus subtilis* strain 168 (indole⁻) to *L*-forms. These *L*-forms could be sub-cultured on so-called "*S.D.*" medium which contains glucose salts, horse serum, sodium succinate and tryptophan, with 0·9 per cent agar. Prolonged incubation (5–10 days) on the normal medium (*S.D.*) or short incubation (48 h) on media with an increased concentration of agar led to equally massive reversion of the *L*-forms back to bacteria. Later[2] the conversion to *L*-forms of both *B. subtilis* and *B. licheniformis* was shown to be accompanied by the extrusion of mesosomes. The authors comment that "Our data on the stimulation of reversion by physical agents have suggested . . . that membrane invagination may be the primary event".

In the present work *L*-forms of *B. licheniformis* strain 6346 were made[1] with 500 μg/ml. benzylpenicillin (they could also be made with *cyclo*serine or methicillin). After conversion to *L*-forms a block of agar containing one colony was streaked on to *S.D.* medium containing 500 μg/ml. benzylpenicillin. Ten successive sub-cultures were made on the same medium and the *L*-forms were then transferred to medium not containing penicillin, and 100 per cent reverted to the bacillary form during 48 h. All incubations were at 35° C. After the thirtieth passage on penicillin media, however, the reversion in the absence of the antibiotic was lower and delayed and, starting from the thirtieth passage, sub-culture was made on penicillin-free media. After the fiftieth passage there was no detectable reversion to bacterial forms, that is to say, the *L* strain appeared to be stable. When the agar concentra-

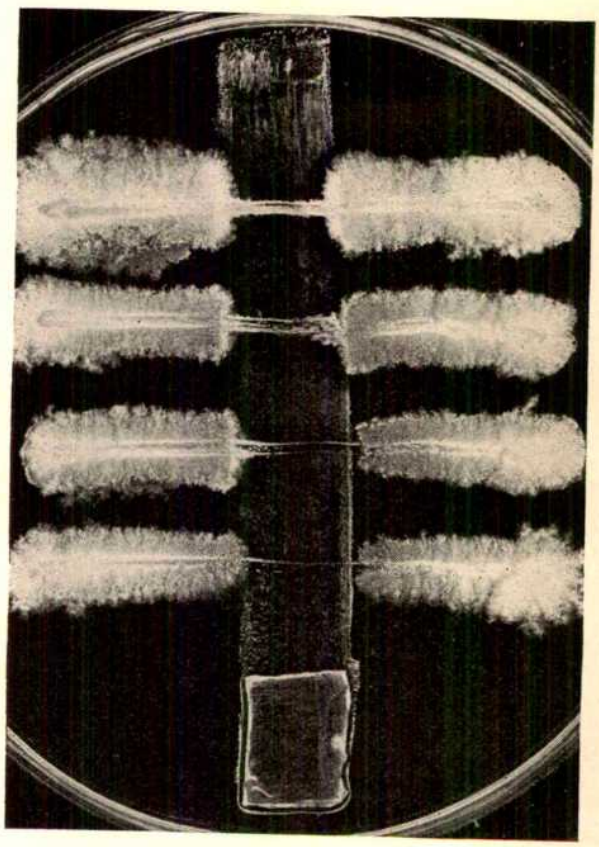


Fig. 1. An *S.D.* agar plate was streaked in the vertical direction with *L*-forms and at right angles with decreasing (from top to bottom) amounts of an *S.D.* liquid culture of vegetative cells of *B. licheniformis* 6346. Clear zones of inhibition both of bacterial and *L*-form growth can be seen

tion in the *S.D.* medium was increased to 2·5 per cent a considerable number of revertants appeared after 48 h growth. The amount of growth of *L*-forms was considerably less on this medium. It seemed that partial inhibition of *L*-form growth occurred; if there were any antagonism between growth of *L*-forms and of bacilli it could well explain the more frequent emergence of revertant colonies.

A plate of *S.D.* agar was first streaked in one direction with a block of agar cut from an *L*-form culture grown on the same medium, and then an overnight culture of the parent bacillus in fluid *S.D.* medium was streaked at right angles to the tracks of the block of agar containing the *L*-forms. The test was also made on other plates with fluid cultures of ten revertants. The plates were incubated at 35° C for 24, 48 and 72 h. Zones of inhibition of the vegetative cells and of the *L*-forms were always seen. Fig. 1 is a photograph of a plate that had been incubated for 72 h. The antagonism demonstrated in Fig. 1 could account for the failure to detect revertants on ordinary *S.D.* agar, when the good growth of *L*-forms would be expected to inhibit the emergence of colonies of revertant bacilli. The higher agar concentration which depresses the growth of the *L*-form may allow the growth of the revertant bacteria into detectable colonies.

Preliminary work has been done on the nature of the inhibition of the growth of *L*-forms by the bacilli. Cultures of *Bacillus licheniformis* 6346, growing on *S.D.* agar medium, were extracted by freezing and thawing, and the extract was shown to be inhibitory to the growth of *L*-forms. The substance is heat-labile and non-dialysable; it could not be detected either in disrupted cells or in the culture fluid from cultures of *B. licheniformis* 6346 grown in fluid *S.D.* medium for 24 h.

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Flagella-assisted Cell Fission in *Escherichia coli*

Hoffman and Frank[1] have recently described what they call "snapping movements" during division of *Escherichia coli*. The evidence presented consists of "a time-lapse series of four photomicrographs . . . inadvertently obtained from a microculture of *E. coli* during the snapping of a cell pair". The whole snapping sequence appears to have taken about one minute seventeen seconds, and is described as follows: "The sister cell on the right side of the photomicrograph (Fig. 2) exhibits a blurring interpreted as owing to a vibrating motion of the cell with its proximal end acting as a moving pivot. The proximal end of the second cell is slightly blurred from movement as well, indicating cell flexion with the midpoint of the cells as the pivot. The right-hand cell at one point during these movements seems to have paused in the long axis of its sister for an appreciable time, since its photographic image in this position is more fully developed than in any other. Fig. 3, photographed at one minute nine seconds from zero-time, indicates continued back and forth motion which was followed by a sudden large hinge movement of the cell in a counterclockwise direction, and a consequent decrease in the obtuseness of the angle formed with its sister . . . ".

The phenomenon described by Hoffman and Frank is not uncommon in *E. coli*, ("an organism not thought to be capable of this manoeuvre") if the right strain is observed under appropriate conditions. During the course of several years study of division in *E. coli* (for methods see ref. 2) several strains were observed in continuous flow microculture which allowed the behaviour during division of motile strains to be clearly observed. What occurs is exemplified by the following experiment within the Fredericq strain *CA* 18.

The organism was grown on nutrient agar for about 20 h at 37° C and some of the growth washed off with 0·5 ml. synthetic medium, mixed with about 5 ml. of synthetic medium and a droplet inoculated onto the prepared membrane of the continuous culture chamber through which a flow of synthetic medium was maintained. Motility was not observed during the first 2·75 h. The inoculum cells settled onto the membrane and remained quite still until the movements described. The division history of five cells (*a–e*) continuously observed for 4 h was as follows. The membrane was inoculated at 11.00 a.m.; at 11.30 a.m. cell *a* divided and the sister offspring (a_1 and a_2) remained non-motile and continued to grow slowly. At 2.10 p.m. cell wall fission of one of these offspring, a_1, appeared to be complete and at about the same time a rotary vibratory motion started at one end of the couplet (with the other apparently firmly adherent to the membrane), which continued intermittently for 3 min when the distal cell broke away abruptly and immediately swam rapidly out of the field of view. The cell remaining behind from this fission was motionless for about 15 min and then began vibrating in the way its partner had done—the vibrating end was the newly formed "fission wall". This would suggest that in this strain the generation of functional flagella under the conditions of culture required 15 min. The first generation cell a_2 divided at 2.25 p.m. and one of the offspring, as before, commenced flagellar lashing at its extremity which proceeded actively with occasional pauses for 'rest' during the next 10 min. For the following 15 min lashing continued with diminishing energy and longer pauses and ceased altogether at 2.40 p.m. when the pair remained at rest (in the relative positions shown in Fig. 1) up to the termination of the experiment, at 3.00 p.m. It would seem that this cell "burnt-out" its energy without achieving a complete "break-away" from its partner (connected probably by capsular material as the walls were obviously separate).

Cell *b* divided at 11.50 a.m. and one partner moved dispiritedly during the course of 5 min, the pair coming to rest side by side as shown in Fig. 1 with no further movement and apparently no growth until 3.00 p.m. These cells were in all probability non-viable. Cell *c* divided at 2.02 p.m. accompanied by flagellar lashing for 8 min when break-away was achieved. Similarly, cell *d* divided at 2.40 p.m. accompanied by lashing until 2.48 p.m., when the motile sister broke away and immediately swam hastily out of the field of observation. Cell *e* divided at 2.45 p.m. only 15 min before break-down of the experiment with weak lashing movement, but the sisters were still apparently adherent when observation ceased.

In addition to these cell movements, from about 2.00 p.m. motile cells were seen to swim actively across the field of view. By 2.30 p.m. the number of motile cells had significantly increased and by 2.45 p.m. about four cells per minute were crossing the field of view. A high proportion of the swimming cells were unseparated couplets which showed a distinctive mode of propulsion which provided the basis for belief that the movements described were caused by flagella. Each couplet had a more or less static leading cell while the trailing propulsive cell showed vigorous rotary gyration in an anticlockwise sense viewed from the rear (Fig. 2), which caused the

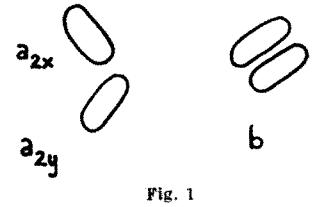


Fig. 1

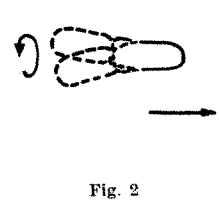


Fig. 2

couplet to move forward so that any point on the surface of the leading cell traced out a helical path.

Hoffman and Frank discount the possibility that snapping movements are due to the action of flagella, which is strange when they claim to have worked with a motile strain and also state that the phenomenon was observed only in the transitional zone of the micro-culture system used[3] where there would be the necessary freedom of movement. In my own experiments there was complete facility for free-swimming as there was no pressure between membrane and cover-slip. Also, the four photomicrographs published might be misleading as problems of depth-of-focus would necessarily dictate that images were obtained only in one plane so that rotary action could not be recorded. Yet none of the recordings of these authors contradict the findings made by direct continuous observation, and are, in fact, wholly compatible with them, even in regard to the periods of rest during lashing activity which is recorded micrographically by the non-blurred image published in Fig. 4 of Hoffman and Frank's paper.

The reported observations would seem to indicate that the flagellation in this strain might be polar, yet Leifson[4] reports that of several dozen strains of *E. coli* examined all were peritrichously flagellate, although some had very few flagella; moreover, these observations on motility fully confirm other well-established views (see Weibull[5]), but we have yet to understand how peritrichously arranged flagella could drive bacteria in the unidirectional manner observed.

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Hydrocarbons as Substrates for Yeasts

Markovetz and Kallio[1] pointed out that the assimilation of hydrocarbons might be of value in the classification of yeasts. Komagata *et al.*[2] found that fifty-six of 498 yeast strains belonging to twenty-eight genera were able to grow on kerosene as the sole source of carbon and energy. All strains except two belonged to the genus *Candida*. In several species some strains grew well on kerosene, while others did not. No growth on kerosene was observed with some species, which were able to assimilate pure hydrocarbons of the kerosene fraction according to other reports[1,3].

We investigated whether the results for the assimilation of *n*-decane and *n*-hexadecane by various yeast species correspond with those on kerosene and, furthermore, whether the utilization of these hydrocarbons may be a specific property of the species. We tested 1,200 yeast strains of the culture collection of the Centraalbureau voor Schimmelcultures. The 244 species represented by these strains belonged to the following genera: *Saccharomyces* (thirty-seven), *Hansenula* (twenty-one), *Pichia* (thirty), *Debaryomyces* (ten), *Candida* (fifty-four), *Torulopsis* (fifty-two), *Brettanomyces* (seven), *Kloeckera* (eight), *Cryptococcus* (ten) and *Rhodotorula* (fifteen).

For the preliminary screening reported here the technique of replica-plating was applied. Thirty cultures were transferred to the initial plate, containing wort agar, and incubated at 25° C for 2 days. The basic medium for the tests was a mineral agar with B-vitamins[4].

To the sterilized basal agar, in which 0·25 per cent taurocholate was incorporated for better emulgation, 1 per cent *n*-hexadecane (99 per cent pure, Fluka) was added. *n*-Decane (99 per cent pure, Fluka) and kerosene (normal Esso) were supplied as vapour. The plates were incubated at 25° C for 1 week. If strains of a species grew both on *n*-decane and *n*-hexadecane as well as on kerosene, one of these strains was retested in shaken cultures with 2 per cent of the same carbon sources.

Table 1. NUMBER OF STRAINS ASSIMILATING *n*-HEXADECANE, *n*-DECANE AND KEROSENE IN SOME SPECIES OF *Pichia*, *Debaryomyces*, *Torulopsis* AND *Candida*

Species	Number of strains tested	Number of strains growing on *n*-Hexadecane	*n*-Decane	Kerosene
**Pichia farinosa*	22	22	22	19
Pichia guilliermondii	37	37	37	33
**Pichia haplophila*	2	2	2	2
**Pichia pastoris*	2	2	2	2
**Pichia polymorpha*	3	3	3	2
**Pichia robertsii*	2	2	2	2
**Pichia scolyti*	5	1	1	1
**Pichia vini*	5	5	5	5
Debaryomyces hansenii	45	45	45	45
**Debaryomyces vanriji*	4	4	4	4
**Torulopsis dattila*	2	2	1	1
**Torulopsis famata*	12	12	12	12
**Torulopsis haemulonii*	2	2	2	2
**Torulopsis saké*	2	1	1	1
**Candida brumptii*	6	6	5	4
**Candida catenulata*	5	5	5	1
Candida intermedia	10	10	10	6
Candida lipolytica	12	12	12	8
**Candida melinii*	4	1	1	1
Candida parapsilosis	11	11	11	9
Candida pulcherrima	17	16	16	11
Candida reukaufii	7	7	5	2
**Candida rhagii*	4	4	3	4
**Candida tenuis*	8	4	3	2
Candida tropicalis	24	24	24	24

* Species not previously known as hydrocarbon utilizers.

In the genus *Kloeckera* no *n*-decane, *n*-hexadecane and kerosene assimilating strains were detected. A few of the *Hansenula*, *Brettanomyces* and *Cryptococcus* strains grew very weakly on *n*-hexadecane. In the genus *Saccharomyces* only the two strains of *Saccharomyces elongasporus* assimilated hydrocarbons readily, but the yeast differs from the typical species of this genus by a slow fermentation and the shape of the spores. In some *Rhodotorula* species *n*-decane-, *n*-hexadecane- and kerosene-utilizing strains occurred, but growth was very slow or weak; negative strains were found in the same species. Many strains, utilizing *n*-decane, *n*-hexadecane and kerosene, were detected in the genera *Pichia*, *Debaryomyces*, *Candida* and *Torulopsis*. Some of the results are shown in Table 1. The species not previously known as hydrocarbon utilizers are indicated by an asterisk.

Generally, growth on kerosene vapour was weaker than on the pure *n*-alkanes; sometimes no growth on kerosene was observed with strains assimilating *n*-decane and *n*-hexadecane. Some strains, which grew well on kerosene vapour, failed to grow in shaken cultures containing kerosene. This suggests that the absence of growth may be due to an inhibitory effect of some components of the kerosene, rather than to the inability of the yeast to attack the *n*-alkanes. The results with the shaken cultures containing *n*-decane and *n*-hexadecane paralleled those obtained on agar, but in liquid medium growth was often better with *n*-hexadecane. In many species all strains utilized the pure *n*-alkanes, indicating that this ability can be a characteristic feature of the species. However, the assimilation of *n*-alkanes by the strains of one species may vary from weak or latent to good. In species, where only some strains were found to assimilate *n*-alkanes, growth was generally poor.

Additional strains utilizing *n*-alkanes could probably be detected with test conditions more favourable to adaptation and selection.

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RADIOBIOLOGY

A Computer Programme for Automatic Scanning of Autoradiographs and Microradiographs of Bone Sections

AUTORADIOGRAPHY of bone has played an important part in the investigation of the distribution of bone-seeking isotopes. A measure of the non-uniformity of this distribution is of paramount importance in determining local radiation doses in bone and adjacent soft tissues. In addition, a comparison of autoradiographic and microradiographic pictures can provide information about bone resorption and bone turnover. Until recently these measurements have been severely limited in scope because of the tedious and time-consuming nature of such analyses. With advances in automatic densitometry and computers, these analyses have now become practicable on a much wider scale.

This report outlines the use of an automatic scanner under the control of a computer which is capable of analysing the distribution and area of blackening on a photographic film. The machine (named *Chloe*) was designed and built for the analysis of spark chamber photographs[1] and pattern recognition of chromosomes[2]. In the present application, a spot of light from a cathode-ray tube is focused on to the autoradiographic plate and the light transmitted through the plate is viewed by a photomultiplier. This light spot ($\sim 15\mu$ in diameter) is driven in both the X and Y direction by two counting registers so as to scan a rectangular area on the plate, the extent of which is determined by the computer programme. The spot does not actually move, but appears in one place for 1 μsec, is blanked out for 3 μsec, and reappears in the adjacent location for 1 μsec. When the photomultiplier detects less than a specified amount of transmitted light, the contents of the registers are entered into the computer memory as the co-ordinates of the point. The beam then moves on until the photomultiplier again detects more than the given amount of light and the co-ordinates of this second point are entered in memory. These two points determine a line segment. Overlapping line segments are later assembled into shapes by the computer programme.

The calibration and stabilization of beam intensity in terms of photographic density are of the utmost importance in the operation of the scanner as an automatic densitometer. Calibration plates are therefore used to act as calibrators both for area and for density. A step wedge containing fifteen steps ranging in density from 0·05 to 2·1 was constructed to fill the scanning area, which was 1 in.^{2} on the photographic plate. The portions of this plate transmitting less light than that corresponding to a fixed photomultiplier discriminator setting were displayed on a separate cathode-ray tube. Beam intensity values corresponding to each density level on the step wedge were determined by increasing the cathode ray beam current in small discrete increments until the apparent area of each step was reduced by half. This information provided a table of beam intensity versus density which was later fed into the computer memory. All beam intensities are corrected for the background blackening of each plate by an automatic background measurement.

A further calibration plate with two spots of known density and a third black spot of known area is inserted in the scanner before each data plate in order to correct for any change in the operating conditions. The computer measures these two densities automatically, compares them with their known values, computes a quadratic correction function, and substitutes a corrected table of net beam intensity versus density into its memory.

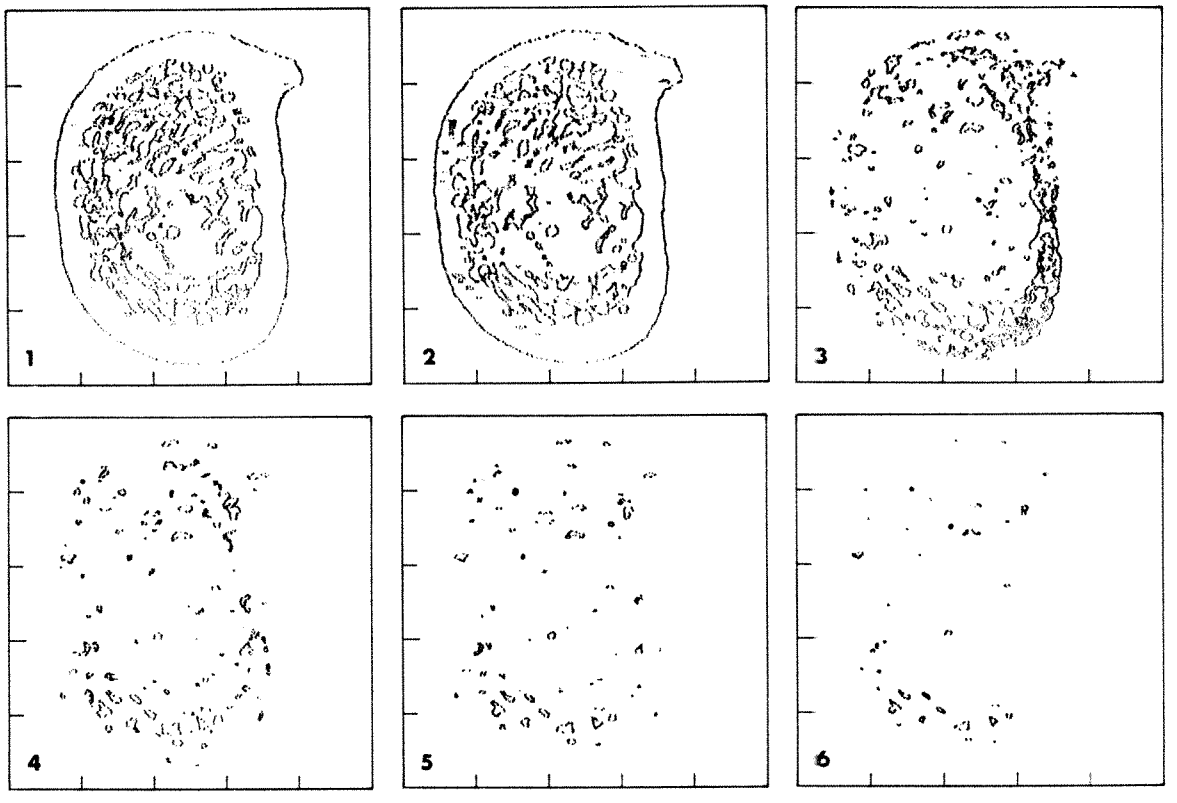


Fig. 1. Pictures, reconstituted from digital information using a *Calcomp* plotting device. Isodensity lines are drawn to indicate areas of the autoradiograph which fall above a certain level of photographic density. The six pictures correspond to six different density levels in steps of 0·08 from picture (1) corresponding to density 0·08 to picture (6) corresponding to density 0·48

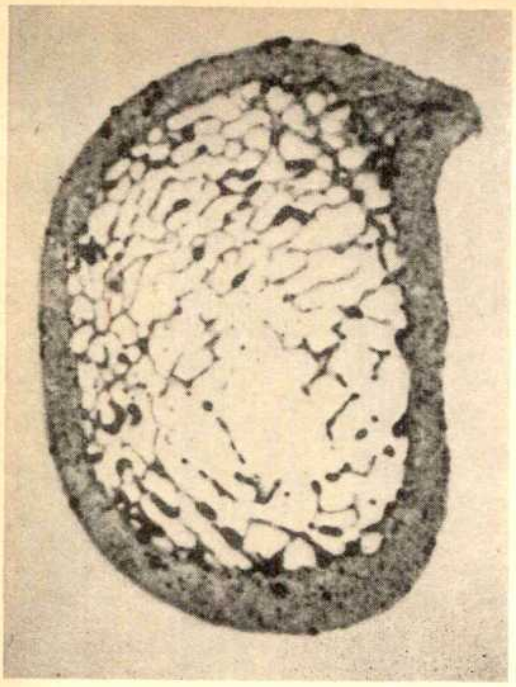


Fig. 2. Autoradiograph of a cross-section of bone taken from the distal femur of an adult dog injected with 500 μc./kg of calcium-45 and killed 17 days later

So far, the computer has been programmed to read-out areas and shapes corresponding to different photographic densities. This information is stored on magnetic tape and the corresponding areas and densities are available in a print-out form. To test the procedure, the autoradiograph can be reconstructed from computer memory to show isodensity lines in the form of pictures. These are shown in Fig. 1, the autoradiograph in Fig. 2.

Other forms of output, such as read-out in terms of isotope concentration in microscopic areas on the autoradiograph, can be obtained by inserting a calibration of the isotope in the form of a radiator on the same photographic plate as the bone section. Investigation of microradiographs to obtain quantitative information such as bone porosity is also possible with the present programme.

Other investigations for which this scanning equipment may prove useful are: (1) investigations of changing distribution of isotopes either as a function of time after administration in animal experiments or as altered by external factors such as drugs; (2) mineral density measurements using microradiography; (3) correlation of autoradiographic and microradiographic pictures of the same bone section to give information on bone resorption. These are just a few of the possible applications of this automatic scanning device to problems associated with bone. However, the scanner described here has basically been designed as an automatic densitometer and it may prove useful in fields of research other than just those pertaining to bone.

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Radiation Recovery Response of Mammalian Tumour Cells *in vivo*

SINCE the original report by Elkind and Sutton[1] that mammalian cells are able to repair sublethal radiation damage *in vitro*, there have been several reports describing similar results in a variety of mammalian cell systems both *in vitro* and *in vivo*[2]. The *P*-388 murine lymphocytic leukaemia maintained as an ascites tumour in the *DBA*/2 mouse is ideal material with which to investigate the recovery response of a malignant mammalian cell *in vivo*. Shortly after intraperitoneal transplantation of 10^6 cells (24–48 h), the radiation response of this tumour is typical for that of an oxygenated population; after 6–7 days of intraperitoneal growth, this tumour demonstrates anoxic radiation response[3]. Consequently, it is possible to investigate the radiation recovery response of oxygenated and hypoxic cells depending on the age of the tumour at the time of the conditioning dose. Fig. 1 shows the survival response for 1-day tumours and 6-day tumours irradiated *in vivo* and assayed for tumour cell viability as previously described[3]. The dose modifying factor for 6-day tumours with respect to 1-day tumours is ~3. In addition, while both curves have an initial shoulder, the extrapolation number for 1-day cells is significantly higher than that for 6-day cells.

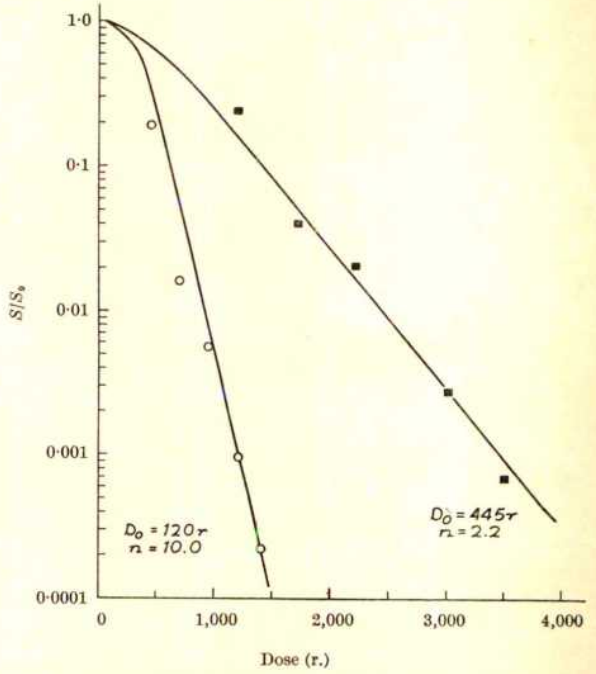


Fig. 1. Survival curves for *P*-388 tumours irradiated and assayed *in vivo*. 1-day tumours (○) and 6-day tumours (■). *n*, Extrapolation number; D_0, dose in roentgens required to reduce the surviving fraction to 1/*e* on the exponential portion of the curve. 6-day tumours are more radio-resistant by a factor of 3·7

A series of experiments was undertaken designed to investigate the radiation recovery response of *P*-388 tumour as a function of tumour-age at the time of the conditioning dose. Animals bearing 1-day or 6-day tumours were irradiated with whole-body air exposure doses of 500 r. or 1,500 r. respectively, at time 0. At intervals of time up to 14 h following this conditioning dose, a second, equal dose was delivered to the same animals. X-radiation was produced at 220 kV, 18 m.amp and 0·5 mm copper and 1·0 mm aluminium filtration (half value layer 1·33 mm copper), and this gave 212 r./min at a target–surface distance of 25 cm. Immediately after the second dose, cells were removed from the abdomen and assayed for reproductive viability; recipient animals

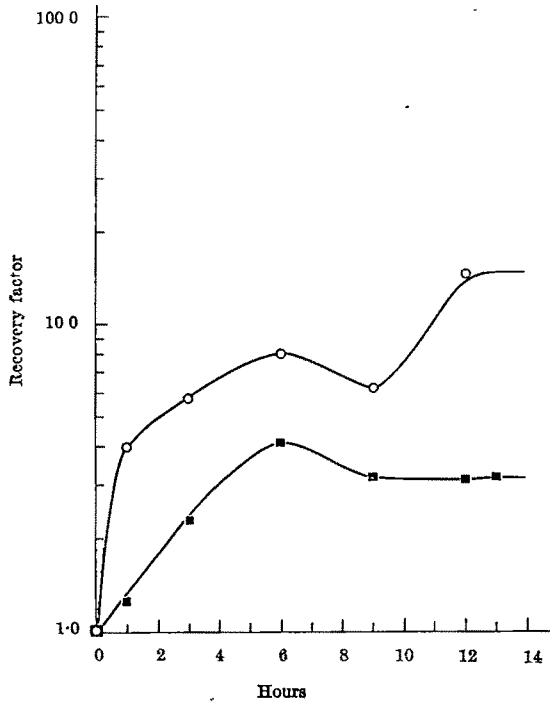


Fig. 2. Recovery response of 6-day tumours (■) and 1-day tumours (○). Recovery factor is the observed survival fraction to two-dose fractions relative to the total dose delivered in one exposure

were inspected for tumour formation and such tumour production data were analysed as described by Belli and Andrews[3].

Net survival fractions to the fractionated dose were expressed relative to the survival fraction which resulted from the total dose without fractionation (recovery factor). A recovery factor of 1·0 indicates no recovery. These data are given in Fig. 2. Initial, rapid repair of sublethal damage is evident in tumours of either age. However, the rate of repair seems to be slower in 6-day (hypoxic) tumours. Initial repair appears to reach a maximum by 6 h following the conditioning dose in both tumours. A subsequent minimum is seen at 9 h which rises to a second maximum by 12 h in 1-day tumours. In the hypoxic condition, however, this minimum appears to persist through 13 h. It is of interest to note that the maximum recovery factor for 1-day tumours (~ 15) and for 6-day tumours (~ 4) are of the same order of magnitude as the extrapolation numbers observed from the acute exposure survival curve for these tumours (ten and two respectively).

The data reported here demonstrate that mammalian tumour cells are capable of repairing sublethal radiation damage *in vivo* regardless of whether the cell is aerobic or hypoxic at the time of the first dose, but that the rate of repair is probably influenced by the state of oxygenation of the tumour cell population at the time of conditioning dose. These data are also compatible with the model first proposed by Elkind *et al.*[4] for the recovery kinetics of irradiated mammalian cells, and later developed by Elkind and Sinclair[5]. The model predicts that, concurrent with repair of sublethal damage, cells surviving the conditioning dose progress through the generation cycle into that portion of the cycle which is radiation sensitive and such cell progression gives rise to the minimum observed in most two-dose recovery curves.

Littbrand and Revesz[2] have reported that Ehrlich ascites tumour cells do not demonstrate recovery when irradiated in the anoxic state *in vitro*. It should be pointed out, however, that the challenge dose was delivered 18 h following the conditioning dose. No other time sequence was reported. Thus, little can be said concerning the early recovery kinetics of this cell line. In addition, cells were not kept anoxic between the fractions and this may have influenced the recovery response.

The differences in recovery kinetics demonstrated by 1-day and 6-day *P*-388 tumours irradiated *in vivo* may result from the fact that 6-day tumours consist of a population of cells which have been allowed to grow into the hypoxic state. This may give rise to a different tumour cell population age density distribution[6] which in turn would be expected to result in variation of the age response function[5]. Such differences in population composition and kinetics can give rise to variation in the recovery responses of such populations.

In summary, it has been demonstrated that malignant mammalian cells are capable of repairing sublethal radiation damage whether in the oxygenated or hypoxic state. The kinetics of the recovery response differ in that hypoxic tumour cells appear to repair sublethal damage at a slower rate than aerobic cells.

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VETERINARY SCIENCE

'Ewe × Side' of Udder Interactions in Milk Yield in Relation to the Number of Lambs suckled

ONE method used to estimate milk yield of ewes at pasture during the course of experiments at Armidale, New South Wales, in 1960, was the oxytocin technique[1]. This involved injections of oxytocin to induce let-down, followed by an "emptying" milking and, 4 h later, a "measuring" milking, milk yield being calculated as a rate (g/h). The good agreement found between this technique and lamb suckling has already been discussed[2].

A ewe has only one major gland on each side, and production from the supernumerary glands is very small[3]. In this study milk production of the two halves of the udder was recorded separately, and supernumerary glands, when present, were included with the main gland. It has been shown by numerous authors[4–7] that ewes are not fully extended in milk yield when they are suckling singles. The milk yield of each gland can then be regarded as the result of an interaction between its potential to produce milk and the sucking stimulus of the lamb. A single lamb may equally stimulate each side of the udder, may consistently suck more on one side of the udder than the other or may change its preference from time to time. The stimulation of the two sides of the udder is likely to be more equal for twins than for singles because both sides will be more fully stimulated.

To investigate this point, analyses of variance (Table 1) on the milk yields of the two sides of the udder were

Table 1. ANALYSES OF VARIANCE OF WEEKLY MILK YIELDS FROM THE TWO SIDES OF THE UDDER FOR MERINO EWES

Source of variation	Milk yield (g/h) Medium-wool Peppin ewes Suckling twins D.F.	M.S.	Suckling singles D.F.	M.S.	Strong-wool ewes Suckling twins D.F.	M.S.	Suckling singles D.F.	M.S.
Sides	1	168 03	1	82 84	1	3·78	1	603·29
Ewes × sides	5	102·83*	11	554·49†	6	23 66*	13	404 94 †
Weeks × sides	9	17 20	9	15·87	9	11·16	9	4·79
Residual	45	24·24	99	27·10	54	6·77	99	8·26

* $P < 0.01$.
† $P < 0.001$.

Table 2. VARIANCE COMPONENTS OF THE 'EWES × SIDES' INTERACTIONS FOR THE FOUR ANALYSES OF TABLE 1

	Medium-wool Peppin ewes Suckling twins	Suckling singles	Strong-wool ewes Suckling twins	Suckling singles
Component σ^2_{ES}	7 9	52·7	1·7	39·7
95 per cent confidence interval	2·1–13·9	25·1–147·8	0·5–2·6	21·1–109 4

carried out for the first 10 weeks after parturition for six medium-wool Peppin and seven strong-wool Merino ewes suckling twins, and twelve Peppin and fourteen strong-wool ewes suckling singles. The main effects, ewes and weeks, and the 'ewes × weeks' interactions, which were very highly significant in all four analyses, are not shown. The 'ewes × sides' interactions are all highly significant, showing that, whether suckling twins or singles, some ewes produce more milk on the left and some more on the right. The variance components for these interactions, together with their 95 per cent confidence intervals, were calculated by Grayhill's method[8]. The 'ewe × sides' interaction component is significantly greater for singles than for twins for both strains (Table 2).

The significant 'ewe × sides' interaction, accompanied by a small and not significant 'weeks × sides' interaction, indicates an uneven stimulation of the sides which is consistent from week to week. If the ewe is fully stimulated by suckling twins, the unevenness can be interpreted as showing that the potential production for some ewes is greater on the left, and for others on the right. Because unevenness between sides is greater for single lambs than for twins it is likely that single lambs develop a consistent preference for the better side.

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SOIL SCIENCE

Hydrolysis of Soil Nitrogen by Strong Acids

NUMEROUS tests have been proposed for determining the "available" nitrogen in soils, but although they have proved successful under some conditions, they have not generally been reliable for predicting field crop response[1–3]. Most of these methods are based on the estimation of nitrogen which can be readily hydrolysed by dilute acids[1] or mineralized by incubation over short periods of time[3]. It seems quite probable that nitrogen left in more resistant fractions of soil organic matter may also play a supporting part in supplying nitrogen that can be used by plants. Recent research at this Station indicates that more vigorous acid hydrolysis and extraction of soil releases considerable nitrogen from resistant organic complexes.

Soils from six plots at the Rhode Island Agricultural Experiment Station, which have a long history of treatment with various types of nitrogen fertilizer, were used in the investigation. The soil, classified as a Bridgehampton silt loam, has received continuous fertilizer, lime and cropping treatment for more than 60 years. Table 1 presents data on these soils pertinent to their nitrogen status and for later use in comparison with the acid hydrolysis investigations.

Table 1. CHEMICAL PROPERTIES OF THE SOILS

Plot No.	Treatment*	pH	Org. C (per cent)	N (per cent)	C/N ratio	PAN† (p.p.m.)	NO_3-N (p.p.m.)‡ 0 Days	14 Days	28 Days
37	No N, lime	6 3	2·83	0·202	14 0	40	7	31	40
60	Manure, lime	6·5	3 72	0·263	14 1	88	10	39	64
61	Chemicals, lime	6 3	3·27	0·259	12·6	65	9	34	57
63	Cont corn, no cover	6·0	1·73	0·126	13·7	36	2	11	19
71	Fertilizer, no lime	5·0	2·52	0·185	13·6	55	3	21	30
72	No fertilizer, no lime	5 0	2·68	0·183	14·6	50	4	13	22

* Complete fertilizer except where otherwise indicated.
† Potentially available nitrogen (ref. 1).
‡ Incubation at 30° C (ref. 3).

Hydrolysates were prepared by means of a modification of Bremner's procedure[4]. Soils were subjected to a series of sequential hydrolyses at 100° C using distilled water and 1, 3, and 6 N hydrochloric acid and sulphuric acid. A 4-in. round-bottom centrifuge tube containing 5 g of soil and 20 ml. extracting solution was placed in a boiling water bath. An 11-in. chromatographic tube containing a glass bead served as a condenser. After an hour, the tube was removed from the bath, allowed to cool and centrifuged. The hydrolysate was decanted and the residue washed with 10 ml. of fresh extractant by mixing well and centrifuging. The supernatant was decanted and combined with the previous hydrolysate. Twenty ml. of fresh solution was added to the residue and this mixture was again hydrolysed for a second hour. The same separation procedure was repeated. The residue was subjected to a similar hydrolysis for a final 2 h. This time the residue was washed with three 10-ml. portions of water. The residue was dried and saved for further analysis. The three acid hydrolysates (1 h, 2 h, and 4 h) were neutralized with sodium hydroxide (5 N and 0·5 N) to *p*H 6·5 ± 0·1 and made up to 100 ml. Ten-ml. portions of the extracts were used for all determinations.

Organic carbon was determined using a modification of the method devised by Tinsley[5]. Two grams of mercuric oxide were added to hydrolysates containing chloride ion to prevent reduction of dichromate[6]. Total nitrogen and alpha-amino nitrogen were determined by the micro-Kjeldahl procedures developed by Bremner[4].

It was found that there was little difference in the percentages of carbon and nitrogen released from the six soils. This was to be expected, as previous work had indicated the similarity of acid hydrolysable soil organic matter independent of the origin of organic residues[7,8]. However, there were large variations in relation to acid treatments (Table 2).

For carbon, an increase in the strength of either hydrochloric acid or sulphuric acid beyond 3 N did not materially increase the amount hydrolysed. A comparison with the water extraction indicates the degree of decarboxylation by acid hydrolysis. Nitrogen recoveries were directly related to increases in acid strength from 1 N to 6 N, resulting in a narrowing of the carbon/nitrogen ratios in the hydrolysates and a sharp rise in the ratios of the residues (Table 3). The wider carbon/nitrogen ratios of the first-hour hydrolysates suggest that easily dissolved materials (for example, carbohydrates) and some amino-acids and amino sugars were being extracted. The narrow ratios in the second hour are suggestive of more effective extraction of compounds containing greater

Table 2 ORGANIC CARBON AND NITROGEN RECOVERIES BY HYDROLYSIS WITH 1, 3 AND 6 N ACIDS AND WATER AT 100° C*

Extractant	Carbon recovered (per cent)† 1 h	2 h	4 h	Residue	Total	Nitrogen recovered (per cent)† 1 h	2 h	4 h	Residue	Total
					1 Normal					
Hydrochloric acid	18·7	6·1	3·3	62 6	90 7	29 2	14·1	9 8	47·9	101 0
Sulphuric acid	18 0	5·3	3 3	65 2	91 8	26 5	11 4	9·9	52 3	100·1
					3 Normal					
Hydrochloric acid	22·9	8·1	2 6	56 6	90·2	40·7	18 5	7·6	34 9	101·7
Sulphuric acid	21·8	7·1	3·9	57 3	90·1	34·6	16·8	9 6	39 4	100·4
					6 Normal					
Hydrochloric acid	22·4	7·5	2 0	57·9	89 8	46·4	19 7	6·5	29 1	101 7
Sulphuric acid	23 0	7·2	3·3	50 7	84 2	41·8	18·4	7 8	31 6	99 6
					Water					
Water	1·0	0 3	0·2	95·0	96·5	3·5	2 0	1·9	92 8	100·2

* Average of six soils.
† Percentage of total carbon or nitrogen.

Table 3. CARBON–NITROGEN RATIOS OF HYDROLYSATES WITH 1, 3 AND 6 N ACIDS AND WATER*

Extractant	1 h	2 h	4 h	Residue
		1 Normal		
Hydrochloric acid	8 8	7 8	3·6	17·9
Sulphuric acid	8 7	6·3	4·6	17·2
		3 Normal		
Hydrochloric acid	7 7	5 9	4·7	22·6
Sulphuric acid	8 6	5·7	5 5	20 1
		6 Normal		
Hydrochloric acid	6·6	5·1	4·4	27·5
Sulphuric acid	7·4	5·3	5·7	22·3
		Water		
Water	6·4	3 8	2·8	13·9

* Average of six soils.

percentages of nitrogen. In general, the narrowest ratios occurred in the fourth hour. This could arise from the presence of resistant compounds in the hydrolysate.

The very wide carbon/nitrogen ratios of the residues might well indicate very resistant heterocyclic nitrogen compounds, such as humic substances. Felbeck[9] has proposed a possible structure for these compounds the carbon/nitrogen ratio of which (25/1) is similar to that found in several of the residues and particularly where hydrochloric acid is used. It has been shown by Stevenson[10] and Dubach and Mehta[11] that the optimum time for the liberation of hexosamines and amino-acids (non-humic substances) from soil, using 6 N hydrochloric acid, is 4 h. The data presented in Table 3 for alpha-amino nitrogen support these observations. It is suggested that a 4-h hydrolysis with 6 N hydrochloric acid extracts a large percentage of non-humic substances leaving behind in the residue essentially humic materials. The 6 N sulphuric acid solution is considerably less effective in its extraction of non-humic materials than 6 N hydrochloric acid. In all cases the carbon/nitrogen ratios of the hydrochloric acid residues are wider than those from sulphuric acid. This is because of the more effective extraction using hydrochloric acid and probably greater decarboxylation with sulphuric acid.

An appreciable fraction of the dissolved nitrogen in the hydrolysates was in the alpha-amino form (Table 4). The largest amounts were liberated in the first hour and decreased rapidly during the second- and fourth-hour periods. Amounts extracted were related directly to acid strength. At concentrations above 3 N, hydrochloric acid proved to be a much more effective extractant than sulphuric acid.

Table 4. ALPHA-AMINO NITROGEN DISSOLVED BY HYDROLYSIS WITH 1, 3 AND 6 N ACIDS (BASED ON TOTAL NITROGEN)*

Extractant	Percentage recovered† 1 h	2 h	4 h	Total
		1 Normal		
Hydrochloric acid	2·7	1 0	0 5	4 2
Sulphuric acid	1 8	0·8	0·8	3·4
		3 Normal		
Hydrochloric acid	6 1	3 9	0·8	10 8
Sulphuric acid	2·2	1 2	0 8	4 2
		6 Normal		
Hydrochloric acid	10 3	5 4	0·8	16·5
Sulphuric acid	6 6	1 3	0 8	8 7

* Average of six soils
† Percentage of total nitrogen.

The average of 16·5 per cent extracted with 6 N hydrochloric acid is somewhat lower than others have reported[12,13]. However, the 4-h hydrolysis used in this investigation is considerably less than that used by others. Bremner[12] found that maximum liberation of amino-acids could be attained in about 12 h and that prolonged hydrolysis caused their destruction. It is believed that extending the time of hydrolysis would liberate a greater percentage of amino-acid, but it is doubtful that these values would be much higher than that obtained during the 4-h period. It is concluded that the soils contain rather limited quantities of alpha-amino nitrogen and essentially all this nitrogen is extracted in a few hours by acid hydrolysis.

Table 5. α-AMINO NITROGEN DISSOLVED BY HYDROLYSIS WITH 1, 3 and 6 N ACIDS (BASED ON HYDROLYSATE NITROGEN)*

Extractant	Percentage recovered† 1 h	2 h	4 h
		1 Normal	
Hydrochloric acid	9 1	7 3	5·5
Sulphuric acid	6·6	7·1	7·8
		3 Normal	
Hydrochloric acid	15·0	21·3	10·7
Sulphuric acid	6·4	6 9	8 3
		6 Normal	
Hydrochloric acid	22 3	27 7	12·9
Sulphuric acid	15·9	7 2	9 9

* Average of six soils.
† Percentage of nitrogen in hydrolysate.

Table 5 shows the amount of alpha-amino nitrogen dissolved based on the nitrogen released to the hydrolysate. With increasing acid strength the hydrochloric acid extracts from the second hour contain the largest amount of alpha-amino nitrogen. This supports the carbon/nitrogen ratio data of Table 3, which show a narrowing carbon/nitrogen relationship in the second hour attributed to the solubilization of nitrogen-rich compounds.

Although the data presented here do not relate nitrogen extracted by strong acid hydrolysis to crop response, it is suggested that such vigorous treatment and subsequent nitrogen measurement may be an approach to the estimation of nitrogen-supplying power of soils.

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Microbial Factor in Short-term Phosphate Uptake Studies with Plant Roots

IN most investigations of the mechanisms of nutrient uptake by plant roots no attempt has been made to grow plants under sterile conditions. A prolific microflora develops on the root surface even in very young plants unless stringent precautions are taken to grow them

aseptically[1]. It could be envisaged that such a population may affect the results of nutrient uptake experiments either by influencing plant metabolism or by adding its own uptake to that of the root. Krasil'nikov[2] has shown that rhizosphere organisms can affect the amino-acid composition of barley.

Krasil'nikov[2] cites an instance in which phosphate uptake over 17 days was doubled by the presence of micro-organisms on barley roots, but the reverse occurred with woody plants. However, in neither instance was uptake examined in relation to root growth which can be affected by micro-organisms[3]. The reported lower total ash contents of non-sterile red clover and sunflower compared with sterile plants[4,5] were not examined in relation to root growth differences caused by the treatments. A recalculation of the data of Welte and Trolldenier[4] as total ash (mineral uptake) per g of root shows that non-sterile plants took up to 64 per cent more nutrients than sterile plants from nutrient culture solution over periods of approximately 30 days. Subba-Rao, Bidwell and Bailey[6] showed that in tomato plants, the roots of which were infested with *Fusarium* sp. or with *Trichoderma viride*, the tops contained less phosphorus-32, sulphur-35 and carbon-14 than sterile plant tops. This was after uptake periods of 48–72 h from nutrient solution containing radioactively labelled phosphate, sulphate and bicarbonate. However, total uptake was not studied and therefore the data refer only to translocation. As none of these authors have calculated the effects of micro-organisms on the efficiency of nutrient uptake, the present investigations were designed to investigate this using techniques commonly used by plant physiologists.

Tomato seeds (*Lycopersicon esculentum* var. 'Grosse Lisse') and subterranean clover seeds (*Trifolium subterraneum* var. 'Bacchus Marsh') were surface sterilized with 7 per cent calcium hypochlorite solution, washed with sterile water and germinated on a nutrient agar to detect contamination. Sterile germinated seeds were transferred to a stainless-steel mesh above 30 ml. of Hoagland and Arnon plant nutrient solution[7] in cotton-plugged test-tubes (3 × 20 cm) using three seeds per tube. Non-sterile treatments received 0·1 ml. of inoculum consisting of a 1 per cent suspension of Urrbrae loam (a red-brown earth) to provide a wide spectrum of micro-organisms. After 4–8 days' growth in a glasshouse, plants were tested for sterility and harvested before extensive branching of roots had occurred. The roots of the intact plants were washed in tap water for 60 sec to remove excess plant nutrient solution, placed in 5×10^{-4} M calcium sulphate for 2 h and then transferred to 5×10^{-4} M calcium sulphate containing 5×10^{-6} M potassium dihydrogen phosphate (*p*H 6·7) and phosphorus-32 as phosphate at 80 μc./l. (less than 10^{-8} M). After uptake periods ranging from 5–60 min the roots were washed in rapidly running water for 5 min and separated from the tops to estimate translocation. Twelve replicate plants were used for each treatment. Liquid scintillation counting was employed using a thixotropic base in which to suspend the plant material.

Root and top data for 5-day-old tomato and for 8-day-old subterranean clover (Table 1) showed little difference between sterile and non-sterile plants in these experiments. No differences in root hair production between sterile and non-sterile plants were detected. Differences in phosphate uptake (Table 2) between these sterile and non-sterile plants can therefore be ascribed directly to the micro-organisms and not to differences in plant morphology.

Table 1. ROOT AND TOP GROWTH OF STERILE AND NON-STERILE PLANTS*

Plant	Condition	Root length (cm)	Root dry weight (mg)	Shoot dry weight (mg)
Tomato	Sterile	7·7	0·24	2·7
	Non-sterile†	7·7	0·24	3·0
Subterranean clover	Sterile	6·5	1·1	7·3
	Non-sterile‡	6·1	1·1	7·8

* Means of 24 plants.
† Mean number of bacteria per root system: $4\,2 \times 10^8$.
‡ Mean number of bacteria per root system: $1{\cdot}7 \times 10^6$.

Table 2. UPTAKE AND TRANSLOCATION OF PHOSPHATE BY STERILE AND NON-STERILE PLANTS

Plant	Uptake period (min)	Condition	Total uptake*	Phosphate* in roots	Phosphate* translocated to tops
Tomato	5	Sterile	131	114	9
		Non-sterile	254†	215	27
	20	Sterile	819	777	36
		Non-sterile	1,453†	1,247†	159§
Subterranean clover	5	Sterile	757	666	44
		Non-sterile	1,214§	1,138‡	59
	20	Sterile	1,225	971	119
		Non-sterile	1,906†	1,718‡	125

* Phosphate (10^{-12} moles) per plant. Means calculated from 12 plants after log transformation.
Differences between sterile and non-sterile: † Significant at 5 per cent level; ‡ significant at 1 per cent level; § significant at 0 1 per cent level.

Calculations made from the data in Table 2 show 77–95 per cent increases in uptake by non-sterile tomato and 55–60 per cent increases by non-sterile clover. The variation in uptake between replicate tomato and clover plants was great despite selection of apparently uniform plant material. In a 5-min uptake experiment with barley using twelve replicate plants, an 18 per cent increase in phosphate uptake was not significant at the 5 per cent level. Variation between non-sterile plants was greater than that between sterile plants.

In the absence of treatment/time interactions, uptakes over all time treatments were averaged in these experiments. Over the two time treatments for tomato, the increases in non-sterile plants in total uptake and root content of phosphorus-32 were significant at the 1 per cent level and the greater translocation in non-sterile plants was significant at the 0·1 per cent level. With clover data averaged over five time periods up to 60 min, the higher total uptake and root content of phosphorus-32 of non-sterile plants were significant at the 0·1 per cent level.

Uptake was greater in the non-sterile tomato by 85 per cent and non-sterile clover by 45 per cent over all time treatments, although there was virtually no difference in root and shoot growth between sterile and non-sterile plants. It is therefore suggested that phosphate uptake may be grossly overestimated by neglecting the need for complete asepsis in growing test material used in short-term uptake experiments. This type of experiment has often been used to postulate mechanisms of uptake and to estimate kinetic "constants" such as carrier concentrations[8–10]. The results refer especially to the use of low concentrations of phosphate; effects at higher concentrations and longer uptake times are under investigation.

Phosphate translocated to the top by tomato in 20 min was 4·4 times greater in non-sterile plants (this was significant at the 0·1 per cent level). A strong trend in the same direction occurred with clover after 60 min. The much higher phosphorus-32 content of tops of non-sterile tomato plants demonstrates conclusively that the increased phosphate uptake by non-sterile plants cannot be attributed solely to absorption by the rhizoplane microflora. A direct effect of micro-organisms on the metabolism of the plant is indicated.

We thank Mr. K. M. Cellier, Division of Mathematical Statistics, C.S.I.R.O., for the statistical analyses.

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Adelaide, South Australia.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned.

ASSISTANT LECTURERS (2) (with an honours degree in pharmacology, physiology, biochemistry or zoology) IN THE DEPARTMENT OF PHARMACOLOGY —The Clerk to the Council, The School of Pharmacy, University of London, Brunswick Square, London, W.C.1 (August 10).

RESEARCH ASSISTANT (with a good honours degree and preferably some research experience, but consideration will be given to applications from recent graduates) IN THE DEPARTMENT OF BIOLOGY, for work on the physiology of stomata—Prof. C. D. Piggott, Department of Biology, The University, St. Leonard's House, Lancaster, Lancs (August 10).

RESEARCH FELLOW (with a good honours degree in the social sciences and some research experience) IN SOCIOLOGY—The Registrar, The University, Sheffield (August 10).

LECTURER (with an honours degree in pharmacology, physiology or biochemistry and also a higher degree or with evidence of original research in an appropriate field) IN PHARMACOLOGY—The Clerk to the Council, School of Pharmacy (University of London), 29/39 Brunswick Square, London, W.C.1 (August 11).

SENIOR TECHNOLOGIST (male, with either a university degree in science or the A.I M.L.T. in bacteriology, and previous experience in microbiology) IN THE DEPARTMENT OF BACTERIOLOGY—Prof. F. S. Stewart, Department of Bacteriology, Trinity College (University of Dublin), Dublin 2, Republic of Ireland (August 12).

TEMPORARY DEMONSTRATOR OR TEMPORARY ASSISTANT LECTURER (with specialist experience in soil bacteriology) IN BOTANY—The Registrar, The University, Liverpool 3, quoting Ref. 170/N (August 13).

DEPUTY CHIEF REGIONAL PHYSICIST, PRINCIPAL PHYSICIST GRADE (with wide experience in medical physics) IN THE REGIONAL MEDICAL PHYSICS DEPARTMENT—The Secretary, Sheffield Regional Hospital Board, Fulwood House, Old Fulwood Road, Sheffield, 10, quoting Ref. N3266/2 (August 15).

JUNIOR/SENIOR RESEARCH ASSOCIATE (graduate in psychology with strong research interests) IN THE DEPARTMENT OF PSYCHOLOGY to work under the supervision of Dr Gerald H. Fisher on a research project concerned with fundamental aspects of perception—The Registrar, The University, Newcastle upon Tyne, 2 (August 15).

RESEARCH STUDENT IN ASTRONOMY in St. Salvator's College to undertake research in observational or theoretical astronomy at the University Observatory under the direction of Prof. D. W. N. Stibbs—The Secretary, University of St. Andrews, College Gate, St. Andrews, Fife (August 15).

SENIOR LECTURER (medically qualified) IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, 3, quoting Ref. OV/169 (August 19).

SENIOR SCIENTIFIC OFFICER/SCIENTIFIC OFFICER (preferably ex-Service candidate, with an honours degree in agriculture, science or food science, preferably with postgraduate experience in bacteriology) IN THE AGRICULTURAL BACTERIOLOGY DIVISION of the Ministry of Agriculture, to undertake research and investigational work in connexion mainly with the bacteriology of meat and meat products, and possibly to undertake some teaching duties in the Faculty of Agriculture, Queen's University, Belfast—The Secretary, Civil Service Commission, Stormont, Belfast 4, Northern Ireland (August 19).

ASSISTANT LECTURER OR LECTURER (with an honours degree in biochemistry, agricultural biochemistry, or some equivalent qualification) IN THE DIVISION OF AGRICULTURAL BIOCHEMISTRY, School of Agriculture, University of Aberdeen and North of Scotland College of Agriculture—The Secretary, The University, Aberdeen (August 20).

ASSISTANT LECTURER (with special qualifications in Greek philosophy) IN PHILOSOPHY—The Registrar (Room 39, O.R.B.), The University, Reading (August 20).

LECTURER OR SENIOR LECTURER IN HISTOLOGY OF NEURO-ANATOMY IN THE DEPARTMENT OF ANATOMY—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (August 20).

RESEARCH ASSISTANTS (with an appropriate honours degree of a British university or exceptionally an equivalent qualification) IN MECHANICAL ENGINEERING; ELECTRICAL ENGINEERING; CIVIL ENGINEERING; POLYMER CHEMISTRY (two posts); PHYSICAL ORGANIC CHEMISTRY; PHYSICAL METALLURGY; MATHEMATICS; PHYSICS; BIOLOGY (Plant Physiology); and a RESEARCH ASSOCIATE IN ELECTRICAL ENGINEERING—The Principal, Lanchester College of Technology, Priory Street, Coventry (August 22).

TEMPORARY ASSISTANT LECTURER (preferably with a special interest in political and moral philosophy) IN PHILOSOPHY—The Registrar, The University, Manchester 13, quoting Ref. 180/66 (August 24).

CHAIR OF PRODUCTION ENGINEERING IN THE DEPARTMENT OF PRODUCTION AND INDUSTRIAL ADMINISTRATION—The Registrar, The College of Aeronautics, Cranfield, Bedford (August 26).

ASSISTANT EDITOR (with experience of research and publication in medical or veterinary entomology, good critical faculty and ability to write lucid and concise English, and preferably a reading knowledge of other European languages) at the Commonwealth Institute of Entomology, London, S.W.7, for duties which will include producing and editing the Institute's publications—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough, Bucks (August 31)

ASSISTANT LECTURER -IN AERONAUTICAL ENGINEERING—Prof. A. V. Stephens, The Queen's University, Belfast, Northern Ireland (August 31).

CURATOR VETERINARIAN (registered veterinary surgeon) to be in charge of the Small Animal Breeding Station—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (August 31).

LECTURER/SENIOR LECTURER (preferably with research activities in one of the following fields: ionization, discharge and plasma physics, solid state physics and ionospheric physics) IN PHYSICS at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, August 31).

POSTDOCTORAL RESEARCH FELLOW IN BIOCHEMISTRY/IMMUNOLOGY for work in connexion with a project under the joint direction of Dr. I. G. Murrey and Dr. W. D. Brighton concerning the extraction and characterization of the antigens of organisms involved in farmer's lung—The Personnel Officer, Central Public Health Laboratory, Colindale, London, N.W.9 (August 31).

RESEARCH OFFICER IN THE DEPARTMENT OF PHYSICS to investigate damage occurring in high-power laser systems—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 31).

SENIOR DEMONSTRATOR IN THE DEPARTMENT OF INORGANIC CHEMISTRY —The Registrar (Appointment E.55), The University of Melbourne, Parkville, N.2, Victoria, Australia (August 31).

DEPARTMENTAL GRADUATE ASSISTANT IN THE DEPARTMENT OF MICROBIOLOGY to assist in the preparatory work for practical classes and demonstrations—The Registrar, The University, Newcastle upon Tyne, 2 (September 1).

SENIOR LECTURER OR LECTURER IN BOTANY (Plant Physiology) at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 2).

SENIOR LECTURER and a LECTURER or ASSISTANT LECTURER IN GEOLOGY at the University of Ife, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 3).

LECTURER IN EXPERIMENTAL ZOOLOGY—The Secretary, The Queen's University, Belfast, Northern Ireland (September 7).

HOLBROOK GASKELL CHAIR OF BOTANY—The Registrar, The University, Liverpool (September 10).

LECTURER or SENIOR LECTURER (with special interests in physical chemistry) IN THE DEPARTMENT OF CHEMISTRY, University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 16).

LECTURER (qualified in one or more of the following fields: biometry, agricultural statistics, biomathematics, mathematical statistics, an agricultural or biological discipline with supplementary experience in its mathematical or statistical aspects) IN BIOMETRY IN THE DEPARTMENT OF AGRICULTURAL BOTANY, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 16).

READER or SENIOR LECTURER IN GEOGRAPHY at the Australian National University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 16).

HARRISON CHAIR OF MECHANICAL ENGINEERING—The Registrar, The University, Liverpool (September 17).

SECOND CHAIR OF CIVIL AND STRUCTURAL ENGINEERING—The Registrar, The University, Sheffield (September 17).

SENIOR LECTURER/LECTURER IN GEOGRAPHY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, September 18).

DIRECTOR (with a veterinary qualification, high scientific attainment in a relevant science, and preferably some experience of administration) OF THE INSTITUTE FOR RESEARCH ON ANIMAL DISEASES, Compton, Newbury, Berkshire—The Secretary, Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1 (September 19).

LECTURER/ASSISTANT LECTURER/SENIOR DEMONSTRATOR (with special qualifications and interests in the field of biogeography) IN THE DEPARTMENT OF GEOGRAPHY, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

PLANT NEMATOLOGIST with a good degree in the biological sciences, experience in the identification of the nematode parasites of economically important crops, and an interest in taxonomy) IN THE COMMONWEALTH BUREAU OF HELMINTHOLOGY, St. Albans—The Secretary, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Bucks (September 30).

SENIOR LECTURER (with an honours degree in electrical engineering (or mechanical and electrical engineering) with industrial and/or research experience in power system analysis or electrical machines (specification of design) preferred) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 1).

LECTURER (with a degree in pure science or agricultural science) IN PLANT PATHOLOGY at Lincoln College (University of Canterbury), New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 31).

CHEMISTS (with a good honours B.Sc. degree, and preferably of Ph.D. standing) to work in fields such as relation between structure and properties in polymeric materials, modification of polymer properties by cross-linking and grafting, the application of reverse osmosis to the desalination of water and to non-aqueous systems, applications of physico-chemical methods, such as light scattering, to determine molecular weights and biomedical applications of new membranes—The Director, Arthur D. Little Research Institute, Inveresk, Musselburgh, Midlothian.

HEAD OF THE DEPARTMENT OF MATHEMATICS AND PHYSICS; and HEAD OF THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary, Wolverhampton College of Technology, Wolverhampton.

HISTOPATHOLOGIST (with a medical or veterinary degree, postgraduate experience in histo-pathology, and preferably general research and/or specialized knowledge of histo-chemistry) IN THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY, Chester Beatty Research Institute, Fulham Road, S.W.3 —The Secretary, Institute of Cancer Research, Royal Cancer Hospital, 34 Sumner Place, London, S.W.7, quoting Ref. 300/G/25.

INSECT PATHOLOGIST (microbiologist, preferably with experience in bacteriology, mycology, virology, or protozoology) to study insect diseases and their possible utilization in pest control—Division of Entomology, University of California, Berkeley, California 94720, U.S.A.

INSTRUMENT TECHNICIAN (with technical college training, some years of experience in instrumental techniques related to chemistry, and fully conversant with the operation of UV and IR equipment) IN THE DEPARTMENT OF CHEMISTRY to undertake routine NMR mass spectrometric, IR and UV spectrophotometric analyses and to interpret simple spectra (training will be given in NMR and mass spectrometry)—Prof. A. Bourns, Chairman, Department of Chemistry, McMaster University, Hamilton, Ontario, Canada.

LECTURER or RESEARCH ASSOCIATE (with qualifications and/or experience in the fields of metallurgy or ceramics) IN THE DEPARTMENT OF PHYSICS— The Registrar, University of Bristol, Senate House, Bristol, 2.

MICROBIOLOGIST (Ph.D. or equivalent) to supervise clinical microbiology and the Diagnostic Chemical Laboratory servicing 285 beds—The Assoc. Director of Research, National Jewish Hospital, 3800 E. Colfax, Denver, Colorado 80206, U.S.A.

ORGANIC CHEMIST (Postdoctoral) for structure elucidation studies on the phenolic constituents responsible for the pigmentation of wheat bran—Prof. D. J. Baisted, Science Research Institute, Oregon State University, Corvallis, Oregon 97331, U.S.A.

PHYSIOLOGIST/BIO-PHYSICIST to study physiological response to controlled changes in environmental conditions—D. C. Page, Head of Personnel Services, The Electricity Council, Research Centre, Capenhurst, Chester, quoting Ref. N/28.

RESEARCH ASSISTANT (preferably with postgraduate biochemical experience) for investigations on calcium metabolism—Dr. M. R. Wills, Department of Chemical Pathology, Royal Free Hospital, Gray's Inn Road, London, W.C.1.

RESEARCH FELLOW IN CHEMISTRY for work which will include the synthesis and the determination of physical properties of heterocyclic boron compounds —The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey.

RESEARCH HISTOPATHOLOGIST in the Paterson Laboratories, with responsibility for the supervision of work on small animal pathology—The Director of Research, Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester, 20, quoting Ref. 5059.

RESEARCH STUDENTS (honours graduates or others having equivalent qualifications in engineering, physics or mathematics) IN THE DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING, to join a group concerned with theoretical, computational and experimental aspects of the formation and noise properties of high-perveance electron beams—The Registrar, The University, Sheffield, 10.

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YOUNG POSTGRADUATE PHYSICISTS, MATERIALS SCIENTISTS, ELECTRICAL ENGINEERS, and MECHANICAL ENGINEERS to work on a new approach to the use of conducting, dielectric, and magnetic materials (also materials with compound properties) in such applications as cabling, wiring, switching (at distribution, industrial and domestic voltages), heat transfer, and electrical machines—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Nr. Chester, quoting Ref. N/35.

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RESEARCH COMPUTERS

THE Computer Board now established by the Secretary of State for Education and Science is an important innovation in the administration of academic research, at least in Britain. Though the questions which are being raised about the adequacy of the plans for providing universities with computers are real enough they are in some ways less important than the issues of principle created by the existence of the Computer Board. For one thing, it is an organization that cuts across the autonomous instincts of British universities. Still more remarkably, its decisions will cut across the boundaries between universities and that important sector of the scientific research which is at present undertaken directly by the research councils. Finally, its work in the next few years is certain to determine a pattern for the use of computers in scientific research for decades to come. In other words, it is a powerful body, and is almost certain to be influential as well.

The first thing to be said is that the complaints now being made about the immediate plans for installing computers are in one sense inevitable and in another irrelevant. The Working Group under Prof. Flowers (see p. 675 of this issue) was always clear that its five year plan would have to be re-modelled every year, and this point has apparently been accepted by the Government. Indeed, the chief task of the Computer Board is to bring the planning up to date. The first thing to be done, now that the core of the membership of the board has been appointed, is to see whether the experience of the past few months is enough to justify a massive expenditure on multiple access computing machinery. Even though it would be a surprise to many people if that brave new world were already here, there is nothing in the constitution of the Computer Board to prevent it saying that the plans of a year ago are now old hat, and must be discarded.

The fact that the work of the Board will cut across the autonomy of the universities is not as fearsome as it sounds. There are sound precedents for a collective decision by the universities that important items of equipment should be acquired and managed collectively. The National Institute for Research in Nuclear Science, with its co-operative accelerators, is the most obvious example. But the decisions of the Computer Board may have wider implications. Some universities may, for example, be told that neighbouring institutions must have access as of right to their computer installations. Others will find themselves being persuaded not to acquire the computer they would prefer to have but some other that will more easily fit into a national network. Although at first sight decisions like this seem to bristle with difficulties, universities as a whole are well aware that they will be better off with a balanced network of computers spread through the country than with an extension of the present system of individual acquisition and management. That, at least, is the present mood. One of the tasks of the Computer Board will be to persuade the universities and the research establishments that future instalments of the computer plan are as sensible as was the Flowers Report a year ago.

The relationship between the Computer Board and the research councils is also important. Though the Computer Board may seem to the universities to be a kind of University Grants Committee with special responsibility for computers, to the public laboratories maintained by the research councils it will be an entirely novel creature. This is one of the ways in which its work may be most rewarding, for the gulf between the universities and the public research laboratories has traditionally been too wide. All kinds of facilities which might be shared are kept too much in isolation. The movement of people between the two kinds of institutions is similarly less fruitful than it might be. So it is tempting to hope that if the Computer Board can establish close liaison over the admitted immediate need of a better computing system, the parties to that shot-gun marriage will recognize that their common interest is really very much more extensive.

It is also to be hoped that the good sense of these arrangements will soon be even more widely appreciated. Although the Computer Board is to help in formulating the forward planning of the public laboratories working under the research councils—the research councils for science, agriculture, medicine, the natural environment and, no doubt in time, social science—the public laboratories now under the wing of the Ministry of Technology will remain apart. One of the more obvious awkwardnesses of this arrangement is that institutions such as the Royal Radar Research Establishment, the Royal Aircraft Establishment and the National Physical Laboratory are at once among the largest and most experienced of British users of computers, and similar to the universities in the character of their need. Fortunately the links between the Atomic Energy Authority—another dependant of the Ministry of Technology—and the universities on computing machinery are close enough, and there is no reason why other establishments should not seek to develop in a similar way. In the long run some formal integration of the two parallel systems will be essential.

Another defect in the present arrangement is that there is no means of restraining the demand of the universities for extra computing power. At this stage, of course, such a restraint would be unwise. British universities and research laboratories are not as well provided for as they should be, and there is probably a need that the Computer Board, like the Flowers Group before it, should do something to force

the pace. But it will not indefinitely be possible to meet an unrestrained demand. Sooner or later it will be necessary for those who use computers to work out more explicit arrangements for making the most economical use of them. One difficulty is that the diversity of the demand on computer power will make it harder to operate the kind of committee system which has proved successful in managing the big accelerating machines. In any case, it is hard to see how a committee would ever be able to decide confidently between the merits of some complicated analysis in demography and a Monte Carlo calculation in nuclear physics.

The question remains of how the Computer Board may serve as a model for the development of sectors of research in British universities. The first need, of course, is that the board should show that it can function effectively. The special characteristics of computers, and the obvious need of integration within the system, also restrict the usefulness of comparison with other fields of research in which development is necessary. It is nevertheless tempting to wonder whether it will not turn out that the need for development in, say, molecular biology can best be met by independent board given the means and the responsibility for seeing that developments at universities and research establishments are sensibly co-ordinated blessed with funds. Evidently the Computer is more important than the narrowness of its of interest would suggest.

WE WUZ ROBBED

THE World Cup which has recently been enacted in Britain may have been fun to watch, but there is no question that it was a thoroughly badly designed experiment. If the intention was to find out which football team is the best in the world, the organizers would have gone about their task quite differently.

Just as the frequency of deaths from kicks by horses in those far-off but now classical stables of the Prussian Army are described by a Poisson distribution, so are the numbers of goals scored by the competing teams in the recent series of World Cup matches. Table 1 shows the numbers of occasions during the series of thirty-two games (each involving two teams) on which specified numbers of goals were scored, together with the frequencies described by a Poisson distribution of the form $P(n) = e^{-q}q^n/n!$ with $q = 1{\cdot}234$, or the mean of the observed scores.

Table 1

Numbers of goals	Observed	Calculated $65.P(n)$
0	18	18·4
1	20	22·8
2	15	14·0
3	7	6·0
4	2	1·8
5	2	0·5
More than 5	0	0·7

In reality an even better agreement between fact and prediction can be obtained by taking $q = 1{\cdot}27$, a value which lies well within the standard deviation of the mean.

The mere fact that a Poisson distribution can describe so well the distribution of scores by individual teams goes a long way to suggest that the teams were much of a muchness in talent and their scores were independent of each other. From this point of view, the decision that the outcome of the whole competition should depend on the outcome of a single game between the two so-called finalists was as much of a farce as a great many West German supporters already know it to have been. If it is assumed that the goal scoring potentiality of the two teams is equally well described by the Poisson distribution already specified, the chance that the result will be a draw is a mere 0·27. In other words, if two teams are equally matched, the chance that the result will be an active injustice to one of them will be 0·73. By the same token, a team which is slightly less skilled than its opponent can nevertheless expect a one in three chance of winning the deciding match.

Chancy outcomes of individual games are given extraordinary importance by the overall design of the World Cup competition, in which small groups of team play an incestuous competition on their own before one team is sent on to another stage in the competition. Obviously a properly randomized block design would be an improvement. But even within the framework of the existing competition, much could be done to reduce the gross sampling error under the present arrangements. Replication of the crucial games by a series of identical trials is one obvious remedy, and the organizers of the World Series of baseball games in the United States have in this spirit arranged that the winner should be the one who wins the most of seven games. The ideal is that in future World Cup competitions, the organizers should fix a certain limit of confidence (say, $P < 0{\cdot}01$) and then that they should require that the finalists go on playing against each other either until the superiority of one or the other of them is properly established, or until both parties agree to negotiate a draw.

A more practicable alternative might be to redesign the parameters of the game of football in such a way that a respectable degree of confidence in the outcome of the competition can be acquired in a reasonable interval of time. If, for example, it were agreed that single cup finals should remain, but that no team should be declared the winner until its score exceeds that of its opponent by three standard deviations of Poisson distribution, it might be necessary to design the game of football so that it would be practicable for one side to score 100 goals or so within the limits of endurance of the spectators. This implies that the parameter q would have to be much greater than under the present rules. Such a change could easily be brought about, possibly by widening the goals or by abolishing goalkeepers. The precise method by which these matters are attended to is, of course, much less important than that they should be dealt with quickly. The whole of South America knows that.

NEWS AND VIEWS

COMSAT's High Hopes

COMSAT, the United States Communications Satellite Corporation, may be pushing its luck too far. In four years, it has done better than anyone could have expected when Congress patched it together in a series of compromises between the advocates of public and private ownership. (Half of COMSAT's shares are owned by public shareholders, the other half by the American communications industry.) Even COMSAT's purpose was two-headed—the corporation was told to set up a global network of communications by satellite as soon as possible, and at the same time to enlist international partners willing to join in a system dominated by Americans.

Yet all this has been done. And quickly too. At home the corporation is a constant favourite with small investors, to the surprise of experts in Wall Street, yet it keeps cordial relations with the American Telephone and Telegraph Company, its biggest corporate shareholder. Abroad, it has set up the International Telecommunications Consortium which fifty-two countries have joined: the Philippines and Indonesia are recent recruits, and Yugoslavia is thinking about it—the first communist country to do so. (The consortium, INTELSAT, is the actual owner of the *Early Bird* satellite; COMSAT is the manager.) *Early Bird*, while by no means working to full capacity, has been successful enough for the transatlantic rates to be reduced. By the end of this year, COMSAT will be able to offer satellite communication over most of the world, as it launches a bigger version of *Early Bird*, capable of reaching ground stations in South America, and a satellite for the National Space and Aeronautics Administration which will provide circuits to the Far East. And by 1968, it will have put into orbit four advanced synchronous satellites for INTELSAT, making a full global network a reality.

But COMSAT is not content to have built so much on the ambiguities of the Communications Satellite Act of 1962. It is now arguing righteously to the Federal Communications Commission (FCC) that Congress gave it a mandate to have a monopoly over satellite communication at home as well as abroad. There is a rich prize at stake: the right to provide communication by satellites between the east and west coasts of the United States, and the country's far-flung hinterlands as well. The issue was raised when the American Broadcasting Company, one of the United States three major television networks, asked the FCC for permission to put up its own satellite, so that it could relay its own programmes. (The three networks now pay the American Telephone and Telegraph Company about $65 million a year from transmitting their shows around the country.) Most likely the FCC would not like to rule in favour of the crowded sky with every newspaper, television network and wire services allowed to have its own satellite. But COMSAT's claim that Congress had domestic satellites in mind in 1962 is laughable.

This has been politely pointed out by the Ford Foundation, in a brilliant brief prepared for the FCC which says, in effect, that COMSAT has had enough favours from the Federal Government to satisfy any corporation and that it should stick to its international business. What the foundation wants is an American satellite system to be run by a private—non-profit, non-governmental—corporation. That would relay *Batman*, *Peyton Place* and other staples for the commercial networks at a cheaper rate than they now pay; with its revenues, the corporation could provide, on five channels in each time zone, a mixture of public affairs and education.

There is no sign that the FCC will be tempted by such an adventure. The pressure from COMSAT is strong; it wants to be as powerful as possible by 1969 when the international agreement which the consortium's members have signed comes up for permanent ratification, so that its partners will not be tempted to join any rival system, and that hold-outs, notably the Soviet Union, will be tempted in. The FCC is in an unenviable spot—it must do what Congress could not do four years ago: make decisions about the future of a technology which is only just coming into existence. Along with its utopian plan, the Ford Foundation gave the FCC some wise advice: do not grant any hasty monopoly before the Carnegie Corporation's enquiry into the future of non-commercial television in the United States comes out. COMSAT could use the time to think whether the international perspective that it will need in 1969 to attract communist partners into a permanent relationship will be helped by its alliance with American commercial television.

Industrial Training

THE first clutch of the reports from the industrial training boards is an unsurprising demonstration that a lot will have to be done before the Industrial Training Act of 1964 brings all the benefits that its sponsors were hoping for. In particular, it is plain that the boards have not yet moved far into the field where most is expected of them—the training of adults, as distinct from juvenile workers, in the techniques of modern industry. Indeed, the three reports which are available, for the engineering, iron and steel and shipbuilding industries (*H.C.* 122, 123 and 124 respectively, H.M.S.O., 1*s.* 9*d.*), show that the past year has been something of a rush for all of them, with the occupying of new premises and the recruitment of staff dominating everything. (The board for the engineering industry has also been able to run up an overdraft of close on half a million pounds.)

The fourteen boards so far established have a right to collect a levy from all except the smallest industrial companies, and a duty to return some of what they collect to those companies with adequate training schemes. The funds thus available will enable the boards to arrange for training facilities for employees not otherwise catered for, either on premises of its own or elsewhere. It is also intended that each of the boards should recruit and train officers who will, in turn, help companies with their training plans. This is where the three boards whose reports have so far appeared seem to have done most in the past twelve months.

This, however, is only a beginning. The scale on which they will be operating in the future can be told from the estimate of the engineering training board—

admittedly the largest—that its annual levy, at a rate of 2·5 per cent of industrial turnover, will amount to £75 million—a sum not very different from the £130 million spent on British universities in a year.

In the administration of this levy and the compensating grants to companies, the boards must necessarily act as judges of what is good practice. But how do they know what is good? The engineering board has taken a step in the right direction by advertising its willingness to make grants for "research projects directed towards improving the effectiveness of training". It will be interesting to see how soon such work can provide an objective evaluation of the benefits (or otherwise) of the industrial training, which has necessarily grown up in a somewhat empirical fashion.

Ornithology in 1966

DR. D. W. SNOW, of the British Trust for Ornithology, writes: "The fourteenth International Ornithological Congress, which took place at Oxford during July 24–30, was the largest ever held, with some 900 members from forty-four countries. The 130 papers presented were ample proof that research in birds continues to make important advances in such diverse fields as systematics, behaviour, reproductive physiology and population dynamics. This has indeed been so for the last 25 years, as shown by such books as Mayr's *Systematics and the Origin of Species*, Tinbergen's *Study of Instinct*, and Lack's *Natural Regulation of Animal Numbers*, which draw more of their examples from birds than from any other group of animals. But the Congress also reflected some newer trends in ornithological research.

"Most strikingly, ethology, in the restricted sense of the study of the causal analysis or motivation of behaviour, was not represented at all. Instead, most of the papers on behaviour dealt with the development and function of song and other vocalizations, a field in which rapid advances have been made in recent years thanks to the sound spectrograph. One paper in particular, by Prof. Niko Tinbergen, Secretary General of the Congress, stressed the adaptive significance of specific behaviour patterns in the context of the whole ecology of the species, an aspect of behaviour which has been rather neglected.

"Evolutionary studies also showed a change of emphasis. Studies of geographical variation and speciation were rather few; in birds this is well-worn ground and the main established principles are not likely to alter. Instead, there were several papers dealing with evolution at the family level or higher, among them an account of Prof. Charles Sibley's studies of avian haemoglobins and egg-white proteins These studies are likely to clear up many of the debated problems of the relationships between the various orders of birds, for which the evidence from morphology is equivocal.

"The paper by the President of the Congress, Dr. David Lack, opened the eyes of many ornithologists to the wider significance of aspects of bird behaviour and ecology which have often been studied in isolation from each other. Entitled "Inter-relationships in Breeding Adaptations", it aimed to show that the type of nesting dispersion (colonial or widely spaced, for example), the nature of the nest site, the incubation and fledging periods, the type of young (whether nidicolous or nidifugous), the ability of the parent birds to defend their nest, and food and feeding habits are all inter-connected. None of them can be fully understood without reference to the others. Dr. Lack drew his examples from sea birds because they have been the subject of many detailed studies in recent years, but the same principles probably apply to all. (Prof. Tinbergen's paper, already mentioned, was planned jointly with Dr. Lack's and dealt with essentially the same theme from the point of view of behaviour.) This approach is likely to prove fruitful, and will make evolutionary sense of many facts which now seem merely isolated details.

"Several contributions at the Congress dealt with new techniques of radio-telemetry which will in coming years undoubtedly lead to great advances in the study of populations and migration and orientation. It is already possible to record for each of fifty-two birds simultaneously one location every 45 sec, and to plot the results automatically on maps. The possibilities are boundless, and more will undoubtedly be heard of them at the next Congress four years hence"

More River Meandering

A FURTHER development in the theory of river meandering has now appeared as *Professional Paper* 422-*H* of the United States Geological Survey. The paper is credited to Dr. Luna Leopold and Mr. W. B. Langbein, respectively the chief hydrologist and a research hydrologist with the U.S. Geological Survey, both of whom have been closely associated with the theory of river meandering for the past decade, particularly since Dr. H. von Schelling showed, in 1951, that the meandering of rivers is really a random walk problem[1,2]. By field studies of actual rivers, it has now been shown that there is a negative correlation between the meandering of rivers and certain other characteristics of the bed of a river which dissipate energy. Briefly, the studies have confirmed what successful fishermen have known for centuries—the more sinuous a river, the smoother the bed and the more uniform the velocity of flow.

The starting point for Von Schelling's theory is the hypothesis that the probability of a river deviating by an angle $d\phi$ from its previous direction in a distance ds is best represented by a Gaussian distribution. This leads to the conclusion that the shapes of rivers should be determined by the condition that:

$$\Sigma(\Delta\phi)^2/\Delta s$$

should be a minimum. With suitable approximations, this boils down to the assertion that the shape of a meandering river can be obtained from an equation:

$$\phi = \omega \sin 2\pi(s/M)$$

which specifies the deviation of the river (ϕ) from the direct path at all distances s along the loop the total length of which is M. The constant ω is a measure of sinuosity. Observation in the past decade has shown that a great many meandering rivers can be adequately accounted for by such a formula.

What Leopold and Langbein have now done is to obtain a correlation between the sinuosity of a river and the variances of depth, velocity, slope and shear, and also with a parameter which is a measure of frictional forces at the bed of the river. They have

worked in detail with five stretches of water in Wyoming, using techniques of recording the level of the water surface to within 0·001 in. on some occasions. They have found that when a reach of river is virtually straight, the variance of the chosen parameters of shear and friction near the bed tends to be much higher than when nearby reaches on the same river are sinuous. These and other correlations tend to confirm what the authors have been suggesting for some time—that the rate of dissipation of energy along the length of a meandering river tends to be a constant.

[1] von Schelling, H., Report No. 64*GL*92 (General Electric Co., Schenectady, 1964).
[2] von Schelling, H., *Trans. Amer. Geophys. Union*, **32**, 222 (1964).

Fisheries Research at Aberdeen

DR. P. T. GRANT writes:

"The new laboratory of the Natural Environment Research Council's Fisheries Biochemical Research Unit was formally opened on April 27 by Sir Rudolph Peters at a ceremony presided over by Dr. E. M. Wright, Principal and Vice-Chancellor of the University of Aberdeen, in the presence of Mr. R. J. H. Beverton, Secretary of the Natural Environment Research Council.

"Completed in November 1965, the new laboratory is located in the Torry district of Aberdeen, overlooking the sea and the estuary of the River Dee. It is adjacent to the Marine Laboratory of the Department of Agriculture and Fisheries for Scotland and to the Torry Research Station of the Ministry of Technology. The building is on two floors with a total area of 10,000 sq. ft. The main and upper floor contains the laboratories, library, cold room and offices. The flexible system of laboratory furniture was designed and manufactured locally. All service rooms are on the ground floor, which includes an animal house for small mammals and an aquarium. A sea-water reservoir of 10,000 gallons capacity serves to maintain marine organisms at controlled temperatures in glass-fibre or plastic tanks. The architects were Mackie, Ramsay and Taylor of Aberdeen and the main contractors, Alexander Hall and Son, Ltd.

"The running of this research laboratory is an interesting venture in co-operation between the University of Aberdeen and the Natural Environment Research Council in that the University employs the staff and administers the unit, with funds provided by the research council. Both bodies are equally represented on the joint management committee and the unit has close links with the Department of Biological Chemistry. As director of the Unit and a reader in the Department of Biological Chemistry, I find that this close association has greatly helped members of staff to take part in teaching activities of the appropriate university departments although, of course, their primary obligation is to research.

"The research programme is concerned with the basic biochemistry of marine and freshwater organisms and the laboratory is equipped to carry out a broad range of metabolic studies. Problems under investigation by the present research staff of five and two research students include studies on the function and biosynthesis of biologically active molecules such as enzymes and hormones. A study of the variants of enzymes present in the tissues of marine organisms should have a bearing on the genetics of fish."

Parliament in Britain

IN the House of Commons on August 3, Mr. H. Jenkins was given leave under the Ten Minute Rule to introduce a Bill which would enable the Postmaster General to establish a Television and Radio Authority, to run the fourth television channel, and to set up a National Popular Radio Programme which would act as a parent station to local radio stations. Moreover, the Bill would enable the Postmaster General to abolish pirate radio stations within or without territorial waters. The new public authority would acquire its main revenue from advertising and its first task would be to replace the pirates in a manner acceptable to their audience and to the writers' and performers' unions and organizations. Mr. Jenkins mentioned that Radio 390, for example, was interfering with Swedish radio broadcasts and with shipping broadcasts on the Medway. The Authority would also support the University of the Air.

IN a statement in the House of Commons on August 3, the Postmaster General, Mr. E. Short, said that the Post Office now faced considerable problems of expansion, modernization and reorganization. In considering the present appropriateness of the Civil Service context in which the Post Office functions, the Government had recently made a fundamental survey of its management, structure and functions. After careful consideration it had been decided that instead of being a Department of State with a Minister at its head, the Post Office should become a public corporation and its members would be appointed by and responsible to a Minister. A final decision on the exact form of the reorganization and of the structure of the internal management must await the report of the Select Committee on Nationalized Industries, which was now examining the Post Office, and the fullest consultation with representatives of the staff which would now take place. A White Paper setting out the Government's final proposals would be presented to the House in due course. Employees would cease to be civil servants, and as regards management he hoped there would be cross-fertilization with industry. The present system of regulations would not obtain, but whether or not the Savings Bank was managed by the new corporation, banking policy would remain in the hands of the Treasury. It was intended to separate the postal and telecommunications services as completely as possible.

IN a statement to the House of Commons on August 4, the Prime Minister, Mr. H. Wilson, announced that it was the Government's firm intention to find time to pass the Parliamentary Commissioner Bill through all its stages in both houses by the end of the year. The Bill was given its first reading on July 20. Meanwhile it had been decided that, without prejudice to full discussion of the Bill itself, it would be prudent to designate the first Parliamentary Commissioner, who would be an Officer of the House of Commons, with a high degree of independence, dealing with complaints transmitted to him by members and reporting back to them individually, to the House as a whole and to whatever Select Committee the House might appoint. Mr. Wilson's announcement of the choice of Sir Edmund Compton was warmly welcomed by the Leader of the Opposition, though the appointment was criticized by Mr. J. Grimond as an encroachment on parliamentary freedom.

University News:

Indiana

PROF. H. GEST, at present professor of microbiology in Washington University, Missouri, has been appointed professor of microbiology and chairman of the Department of Microbiology in Indiana University.

Washington

DR. J. L. DEC. DOWNER has been appointed professor of ophthalmology in the University of Washington Medical School, Seattle, and is therefore resigning his position as director of the Medical Research Council's Cerebral Functions Research Group. It is expected that the activities of the Group will continue under a new director. Over the past few years the work of the Group has been concerned with learning and memory functions in monkeys utilizing the "split-brain" technique.

Appointments

MR. DUDLEY SEERS, director-general of Economic Planning at the Ministry of Overseas Development, will be the first director of the Institute of Development Studies at the University of Sussex. The Institute was founded a year ago by the Ministry of Overseas Development and is intended to engage in teaching and research linked with problems of development in countries overseas. It is an autonomous institute sited on the campus of the university.

MR. J. P. EBERHARD has been appointed director of the National Bureau of Standards Institute for Applied Technology on the resignation of Dr. D. A. Schon, who has left to direct the Organization for Social and Technical Innovation, Cambridge, Mass. Mr. Eberhard has been deputy director of the Institute since its establishment in 1964. Dr. L. M. Kushner, former chief of the NBS Metallurgy Division, has been appointed deputy director.

DR. C. M. SINGH, dean of the College of Veterinary and Animal Sciences, Punjab Agricultural University, Hissar, has been appointed director of the Indian Veterinary Research Institute, Izatnagar and Mukteswar. Dr. M. R. Dhanda has been appointed dean of the new Veterinary Faculty at the Agricultural University, Hyderabad.

THE Medical Research Council is assisted in the formulation and execution of its research programme by three advisory Boards which cover the main fields of its work, and have the principal function of overseeing the progress of work supported by the Council and advising on proposals for such support in their respective fields. The following have been appointed to the Boards by the Council: *Biological Research Board*, Dr. D. E. Broadbent (M.R.C. Applied Psychology Research Unit, Cambridge); Prof. W. T. J. Morgan (Department of Biochemistry, Lister Institute of Preventive Medicine); *Clinical Research Board*, Prof. D. A. K. Black (University Department of Medicine, The Royal Infirmary, Manchester); Prof. R. S. Illingworth (Department of Child Health, University of Sheffield); *Tropical Medicine Research Board*, Prof. B. G. Maegraith (Department of Tropical Medicine, Liverpool School of Tropical Medicine); Dr. C. E. Gordon Smith (Microbiological Research Establishment, Porton, Wilts). Prof. W. D. M. Paton has been appointed chairman of the Biological Research Board, in succession to Prof. A. Neuberger.

Announcements

THE Deutsche Akademie der Naturforscher Leopoldina, Halle, recently elected the following new members in the sections indicated: *Physiology*, Prof. H. Drischel (Leipzig); Prof. L. Lubinska (Warsaw); *Physiological Chemistry*, Dr. E. Mikulaszek (Warsaw); *Orthopaedics*, Dr. M. Jaroš (Prague); *Surgery*, Prof. J. J. Navrátil (Brünn); Prof. B. W. Petrovskij (Moscow); *Physics*, Prof. E. Hückel (Marburg); *Chemistry*, Prof. E. Schmitz (Berlin); Prof. K. Schreiber (Gatersleben); Prof. F. Sondheimer (Cambridge). *Mineralogy, Petrology and Crystallography*, Prof. J. Wyart (Paris).

A SYMPOSIUM on "Potential Industrial Applications for Isotopic Power Generators" will be held at the Atomic Energy Research Establishment, Harwell, during September 28–30. Further information can be obtained from the Post Graduate Education Centre, Building 455, A.E.R.E., Harwell, Didcot, Berkshire.

THE eighteenth Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Inc., will be held at the Penn-Sheraton Hotel in Pittsburgh, Pennsylvania, during March 6–10, 1967. Further information can be obtained from Dr. Gerald L. Carlson, program chairman, Eighteenth Pittsburgh Conference, Inc., Mellon Institute, 4400 Fifth Avenue, Pittsburgh, Pennsylvania.

AN international symposium on "Marine Geodesy, Present and Future", sponsored jointly by the U.S. Coast and Geodetic Survey and the Columbus Laboratories of Battelle Memorial Institute, will be held in Columbus, Ohio, during September 28–30. Further information can be obtained from A. G. Mourad, Columbus Laboratories, Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio.

CORRIGENDUM. In the communication entitled "Effect of Altered Perfusion Rates on the Retention of Noradrenaline by the Spleen", by Dr. D. M. Paton and Prof. C. N. Gillis, which appeared on p. 391 of the October 23, 1965, issue of *Nature*, in line 2 of p. 392, "4·8 mg/ml." should read "4·8 ng/ml.", and in the last line of Table 1 "weighted ± test" should read "weighted *t* test".

CORRIGENDUM. In the article entitled "Uptake of Potassium at Low Temperatures in Kidney Cortex Slices of Hibernating Mammals", by Prof. J. S. Willis, which was published on p. 691 of the November 14, 1964, issue of *Nature*, the concentrations of inhibitors used were incorrectly stated (p. 692, column 1, lines 46, 47; Table 1, lines 4, 5 of legend). These should have read "dinitrophenol (0·2 mM) and iodoacetate (1·0 mM)" instead of 0·02 mM and 0·1 mM, respectively.

CORRESPONDENCE

Stonehenge

SIR,—Prof. Hoyle's authority in cosmology is unquestionable and his theories about the use of Stonehenge are ingenious and mathematically sophisticated—perhaps too sophisticated for the culture which produced it. Unfortunately, the same cannot be said of his speculations, which must appall anyone with the slightest acquaintance with the archaeology and history of religions, that the assumed use of the Aubrey holes perhaps gave rise to the idea of Trinity and the concept of an unseen God.

2,000 B.C. is the earliest date assigned to Stonehenge and some authorities date it somewhat later. The worship of the neolithic Mother Goddess, whose cult was widespread over the Western world, and to which Stonehenge is linked through the megalithic tradition associated with it, is dated, in origin, not later than 7,000 B.C. Her triple aspect associated with the ancient three-season year, presumably in use by the builders of Stonehenge, seems to have been the protohistorical origin of the three-in-one concept of Trinity, long before the Bible or Stonehenge. So any symbolic significance the number three might have would be derived from the old religious tradition, and not the other way round.

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COMPUTERS FOR BRITISH RESEARCH

THE formation of the Computer Board by the Secretary of State for Education and Science has not brought a great wave of congratulation from British universities and research establishments, but instead a tendency to complain that it is not enough, and not soon enough. Even the fact that Mr. Anthony Crosland has somehow persuaded the Treasury to part with £3 million a year at a time like this has not silenced all the critics. This may therefore be the right time to ask what are the prospects for computers in the universities and other research establishments in Britain.

The origins of current arguments about the scale on which computing facilities should be provided in British universities go back to the appointment in March 1965 of a working group under Prof. B. H. Flowers. The working group itself was born of discussions within the Council for Scientific Policy (under Sir Harrie Massey), and between the council and the University Grants Committee. Professor Flowers is himself a member of Sir Harrie Massey's committee. His working group moved quickly, and reported to the Minister in July 1965. The report was eventually published in December 1965, together with a general statement by the British Government that its recommendations had won "general approval".

The essential feature of the recommendations was that computing facilities at universities and research establishments should be integrated into a regional framework, with universities and research councils having access as of right to whatever facilities are available. Edinburgh, Manchester and London were singled out as regional centres, to be equipped with powerful computers (rented in the first instance from suppliers in the United States) with the possibility that other universities in the Midlands and South-West might graduate into regional centres later on. It was also argued that money should be spent immediately on making the fullest use of the English Electric *KDF* 9 computers at seven British universities.

Finally, the working group argued the case for spending money on a number of installations at a further twenty-one institutions, either to improve existing machines or to buy new ones. All these developments, according to the report, should be carried out on a regional basis, with universities and the research institutions responsible to the five research councils having a right to use all nearby computers and the regional computing centre.

The estimates in the Flowers Report on future demand for computing power are based on estimates by the universities themselves, though the working group did on some occasions point out that demand was likely to grow more quickly than universities themselves foresaw. Its estimates are, however, supported by the experience of large university installations in the United States where the demand for computer time appears to have been doubling every year for some years now. Taking the computing power of an IBM 7090 as a yardstick (which means that an improved *KDF* 9 with a 36*K* store is equivalent to 1·2 7090 machines), the group estimated that its proposals would increase the computer power of British research institutions from roughly 15 units in March 1965 to roughly 135 in March 1970.

The response of the British Government to these proposals has been surprisingly openhanded, given the economic difficulties of the past twelve months. The total cost of the programme has, however, been stretched out over six years and not five. Orders for the improvements intended at the *KDF* installations and the other twenty-one universities with less sophisticated equipment have already been placed, chiefly through a panel of the University Grants Committee under Sir Willis Jackson. Moreover, a regional centre at Edinburgh has already been formed.

Why, then, has the whole project encountered widespread criticism in recent weeks? Three separate lines of complaint are evident. First, people complain of the delay, both in setting up the computer board and in implementing the other proposals of the Flowers Group. Then there is a complaint that in the interval between the report a year ago and the present, multiple access computer systems have been shown in the United States not to be fanciful experimental tools but essential building bricks in any modern computer system, so that the degree of integration implicit in the Flowers proposals is now inadequate. Finally, it is held that the preoccupation of the Flowers Report with the needs of universities and research councils has made even sharper the gulf between the comparatively academic affairs of the Department of Education and Science and the more industrial preoccupations of the Ministry of Technology.

The nub of the argument on delay is that the investments proposed by the Flowers Group were intended to be spent in the period July 1965 to July 1970, while the present plans of the Government would start a year later and finish two years later. Moreover, the criticism continues, the delay in the setting up of the Computer Board has meant further delays in beginning serious work on the proposals. The rejoinder to this is that Sir Willis Jackson and his panel have been hard at work spending their £3 million a year on the improvement of university computing, and that the panel has even gone somewhat further than the Flowers Group foresaw by placing a firm order for an English Electric multiple access computer at Edinburgh for delivery in roughly two years.

On the character of the computing system to be developed there seems to be genuine disagreement. There are those who argue that the time has now come for a closely integrated system of computers within a national network, with access by means of individual consoles. It is held that experience in the United States, and the character of forward planning in France, make such a system feasible and desirable in Britain. On the other hand are those who argue that the experience with systems such as Project MAC at the Massachusetts Institute of Technology (*Nature*, 209, 1161; 1966) is not yet sufficient to demonstrate that a multiple access system on a national scale will really work. In any event, they argue, the universities and research establishments will always need a much more personal form of access to computers, for their need of these machines is not simply their need of computation but also their need of opportunities for teaching and research.

The complaint about the gulf between science and technology is linked with the way in which the Ministry of Technology is apparently pursuing an independent policy in the development of computing centres. Thus it has recently established a National Computing Centre at the Manchester College of Science and Technology, with Professor Gordon Black as director. The centre will be a more or less commercial venture, drawing support by way of fees from industrial manufacturers and users of computers, and also by means of grants from the Ministry of Technology. The decisive role of the centre can be in the development of software both for use with machines now being developed by British companies and for improving the efficiency of systems already in use.

DID THE ATLANTIC CLOSE AND THEN RE-OPEN?

By PROF. J. TUZO WILSON

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FOR more than a century it has been recognized that an unusual feature of the shallow water marine faunas of Lower Palaeozoic time is their division into two clearly marked geographic regions, which are commonly referred to as faunal realms. "The faunal assemblages are amazingly uniform throughout each realm so that correlation of any Cambrian section with another in the same realm is usually easy; on the other hand, the difference between the faunas in the two separate realms is so great as to make correlation between them very difficult"[1].

Two aspects of the distribution of these realms are remarkable. For one thing, some regions of similar faunas are separated by the whole width of the Atlantic Ocean; then, on the other hand, some regions of dissimilar faunas lie adjacent to one another. This is illustrated by Fig. 1, which is based on work by Cowie[2], Grabau[3] and Hutchinson[4].

Grabau showed that, if Europe and North America had become separated by continental drift, a simple reconstruction could explain the first anomaly in the distribution of the faunal realms in that, before the opening of the Atlantic Ocean, each realm would have been continuous, with no large gaps between outcrops of similar facies (Fig. 2).

It is the object of this article to show that drift can also explain the second anomaly. It is proposed that, in Lower Palaeozoic time, a proto-Atlantic Ocean existed so as to form the boundary between the two realms, and that during Middle and Upper Palaeozoic time the ocean closed by stages, so bringing dissimilar facies together (Fig. 3). The supposed closing of the Tethys Sea by northward movement of India into contact with the rest of Asia, and the partial closing of the Mediterranean by northward movement of Africa, can be regarded as a similar but more recent event. The figures are based on a reconstruction by Bullard, Everett and Smith[5], but because those authors pointed out that no allowance had been made for the construction of post-Jurassic shelves, the continents have been brought more closely together.

Four lines of evidence suggest that this proposal is reasonable. (Unfortunately, so far as I can ascertain, palaeomagnetic evidence which might bear on this problem does not exist.)

First, this reconstruction of geological history is held to provide a unified explanation of the changes in rock types, fossils, mountain building episodes and palaeoclimates represented by the rocks of the Atlantic region.

Second, wherever the junction between contiguous parts of different realms is exposed, it is marked by extensive faulting, thrusting and crushing.

Third, there is evidence that the junction is everywhere along the eastern side of a series of ancient island arcs (Fig. 3).

Fourth, the fit appears to meet the geometric requirement that during a single cycle of closing and

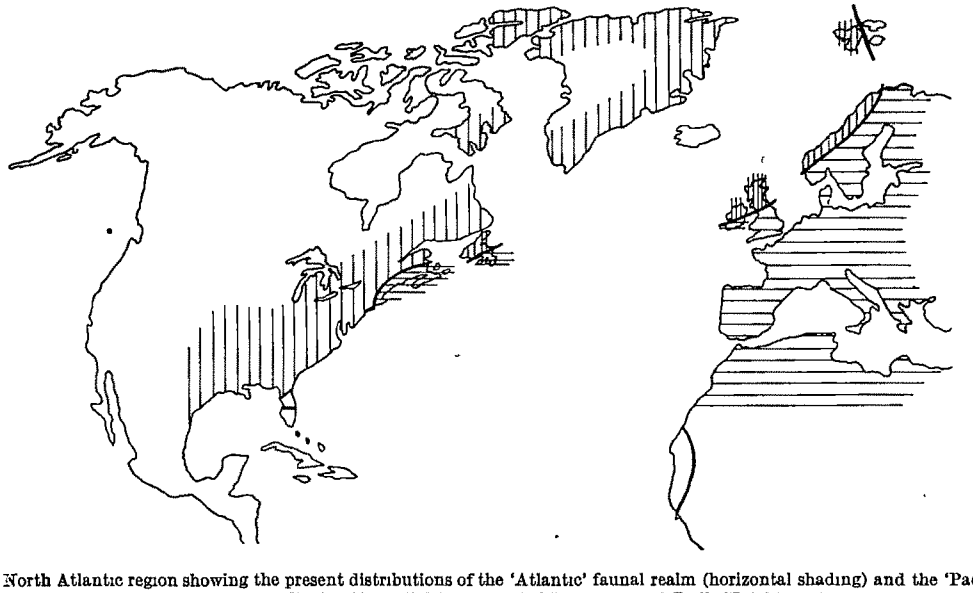


Fig. 1. The North Atlantic region showing the present distributions of the 'Atlantic' faunal realm (horizontal shading) and the 'Pacific' faunal realm (vertical shading). (After J. W. Cowie, A. W. Grabau and R. D. Hutchinson)

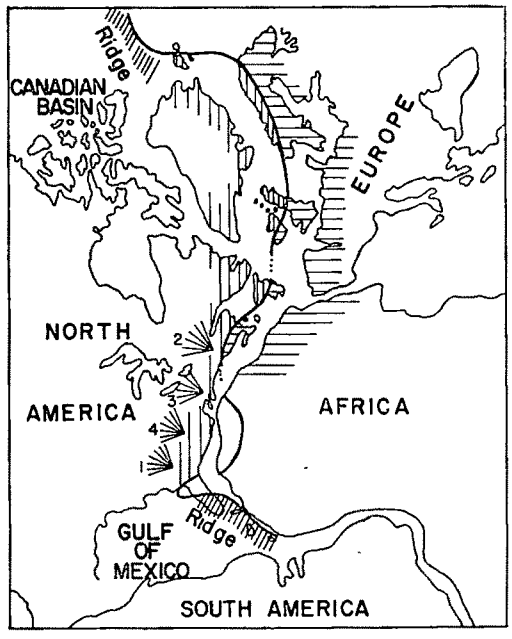


Fig. 2. The North Atlantic region in Upper Palaeozoic and Lower Mesozoic time showing that of the present Atlantic Ocean only the Canadian Basin and the Gulf of Mexico then existed. Four fans are shown which were formed: (1) during Middle Ordovician; (2) during Upper Ordovician, (3) during Upper Devonian; (4) during Pennsylvanian. The heavy line separates 'Pacific' and 'Atlantic' faunal realms. The two ridges are considered to have formed when the modern Atlantic started to open

reopening of an ocean, and in any latitudinal belt of the ocean, only one of the pair of opposing coasts can change sides (Fig. 4).

Recurrent Drift in the North Atlantic

The history proposed for the North Atlantic region can be stated very briefly as follows: (*a*) From the Late Pre-Cambrian to the close of Middle Ordovician time an open ocean existed in approximately, but not precisely, the same location as the present North Atlantic (Fig. 3). (*b*) From the Upper Ordovician to Carboniferous time, this ocean closed by stages. (*c*) From Permian to Jurassic time there was no deep ocean in the North Atlantic region. The only marine deposits of that time are those connected with the Tethys Sea, with a shallow Jurassic invasion of Europe and with deeper Jurassic seas in the Gulf of Mexico and in the western Arctic Basin (Fig. 2). (*d*) Since the beginning of the Cretaceous period the present Atlantic Ocean has been opening, but this reopening did not follow the precise line of junction formed by the closing of the early Palaeozoic Atlantic Ocean; the result is that some coastal regions have been transposed (Fig. 1).

The Lower Palaeozoic continents may have first touched each other at the end of Middle Ordovician time, for thereafter the distinction between 'Atlantic' and 'Pacific' faunal realms ceases to be marked, but the complete closing of the ancient Atlantic may have required several periods.

For each continent, union meant replacing the open ocean by the other continent. This is offered as an explanation of the borderlands of J. Barrell and C. Schuchert for which there is no clear evidence until Upper Ordovician time. As Kay has suggested[6] concerning Eastern North America: "There has been little discussion of the evidence for borderlands in earlier Paleozoic time, though some have expressed scepticism". Kay's own support for island arcs is muted after Lower Palaeozoic time and he accepts the view that the sediments of the "Late Devonian and Early Mississippian came from the land of Appalachia", —a borderland.

This view that extensive upland source areas lay to the east of the Appalachian geosyncline in the sites of the present coastal plain or ocean has been fully supported by recent work[7–9]. Tens of thousands of cubic miles of quartz-rich sediments, derived from the east, were deposited in shallow marine to sub-aerial deltas.

When the continents were pushed together, they would have touched first at one promontory and then at another. It can be expected that high mountains would have been formed locally and that they would have produced alluvial fans on both continents. As the ocean diminished the climate would have become increasingly arid. Such drastic alterations in the physiography would explain the change from predominantly marine and island arc deposition in the Lower Palaeozoic to conspicuous fans of Queenston, Catskill, Old Red Sandstone, and other deltas of Middle and Upper Palaeozoic time (see refs. 10 and 11, and Fig. 2). It can also be expected that the collision of continents would have produced great local uplifts which, if one continent overrode the other, would have migrated inland, perhaps pushing the Taconic and northern Newfoundland klippen[12] before them.

It would seem that by Permian and Triassic times the Atlantic Ocean was completely closed, because only continental beds, such as the Dunkard and Newark series, are found in North America. In Great Britain the New Red Sandstone is also continental as is the Permian of the Oslo district.

No Jurassic beds are known in eastern North America except in the Gulf of Mexico. Those of Europe were formed by a shallow marine invasion of the continent and are said by Hallam[13] to have fossils that "include many neritic forms that could not have crossed a deep ocean. The paleogeography for the Scottish Jurassic gives no hint of increasingly marine conditions to the west" (personal communication). Most of the available geological evidence suggests that the present Atlantic Ocean started to open at the beginning of Cretaceous time[14,15]. Although objections to this view are still being raised, they seem to be minor in comparison with the other evidence, and it is possible that they can be explained in other ways.

Faulted Contact between Faunal Realms

Starting our considerations in the north, the island of West Spitsbergen is underlain by a thick eugeosynclinal section of Lower Palaeozoic rocks named the Hecla Hoek succession. These strata rest on no known basement and were deformed, metamorphosed and intruded by granites during the Caledonian orogeny[16,17]. They contain fossils of the 'Pacific' fauna similar to those of Scotland and North America[18].

In Nordaustlandet, the adjacent, eastern island of the Spitsbergen group, Kulling[19] and Sandford[20] have mapped a thin section of unmetamorphosed and gently folded strata which a few fossils indicate to be of about the same age. These beds lie uncomformably on a basement which is regarded as part of the Baltic Shield. These strata do not thicken to the west as the much thicker section of West Spitsbergen is approached, nor do the few fossils necessarily belong to the 'Pacific' faunal realm.

Despite the considerable number of attempts to compare the sections in the adjacent islands, the correlation is not good. Changes in thickness, facies, and degree of metamorphism, basement and type of intrusives are all abrupt and striking. Orvin[21] and others have mapped faults in Hinlopen Strait between the islands. Klitin[22] summarizes the situation thus: "Of particular interest is the junction zone of the alleged Caledonian platform and Caledonian fold system. The transition to typical caledonids takes place in a zone not over 15 to 20 km wide, in the Hinlopen

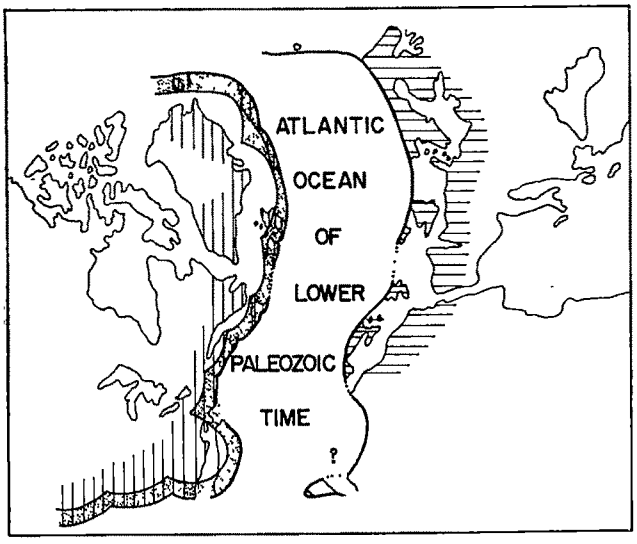


Fig. 3. The North Atlantic region in Lower Palaeozoic time. The proto-Atlantic Ocean would have formed a complete barrier between two faunal realms (shaded). Island arcs (dotted) probably lay along the North American coast. The floor of this ocean could have been absorbed in the trenches associated with these arcs as the ocean closed

Strait area, where the Hecla Hoek section abruptly increases in thickness, by a factor of four, with the appearance of extrusives in its metamorphosed and linearly folded beds. Such radical changes in thickness suggest a fault junction between an ancient platform and the caledonids." Following this interpretation I suggest that the Lower Palaeozoic ocean once separated the two islands and that, whereas Nordaustland formed part of the Baltic shield and shelf, West Spitsbergen was the site of a North American island arc.

In Scandinavia it is well established that the peninsula is divided longitudinally into two different provinces separated by a great zone of nappes and faults overthrust towards the east. According to the descriptions of Holtedahl[23], south-eastern Norway and most of Sweden are underlain by an extension of the Baltic Platform on which lie nearly flat, unfossiliferous rocks and "the eastern facies of the Cambro-Silurian (which) can be classed as miogeosynclinal in the terminology of Stille. The thickness is rather large and there is much terrigeneous material. Caledonian volcanic and intrusive igneous rocks are not found in the deposits of this type. The rocks are unmetamorphosed in the east and are of low metamorphic grade farther to the north-west. The deposits occur in an autochthonous or parautochthonous position above the original Archean basement."

The fossils are repeatedly referred to as being similar to those of the Baltic region, England and Wales. On the other hand, in the Trondheim area of western Norway, a thick succession of pillow lavas, shale and limestone with serpentinites in the lower part of the sequence contains a fauna "of American affinities . . . the limestone of Smøla is similar to the Durness in Scotland and to more or less contemporaneous limestone in Newfoundland, Bear Island and Spitsbergen. The limestone in Smøla thus seems to represent an American-Arctic facies of the Ordovician." In these "mainly pelitic sediments . . . we thus have a eugeosynclinal facies characteristic of the central parts of a geosyncline . . . probably all rocks of the present facies occur in allochthonous positions." An important event in central southern Norway was the close of marine deposition "in Late Ordovician or Early Silurian time brought about by the thrusting of nappes and deposition of the Valdres sparagmite (arkose), which was considered as a deposit of flysch type by Goldschmidt". The zone of nappes is, therefore, held to be the boundary between faunal provinces and the line of closure of the Lower Palaeozoic ocean.

The geological relationship between Spitsbergen, Scandinavia and the British Isles has been discussed by Bailey and Holtedahl[24]. Of the Caledonian structures they state: " . . . in the present land area of Scandinavia we find the eastern part of the orogenetic belt only, while in Great Britain the whole of the orogenetic zone is represented. The Spitsbergen Group seems to lie rather centrally in the zone of deformation." Following Wegener, they consider that Greenland may have been formerly connected with Europe and suggest that, if so, it would have completed the western side of the mountain belt opposite Norway. This relationship has found support in recent years from several authors[14] including Umbgrove[25].

That the boundary between 'Atlantic' and 'Pacific' faunal realms crosses northern England between Scotland and Wales is supported by Walton, who writes of the Scottish occurrences: "The close affinity of the Durness and North America rocks was recognised long ago . . . . The long-recognised affinity of the Girvan Caradocian and Appalachian Mohawkian faunas was re-emphasized by Williams . . . . By contrast the faunas are only remotely connected with the Welsh Ordovician rocks"[26]. George[27] describes the relations thus: "The Salopian geosyncline may thus have extended unbrokenly from conjectural north-western limits in the Highlands to a south-eastern margin or marginal shelf in the English Midlands". It does not require much change to regard this geosyncline as a former ocean.

In Newfoundland the geology of the north-eastern coast has recently been described by Williams[28]. He suggests that in Cambrian time a deep basin or ocean, not underlain by continental crust, crossed the central part of the island and separated two shelves. The north-western shelf underlying the Long Peninsula has a basement of Grenville age overlain by strata with 'Pacific' faunas like those of Scotland, while the south-eastern shelf, which forms the Avalon peninsula, has a younger Pre-Cambrian basement overlain by strata with 'Atlantic' faunas like those of Wales. During Ordovician and subsequent time the intervening sea became filled with eugeosynclinal and volcanic sedimentary rocks, probably representing a former island arc and mountain belt.

Anderson[29] believes that faulting in Hermitage Bay on the south coast of Newfoundland not only separates the rocks of the south-eastern shelf from the central geosyncline, but may also completely divide the two faunas. On the north coast the corresponding fault zone may be in Freshwater Bay, but this point has not been settled as yet[30].

Among those who have recently correlated the Newfoundland and British sections are Dewey and Church[31]. Although Church does not favour continental drift, both he and Dewey make the same correlation as that already given here and extend the Caledonian mobile belt from northern England and central Ireland to central Newfoundland.

South of Newfoundland, the Gulf of St. Lawrence and younger rocks cover the key areas of Lower Palaeozoic formations almost as far as the Maine border. Little can be said except that the faunas of Cape Breton Island and St. John, New Brunswick, have European affinities while those of Gaspé and the Eastern Township of Quebec are typical of Scotland and most of North America[4].

In northern and eastern Maine the older literature is sparse and generalized. Recently a combined group of government and university geologists, including W. B. N. Berry, A. J. Boucot, E. Mencher, R. S. Naylor and L. Pavlides, have discovered new fossil localities there with both European and North American affinities, important Caledonian uplifts and large pre-Silurian faults[32]. Much

of the state has been remapped, but for the reason that little of this work has yet been published (R. G. Doyle, personal communication), and because the structure is clearly much more complex than shown on early maps, this is not an opportune time to consider the area in detail.

From the southern part of Maine south across New Hampshire, Massachusetts and Connecticut a major zone can be traced from published accounts. Novotny[33] has described this "major fault zone" where it crosses the New Hampshire–Massachusetts boundary (Fig. 5). To the north it connects with several faults and silicified zones shown on the map of New Hampshire[34,35]. These lie along the line which separates those formations which underlie the greater part of New Hampshire from a suite of completely different formations, underlying south-eastern New Hampshire. This line may be continued northward into south-eastern Maine by the faults which Katz[36] has suggested bound the Berwich gneiss, itself crumpled, "closely folded and overturned". In Massachusetts, Novotny's fault may be exposed in the abandoned Worcester "coal" mine. The description suggests that much of the rock may be carbonaceous mylonite[37]. South of Worcester this fault connects with a major change in formations evident on the geological map of Massachusetts[38].

In Connecticut this boundary appears to join the Honey Hill fault and its northern continuation which bisect the state and separate two major rock sequences. Where best described in the south it "has been mapped for 25 miles eastward from Chester nearly to Preston without apparent repetition of the stratigraphy on either side of the fault. The fault plane dips 10°–35° N parallel to the underlying metasedimentary rocks .... The fault is marked by a zone a mile wide of mylonitized and crushed rocks .... Displacement must have been many miles"[39]. As in Norway, the orogenic belt appears to have been thrust eastwards over the eastern platform. To the south this major fault seems to strike into the Atlantic Ocean and the Appalachian belt, then narrows conspicuously. I have recently learned from L. R. Page and J. W. Peoples that mapping (for the most unpublished) by the federal and state surveys has defined this fault zone more satisfactorily, and that published aeromagnetic maps show a change in the strike of anomalies across it. This evidence suggests that New England is divided by a major fault zone into two provinces underlain by quite different rock formations. Of the few occurrences of the Lower Palaeozoic faunas, all those with European affinities lie to the east of the fault zone; all those typical of North America to the west[40,41].

In the light of this evidence it seems reasonable to suggest that this fault zone marks the line of closure of the Lower Palaeozoic Atlantic Ocean. It may seem strange to propose that a former position of the Atlantic Ocean lies through New England and that its full significance has not been realized, but it must be remembered that throughout the area outcrops are poor and that most of the mapping is old. Surface mapping reveals few faults, but new tunnels have shown that faults abound under the drift-filled valleys and that some of these are major (J. W. Skehan and A. Quinn, personal communications).

Most North American geologists have not accepted the idea of continental drift. Instead they have sought to explain the changes in faunas in terms of different environmental conditions. This has certainly been a factor and no doubt was responsible for the differences between the Durness, Girvan and Moffatt facies in Scotland, although those facies belong to the same faunal realm. In another example, G. Theokritoff (personal communication) has directed my attention to a possible mixing of Atlantic, Pacific and endemic faunas in the Taconic sequence of New York[42]. Christina Lochman[43] has emphasized the difficulties incurred in accepting such an interpretation. She states that the areas of mixed faunas

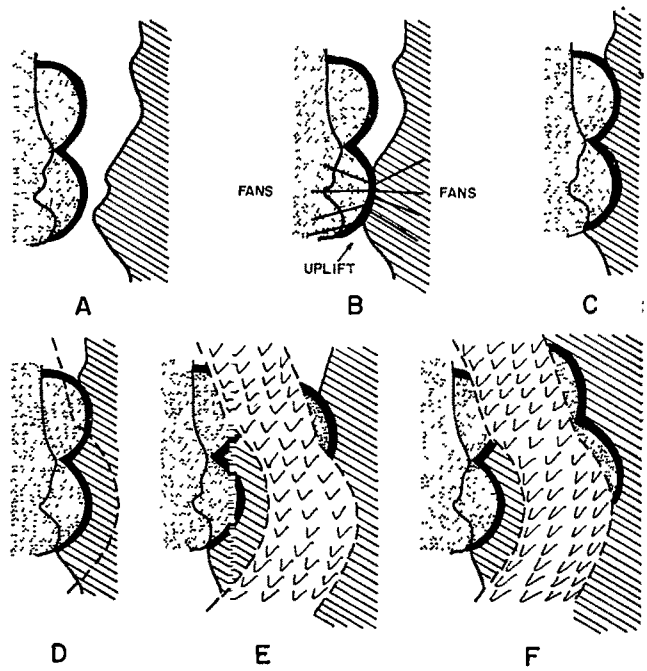


Fig. 4. *A*, A closing ocean, with island arcs on one coast, separating two different faunal realms. *B*, First contact between two opposite sides of a closing ocean. *C*, The ocean closed by overlap of the opposite coasts. *D*, A possible line (dashed) along which a younger ocean could reopen. *E*, A new ocean (checked) opening in an old continent. *F*, A geometrically impossible way for a younger ocean to open. (Note how the arcs overlap.)

lie in deeply down-warped basins between two shallow shelf deposits containing respectively Atlantic and Pacific faunas. These basins, she remarks, had connexions with the Atlantic Ocean and had a benthonic environment "similar to that of the open ocean .... Few normal benthonic species of the coastal shelf could establish themselves in such an alien environment, although, because of the geographic proximity of the two areas, sporadically drifted individuals might be found." She also refers to deep basins separating different faunas and to the evidence for a "biofacies regime ordinarily found on the floors of the continental shelf beyond the inner islands of the volcanic archipelago". These views would seem to admit the possibility of interpreting the palaeogeography as has been done in this article. It is suggested that one of the deep basins, instead of lying on a shelf, might have been an open ocean in Lower Palaeozoic time and that the mixed fauna may have come from the extreme edge of one continent.

The reconstruction (Fig. 2) then leads to Africa. Sougy[44] has described a large plate of metamorphic rocks east of Dakar thrust eastwards over Upper Devonian strata. Farther north in Spanish Morocco there is a folded belt. A large Cretaceous overlap along the coast and lack of diagnostic fossils in the overthrust block make correlation difficult, but Sougy concludes that: "In the future, when geologists study the relationships between America and Africa for evidence bearing on permanence of continents and oceans *versus* continental drift, they will have to consider that the western rim of Africa is made, from Guinea to Morocco, not of a Pre-Cambrian basement but of a mainly Hercynian orogenic belt, in some respects symmetrical to the Appalachian belt." His mention of drift suggests, as do most reconstructions[5], that this West African belt was formerly part of the Appalachians. This view is supported by P. A. Mohr, who writes: "the Cambrian manganese ores of Newfoundland, Wales and Morocco ... I think were formed in a common geosynclinal trough" (personal communication). The view that these regions were

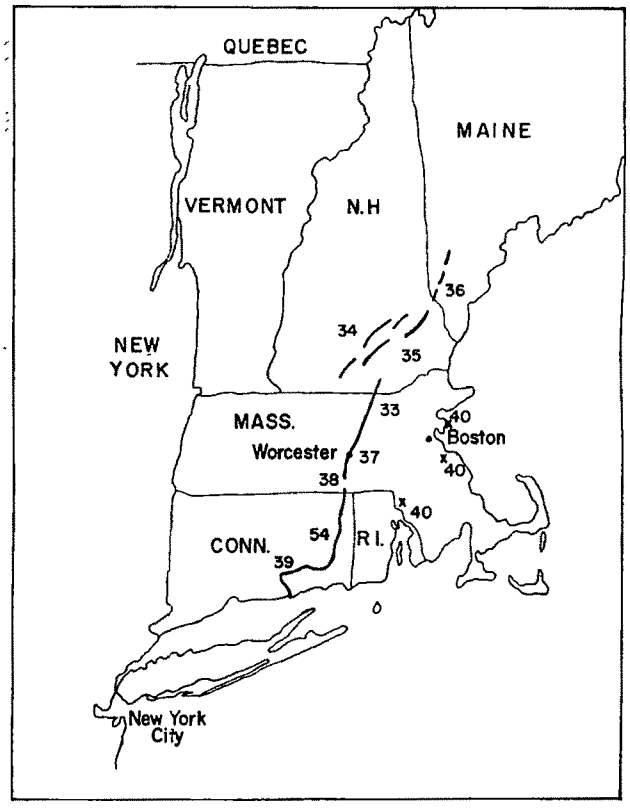


Fig. 5. Sketch map of New England showing location of some major faults and three fossil localities of the 'Atlantic' faunal realm (*X*). The numbers refer to papers in the list of references from which information was obtained

together until Cretaceous time is supported by the close similarity of Cretaceous fossils from these two regions now on opposite sides of the ocean[45]. Only further investigation, aided by extensive seismic investigations or drilling through the Cretaceous of both continental coasts, can show whether it is possible or likely that West Africa ever fitted into the central east coast of the United States. If so, some rotation of Africa clockwise and a closer fit than that shown by Bullard, Everett and Smith[5] may be indicated.

Although the northern termination of the West African fold belt is covered, its termination to the south is definite; the Pre-Cambrian of the African Shield extends to the Atlantic near Conakry[46]. The reconstruction suggests that this contact might reach Florida. In Florida and Georgia a cross-section of the Appalachians resembles that of Newfoundland in that these are the only parts of the Appalachians with a platform on both sides of the mobile belt. The platform on the south-eastern side of the mobile belt which lies beneath northern Florida and adjacent states is only known from well cores. These consist of Early Ordovician to Middle Devonian sandstones and shales said to have been deposited in shallow water[47]. Published accounts do not correlate the strata or fossils with formations elsewhere. It would seem that the basement has not been penetrated. Thus it is only speculative to suggest that this region was formerly part of Africa.

Island Arcs of Lower Palaeozoic Atlantic

According to a widely adopted hypothesis, island arcs and mountains represent places where the lithosphere is being compressed, while mid-ocean ridges represent places where it is being pulled apart and where new crust is being created. Thus the present Atlantic Ocean is expanding from the Mid-Atlantic Ridge.

For a Lower Palaeozoic Atlantic Ocean to have closed, it must, according to this hypothesis, have been marked not by a central ridge, but by a continuous system of island arcs and mountains. Observation shows that such systems commonly lie at the sides and not in the centre of oceans. Kay's view, that a system of island arcs existed off the eastern and southern coasts of North America in Lower Palaeozoic time, is entirely compatible with this hypothesis. This would have allowed the former Atlantic Ocean to close, and thus to convert the offshore island arcs of Lower Palaeozoic time into the intercontinental Appalachian mountains of Middle and Upper Palaeozoic time. Following Keith, Kay and King, I have sketched the location of seven former arcs in the Appalachians and their south-western continuation past the Ouachita and Marathon Mountains[48]. I see no reason to change this view.

The situation in Scotland has been described by Walton: "It seems likely that both the north-west Highlands and the Appalachians formed part of a very wide stable shelf which also included Greenland and other 'Boreal' regions having very similar Cambrian rock types. . . . it is probable that sedimentation in the area of the southern Highlands during the Cambrian period was mainly of a greywacke, geosynclinal type and contrasted strongly with that in the north-west Highlands . . . it is probable that Cambrian sedimentation stretched unbroken to an unknown distance south of the Highland Boundary fault"[26]. Ordovician volcanism followed and these correlations and descriptions suggest that, during Lower Palaeozoic time, arcs associated with a western land extended across Scotland. That there may have been contemporaneous volcanism and islands along the eastern coast in what is now Wales, merely adds complexity without affecting the history in Scotland. In the Silurian period conditions changed and the marine conditions "gave way to mixed environments . . . at least partly in fluvio-deltaic environments with periods of emergence"[26].

Enough has been described of the conditions in Norway and West Spitsbergen and of their correlations with Scotland to show that they too were the sites of Lower Palaeozoic island arcs associated with a western continent. Thus the Caledonian–Appalachian arcs seem to have formed a continuous system along the western side of the former ocean. It is suggested that it was in the trenches of these arcs that the floor of the Lower Palaeozoic Ocean was swallowed up as that ocean closed.

Geometrical Control of Transposition

If two bodies are brought together so that they unite, and if they are later pulled apart so that they break on a different line from the line of union, then one important geometrical consideration holds. It is that, along any one stretch of the junction, only one fragment can change sides: one cannot transpose pieces from each margin at the same place. This is illustrated in Fig. 4 and a comparison with the other figures shows that our reconstruction obeys this principle. This does not prove that the reconstruction is correct, but the neat fashion in which fragments from either side are alternately transposed meets the geometrical requirements.

Some Possible Extensions

When, as is believed, the present Atlantic Ocean started to open at the beginning of Cretaceous time, it did so by breaking open a continent which was then continuous from West Spitsbergen to Florida (Fig. 2). North of Spitsbergen the coasts of North America and Siberia at that time diverged and the opening ceased to lie wholly within a continent and to have continental blocks on both sides. Following the descriptions of B. C. Heezen

and M. Ewing, and Ya. Ya. Hakkel and N. A. Ostenso, I have suggested that the fracture followed the coast of Siberia forming the Lomonosov Ridge on the other side of the opening. Thus the Lomonosov Ridge separates an older Canadian ocean basin from a younger Siberian ocean basin in the Arctic Sea[14].

In the south the situation appears to be similar, but to understand it one must clearly separate the platform of northern Florida from the different geology of southern Florida. South of central Florida a southern Florida–Bahamas Ridge separates a main Atlantic, apparently of Cretaceous age, from a Gulf of Mexico which seems to have been a deep ocean and evaporite basin during the Jurassic period.

Drake, Heirtzler and Hirschman[49] emphasize the importance of the Florida-Bahamas Ridge for sealing off the Gulf of Mexico from the open ocean so that the Jurassic salt deposits could form. They suggest that this ridge is an "extension of the Ouachita system" and that it forms the "foundation for the entire chain of islands and banks". This ignores the earlier interpretations of magnetic and gravity anomaly maps made by Miller and Ewing[50] and by Lee[51]. Both papers suggested that southern Florida and the Bahamas are coral banks built on deeply submerged volcanoes. The Palaeozoic platform-type of sedimentary rocks, described from drill cores in northern Florida, are not like the Ouachita folds. Drilling has revealed no Palaeozoic rocks beneath southern Florida and the Bahamas. The magnetic anomaly map shows a marked change in central Florida. Drake *et al.*, in their Fig. 5, show three trend lines connecting southern Florida with the Bahamas and only one connecting it with northern Florida. It is suggested that the Florida-Bahamas Ridge, like the Lomonosov Ridge, formed when the main Atlantic Ocean started to open at the beginning of Cretaceous time and separated an ocean basin of Cretaceous age from an older ocean basin.

Drake *et al.* hold that the Florida-Bahamas Ridge extends to Navidad Bank, north of Hispaniola. It thus ends at the major zone of faulting which, according to Hess and Maxwell[52], and others, extends from Central America to the northern end of the West Indies arc. The Caribbean Sea and West Indies arc have often been regarded as associated with the Pacific Ocean, and I have discussed elsewhere their possible origin as a tongue, thrust from the Pacific and bounded by transform faults[53].

A complete discussion would require consideration of the Hercynian orogeny and faulting and post-Triassic faulting. This seems feasible but will not be attempted here.

It has been suggested in this article that during Lower Palaeozoic time North America and Europe were approaching each other, that this motion stopped and that it later reversed. If this is true, the onset of the reverse motion and the start of reopening of the Atlantic Ocean must have been an event of very major significance in world geology. The evidence suggests that it occurred at about the close of the Jurassic and the beginning of the Cretaceous periods. It seems reasonable to link it with other major events of that age in the Americas.

McLearn[55] has pointed out that at that time the drainage of much of western North America reversed its direction so that rivers which had been flowing west and building a great shelf along the Pacific coast were interrupted by the rise of the Cordillera and began to follow their present directions. Gilluly[56] has pointed out that "In Cretaceous time plutons probably a thousand times larger than those of all the rest of the Phanerozoic were emplaced". The onset of a relative advance of the Americas over the Pacific Ocean floor might well have caused a crumpling of the shelves along that coast with the creation and rise of extensive batholiths. Gilluly has pointed this out and concluded that the likely cause was that "it is probable that the continent as a whole is moving away from a widening Atlantic".

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BOOK REVIEWS

RADIOACTIVE FALL-OUT

Radioactive Fallout from Nuclear Weapons Tests
Edited by Alfred W. Klement, jun. (Proceedings of the Second Conference, Germantown, Maryland, November 3–6, 1964. AEC Symposium Series 5.) Pp. ix + 953. (Oak Ridge, Tennessee: U.S. Atomic Energy Commission, 1965. Available from Clearinghouse for Federal Scientific and Technical Information, National Bureau of Standards, U.S. Department of Commerce, Springfield, Virginia.) 6.50 dollars.

THE second fall-out conference at Germantown was convened by the U.S. Atomic Energy Commission in November 1964. The size of the programme of about sixty papers presented by research groups sponsored by the Atomic Energy Commission indicates the magnitude of the sponsor's interest and investment in the subject.

The conference took place 2 years after the end of the second phase of massive nuclear testing during 1961–62 and was aimed at "elucidating fallout mechanisms from the source of nuclear debris to its contact with man". The scope was therefore large, ranging from the fractionation of fission products and the formation of particles in the explosion, through atmospheric transport over short and long distances, atmospheric storage and transfer, observation of the concentrations of radioactive debris at altitude and near the surface, to deposition on the ground and incorporation in foodstuff and eventually in man.

The introductory paper by E. C. Freiling on "Nuclear Debris Formation" ascribed the theoretical distribution in the case of a land-surface explosion of the refractory nuclides such as zirconium-95 largely to local deposition, and the more volatile mass-chains, represented by caesium-137 and strontium-90, mainly to world-wide fall-out. Qualitatively, agreement with observation was good despite differences in the particle-size distributions. Papers by P. Loysen and P. J. Drevinsky dealt with characteristics of radioactive particles collected at altitudes up to 30 km. Most of the radioactivity is carried on particles of less than $\sim 0{\cdot}1\mu$ diameter with a peak concentration between 15 and 21 km. This is consistent with the altitude of the maximum (number) concentration of natural aerosol observed by Junge, although the size is at the lower end of the natural range. Such particles of nuclear debris from a stratospheric source become associated with relatively large non-active aerosols in the troposphere. This is clearly shown by the results of L. B. Lockhart, who reported a diameter range near ground level between 0·3 and 1μ for an assumed density of 1·8 g/cm^3.

H. W. Feely presented the latest analysis of the stratospheric distribution of strontium-90 based on sampling by aircraft and balloon. He estimated that by January 1964 the stratospheric burden had fallen to 4 Mc. of strontium-90 (from a total production of 19 Mc.). The vertical distribution of strontium-90 and other tracers suggested that little debris had stabilized above 21 km in the polar stratosphere although a movement from the northern to the southern hemisphere had occurred, preferentially in the southern autumn and winter. The paper of L. Machta took a similar global view and summarized the fate of debris injected into the troposphere and stratosphere, the global inventory of strontium-90 and carbon-14 and the partition between the stratosphere, troposphere and Earth's surface. He also discussed the concept of atmospheric residence time—in the simplest treatment apparently variable with time—the validity of an exponential removal rate and the need for theoretical meteorological models to aid prediction. R. E. Newell reviewed the temperature and wind structure of the atmosphere by investigating the interaction between radiative and dynamic processes. It appears that both the mesosphere and lower stratosphere are driven by energy received from the regions immediately below, and that mass transfer in the lower stratosphere is primarily accomplished by large-scale eddy processes, although forced mean circulations arise from these processes.

The fate of cadmium-109 as a specific tracer is described in papers by M. I. Kalkstein and L. P. Salter. There is evidence that this material injected at very high altitude near the equator spread to both hemispheres, as did a portion of the fission products from the more energetic polar injections.

At a lower altitude, E. F. Danielsen developed his concept of "tropopause folding" as a mechanism of exchange between stratosphere and troposphere by showing that layers could form in the troposphere having radioactivity and potential vorticity characteristic of the stratosphere. In the same region of the atmosphere, J. D. Mahlman established a relationship between cyclogenesis at the tropopause and short-period fall-out peaks, although not with the broad spring maxima.

Since 1957, the United States has established an extensive air sampling network roughly along the eightieth meridian. The results of this work were presented by H. L. Volchok, who confirmed that debris was well and rapidly mixed but remained within the northern hemisphere. This implies an effective equatorial barrier to interhemispheric exchange in the lower atmosphere and is contrary to the situation noted for high-altitude injections. The behaviour of the hemispheres was thought to be symmetrical after allowing for a four-month difference in phase, although Danielsen, in discussion, proposed that asymmetry in the lag and in the seasonal variation could be attributed to asymmetry in the land masses of the two hemispheres. This hypothesis may be tested by French nuclear tests in the southern hemisphere, perhaps during 1966. On a much smaller scale, several papers dealt with the cleansing of the atmosphere by rain using intensive rain sampling networks.

The final section of the conference report is concerned with the distribution of radionuclides in food and retention in man. Various empirical formulae were derived to predict fall-out deposition from the concentration in air and also to determine the content in milk. The paper by J. Rivera contained estimates, for residents of New York City, of the strontium-90/calcium ratio in diet and in human bone. The derived bone dose already received from nuclear tests was seen to be very low compared with that delivered from natural radioactivity.

No compilation of conference papers can hope to give a connected and consecutive account of the chosen subject: nevertheless, the Germantown proceedings present to the informed reader a survey of the field. One would have welcomed a discussion of the mechanism of dry fall-out, and a short but authoritative analysis of the hazard to the world's population.

The aims of research into fall-out are really twofold. On the one hand, the objective is to determine the effect on human health and life, and on the other to utilize the nuclear debris as tracers of atmospheric movement. There is probably sufficient information from experimental

observation to assess the effect of fall-out on human life, not absolutely, but in comparison with the effects of natural radiation. On the meteorological side, sampling and measurement have established or confirmed the existence of interesting phenomena such as the seasonal and latitudinal variations of fall-out and provided a measure of the exchange between the hemispheres, but there have as yet been no decisive links with meteorological theory. The *Proceedings of the Second Germantown Conference* reflect the early stages of development of this branch of science by defining, more or less, the problems but not the solutions. D. H. PEIRSON

SYNTHESIZING PEPTIDES

The Peptides

By Eberhard Schroder and Klaus Lübke. Translated by Erhard Gross. Vol. 1: Methods of Peptide Synthesis. Pp. xxix+481. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 160*s*.

Peptides

Edited by L. Zervas. (Proceedings of the Sixth European Symposium, Athens, September 1963.) Pp. xvi+386. (London and New York: Pergamon Press, Ltd., 1966.) 105*s*. net; $17.50.

THE modern era in peptide synthesis began in 1932, when Bergmann and Zervas introduced the benzyloxycarbonyl ('carbobenzoxy') group for the protection of amino-groups in such syntheses. The subsequent years have seen a remarkable growth of interest in the field and of methods available for the synthesis of peptides. Increased interest in the field has resulted mainly from the discovery of numerous biologically highly active peptide hormones and the desire to synthesize not only the hormones themselves, but also analogues in the hope of finding more active substitutes for, or antagonists to, the natural hormones which might be of value in medicine. Major landmarks in the progress of peptide synthesis during this period include Harington's synthesis of glutathione (1935), du Vigneaud's synthesis of oxytocin (1953) and Schwyzer's synthesis of ACTH (1963). The even more recent syntheses, by groups of workers in China, Germany and the United States, of the two peptide chains of insulin and their oxidative combination to give insulin, albeit only in poor yield, perhaps open a new era which may well see the total synthesis of true proteins, with all that this implies for biochemical knowledge.

To the uninitiated, peptide chemistry may sometimes seem to involve the expenditure of a quite disproportionate amount of effort to achieve a very limited chemical goal, namely, the condensation of an amino-group with a carboxyl group to give a substituted amide. Inspection of the present two books should, however, convince such critics that the real position is quite otherwise, mainly owing to the presence in amino-acid side-chains of highly reactive functional groups and the large number of steps necessarily involved in the synthesis of even a relatively small peptide. The effort that has gone into the improvement of peptide syntheses in recent years has led to remarkable advances, and many peptides which it is now possible to synthesize comparatively easily would have presented almost insuperable difficulties a few years ago. The most noteworthy landmarks in this technical advance include the introduction of the *t*-butoxycarbonyl group for amino-group protection, of the *t*-butyl ester group for carboxyl protection, and of the mixed anhydride, carbodi-imide and *p*-nitrophenyl ester coupling methods.

There are signs of some slackening of pace in the development of new methods and the present time is very opportune for the appearance of an authoritative and comprehensive review of methods of peptide synthesis. The first volume of *The Peptides*, by Schröder and Lübke, is just such a review and is clearly destined to become the standard work on the subject. The authors are well known, active workers in the field and their mastery of, and feeling for, the subject are evident on every page of their book.

The first three chapters of the volume, which deal generally with amino- and carboxyl-group protection and with coupling methods, are perhaps the most valuable; the very many available methods are fully and critically discussed and there are clear indications which will be particularly useful to newcomers to the field of those methods which are of most general use. At the end of the second chapter there is a useful table showing the behaviour of the principal protecting groups towards a graded series of reagents. Chapter 4 is concerned with the application of these general methods to the synthesis of peptides of individual amino-acids, particularly those containing reactive side-chain functional groups; this chapter contains valuable tables listing the known synthetically useful derivatives of the more important trifunctional amino-acids. The volume ends with six short chapters on specialized topics, a very comprehensive list of literature references (well over 3,000) and good author and subject indexes. The literature coverage is claimed to be complete until nearly the end of 1964; in the main, this claim is justified although there are some surprising omissions. The translation is more than adequate and traces of the original German can only be detected here and there.

This book has been in almost daily use in my laboratory for the past six months. It has proved invaluable as a reference book, as a bibliographical source and as an aid in planning peptide syntheses and is now regarded as indispensable. The appearance of the second volume, which will deal with biologically active peptides, is awaited with great expectations. Every peptide chemist should have his own copy of this book and there should be one in every good chemical library. Unfortunately, the rather high price seems likely to restrict the book's circulation; perhaps, when a second edition is required, the price might be reduced by abandoning the policy of printing the full bibliography in both volumes.

Peptides, edited by Prof. Zervas, is in no sense a rival to, or substitute for Schröder and Lübke's book. It is more correctly described by its sub-title and contains most of the forty-six papers read at the Sixth European Symposium, held in Athens in September 1963, together with much of the discussion. Noteworthy omissions are the papers by Sieber and Schwyzer on the total synthesis of ACTH and that by Boissonnas on structure–activity relationships in analogues of the post-pituitary hormones, both of which have been published elsewhere. The majority of the papers deal with peptide synthesis although there are a few on the degradation and physical properties of peptides. The first European Peptide Symposium was held in Prague in September 1958 and the seventh is being held in Holland this September. Many new advances in peptide chemistry have been first announced at these meetings and there is no doubt that the free discussion and interchange of ideas at these meetings of a relatively small group of active workers have greatly helped progress in the field. However, the advance information given at these meetings ought not to be restricted to participants but should be made as widely available as possible. This calls for rapid publication and it is a great pity that no less than two and a half years have elapsed between the Athens meeting and the publication of these *Proceedings*, especially since the proceedings of the seventh Symposium held in Budapest in September 1964, were published (*Acta Chim. Acad. Sci. Hung.*, 44, No. 1–2) in August 1965. A real effort should be made to publish symposium proceedings more rapidly and in some less elaborate form, such as offset photolithography. It is probable that the

majority of the papers presented at the Athens meeting and collected in this volume have now appeared in full elsewhere; for this reason these *Proceedings* will be of much less general value than they might have been and may, indeed, only appeal to those who already possess the proceedings of the other meetings and wish to make their collections complete. H. N. RYDON

NEMATODA *et al.*

Traité de Zoologie

Anatomie, Systématique, Biologie. Publié sous la direction de Pierre-P. Grassé. Tome 4 (Deuxième Fascicule): Némathelminthes (Nématodes). Pp. 1–732. Tome 4 (Troisième Fascicule): Némethelminthes (Nématodes, Gordiacés), Rotifères, Gastrotriches, Kinorhynques. Pp. 733–1498. (Paris: Masson et Cie., 1965.) Br. 320 francs; Cart. 344 francs deux volumes.

Traité de Zoologie has already earned distinction and represents the conjoint efforts of many distinguished specialists. The first part of Volume 4 dealt with the Platyhelminthes, Mesozoa and Nemertini and the second and third parts extend the treatment of helminths. About five-sixths of these two large books is concerned with the Nematoda, one-sixth with the Gordiacea, Rotifera, Gastrotricha and Kinorhyncha, the rotifers taking up most of this remainder. The first half of the section on the Nematoda is devoted to structure, development, reproduction, ecology, host-parasite relationships and adaptations to the parasitic habit, the other half to systematics. Under the heading of "External Morphology" there is a wealth of information concerning form, size, colour, symmetry relations, cephalization, modifications of the two ends of the body, and the organization and arrangements of amphids and phasmids, the cuticle, spines, scales, tubercles, alae, bursae and suckers. The finer structure and composition of the cuticle come under the heading "Internal Morphology", along with the consideration of glands, musculature, the parenteric cavity, sense organs and nervous tissue, and accounts of the arrangements within the digestive, nervous, excretory and reproductive systems.

Development and reproduction next come under consideration. Embryonic development shows some homogeneity, notably in respect of rigorous cell lineage, and the first difference between species of nematodes concerns the form of the blastula and various modes of gastrulation. Segmentation and gastrulation are treated in some detail for *Parascaris equorum*, likewise the organization of the ovum and details of the first three divisions. The possibility of regulation in nematode development is considered, and various theories concerning the structure and development of the egg are discussed. Early stages of organogenesis are little known and are therefore treated briefly, but the influence of external factors is noted. The processes of growth, moulting and encystment are specially considered and information on organogenesis is supplemented by a discussion of the phylogenetic implications of this stage of development. Other matters which are treated and discussed include gametogenesis, fecundation, the structure of sperms and egg membranes, sexuality and intersexuality, the proportions of the sexes, hermaphroditism and pseudogamous species. Topics such as the epigenetic determination of sex, alternation of generations, polymorphism of heterogenic species and modes of reproduction are also discussed, as are the transmission of various characters and speciation in the Nematoda.

A short section on the ecology of free-living nematodes includes useful details of techniques for their investigation. This is followed by separate accounts of the parasites of invertebrates and vertebrates, and also plant-parasitic nematodes. Parasitologists will be specially interested at this point and in what follows, when the physiological peculiarities of nematodes are discussed in terms of their action on the hosts and considered as examples of Brumpt's five categories of action—spoliatory, toxic, traumatic, mechanical and irritative—although it is properly emphasized that the pathogenic effects of nematodes are usually complex. A brief statement on the principal symptoms of nematode infections of man and animals is followed by discussion of manifestations of immunity in vertebrate hosts. To conclude this part of the work there is a succinct account of the morphological adaptations of nematodes to parasitic modes of life.

The taxonomic scheme which is adopted includes subclasses, infra-classes, orders, sub-orders, super-families and families. The class Nematoda is divided into two sub-classes, Adenophorea (Aphasmidia) and Secernentea (Phasmidia). The two infra-classes of Adenophorea are the Chromadoria, with thirty-one families arranged in five orders (Araeolaimida, Monhysterida, Desmodorida, Chromadorida and Desmoscolecida) and the Enoplia, with about thirty-two families arranged in three orders (Enoplida, Dorylaimida and Enoplides). The sub-class Secernentea has about sixty-seven families arranged in five orders (Rhabditides, Tylenchides, Strongylida, Ascaridida and Spirurida). The entire class of nematodes is thus credited with about one hundred and thirty families arranged in thirteen orders. The great virtue of this scheme is that it is comprehensive, including free-living and plant-parasitic nematodes as well as the more familiar parasites of animals. Until fairly recently, parasitologists have been content with schemes which take only the parasitic nematodes of vertebrates into consideration, and, in 1961, Yamaguti arranged these forms in eleven orders and sixty-two families, although most of them are contained in three or four orders of this French scheme. Students and teachers alike become uneasy when major taxonomic schemes do not agree, and they may be dismayed by the great contrast between these two schemes. To discuss details is out of the question but, to make a point briefly, the order Strongylida (Strongylidea) contains eleven families in each of the two schemes, but six families in the French scheme (Amidostomatidae, Dictyocaulidae, Heligsomatidae, Metastrongylidae, Ollulanidae and Strongylacanthidae) are represented by subfamilies in Yamaguti's scheme, which contains six families (Cloacinidae, Cyathostomidae, Pharyngostrongylidae, Protostrongylidae, Pseudaliidae and Stephanuridae) that do not appear in the rival scheme. The order Spirurida (Spiruridea) contains sixteen families in each of the two schemes but Yamaguti's includes seven families (Ancyracanthidae, Haplonematidae, Rhabdochonidae, Rictulariidae, Sallobrellidae, Seuratidae and Skrjabinuridae) that are not included in the French scheme. The Tetrameridae is given the new name Tropisuridae, the Anguillicolidae and Dracunculidae are placed in the order Philometridea, and four families (Diplotriaenidae, Filariidae, Setariidae and Onchocercidae) are assigned by Yamaguti to the order Filaridea. The order Ascaridida (Ascaridideа) has sixteen families in the French scheme but only four in Yamaguti's, some families being placed in the order Oxyuridea. It is certain that much closer international co-operation will be required before finality is reached in the classification of the Nematoda and other classes of helminths. At present there is no consistency even in respect of word endings chosen to denote various taxons.

However, the high standard of the volumes of *Traité de Zoologie* has been maintained. Misprints are rare but some family names are not shown in the contents and, presumably because of the long period spent in the press, references to the original literature for the 1960's are very sparse. The exquisite line diagrams are notable, although more annotation is required in many of them. Perhaps this is the finest exposition of helminth structure which has yet appeared but more information might have been

given on the fine structure of the cuticle and other parts. Zoologists and parasitologists alike will be delighted by information and ideas here consolidated. Without the complete coverage here afforded any attempts to deal with the affinities of the Nemathelminthes (Aschelminthes) are futile. This topic is treated adequately, various theories are mentioned and the arguments of protagonists of the theory that the nematodes are derived from arthropods are stated fully but reasons are given for their derivation from Dalyellian Turbellaria. It is shown that the various classes considered have such affinities as merit their grouping as Aschelminthes and it is suggested that paedomorphosis (neoteny), which has sometimes been held to account for the relative simplicity of nematode structure, has played an important part in giving origin to the various groups. BEN DAWES

GRAFTS FOR MAN

Histocompatibility Testing

Report of a Conference and Workshop sponsored by the Division of Medical Sciences, National Academy of Sciences, National Research Council, 7–12 June, 1964. (Publication 1229.) Pp. 192. (Washington: National Academy of Sciences–National Research Council, 1965.) $6.00.

The Biology of Tissue Transplantation

By Paul S. Russell and Anthony P. Monaco. (New England Journal of Medicine Medical Progress Series.) Pp. viii+207. (Boston, Mass.: Little, Brown and Company, 1965.) $6.75.

IF the transplantation of tissues and organs between one human being and another is to become a routine surgical procedure, then the graft donor must be chosen so that he has as many antigens as possible in common with the prospective recipient and possesses as few antigens as possible which are absent in the recipient. To make such matching possible, methods must be sought to determine what antigens are present on human cells. *Histocompatibility Testing* is a testimony to the amount of research at present being done in this field and to the ingenuity of the workers concerned. The first part of the report is devoted to the results of the various contributors trying to "type" or antigenically analyse cells *in vivo* and *in vitro*. This section is so well summarized by H. Sherwood Lawrence that an attempt to do so in this review would be superfluous. The second part of the report is devoted to the description and practical details of the tests used to obtain the results presented earlier. Most authors have made a valuable contribution to this section, describing their tests in detail, together with possible pitfalls. There are, however, certain annoying deficiencies which could easily have been avoided. Some authors have sacrificed clarity for brevity; others do not always quote the exact concentrations of reagents; one author almost completely neglects to describe his test at all; yet another describes a standardization procedure which he certainly could not carry out himself (nor, it might be added, could anyone else). However, this book does provide, for those interested in tissue typing, a good progress report.

The Biology of Tissue Transplantation, by two surgeons who practise both applied and experimental tissue transplantation, represents an up-to-date review of the field of transplantation immunology. As such it is of interest both to the surgeon and to the experimental biologist and deserves to be read by both. The authors have presented both the old-established facts and present research trends in a readable form. The sections on the nature of the homograft reaction, the alteration of the immunological reactivity of the graft recipient, and the clinical aspects of transplantation are excellent. On the other hand, transplantation antigens deserved to be treated much more critically. The one general criticism of an otherwise excellent book is that the authors have been a little too prone on occasions to take at face value some of the claims made in the literature quoted. As some 600 references are cited, this is perhaps, to a certain extent, understandable. G. GOWLAND

REMARKABLE AND FEARSOME

Foundations of Anesthesiology

By Albert Faulconer and Thomas E. Keys. Vol. 1: Pp. 1+1–716. Vol. 2: Pp. 1+717–1337. (Springfield, Ill.: Charles C. Thomas, 1965.) $38.50.

ANAESTHESIA is one of the fastest growing fields of modern medicine. This is true not only of its technology, but of the understanding of the fundamental processes which it involves both at the level of the organ and at that of the cell. It is natural that amidst the welter of new knowledge, the origins of the old on which the new is built might be overlooked or even misunderstood. Sometimes the full implication of new observations is not realized through lack of care in reading the original accounts of earlier experiments, and much time and effort may thereby be needlessly wasted. Albert Faulconer, an anaesthesiologist in the Mayo Clinic, has joined forces with Thomas Keys, a librarian and historian particularly interested in anaesthesia, to gather together a large series of papers which they rightly consider to be the foundation stones of modern anaesthetic practice and understanding. Their volumes will both educate and make humble any anaesthetist who reads them and they will provide a long lasting source of information for any physician or biologist interested in this field. They make clear that while modern technology is of great importance in anaesthetic research, essentially intellectual processes of reasoning count for as much, and were particularly important when deductions were made from data which were often obtained with the simplest of equipment.

The 150 papers which are collected in these two volumes cover most of the wide range of the anaesthetic field. The majority would be difficult to find in the original, and many are translated into English for the first time.

Almost any page opened at random produces a gem. What better example than this quotation from Sir Humphry Davy in 1800, "Supposing that no part of the water evolved in solution by the expired gas of common air is formed immediately in respiration, it will follow that a very considerable quantity of oxygene must be constantly *combined* with the red particles, even allowing the consumption of a certain portion of it to form carbonic acid; for the carbonic acid evolved, rarely amounts to more than three fourths of the volume of the oxygene consumed", or this from Flourens in 1847, when ether was being increasingly used, mostly by completely unqualified people who regarded it as an innocuous substance: "When the surgeon contemplates the possibility of the use of ether in an operation, the prime consideration must also be borne in mind: ether which possesses the power to abolish pain can also endanger life. Hence the new agent which has just been added to the resources of surgery is at once remarkable and fearsome." This statement is as true to-day of the wide range of anaesthetic drugs now available as it was then of ether, and is the basic reason why anaesthesia has become such a highly developed specialty within the general field of medicine. Many of the papers in these volumes could be regarded as of historical importance only but others are of quite immediate importance.

These books will provide good reading, intellectual delight, and scientific education for as long as they are possessed, for their contents will never age.

W. W. MUSHIN

BRIDGING THE GAP

Neurohistochemistry

Edited by C. W. M. Adams. Pp. vii+753. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 160*s*.

THE title of this book is slightly misleading as the contents cover a much wider field. Although the greater part has been written by the editor, there are ten other contributors. The work is divided into three sections, in which histochemistry is dealt with as a technique, in relation to the investigation of normal nervous tissue, and in connexion with neuropathology.

The editor's introduction emphasizes the importance of histochemistry in bridging the gap between conventional histology and the biochemistry of homogenized tissues. He appears to regard it as more closely orientated towards histology and thus as an extension of a staining technique. This is far too limited, for as Glenner points out in his chapter: "the potential of histochemistry is great for it offers an unexcelled opportunity to relate cellular and intracellular structure to physiologic function". Furthermore, most pathologists would hope that modern histochemistry might be able to reveal cellular abnormalities underlying "biochemical lesions" in tissues which are known to be functioning abnormally but in which no abnormality of structure can be detected by conventional histological methods.

The opening part of the book is devoted to histochemical methods and is not intended to be exhaustive. It thus gives a very useful introduction to the subject and an adequate bibliography is available for those who wish to delve deeper into the subject. In addition to histochemical methods, techniques for single-cell examination and autoradiography are included and future developments taken care of by a chapter on electron histochemistry by Torack, and all of this is very valuable. The distinction between histochemistry and cytochemistry is made very sharp chiefly because of the different methods of preparation. In the broader search for knowledge this distinction is artificial, for each technique makes its own contribution to the final composite picture of a pathological lesion. On the whole, this histochemical section is satisfactory, though there are a number of minor points which call for comment. For example, it is stated on page 11 that acetylation of Sudan black gives rise to "improved staining" though this is by no means certain; on page 85 Deitch[1] is credited with the introduction of 2-4-dichlorophenol in the Sakaguchi reaction, though this was recommended by McLeish *et al.* in 1957 (ref. 2). On page 88, glycogen is said to be bound to protein (desmoglycogen), yet Meyer and Jeanloz[3] proved that although it may be mechanically included in coagulated protein it is never chemically bound to it. On page 91, it is stated that there is uncertainty about the mechanism of metachromasia, although the physical chemistry of this subject has been worked out by Michaelis several years ago. Glenner deals very fully with enzyme histochemistry, and his discussions on factors affecting enzyme localization and characterization are most timely. Quantitative methods are discussed by Glenner and also by Davidson and Gregson in a later chapter on the cytochemistry of the nervous system. In the first section of the book there is a large amount of useful information on lipids and this embodies much work done by Prof. Adams and his colleagues.

The future of histochemistry is so closely bound up with pathology that the last section is particularly welcome. It is in many ways, perhaps not surprisingly, the most interesting part and the editor is able to convey his enthusiasm for the subject of myelin and the demyelinating diseases. So often in the past histochemistry has been dealt with as a subject on its own and often neglected by pathologists. The chief value of this work will be to encourage the neuropathologist to supplement conventional techniques with modern chemical methods, and the general background of histochemistry in the book will be sufficient to enable him to embark on a new discipline. For the painstaking production of this excellent work Prof. Adams is greatly to be congratulated, and he can feel assured that it will remain the standard work on this subject for many years to come. It may well result in the appearance of similar volumes on other specialized branches of pathology.

The production of the book is of high quality and attractive, and the illustrations of an equally high standard. Errors are few, though one mentions somewhat reluctantly that histochemistry is spelled "hystochemistry" on the page of contents. G. J. CUNNINGHAM

[1] Deitch, A. D., *J. Histochem. Cytochem.*, **9**, 477 (1961).
[2] McLeish, J., Bell, L. G. E., La Cour, L. F., and Chayen, J., *Exp. Cell Res.*, 12, 120 (1957).
[3] Meyer, K. H., and Jeanloz, R. W., *Helv. Chim. Acta*, **26**, 1784 (1943).

Space Travel and Exploration

By Harrie Massey. (A *Contemporary Physics* Reprint.) Pp. iv+117. (London: Taylor and Francis, Ltd., 1966.) 18*s*.; $2.60.

BETWEEN February 1964 and June 1965 Sir Harrie Massey contributed a series of articles on space research and exploration to the journal *Contemporary Physics*. These articles have now been collected and published as a paperback volume, with notes on some more recent developments. The book begins with a swift survey of the main uses of artificial satellites and space vehicles. Then comes discussion of "Ways and Means", covering the velocity requirements for space missions, the types of rocket, methods of tracking, and the design of solar cells. The third section gives some results of scientific studies of the Earth and its upper atmosphere, and the fourth section is concerned chiefly with the Sun's influence over the Earth's atmosphere. Section 5 begins with lunar and planetary probes, and then returns to the mundane applications of satellites in meteorology and communications. The next section has biology as its theme and, appropriately enough, splits neatly into two halves: (*a*) "can there be micro-organisms living in space or on planets?" and (*b*) "can we keep macro-organisms (especially *homo sapiens*) living happily in space?" Finally there are the notes on recent advances, up to late 1965.

No one has a better appreciation of space research in all its aspects than Sir Harrie Massey, and his book provides a clear and well-balanced review of the major developments up to 1965. Much of the detail had to be left out, of course, and readers who wish to pursue particular items in greater detail will find it difficult to trace the original papers, since no references are given. The book list at the end is not really adequate as a substitute and is indeed sometimes slightly capricious in its choices. Apart from this very slight imperfection, however, the book can be strongly recommended as a sound introduction to the present state of space research.

D. G. KING-HELE

Friction and Wear in Machinery

Edited by G. Herrmann. Translated from the Russian. Pp. x+334. (New York: The American Society of Mechanical Engineers: London: Pergamon Press, Ltd., 1960.) n.p.

THIS is a collection of papers on wear, friction and lubrication which describe work carried out at the Institute for the Science of Machines of the Academy of Sciences in Moscow. The papers were presented at a seminar there in 1955–56. The English translation, which is well done under the editorship of Dr. G. Herrmann, was made under the auspices of the American Society of Mechanical Engineers. Some fifteen papers are presented. The

preface to the Russian edition (1958) is written by Prof. M. M. Krushchov and the first four papers deal with his investigations with his colleagues of the wear of non-homogeneous materials including steel and cast iron. It includes the use of replica electron microscopy to examine the damage to the surfaces. The emphasis is on materials which are of engineering and practical importance. The second series of papers is by Prof. I. V. Kragel'skii and his associates on the friction of solids, the effect of surface roughness, relaxation oscillations in elastic friction systems and a useful practical paper on the development of high frictional composite materials which are valuable as brake materials. The rest of the papers deal with fluid lubrication, with the behaviour of dynamically loaded bearings and with the influence of temperature and of viscosity. Some experiments with a four-ball machine for testing lubricants are also described. At the end of the book there is an extensive and useful bibliography of relevant papers published both in Russia and elsewhere during the years 1954–55.

Because of the time which has elapsed since the papers were first written much of the work is necessarily now out of date, but the book can serve as a source of reference and it is interesting to see collected together this account of the able and effective work which the Russian scientists and engineers have been doing in this field.

F. P. BOWDEN

Gas Chromatography of Metal Chelates

By Ross W. Moshier and Robert E. Sievers. (International Series of Monographs in Analytical Chemistry, Vol. 23.) Pp. viii+163. (London and New York: Pergamon Press, Ltd., 1965.) 35*s*. net.

THIS book has been written for the mutual interest of both gas chromatographers and inorganic chemists and should prove of considerable value to both. Liberally supplied with references, it not only constitutes a comprehensive survey of the literature of metal chelate gas chromatography but also indicates several potential avenues for future investigation.

In discussing the general conditions required for successful gas–liquid chromatography of metal complexes, volatility and ease of formation are considered. Among the special complexes singled out are the metal acetylacetonates and their fluorocarbon derivatives. In the former case, their value is diminished by some lack of stability at the column temperatures required for gas–liquid chromatography. The greater volatility of the fluoro-derivatives (the most thoroughly studied of which are the tri- and hexa-fluoro compounds) makes possible separation at lower temperatures, which provides more suitable conditions for quantitative analysis. Thus for hexa-fluoro complexes, which can be eluted at little above room temperature, thermal degradation gives no cause for concern.

As a general indication of viability for gas–liquid chromatography, sublimation behaviour is discussed; for example, while the neodymium chelate with trifluoroacetyl acetone resists chromatography, the hexafluoro complex which can be sublimed slowly at 100° C/0·05 mm presents a more hopeful possibility.

After general considerations there follows a sufficiently comprehensive description of gas chromatographic technique, in the context of the compounds discussed, to initiate those unacquainted with this technique and to remind the esoteric of the special considerations which must be borne in mind when chromatographing these compounds. The relative merits of several applicable detectors is discussed. A chapter on "Analytical Determinations" deals with the practical problems of metal chelate preparation, extraction and chromatography. Useful detailed examples are included together with information on retention times. The authors conclude with a consideration of some of the applications of gas chromatography of the metal chelates, including the resolution of optical and geometrical isomers and its application to kinetic studies. An appendix of metal compounds of β-diketones is also included.

E. A. WALKER

Beiträge zur Biochemie und Physiologie von Naturstoffen

(Festschrift Kurt Mothes zum 65 Geburtstag.) Pp. 581. (Jena: Veb Gustav Fischer Verlag, 1965.) 105 MDN.; 179*s*. 2*d*.

THIS volume celebrates the sixty-fifth birthday anniversary of Kurt Mothes. It includes an introduction by Prof. A. Butenandt, followed by fifty articles on subjects concerned mostly with plant biochemistry, and particularly with the biosynthesis of plant constituents, including alkaloids. Fifteen of these articles are in English, one is in French, and the rest in German.

An article by Sir Robert Robinson entitled "Retrospect" surveys some of the author's many contributions to ideas and experiment in the field of plant biosynthesis, including the celebrated *in vitro* synthesis of tropinone. Prof. M. Florkin, of Liège, under the title "Paleoproteins", surveys briefly the evidence for the presence and nature of proteins in fossils, while the veteran Prof. K. Freudenberg writes about the biogenesis of lignins in a most interesting article.

The collection of essays in this volume emphasizes how successful in many instances has been the purely chemical approach to the problem of biosynthesis in plants. Although any generalization is dangerous, animal tissues, with their greater complexity and in general more rapidly effective synthetic processes, are firmly dependent on enzyme-catalysed and directed processes in a manner that does not hold so completely for plant tissues. Be that as it may, the subject of phytogenesis is one the lines of which were foreshadowed and elucidated by the methods of organic chemistry in a way that has not in general held for zoogenesis. And the contributions of organic chemistry to phytogenesis are widely and interestingly surveyed in this book.

F. G. YOUNG

The Thread of Life

An Introduction to Molecular Biology. By John C. Kendrew. (Based on the series of B.B.C. Television Lectures.) Pp. 110+52 photographs. (London: G. Bell and Sons, Ltd., 196€.) 21*s*. net.

THIS book sets out to tell the story of the "revolution in biology" represented by our new understanding of the relationship between molecular structure and biological function—in short, molecular biology. In the informal personal style preferred by television audiences (or, anyway, by producers) Dr. Kendrew outlines the way proteins are constructed, moves on to nucleic acids and the idea of coding, and ends with viruses. The last chapter speculates on how genes (operons) are switched on and off.

Needless to say, no one is better qualified for the task. The only doubts which rise concern the adaptation from a visual to a written medium. Though Dr. Kendrew writes "You can easily see . . .", the fifty-two photographs—excellent in themselves—are all placed at the end of the book. Since they are an essential part of the explanation, they should have been married with the text. Moreover, the density of information is pretty high: the whole story in 30,000 words. On paper, one might relax the pace a bit. The lectures were given early in 1964: so, though footnotes have been added to cover Holley's structure for RNA and Phillips's work on lysozyme, the publishing delay has made the story not fully up to date. In short, an opportunity to produce something exceptional missed through lack of publishing enterprise. Even so, Dr. Kendrew's lucid summary will be welcome to any layman who seriously wants to try to grasp this fascinating subject.

G. RATTRAY TAYLOR

MEETINGS

NEW STRUCTURAL METHODS

OXFORD—1966

A SYMPOSIUM on "Newer Physical Methods in Structural Chemistry" was held during July 18–21 in New College, Oxford. The meeting was somewhat out of the ordinary in that it was organized not by a scientific society but by the journal *Laboratory Practice*, and running alongside the conference a small and most useful exhibition of equipment relevant to the main themes had been arranged. The experiment was successful, and it is tempting to think that it would be welcome in other areas.

The meeting provided an opportunity, taken by more than 120 delegates, to catch up on and discuss recent developments in two areas—first, mass spectrometry and, then, optical rotatory dispersion and circular dichroism. These topics had been suggested by the president, Sir Robert Robinson, and he, together with Prof. Carl Djerassi and Prof. W. Klyne, had been largely responsible for indicating the direction that the symposium should take.

The thirty-one papers presented divided somewhat in favour of mass spectrometry (by 19 to 12). To some degree this division of interest was real, but Prof. Djerassi, who gave the first paper, has been a pioneer in both fields. He dealt with the migration of alkyl groups and oxygen functions in fragmentation processes. It was apparent in this, as in other papers, that the mapping of a fragmentation pattern requires extensive studies (accurate fragment masses, metastable peak observations, isotopic labelling) if a reasonably certain interpretation is to emerge. In this area some particularly elegant experiments involving labelling by both deuterium and carbon-13 were described by Dr. B. J. Millard (Liverpool). Dr. D. Williams (Cambridge) described many other examples of rearrangements, and the story was taken up by Prof. M. Fetizon (Paris) and Prof. A. Maccoll (University College, London), who discussed respectively the relation of fragmentation pattern to stereochemistry and the behaviour of ureas and thioureas on electron impact.

Computer techniques are popping up practically everywhere, and it was no surprise that when attention turned to rotatory dispersion, two contributors discussed the application of such techniques in the interpretation of optical rotatory dispersion curves of polypeptides and proteins. The Cotton effects of aromatic compounds are also attracting a good deal of attention. Prof. Klyne and Mr. R. J. Swan (Westfield College, London) presented some new results in this area and Dr. K. Kotera (Osaka) put forward a rule of the octant type to correlate his observations on a series of lycorine alkaloids and related compounds. But then Dr. G. Snatzke (Bonn) presented a different treatment, and it was evident that something of a controversy is in the making. In the open forum which followed, Prof. Djerassi emphasized the difficulties involved: since guesswork has a fifty per cent chance of predicting the sign of the Cotton effect of a given compound, an especially extensive range of examples is required before a "rule" can graduate to the status of a rule.

One of the most elegant papers in the second section was that given by Prof. S. F. Mason (East Anglia) on the non-empirical determination of absolute configuration using circular dichroism. Aromatic chromophores were again concerned, and the treatment based on coupled oscillators which has already been published for calycanthine was extended to several related compounds, to Tröger's base, and to diphenyl systems of the aporphine alkaloid series.

The conference was set at a cracking pace and although the open forum was a valuable session, longer breaks for discussion between papers—when points at issue were still fresh in the mind—would have been welcome.

R. BONNETT

MICROCIRCULATION METHODS

IN connexion with the fourth European Conference on Microcirculation recently held in Cambridge (June 26–July 2) a four-day symposium on methods suitable for use in the study of the microcirculation of the blood was organized by Prof. H. Wayland of the California Institute of Technology. The number of participants was restricted to twenty-seven so that workers with scientific, medical and engineering interests were brought closely into contact during a series of meetings and short specialized lectures.

During the past thirty years a great many disciplines have become involved in the study of the microcirculation but knowledge of the processes of flow and exchange which occur is fragmentary and largely qualitative. Most of the effort at the meetings was concentrated on techniques for measuring four microcirculatory parameters—microvascular geometry, exchange across the capillary wall, intracapillary pressure, and the velocity of blood and its components. The choice of ancillary equipment and methods of data processing was also discussed.

There was agreement at the meeting on the desirability of knowing the total geometry of the vascular pattern in each tissue and organ, the fluctuation with time of the distribution of blood, and the variations under physiological and specific pathological conditions. The quantitative description of the vascular pattern should include the number, length and diameter of each type of blood vessel and, if possible, the thickness and composition of the walls, the branching angles, the distance between branches and the presence or absence of specific peripheral networks. Data on the endothelial surface area for each type of blood vessel, the volume density of capillaries and the maximum diffusion distances were also required. Such information, which is not at present available, would permit better and more detailed correlation of structure with function and the construction of vascular models for analysis of variables. Dr. C. Piovella (Pavia, Italy), Prof. E. H. Bloch (Cleveland, U.S.A.), Dr. H. J. Berman (Boston, U.S.A.), Dr. W. Bolt (Cologne, Germany) and Dr. S. S. Sobin (Los Angeles, U.S.A.) divided the methods available for such studies into three categories; first come the '*in vivo*' techniques of angiography, microscopic visualization of the blood vessel at the surface of tissues and organs, and the introduction of optical fibres into the depths of a living tissue. The second category includes perfusion after death with a dye or a fluid medium that hardens into a cast, and the third the immediate capture of the living state for subsequent analysis by rapidly freezing the tissue or injecting silicone elastomers into the living preparation. In all cases, much patient and often tedious routine measurement will be needed in order to provide the required data. Two simple but potentially useful suggestions were, first, that investigators who work with a particular organ, tissue or membrane preparation should describe its vascular pattern in a quantitative and reproducible manner and, secondly, that a reference marker of a stated length (preferably placed initially in the optical path) should be incorporated into all figures and illustrations of the microvascular system.

Four methods of determining the exchange across the capillary wall were introduced by Prof. C. Weiderhielm (Seattle, U.S.A.), Prof. B. W. Zweifach (New York, U.S.A.), Prof. S. Witte (Erlangen, Germany), and Dr. G. Freytag (Hamburg, Germany). They are the video scanning of dye movement, the Landis technique for calculating fluid exchange, the visualization of the movement of plasma constituents by tagging with fluorescent dyes and the use of tritium-labelled material in autoradiographic studies. In summing up, Prof. A. Silberberg (Rehovoth, Israel) pointed out that deficiencies common to the first three are that data have so far been obtained solely on the circulation in the mesentery, and that no allowance is made in them for lymph drainage which undoubtedly is a significant factor in protein and water movement. The autoradiographic technique would appear to be most suitable for the analysis of the structural elements involved in the exchange process but a major disadvantage in the present state of development is its inability to yield quantitative information. It was emphasized that there is a strong possibility that even in a given tissue the phenomenon of exchange may not be uniform either in time or in different parts of the bed so that the problem of sampling is a major consideration.

The desire to measure the hydraulic forces existing in the microcirculation results primarily from the study of fluid exchange across vessel walls and the need to quantify flow in small vessels. Methods presently in use were discussed by Dr. J. W. Irwin (Boston, U.S.A.), Dr. M. Intaglietta (Los Angeles, U.S.A.), Prof. J. F. Merlen (Lille, France) and Dr. A. M. Benis (Cambridge). They can be divided into two types depending on whether or not the vessel wall is penetrated. In the non-puncture type the measurement is made by deforming part of the vessel wall and relating the force necessary for a given deformation to the transmural pressure existing in the vessel. An inflatable diaphragm applied against the vessel can also be used, when the pressure needed to stop flow in a given vessel is related to the internal pressure. Both methods are subject to uncertainty because tissue and vessel wall resistance may influence the values obtained and pneumatic techniques for compressing the nail bed, followed by stepwise pressure reduction and observation of the resulting flow changes, were considered worthy of further development. Other non-puncture methods which were suggested included the analysis of red cell movement during the occlusion of the capillary with a fine probe and the injection into the vessel either of elastic microspheres (when changes in shape could be followed) or of filled red cell ghosts acting as osmometers with pressure-sensitive optical properties. For pressure measurements requiring the vessel wall to be penetrated by a hollow probe, two types of gauge are at present in use. In the active gauge, the pressure in the probe reservoir is continually adjusted by a servo system to balance the pressure in the vessel. Tip diameters range from 1 to 5μ and because the method requires practically no flow through the probe a response can be obtained very rapidly. The passive system must be designed to operate from the energy obtainable from the fluid inside the vessel. The problem is the conversion of the fluid power to an electrical signal through the choice of a proper transducer system. It was felt that the absolute lower limit of the tip diameter was about 5μ although a lower size might be used if only average pressure values were needed. The passive gauge should be of most value in vessels larger than 30μ in diameter; the active system extends measurements to the 8–10μ level. One serious limitation to all the methods is that they can be used only for vessels near the surface of tissue.

At normal haematocrits it is very difficult to resolve individual cells at the centre of mammalian vessels with diameters greater than about 40μ so that the main attention is at present directed towards the measurement of red cell velocity in capillaries. The main reasons for making the measurement are that it can be used to calculate oxygen transport and also to deduce the velocity profile of the blood, thus providing a measure of the total volume flow through the vessel. In the limiting case where single cells completely fill the vessel lumen the red cell velocity probably gives a true measure of the total volume flow and in principle may also yield an average of the haematocrit of the blood traversing that vessel. A variety of techniques was introduced by Prof. P. C. Johnson (Pasadena, U.S.A.), Dr. P. A. G. Monro (Cambridge), Dr. M. Steinhausen (Heidelberg, Germany), Dr. H. Meiselman (Pasadena, U.S.A.), Dr. A. A. Palmer (Sydney, Australia) and Dr. H. W. Thomas (Reading). They included streak photographs, analysis of ciné film sequences, analysis of the frequency spectrum of the variation of light intensity as cells cross a slit, and flying spot techniques. Methods for measuring whole blood velocity such as thermal dilution and the installation of electromagnetic flow meters were considered but there would obviously be severe practical difficulties in adapting them to the small vessels of the microcirculation.

The large amount of information which correctly designed measuring heads can produce calls for proper methods of data handling. These, it was felt, should be part of the experiment as a whole and improvements in measuring techniques will demand more complex methods of analysis of the data, involving analogue or digital computers or both. In summarizing the discussions, Prof. M. G. Taylor (Sydney, Australia) and Dr. R. L. Whitmore (Nottingham) suggested that the former would seem to be the method of choice for relatively simple procedures where a machine of modest size would suffice and permit the data to be analysed quickly under the direct control of the experimenter. It was also felt that an analogue computer might provide an easier introduction to data processing methods than the immediate use of a digital machine. On the other hand, when large amounts of data required editing and adjustment before analysis, or the number of variables was beyond the capacity of an analogue computer, or complex statistical methods were to be applied, the digital computer was the correct choice. It was generally agreed that as much experimental data as possible should be recorded on magnetic tape but that the amount of analysis which should be carried out "on line" must inevitably depend on the type of experiment. Small digital computers of the *LINC* type have been successfully used in neurophysiological experiments and their use in microcirculation work should be explored.

The needs of the clinician in requiring more detailed and exact information on conditions in the microcirculation were not forgotten, and Dr. E. Davis (Jerusalem, Israel), Drs. S. and S. C. Bunnag (Bangkok, Thailand) and Dr. W. G. Frasher, jun. (Los Angeles, U.S.A.), took active parts in bringing these requirements to the attention of the meeting. It was felt that the ingenuity and resources of scientists and engineers should be exploited by the development of appropriate instruments for routine clinical use. This, however, is scarcely possible until more is known of the variables which are of clinical importance in the microcirculation.

There is no doubt that we are about to see the large-scale application of quantified measuring techniques to the study of the microcirculation. This will call not only for improvements in existing research methods but for the development or invention of equipment which can be satisfactorily used in the tiny vessels of the microcirculation. Automated methods of data recording and analysis will also need adapting to the same ends. The symposium was particularly valuable in pointing out the probable directions of progress in microcirculation research over the next few years, and Prof. Wayland deserves the thanks of all who were fortunate enough to be present, for his initiative in organizing the meeting.

R. L. WHITMORE

EVIDENCE FOR A TWO-WAY SIDEREAL ANISOTROPY IN THE CHARGED PRIMARY COSMIC RADIATION

By R. M. JACKLYN

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FOR several years now the sidereal daily variation of intensity of cosmic ray mesons at the Hobart underground laboratory has been closely investigated[1,2]. In common with earlier investigations of this kind the objective has been to obtain decisive evidence either for or against a maximum of intensity among the charged parent primary particles from some fixed direction in space. The most important observational problem has been the possibility that the annual observed daily variation of meson intensity in sidereal time may contain a large spurious component, due to seasonal modulation of the solar daily variation of intensity. Therefore it will be necessary to outline the problem here briefly and show how peculiarly relevant it is to the observations. However, it is because of the distinctive nature of the observations and of the spurious effect that might be expected that it has been possible to design an experiment which makes it possible to decide whether the observed sidereal daily variation is in the main genuine or spurious. The first results of this experiment are presented in this article and confirm the earlier indications that there is a genuine two-way sidereal anisotropy among the charged cosmic ray primaries.

Although the charged primaries in the energy range 10^{10}–10^{12} eV are thought to be on the average very nearly isotropic in the vicinity of the Earth's orbit, a solar anisotropy has been detected[3], the intensity maximum as observed from the Earth having a fixed direction relative to the Earth–Sun line. It produces an observed solar daily variation whose average amplitude may be as great as 0·5 per cent. It might seem that if a comparable sidereal anisotropy existed among the charged primaries it could also be detected by arranging cosmic ray data in sidereal time. Such analyses have indicated that if there is a genuine sidereal component in the observed daily variation the amplitude must be less than about 0·1 per cent. Consequently, if a sidereal component exists it must usually be superimposed on a much larger solar component of the daily variation. In the simplest case this observational difficulty is overcome by considering annual averages: a solar component of constant amplitude and phase would be completely averaged out if the data were arranged in sidereal time, say month by month, and summed over a complete year. Unfortunately, the solar component may vary considerably from time to time and in particular may exhibit seasonal modulations of amplitude and phase. To illustrate the serious difficulties of interpretation that follow from this, let us consider a solar diurnal variation on which is impressed an annual sinusoidal variation of amplitude. The unmodulated diurnal wave can be regarded as a carrier, of frequency one cycle per day or 365 cycles per year. Amplitude modulation of one cycle per year produces matching sideband components of frequency 364 cycles per year and 366 cycles per year. The latter is the sidereal frequency, there being 366 sidereal days in a complete year. It follows that if a solar daily variation with impressed annual modulation of amplitude is arranged in sidereal time and averaged over one year, we observe an annual daily variation in sidereal time that is a completely spurious sideband effect. The time of maximum, or phase, of this spurious sidereal daily variation depends on the month of the year when the solar daily variation has maximum amplitude[4,5]. Therefore, on our assumption that maximum amplitude occurs during a particular season of the year, the phase of the spurious annual sidereal effect observed in the northern hemisphere will differ by 12 h from that observed in the southern hemisphere. This 12 h phase shift should hold at least approximately in practice even if the seasonal modulation of the solar component is rather complex. We shall see how important it is to be aware of this problem of the spurious effect when interpreting the observational data, although the material presented here relates in the main to daily variations of intensity underground, where seasonal changes of the solar component that are thought to be due to contributions of atmospheric origin should be considerably smaller than they are above ground.

Late in 1957 two semi-cubical vertical counter telescopes were installed at a depth of 40 m.w.e. (metres water equivalent) underground near Hobart (42·9° S geographic)[6]. Continuous records have been obtained of the intensity of mesons, the mean counting rate being about 70,000 particles per h. (It has been calculated that more than 90 per cent of this counting rate is due to primary protons of energy greater than 50 GeV and that the primary energy of maximum response is about 100 GeV)[7]. The time of maximum of the annual mean daily variation in sidereal time that has been obtained each year from the pressure corrected data has remained within the limits 0500–0700 local sidereal time[2]. Seasonal modulation of the solar component of the daily variation observed at this site appeared to have fallen to a very low level in the later years, so that from this point of view there was reason to believe that the sidereal effect might be genuine. Therefore it was of interest to compare the Hobart underground results with observations from similar equipment at a similar location in the northern hemisphere. Data from two (and later, three) vertical semi-cubical telescopes located at a depth of 40 m.w.e. at Budapest (47·5° N geographic) were obtained from the World Data Centre *WDC* 2 for the complete years 1959 and 1961. Fig. 1 shows that over the period the times of maximum of the apparent sidereal effect at the two places differed by approximately 12 h, whether judged by the first harmonics of best fit to the intensity deviations, or the sum of the harmonics, since the first and second harmonics are in phase. We have seen that this is what one would expect from a spurious sidereal effect generated by seasonal modulation of the solar component—a 12 h phase difference at places in opposite hemispheres. However, it is worth noting at the outset that this conclusion is not in accord with the evidence that the effect was still present when seasonal modulation was low. More than this, two cosmic ray phenomena of a somewhat different character that have been reported in recent years support the view that the sidereal effects shown in Fig. 1 are genuine. It may be of interest to describe them briefly, before proceeding to the more recent experimental results from Hobart.

In an investigation of monthly average daily variations obtained from neutron monitors and counter telescopes in the northern hemisphere during 1954, a year of minimum solar activity, Conforto and Simpson[8] found that over a period of some 6 months, the time of maximum expressed in solar time was becoming progressively earlier at the rate of approximately 2 h each month, in

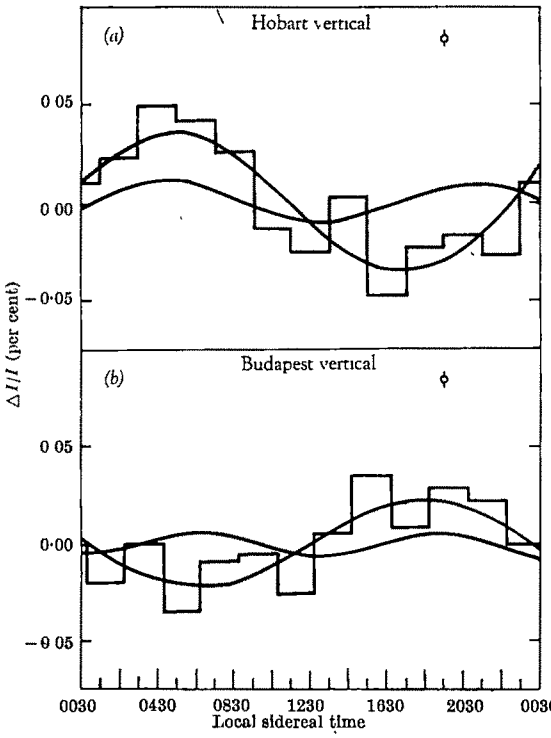


Fig. 1. The observed daily variation in sidereal time, averaged over the years 1959 and 1961, of pressure-corrected meson intensity from vertical semi-cubical telescopes at a depth of approximately 40 m.w.e. (*a*) At Hobart, scanning asymptotic latitudes in the vicinity of 39° S. geographic; (*b*) at Budapest, scanning asymptotic latitudes in the vicinity of 44° N. geographic. The error tails shown are the *S.E.*'s of amplitude associated with the first and second harmonics of best fit

the manner of a genuine sidereal daily variation with a maximum at approximately 2000 local sidereal time; however, it is most important to note that in their terminology this would be 0800 local sidereal time. As Baliga and Thambyahpillai[9] have pointed out, the authors had synchronized solar and sidereal time at the March equinox instead of the September equinox, the customary date, so that 12 h must be added to their published sidereal times of maximum to give the correct values. (This error also appears in a notable summary of the 1954 phase anomaly presented by Dorman[10]). The phenomenon was not detected in the southern hemisphere at that time, although the high counting rate data from the Hobart vertical meson telescope at sea level was carefully examined for the effect by Baliga and Thambyahpillai and by Parsons[11]. However, in 1961 a phase anomaly did occur at the Hobart underground site when the amplitude of the solar daily variation had reached a very low level, the maximum of the monthly mean daily variation in solar time becoming progressively earlier at the rate of 2 h each month, in the manner of a sidereal component with a maximum at 0700 local sidereal time. This time, so far as I am aware, the phenomenon was not detected in the northern hemisphere. In other words, it seems so far to have occurred in only one or the other hemisphere. This point will be reconsidered later. The event at Hobart has been described more fully in earlier publications[12]. Fig. 2 compares it with a typical example of the phase anomaly observed at northern latitudes in 1954, and we note the 12 h phase difference between the respective apparent sidereal maxima.

If, as it seems, the phase anomalies had been produced by genuine sidereal components of the daily variation, there would be no conflict of evidence with the annual apparent sidereal effects observed underground in 1959 and 1961 (Fig. 1) and with the longer term trends which indicated that the effect at the Hobart site was genuine. The inference would be that the direction of maximum intensity of the primaries observable in the northern sky differed from that observable in the southern sky by 180°, that is, a two-way sidereal effect exists. On the other hand, if the argument that the whole effect is spurious were to be sustained, the required seasonally modulated solar component of the daily variation would naturally have to be such as to resemble a genuine sidereal daily variation. To explain the phase anomalies, this would have to be a truly extraordinary seasonal phenomenon—a solar component, of constant amplitude, with a time of maximum which progressed anti-clockwise from noon in midwinter to midnight in midsummer. Nevertheless the evidence so far does not expressly forbid such a possibility.

What is now needed is a simple experimental result exhibiting the 12 h phase shift but explicitly ruling out an explanation based on seasonal changes of the solar daily variation. The first results from such an experiment are reported here.

In October, 1963, a narrow-angle telescope was installed at the Hobart underground site. It consisted of three standard metre-square trays in triple coincidence, the separation between the two extreme trays being approximately 10 ft., giving a total geometric aperture of 36°. The telescope was inclined at an angle of 70° to the north of zenith, so that the latitude of viewing along the axis was 27° N. geographic. Since the telescope responds in the main to primaries of rigidity greater than about 100 GV, deflexions of the trajectories of the primaries in the Earth's magnetic field would be no more than about 5°, so that one could be confident that the asymptotic cone of viewing was located in the northern hemisphere. On the other hand, the asymptotic cones of viewing for the vertical semi-cubes at the underground site are centred at about 39° S. geographic latitude and not more than about 15° E. of the meridian, depending somewhat on the nature of the primary phenomenon to which the telescopes are responding. It is concluded that the vertical and the narrow-angle inclined telescopes view within about 10° of each other in longitude when deflexions of the primary protons in the Earth's magnetic field are taken into account. The reader will now appreciate the purpose of this joint vertical-inclined experiment—to find out whether the apparent sidereal effect obtained from the inclined direction, scanning latitudes in the northern hemisphere, differs by about 12 h from the effect observed with the

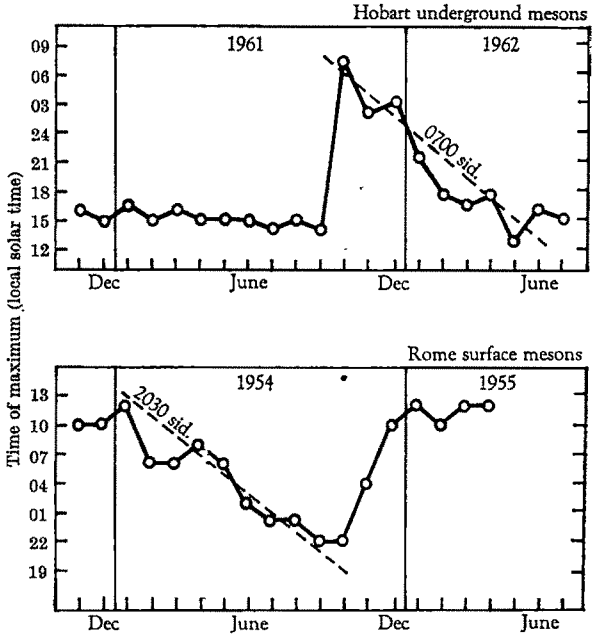


Fig. 2. Monthly averages of the time of maximum, in solar time, of the pressure-corrected daily variation of vertical meson intensity at the Hobart underground site (1961–1962) and at Rome (1954). The dashed lines represent the solar time equivalents of 0700 local sidereal time and 2030 local sidereal time as shown

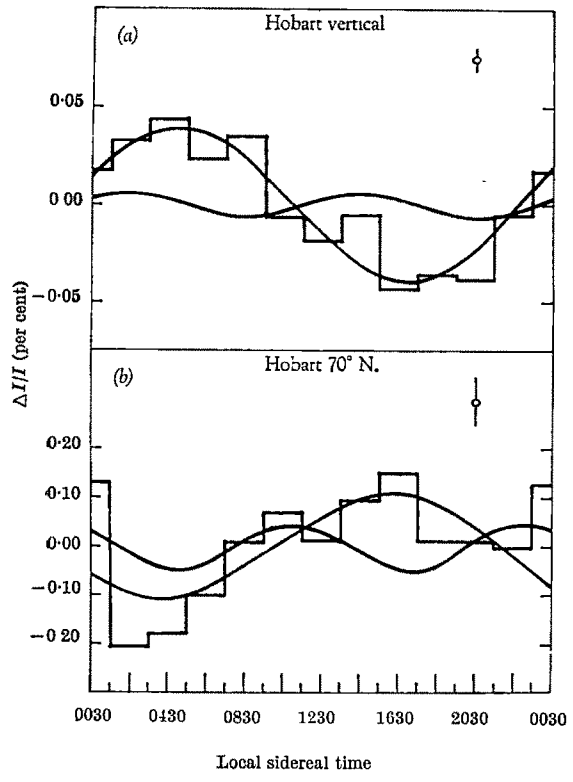


Fig. 3. The observed daily variation in sidereal time, averaged over the year 1964, of pressure-corrected meson intensity at a depth of approximately 40 m.w.e. at Hobart, (*a*) from two vertical semi-cubical telescopes, scanning asymptotic latitudes in the vicinity of 39° S. geographic, (*b*) from a narrow angle telescope inclined 70° to the north of zenith, scanning asymptotic latitudes in the vicinity of 20° N. geographic. The error tails shown are the *S.E.*'s of amplitude, associated with the first and second harmonics of best fit

vertical telescopes, scanning mid-southern latitudes. A 12-h phase difference could not be produced at the one place by any local (that is, atmospheric) process of seasonal modulation, usually thought to be responsible for spurious sidereal effects.

The histograms relating to the first year of operation, 1964, are shown in Fig. 3 with their first and second harmonics of best fit. It is evident that the sidereal daily variation in the vertical direction was much the same in 1964 as it was during 1959 and 1961. In the inclined direction the amplitude of the first harmonic is statistically significant at the 95 per cent level and the time of maximum differs by approximately 12 h from the vertical result. The respective times of maximum are in fact compatible with the Hobart–Budapest findings (Fig. 1) and with the values derived from the phase anomalies (Fig. 2).

We have seen that this latest result, while remaining to be confirmed over several years of observation, was the one to be expected from a sequence which as a whole indicated the existence of a two-way sidereal anisotropy in the primary radiation. The type of anisotropy is depicted schematically in Fig. 4. However, the second objective of the experiment was perhaps the most important one. It now seems scarcely possible that the sidereal effect could be spurious. (Elsewhere we shall also conclude this from considerations of the observed harmonics.)

It is of interest to note that the inward direction of the galactic magnetic field would be expected to pass overhead at about latitude 35° N. at 2000 local sidereal time, while the outward direction (looking towards the periphery of the spiral arm) would pass overhead at about latitude 35° S. at 0800 (ref. 13). These times roughly agree with the observed times of maximum of the sidereal effect. Corrections for deflexions of the primary trajectories in the Earth's magnetic field would improve the agreement. If it should happen that the galactic magnetic field is in fact the seat of adiabatic motions of the charged primaries

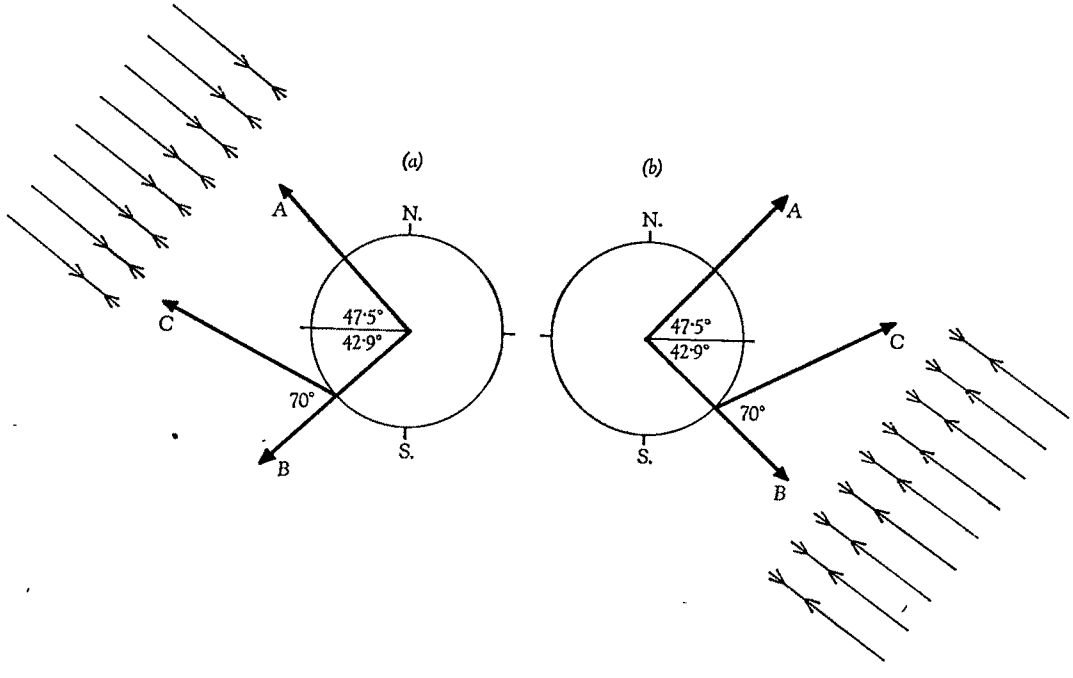


Fig. 4. Schematic diagrams showing the geographic headings of the vertical telescopes underground at Budapest (*A*) and the vertical (*B*) and inclined (*C*) telescopes underground at Hobart, (*a*) at the local sidereal time T h, (*b*) at the local sidereal time $T+12$ h. The parallel lines indicate primary intensity maxima both backwards and forwards along some fixed direction, having declination d, which passes locally overhead at the latitude d in the northern hemisphere at time T and at the same latitude in the southern hemisphere at time $T+12$

in both the backward and forward directions and that these are being detected as intensity maxima at the Earth, then the declination of the direction of the anisotropy should be that of the galactic field in the vicinity of the solar system. An estimate of the declination from cosmic ray intensity measurements will be presented elsewhere. It is based on a simple empirical model for the anisotropy and makes use of observations obtained in other directions in the plane of the meridian at the Hobart underground station.

It should be pointed out that in the model both the first and second harmonics of the sidereal daily variation have physical meaning. It is clear, for example, that at some latitude of viewing between Hobart and Budapest only a semi-diurnal effect should be observed, and that if the primary intensity maxima in opposite directions were equal, the first harmonic would disappear when the asymptotic cone of viewing was centred on the equator.

Finally, it is emphasized that although the first harmonic now being observed underground at Hobart is much larger than at Budapest (Fig. 1), this appears to be only temporary. Long-term trends of the apparent sidereal effect in the ion chamber data from Cheltenham (38·7° N. geographic) and Christchurch (43·5° S. geographic) indicate that the amplitudes were much larger in the northern than in the southern hemisphere during the early 1950's. This could be the main reason why a phase anomaly was detected only in the northern hemisphere in 1954 and only in the southern hemisphere in 1961. As to the changes in the relative amplitudes with time, the following suggestion is offered for what it is worth: when a sidereal effect is viewed through the region of interplanetary solar modulation, asymmetries in the modulating region with respect to the plane of the ecliptic might be responsible for different amplitudes in the northern and southern hemispheres. The amplitude differences would change from time to time as the asymmetry itself changed.

I thank Dr. P. G. Law, Prof. A. L. McAulay and his successor, Prof. G. R. A. Ellis, for their interest and support. I also thank especially Dr. A. G. Fenton, who has been closely associated with the project at all stages, for advice and discussions. I thank the Director of the Central Research Institute of Physics of the Hungarian Academy of Sciences (Cosmic Ray Department) for the underground cosmic ray data from Budapest, made available through the World Data Centre *WDC* 2.

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SYMMETRY OF THE TIME AXIS

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INTERACTIONS are governed by laws of physics that may be conveniently divided into two classes. Laws of the first type, such as those of energy and momentum conservation, must be rigorously obeyed in each interaction. These laws show no preferred direction of time, for reversal of the sign of t wherever it appears in the equations does not alter their validity. In the second class we have laws of a statistical character which dictate the probable outcome of many interactions considered as a whole. In this class we include the second law of thermodynamics and statements concerning the cross-sections for reactions. It is these laws which, while not distinguishing between any direction in three-dimensional space, nevertheless have an asymmetric behaviour with respect to the time axis. Thus, for example, the cross-section for a forward reaction in general differs from that of the reversed process. This is not because of any difference in the strengths of the two interactions, but arises as a result of the density of final states factors. Similarly, entropy is well known to vary in a systematic fashion with time.

Why the otherwise perfect symmetry of the space-time framework should be flawed by a restricted class of laws and in one dimension only has long been a subject of discussion. In this article I shall try a somewhat different approach to the problem. The question asked is: "If the time axis were perfectly symmetric in respect of *all* the laws of Nature, how would the world appear to us?"

The effect of imposing such a symmetry is best illustrated by taking a particular interaction as an example. Let us therefore consider the coupling between the neutron state and the $(p+e^-+\bar{\nu})$ state. The world lines of the particles involved in any such interaction may adopt the general configuration of Fig. 1 or Fig. 2. If the two time directions are indistinguishable there is no reason why either configuration should be favoured. Nevertheless, our observations do indicate a preference for one particular pattern of world lines, say those of Fig. 1, and these we refer to as representing the decay of a neutron, while Fig. 2 shows the creation of a neutron from a triple collision between a proton, electron and anti-neutrino. Once given such an asymmetric pattern of world lines, a direction in time can be defined, and laws formulated which describe the asymmetry in terms of the density of final states factors. Discussions on the symmetry properties of time generally do not seek to deny the existence of such laws and asymmetries, but centre rather on the nature of the contingency that allowed our world to choose between two equally possible sets of world line configurations.

The line of thought we are pursuing here is that no arbitrary choice has been made: both patterns of world lines are equally allowed at all times. This means that in addition to the neutron states with which we are familiar, there exist others such that the transitions from them to the $(p+e^-+\bar{\nu})$ states occur with the greater probability in the opposite direction of time, as in Fig. 2. So that we may distinguish between the two particle states, we introduce the concept of intrinsic time sense. Thus all particles besides carrying basic properties, such as mass, charge, isotopic spin, baryon number, and intrinsic parity, are also to be further sub-divided according to whether they have a positive or negative intrinsic time sense. So far as we know, all matter of which we have experience belongs only to one time sense category. Matter of the opposite time sense we shall designate as "faustian". Interactions between particles in faustian states would be identical in every respect to interactions between our own particles, except that their time asymmetry would be reversed.

Perhaps it should be emphasized that particles of a reversed time sense are not to be confused with particles moving backwards in time. This latter phrase is custom-

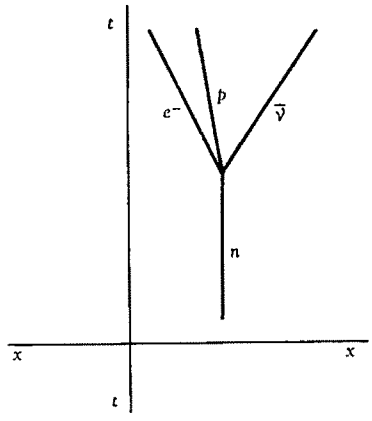


Fig. 1

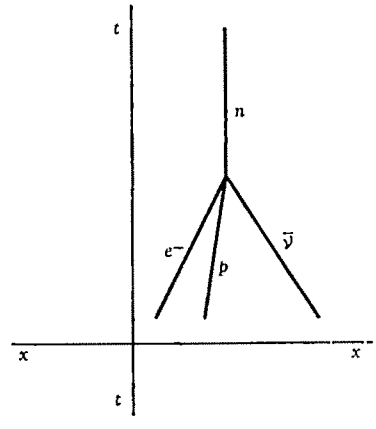


Fig. 2

arily reserved for equivalent anti-particles moving forward in time. As anti-particles obey the same dynamical laws as ordinary particles our proposal is for a further sub-division into particles of opposite time senses.

The question of whether we should be able to observe particles occupying faustian states clearly depends on the type of interaction to be expected between quanta of opposite time senses. There is in fact a strong probability that the two sets of particle states are independent of each other. This can readily be seen by imagining an experiment in which we observe interactions between a beam of our protons and a beam of faustian protons colliding with given energies and at a certain relative angle. There would certainly be some particles that would continue undeviated having failed to interact, and one might expect that the remainder would scatter through various angles with or without the production of other particles. Let us now perform the experiment in reverse. Beginning from a wide variety of states we would clearly find it difficult to produce, as a result of the interaction, only one final state, that is, the state originally regarded as the initial state. (This assumes, of course, that we do not artificially arrange the phases of the beams to cause destructive interference in all states except one.) The interaction as described therefore possesses an asymmetric behaviour with respect to the two directions of time. This is manifestly impossible for it was specified that we were dealing with particles of equal and opposite time sense. The overall time sense of the initial and final states is zero so there can be no way for the interaction to distinguish between the two time directions. There can in fact be no interaction.

In the case of non-corresponding particles, for example a beam of faustian electrons colliding with our protons, symmetry arguments tell us only that the time asymmetry of the interaction (if any) would be of the opposite sense to that of an interaction involving a beam of our electrons and faustian protons. It requires an additional assumption about the quantitative nature of the time sense before we can proceed further. If it is thought reasonable that all particles should have the same magnitude of time sense, in other words the significance is merely one of sign, we may now take as established that there would be no interaction between our matter and faustian matter. We shall discuss this assumption again later.

It would appear then that in a world that was perfectly symmetric with respect to the time axis, matter of opposite time senses would decouple. The faustian universe would be embedded in the same space-time framework, and yet would remain unobservable to us. According to our experience of time, the direction of which is dictated by the intrinsic time sense of the matter from which we are constructed, the faustian world would consist of galaxies absorbing their light rather than emitting it, living organisms growing younger, neutrons being created in triple collisions between protons, electrons and anti-neutrinos, and thereafter being absorbed in nuclei, etc. It would be a universe that was in a state of contraction, and its entropy would be decreasing, and yet the faustian observers would not be aware of anything strange in their environment. Being constructed of faustian matter their subjective experience of time is reversed, so they would be equally convinced that it was they who grew older and their entropy that increased. They would infer that it was our world that showed the unfamiliar behaviour. This rather odd situation, it will be recalled, has a close parallel in the special theory of relativity where two observers in relative motion are equally convinced that it is the other observer's clock that is going slowly.

Thus in a perfectly symmetric universe all observers would be aware of an apparent asymmetry in time, an asymmetry that was merely a measure of their ignorance of the other half of the universe. Perhaps one ought to conclude at this point. Having devised such a universe, however, one is tempted to venture a few speculations.

First we note that there appear to be some striking resemblances between the faustian world introduced here, and the "shadow universe" of Nishijima and Saffouri[1]. The latter was postulated in an attempt to save CP invariance in the long-lived $K^0 \rightarrow 2\pi$ decay experiment[2]. The mechanism invoked was that some K_1^0s became shadow K_1^0s by a CP conserving interaction, and at a later time reappeared to give the observed long-lived 2π decay. One undesirable feature of the theory was that, in order to prevent shadow K_1^0s decaying to two shadow pions, this interaction had to be arbitrarily suppressed. This assumption is not necessary, however, if one is thinking in terms of faustian K_1^0s. We, the observers, must scan the faustian K_1^0 world line according to our time direction, and this takes us away from the end that is coupled to the two-pion state with the greater probability. A faustian K_1^0 created in a time sense flip interaction would remain stable until it once again became one of our K_1^0s. A second difficulty encountered by the shadow universe hypothesis was that there had to be no strong interactions between the two universes so as to account for the non-observation of shadow particles in other experiments. Okun and Pomeranchuk[3] have pointed out that this would mean that shadow K_1^0s produced in the neutrino experiments would have been able to pass through the shielding and decay in copious numbers in the detectors. In fact the number of such decays was not abnormally high. The same criticism, however, cannot be levelled so readily at faustian K_1^0s. As we have seen, there can be a strong interaction between non-corresponding quanta. Furthermore, the existence of such an interaction involving states with particles of both time senses does not require that there should also be interactions linking a state of one time sense only to a state of mixed time sense. There is thus no reason to

expect that in collisions between our particles we should have produced faustian particles.

Recently, experiments have been performed in which the interference between the long-lived K^0 decay and the decay of K_1^0s regenerated by K_2^0 interactions has been studied[4]. A method of testing whether the faustian universe theory has any bearing on the K^0 decay problem is to repeat these investigations with K mesons created as $\bar{K}^0$s. If CP is violated the same results will be obtained as before; if our alternative is correct the sign of the interference will be reversed[5]. The evidence from the interference experiments so far performed is somewhat against our alternative, but it cannot be taken as definitive because it is strongly dependent on an assumption that had to be made regarding the K^0,$\bar{K}^0$ content of the beam used. A second prediction we can make is that the charged to neutral branching ratio of the long-lived $K^0 \rightarrow 2\pi$ decay should have the same value as that of the normal K_1^0 decay. Finally, from the observed ratio of the numbers of long-lived to short-lived K^0s decaying to two pions we calculate the lifetime of the former to be 4×10^{-8} sec, that is, it lies very close to that of the K_2^0.

It might be thought that to invoke a time sense flip for the K_1^0 was unreasonable because the temporary disappearance of other particles from our universe has not been observed. However, it must be noted that a particle experiencing a nuclear or electromagnetic interaction will in general be in a different energy state to that of the corresponding faustian particle situated in the same field. A time sense flip links particle states of equal energy so any significant difference in the energies of the states concerned will lead to a strong suppression of the transition. In effect, the interaction can occur only for free neutral particles. If it is further required that the two lifetimes of the neutral particle should have values lying in a range that makes them accessible to measurement, one is virtually reduced to considering only the K_1^0, Λ^0, and Ξ^0 particles.

If the Λ^0 particle undergoes a time sense flip, the free hyperon will contain both types of particle. Two mixed states will be formed each with its own characteristic lifetime. A bound Λ^0 in a hyperfragment, on the other hand, would be in the nuclear field of the surrounding nucleons and thus be prevented from converting to a faustian Λ^0. It will therefore have the lifetime characteristic of our pure single time-sense state rather than of either of the two mixtures. Because the lifetime of a pure faustian Λ^0 would be infinite if it were prevented from returning to our world, we require the lifetime of the bound Λ^0 to be less than that of either mixture. Dalitz and Rajasekharan[6] have made theoretical studies of the lifetimes of hyperfragments, taking into account the modification to the lifetime of the Λ^0 expected because of the existence of bound states for the decay proton and the effects of the Pauli exclusion principle and the stimulated non-mesonic decay modes. In the case of ${}_{\Lambda}H^3$, which is the system that lends itself most readily to this type of treatment as well as to experimental investigation, the observed lifetime is found to be only about half the theoretical value, and so far no explanation of the discrepancy has been forthcoming. If the experimental data on hyperfragments are used to determine the lifetime of our pure Λ^0, the behaviour of the long-lived free Λ^0 mixture can be predicted. In particular it is noted that if our pure Λ^0 has *exactly* one-half the lifetime of the short-lived free Λ^0, then the two free Λ^0 mixtures have equal lifetimes. For several years now the Λ^0 lifetime measurements[7] have been in disagreement. We remark that this does not necessarily arise from faulty experimentation, but instead may be an indication that the Λ^0 is a particle mixture with two lifetimes, almost but not quite degenerate. Depending on the fiducial criteria used, so experimenters will include varying proportions of long- and short-lived Λ^0s in their samples.

A somewhat similar confusion also exists for the Ξ^0 particle[8]. This, however, could be due to poor statistics and cannot be taken as evidence of a Ξ^0 particle mixture.

Earlier it was pointed out that the decoupling of the two halves of the universe was dependent on the assumption that non-corresponding quanta had no interaction, in the same way as there was no interaction between corresponding quanta. Should this not be the case, the possibility opens up that one might be able to establish contact with faustian cosmic systems. Observations of these systems would pose unusual problems for, as was pointed out earlier according to our time reckoning, they will absorb radiation from their surroundings, not emit it. They will therefore not give rise to images on photographic plates One could, however, systematically scan a region of the heavens with a telescope fitted with a small sensitive thermal device in its focal plane, and see if it suddenly starts radiating energy to a point in space.

Finally, we mention the interesting possibility that if some means exists for the transfer of matter between the faustian universe and ours, a perpetual state of mutual regeneration could be established.

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MODEL OF THE HIGH-ENERGY PHASE OF SOLAR FLARES

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The solar flare is a highly complex phenomenon of considerable importance in solar physics and geophysics[1,2]. Most theoretical attempts at understanding flares have centred on the flash phase. It has recently been argued[3] that the flash phase may be interpreted as a resistive gravitational plasma instability[4] associated with an anomalous density gradient of chromospheric gas supported by a sheared magnetic field. This suggestion fits well with a proposed model of quiescent filaments[5] with which solar flares are known to be closely associated.

In pursuing this model, it seems that the "main" phase of a flare is the manifestation of a "tearing-mode" instability[6] which spreads across successive shells of the sheared magnetic field, releasing the excess magnetic energy and returning the field to a current-free state. This is indicated schematically in Fig. 1, and agrees well with the "rising mound" appearance of limb flares[7] and the "receding double-filament" appearance of disk flares[8].

Although this model goes a long way towards explaining the principal optical features of most flares, it does not explain the remarkable high energy phenomena which some flares exhibit: Type II and Type IV radio bursts[9], proton acceleration[10] and the ejection of plasma clouds

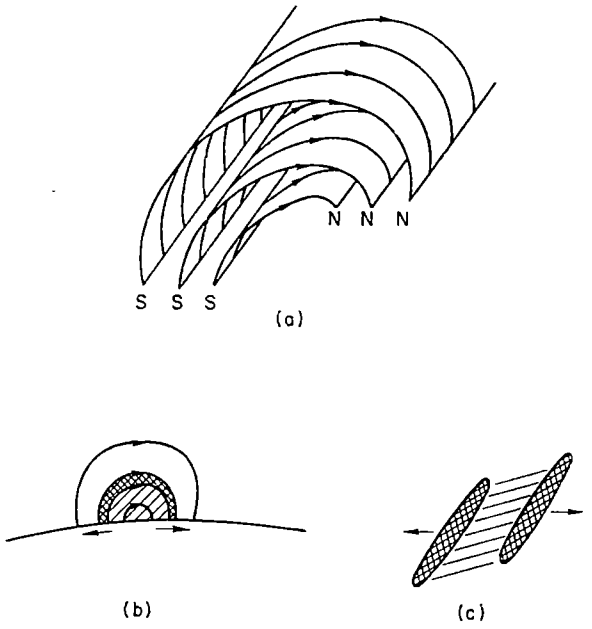


Fig. 1. *a*, Schematic representation of sheared magnetic-field pattern due to displaced line dipole. *b*, Limb view, and *c*, disk view of flare as it spreads across successive shells

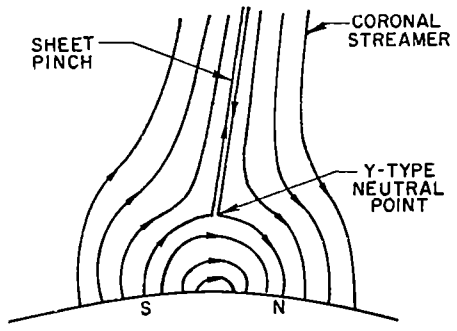


Fig. 2. Model of coronal streamer as bipolar flux tube formed from open-field configuration above closed-field region of bipolar field pattern

which may later produce a geomagnetic storm[11]. The purpose of this article is to offer a model which seems to account for this stage of solar flares.

The nature of coronal streamers[12] is of considerable significance for the flare phenomenon. For example, investigations of Type II and Type III radio bursts indicate that the exciters of these types of radiation move through regions of anomalously high density which are generally interpreted as being streamers[9]. Because Type III radio bursts are caused by clouds of electrons moving through the corona, and because these electrons must follow magnetic field lines, it follows that magnetic field lines run along the length of coronal streamers. Flares are normally produced by bipolar magnetic regions, and for this reason it is likely that the streamer contains flux of both polarity. This suggests that the relevant magnetic field configuration is that shown in Fig. 2, which is topologically identical with the "double-barrelled" tail of the Earth's magnetosphere[13] and can be termed a "bipolar flux tube". This interpretation of coronal streamers is supported by a recent coronal eclipse photograph taken by Dr. Sheldon Smith of the NASA Ames Research Center (to be published). It is to be noted that, as in the magnetosphere, there is a "*Y*-type neutral point" (or "line") which lies on the separatrix of the closed and open field lines, when it meets the "neutral sheet" or "sheet pinch" which runs the length of the coronal streamer.

The field configuration in the neighbourhood of the *Y*-type neutral point seems to be very sensitive to differences of plasma pressure between the closed-field region and the open-field region, having the form of an inverted "*T*" if the "external" gas pressure is the higher, and that of a cusp if the "internal" gas pressure is the higher. This property is probably responsible for the development of the bipolar flux tube from a closed bipolar field pattern. An excess of pressure in the closed field region will produce a cusp and then drive field lines out of the cusp along the sheet pinch, so augmenting the bipolar flux tube. When closed field lines are converted in this way to open field lines, gas "attached" to these field lines may escape into the solar wind, so that the gas pressure at coronal heights will drop, maintaining the pressure differential between closed and open field lines. If this is indeed the mechanism for the formation of bipolar flux tubes, the associated stored magnetic energy is derived from the non-thermal energy flux which heats the chromosphere and corona[14]. On an earlier occasion[15], it was noted that this energy flux is sufficient to explain the energy accumulated between homologous flares. (It is possible that the tail of the magnetosphere develops in a similar way, driven by the pressure of trapped high-energy particles in the radiation belts.)

Although, as is evident from the existence of the magnetospheric tail, the sheet pinch is metastable, it clearly represents a store of available energy which can be released by an appropriate instability. The relevant instability is generally regarded to be the tearing-mode instability[6], the steady-state non-linear stage of which has been analysed by Petschek[16]. Petschek's analysis indicates that the non-linear stage proceeds more rapidly than the linear stage, which implies that the tearing-mode instability is "explosive"[17]. If this instability is indeed explosive, this model offers ready explanations of the high-energy phenomena associated with solar flares.

When the tearing-mode instability (that is, the flare) extends into the open-field region, beyond the *Y* type neutral point, it will give rise to a reconnexion of magnetic field lines, as indicated in Fig. 3. The lower parts of the reconnected field lines will continue the process indicated in Figs. 1*b* and 1*c*. The upper parts of the U-shaped field

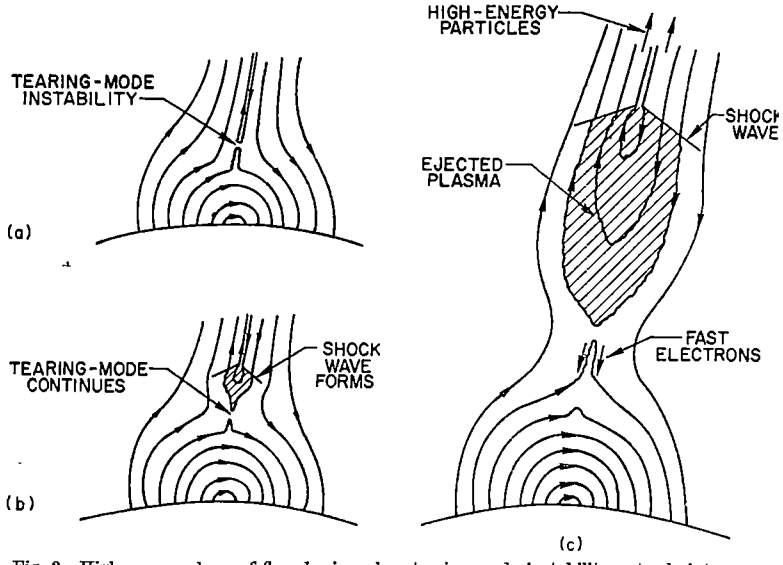


Fig. 3. High-energy phase of flare begins when tearing-mode instability extends into open-field region. (*a*), (*b*) and (*c*) are successive stages in formation of detached pocket of heated plasma and high-energy particles and its shock-wave interface with "pre-flare" coronal gas

lines now exert a tension on the plasma associated with it, so that this plasma will be ejected from the Sun as from a slingshot. Possible values of the area and magnetic field strength of a coronal streamer are 10^{20} cm^2 and $10^{1.5}$ gauss. Since the "scale height" of the field pattern would be comparable with the solar radius $10^{10.8}$ cm, the stored magnetic energy which would be released by the tearing-mode instability amounts to $10^{32.4}$ ergs. If the number density of the electrons and protons at the base the of streamer is taken as 10^{10} cm^{-3} and the density scale height as $10^{8.5}$ cm, the mass which will be ejected is $10^{14.7}$ g. If this mass were ejected into free space, it would acquire a velocity of 10^9 cm sec^{-1}, but this would be reduced by the interaction of the ejected gas with the pre-existing solar wind. Indeed, as Parker[11] has pointed out, the asymptotic form of the blast wave will be almost independent of the form of energy release. This estimate of the energy release is comparable with Parker's estimate of the energy release necessary to explain an intense geomagnetic storm.

The tearing-mode instability leads to the development of strong electric fields which, by a stochastic process[18], will accelerate both electrons and protons. Calculation of the rate of acceleration and final spectrum is a formidable problem, but some simplified calculations[19,20] have been made which corroborate the reality of the process. Electrons which, after acceleration, are caught in closed field lines will stream towards the surface of the Sun. If these electrons are deflected by the magnetic-mirror mechanism, the bulk of their synchrotron radiation will originate at the reflexion points: this offers an explanation of microwave Type IV radio bursts[9]. If at some height there is a favourable relationship between plasma and gyro-frequencies, the same two-stream instability may occur that has been observed by Waniek, Swanson and Grannan[21] and others, which gives rise to intense highly directional circularly polarized radiation. This would seem to explain stationary Type IV*m* radio bursts[9]. Electrons which, after acceleration, are in the "open" magnetic field configuration are likely to remain trapped in the moving "pocket" containing turbulent plasma and magnetic field. Synchrotron radiation by these electrons would comprise a moving Type IV*m* radio burst[9]. Accelerated protons in the closed-field region may go unnoticed, except for producing X-rays at the photosphere, but high-energy protons in the open-field region will leave the Sun and constitute "solar cosmic rays".

As the "magnetic pocket" is ejected from the surface of the Sun it moves into a region of lower magnetic pressure and lower gas pressure, and for this reason it is likely to expand transversely. It will also lengthen, partly due to the heating associated with the tearing instability and partly because the pocket is collecting coronal gas which is shock heated. Because the velocity of the ejected gas will be approximately the Alfvén velocity, the shock will move at a higher velocity. In consequence, a (collisionless) magnetohydrodynamic shock wave will trail from the "leading edge" such a shock wave, moving at just such a speed, is necessary to explain Type II solar radio bursts. These radio bursts are excited by kilovolt electrons accelerated in this shock front, just as electrons are observed to be accelerated in the Earth's bow shock[22].

It is clear that this model involves a number of complex plasma processes, each of which calls for lengthy detailed calculation. While such calculation is essential to confirm the model now proposed, the qualitative and gross quantitative agreements between this model and various high-energy phenomena of solar flares seem sufficiently striking to justify this preliminary publication.

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A MODEL OF QUASI-STELLAR RADIO SOURCES

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The nature of quasi-stellar objects[1], originally identified by association with strong radio sources, remains an open question. It was remarked earlier[2] that the explosions which occur in quasi-stellar objects and those which occur in radio galaxies are similar not only to each other, but also to solar flares[3]. The proposal that the explosions of galaxies and of quasars, leading to radio clouds, could be interpreted as "galactic flares" could not at that time be developed in more detail because the mechanism of solar flares was not adequately understood. The model for the high-energy phase of solar flares, described in the preceding article[4], now makes it possible to construct a more detailed model of quasi-stellar objects.

One may argue very simply in favour of the explosions being flares. Ultra-relativistic electrons must have been accelerated by an electric field. A sudden spontaneous conversion of energy from one form to another is an instability. The kind of instability which generates electrical fields is a plasma instability. If there is to be more acceleration than heating, the gas density must be low. As a consequence, the energy released by the instability will be mainly magnetic. This is precisely what is generally believed to happen in the main and high-energy phases of solar flares.

Hoyle and Fowler[5] have argued that the energy released in some galactic explosions is so great (10^{59} ergs or more) that it is probably derived from the nuclear and gravitational energy of a compact body of gas of mass $10^5 M_\odot$–$10^8 M_\odot$ and small size, generically related to the nucleus of a Seyfert galaxy. If the mass is supporte

by gas and radiation pressure, it is prone to instabilities, including some of a relativistic nature[6], but these do not necessarily release energy of the required magnitude[7]. Moreover, a non-electromagnetic model cannot, of itself, yield particle acceleration.

These difficulties are overcome if it is assumed that the mass is supported principally by magnetic stresses. The stored magnetic energy will then be comparable with the gravitational binding energy so that the model contains enough energy in such a form that it can readily be converted into high-energy particles. The assumption that the magnetic field is closed presents certain difficulties, not the least of which is that of explaining the origin of the field. The assumption that the magnetic field is open circumvents these difficulties. The magnetic field must now be assumed to be primeval.

The nucleus of a quasar is in this model coupled magnetically to inter-galactic space. If there is a sharp drop in density at the surface of the central mass, there will be a field reversal, constituting a "sheet pinch", at the "ecliptic plane" of the system, as shown in Fig. 1. The sheet pinch will terminate in a circular Y-type neutral line outside which there will be a ring-shaped trap for accreting gas. The field lines coupled directly to the nucleus constitute a pair of "funnels" for accreting gas. Photographic evidence that such rings and funnels actually occur is provided by plates 143, 146 and other plates of Arp's *Atlas of Peculiar Galaxies*[8]. It is now tempting to re-interpret the observed gas flux of both *M*82[9] and *NGC* 1275[10] as accretion rather than explosion. (Dr. George Field has independently suggested, in private conversation, that the gas flux of *M*82 may be an influx.)

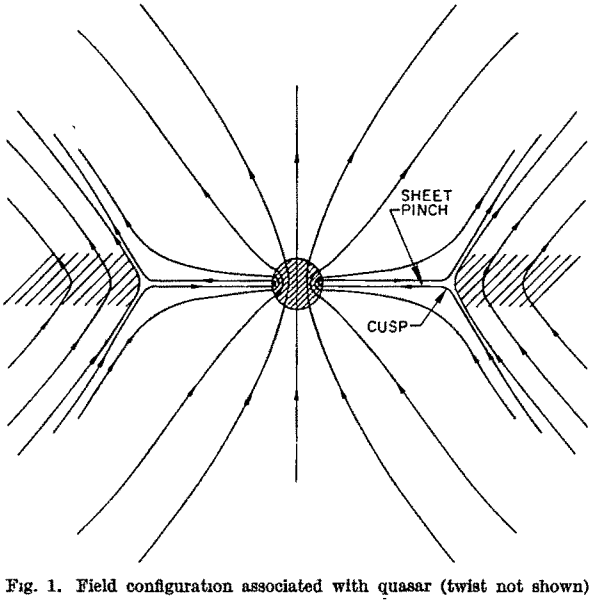


Fig. 1. Field configuration associated with quasar (twist not shown)

On balancing the magnetic energy and gravitational binding energy of a simple model, not too far from spherical[2], a mass-magnetic-flux relation is obtained of the form:

$$\Phi \approx 10^{-3} M \qquad (1)$$

where Φ is in gauss cm^2, M in grams, and the exact coefficient depends on the geometry. It is noteworthy that a typical sample of radio clouds, discussed by Maltby, Matthews and Moffet[11], are calculated to have fluxes in the range 10^{34}–10^{38} gauss cm^2, in excellent agreement with equation (1) and the Hoyle–Fowler hypothesis[5] that the explosions originate in galactic nuclei, for which $10^4\ M_\odot$–$10^8\ M_\odot$ is a reasonable mass range.

It appears that a magnetic field can offer only gross neutral stability. However, magnetohydrodynamic turbulence has an effective γ of 3/2 and so can provide gross stability. For a certain simple model, one finds that the oscillation frequency for radial pulsations ω may be related to the mean density ρ, the radius R and the Schwarzschild radius $R_s(= 2GMc^{-2})$ by:

$$\omega^2 \approx \pi G\rho\,(A^2 - 0{\cdot}55\,[R_s/R]) \qquad (2)$$

where A is the Alfvén number, that is, the ratio of the turbulent velocity v_t to the Alfvén velocity $v_A[= B(4\pi\rho)^{-\frac{1}{2}}]$.

The above and related equations make it possible to estimate parameters for the nucleus of the quasar 3*C* 273, if we interpret the observed 13 year periodicity[12] as a radial pulsation and the dispersion in gas velocity of "half-width" $10^{8\cdot3}$ cm sec^{-1}, inferred from emission line widths[13], as the turbulent velocity v_t. With the choice $A = 10^{-1}$, we then obtain $M = 10^{42\cdot3}$ g $= 10^9\ M_\odot$, $R = 10^{16\cdot2}$ cm (= 5 light days), $\rho = 10^{7\cdot1}$ g cm^{-3}, $\Phi = 10^{39\cdot3}$ gauss cm^2, $B = 10^{6\cdot4}$ gauss, $v_A = 10^{9\cdot3}$ cm sec^{-1}. The escape velocity is approximately v_A.

As was observed earlier[2], the likelihood of anomalous density gradients provides for the occurrence of flare flashes[14], which may explain the observed flashes in both optical and radio emission. It is also possible that magnetohydrodynamic instabilities may occur which could produce sudden changes in radius, either increasing or decreasing the optical luminosity.

We may now seek, in the present model, the analogue of the high-energy phase of solar flares described in the preceding article. The site for this phenomenon will be the sheet pinch which lies in the "ecliptic plane", and the mechanism will be the tearing-mode instability. The result will be a "pocket" of turbulent magnetic field containing high-energy particles being ejected by the tension of the magnetic field, as indicated in Fig. 2. If this occurs over only a small range of longitude, it will constitute a jet, and it is proposed that the jet of 3*C* 273 and the jet of *M* 87 (ref. 15) are manifestations of this mechanism. The fact that the synchrotron radiation of the jet of 3*C* 273 is detectable optically but not in the radio wave-lengths indicates that the spectrum is somewhat flat, implying that acceleration is in progress.

The hypothesis that radiation from the jet of 3*C* 273 is the result of continuing acceleration enables one to estimate the strength of the magnetic field at the tip of the jet. The rate of emission is about $10^{44\cdot3}$ ergs sec^{-1} and the cross-sectional area of the jet is about $10^{43\cdot9}$ cm^2. We make a conservative estimate of the field strength by assuming that the shock front at the tip of the jet is relativistic, advancing at the speed of light. On equating the rate of radiation to the rate of "dissolution" of magnetic energy, one obtains the estimate $B = 10^{5\cdot6}$ gauss at the

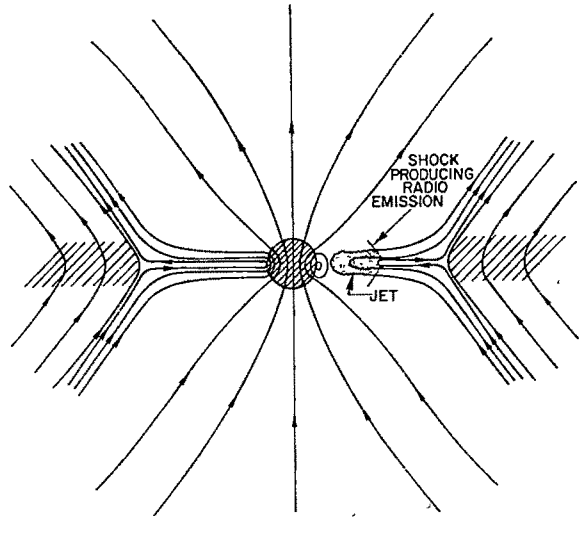


Fig. 2. Field configuration associated with quasar following flare, leading to jet formation

tip of the jet. Because the tip of the jet is at least $10^{23\cdot2}$ cm from the nucleus, the magnetic flux through each hemisphere amounts to $10^{42\cdot8}$ gauss cm^2. If we now refer to the mass-magnetic-flux relation, we see that this magnetic flux must be associated with a mass of $10^{45\cdot8}$ g, that is, $10^{12\cdot5}\ M_\odot$. Our model will not permit the visible nucleus *3C 273B* to have so great a mass. We are therefore led to the conclusion that surrounding the visible object *3C 273* is a much larger mass of gas which is invisible, presumably because it is fully ionized. We may note with some satisfaction that the presence of a mass of this magnitude will make it possible for the quasar to evolve into a galaxy as massive as *M87*, or conceivably into a cluster of galaxies.

It is interesting to enquire further into the likely fate of the jet of *3C 273*. It will continue as a jet until it arrives at the ring-shaped neutral line. At this point, the supply of magnetic energy will cease, and there will be no further acceleration. The cloud of electrons and ions, which have relativistic energies, will now divide into two clouds, about equal in size, one moving "northwards" and the other "southwards". This is indicated in Fig. 3. It is notable that a single explosion eventually produces two similar radio clouds at equal distances from the nucleus. In the case of *3C 273*, the nucleus will not be on the centre line of the double radio source when it forms (although it may seem so to other observers). However if, as seems quite possible, the flare were to extend over all longitudes, the end result would be two "smoke ring" radio clouds diametrically opposite with respect to the nucleus. We see that this model offers a simple explanation of the most striking characteristic of radio galaxies—their double structure.

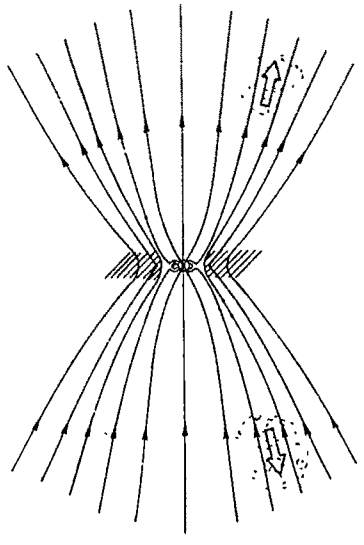


Fig. 3. Later stage, showing division of jet into two radio clouds (twist and effect of twist not shown)

Two possible interpretations of the radio source *3C 273A* present themselves. One is that the electrons responsible for the radio emission have been accelerated in a shock front at the tip of the jet . . . the same mechanism as is responsible for a Type II solar radio burst, although the electron energies would be greater so that we are seeing synchrotron radiation rather than radiation from plasma oscillations. The other alternative is that the jet has just arrived at the cusp so that we are seeing radiation from the first batch of electrons to move out of the acceleration region of the sheet pinch.

As was noted earlier, the funnelling action of the magnetic field has the result that intergalactic gas streams directly into the nucleus. Although the Jeans criterion is open to criticism[16], it will be used to provide an estimate of the radius of the sphere of intergalactic gas which has condensed to form the quasar. If in the formula $R_c = a(4\pi\ G\rho_0)^{-\frac{1}{2}}$, where a is the speed of sound and ρ_0 the density, we adopt the current estimate[17] $\rho_0 \approx 10^{-28}$ g cm^{-3}, we find the required temperature to be $T_0 = 19^{6\cdot2}$ °K; this may seem high, but it has recently been shown that the density of neutral hydrogen in intergalactic space is exceedingly low[18]. The corresponding value of R_c is $10^{24\cdot4}$ cm (800 kiloparsecs). This estimate enables us to calculate the intensity of the primeval magnetic field. As the flux of $10^{42\cdot8}$ gauss cm^2 originally threaded a sphere of radius $10^{24\cdot4}$ cm, the primeval magnetic field is of intensity $10^{7\cdot5}$ gauss. The primeval magnetic pressure was therefore comparable with the gas pressure. However, as Chandrasekhar[19] has noted, this would not have inhibited the gravitational condensation.

It was noted previously that the funnelling action of the magnetic field permits a high accretion rate leading to rapid evolution of the quasar. If we identify the optical radiation from the nucleus of *3C 273*, 10^{46} ergs sec^{-1}, as being due to accretion, the gas influx will be $10^{27\cdot2}$ g sec^{-1} or more, so that the evolution time (the time for the nucleus to double its mass) is 10^{15} sec ($10^{7\cdot5}$ y) or less. The inequality quoted in the previous sentence is based on the expectation that the inflowing gas will give up the bulk of its energy at a shock front (or series of shock fronts) at which the temperature is likely to be much higher than that of the visible surface of the nucleus. If the shock reheats the gas to a temperature comparable with the original (intergalactic) temperature, the radiation would be primarily in the X-ray band so that the X-ray output from the nucleus of *3C 273* may well exceed the visible radiation.

The main "galactic" mass of gas should also be a source of X-rays. Whether or not it is more intense than the X-rays originating near the nucleus depends on the size of the galactic mass (and weakly on its temperature). If the temperature is about 10^6 °K, X-ray emission from the galactic mass will exceed 10^{46} ergs sec^{-1} if the radius of the mass is less than $10^{21\cdot9}$ cm, that is, 2·5 kpc.

The present model suggests answers to many questions concerning quasars. In addition to those already mentioned, it is possible to explain why the formation of a small heavy object has not been inhibited by angular momentum: the 'open" magnetic field configuration provides a mechanism for retaining a low angular velocity during condensation by transferring angular momentum from the inner regions to the outer regions. One may also note that, with the high magnetic field quoted, the inverse Compton effect will be negligible in comparison with synchrotron radiation, so avoiding a difficulty which has led Hoyle, Burbidge and Sargent[20] to suggest that quasars are not at cosmological distances. Further, one may note that the explosion mechanism suggested for quasars will be applicable also to radio galaxies, if one assumes that the magnetic field of the nucleus of such a galaxy is similar to that which we associate with a quasar. Such a galaxy would indeed evolve from each quasar, according to the present model.

This chain of thought now offers an explanation of the recent remarkable observations of Byram, Chubb and Friedman[21], that certain radio galaxies emit X-rays at a power 10–100 times greater than the combined optical and radio output. This X-ray emission may be ascribed to an influx of intergalactic gas into the nucleus.

In view of the high magnetic field strength of the nucleus of *3C 273* (which is expected to be typical of quasars), the great mass of plasma surrounding the nucleus, and the various plasma phenomena which may arise, analysis of the radiation (including radio emission) from the nucleus appears a complex problem. One will need to bear in mind the possibility of proton synchrotron radiation and also of coherent radiation processes, as has recently been suggested by Ginzburg and Ozernoy[22].

Finally, we may suspect from the fact that solar flares produce high-energy protons ("solar cosmic rays") of

energies ($\sim$ 1 beV) much greater than that of electrons responsible for the radio emission (a few meV), that galactic flares should accelerate some ions to energies which are much greater than the energy of electrons in, say, the jet of 3*C* 273. The jet must contain electrons with energies of about $10^{12\cdot3}$ eV in order to give optical radiation in a magnetic field of strength $10^{5\cdot6}$ gauss. These electrons are decelerated by synchrotron radiation at a rate 10^6 eV sec^{-1}. Thus the stochastic acceleration process[23], which arises from the electric-field spectrum produced by the instability, has a maximum acceleration rate of at least this value. Protons are subject to the same acceleration, without experiencing significant deceleration. Any protons which have been accelerated at the maximum rate for the life-time of the jet (which must be at least $10^{12\cdot4}$ sec, as its length is 10^{23} cm) will have attained an energy of at least $10^{18\cdot5}$ eV. Multiply charged ions may have achieved a greater energy. Thus, it is possible that galactic flares are responsible for the high-energy component of cosmic rays.

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THERMOSPHERIC ENERGETICS AND A POSSIBLE EXPLANATION OF SOME OBSERVATIONS OF GEOMAGNETIC DISTURBANCES AND RADIO AURORAE

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ONE approach to the study of large scale atmospheric circulations pursued extensively during the past few years has been the examination of the atmospheric budgets of angular momentum, energy and mass. One asks how the transfer of zonal angular momentum into the regions with westerly winds and out of the regions with easterly winds is accomplished, how heat energy is transferred from regions of surplus to regions of deficit, and how water vapour is transported from regions where evaporation greatly exceeds precipitation to regions where the opposite occurs. The answers to such questions based on the observations now available have led us to picture the atmosphere as a series of superposed and interconnected heat engines and refrigerators. Investigations have concentrated on the following seven items: (*a*) meridional cross-sections of the zonal wind component; (*b*) the horizontal transport of angular momentum towards the poles, proportional to the covariance between the zonal and meridional wind components; (*c*) meridional cross-sections of temperature; (*d*) the horizontal transport of heat energy towards the poles, proportional to the covariance between the meridional wind component and the temperature; (*e*) meridional cross-sections of radiative heating and cooling rates; (*f*) meridional cross-sections of the distribution of trace substances; (*g*) the horizontal transport of trace substances towards the poles, proportional to the covariance between the meridional wind component and the concentration of the trace substances.

For the region of the atmosphere below 30 km items (*a*)–(*g*) can be obtained from the balloon sounding network data. No systematic experimental survey of item (*e*) has been conducted although estimates are available based on items (*c*) and (*f*). For the 30–65 km region, meteorological rocket network data have provided limited information about items (*a*)–(*c*), and the rocket grenade experiment has extended items (*a*) and (*c*) to about 80 km. Items (*d*) and (*g*) are not known. Item (*e*) is computed with fairly strong assumptions made about item (*f*), itself not yet properly measured. Nevertheless, the broad outline of the momentum and energy budget of the region below 80 km has been synthesized[1–5]. In the heat engine regions, radiative processes produce a meridional temperature gradient and the large-scale quasi-horizontal motions carry heat down this gradient. In the refrigerator regions the large-scale motions produce a temperature gradient, essentially through their vertical components, and radiative processes act to reduce this gradient; the motions themselves are forced by energy received from the heat engines. The same line of reasoning is applied here to the thermosphere above 80 km with particular emphasis on some geophysical consequences which have emerged.

The Thermosphere

Item (*a*) has been obtained from the tracks of meteor trails, ionospheric discontinuities, falling spheres and chemical clouds. Large scale circulation systems, small-scale waves and tides all influence the results. Fedor[6] and Murgatroyd[7] have made use of the ionospheric drift results with tides eliminated to extend zonal wind meridional cross-sections to about 120 km. Item (*b*) has been estimated from meteor trail data by Jones[8] and from ionospheric drift data[5]; tidal motions apparently play a significant part in the momentum budget. Item (*c*) has been derived from (*a*) by Murgatroyd[7] by means of the thermal wind equation and there is good agreement with the overall pattern as derived from falling sphere experiments. Whereas high middle latitudes at 80 km are warm in winter and cold in summer, they are cold in winter and warm in summer at 120 km. Items (*d*) and (*g*) cannot be derived from present observations. Mahoney

and Lagos[9,10] have recently presented computations of item (*e*), based on heating which results from absorption of ultra-violet and extreme ultra-violet radiation, conduction and infra-red emission by atomic oxygen. Their results show that the large-scale motions must transfer heat down the temperature gradient in the lower thermosphere in the direction of the poles at the equinoxes and towards the winter pole over most of the region at the solstices. Item (*f*) is available for the bottomside ionosphere from surface ionospheric stations and for the topside from *Alouette*, but only for electron concentrations.

Combining items (*c*) and (*e*), it is noted that diabatic heating occurs where the air is warm and cooling where it is cold, thus zonal available potential energy is generated. This energy is the maximum amount of total potential energy available for conversion into kinetic energy under any adiabatic redistribution of mass. The concept was introduced into meteorology by Lorenz[11] and has been extensively discussed by him and by Van Mieghem[12]. The situation in the 100–150 km layer is somewhat similar to that in the 30–50 km region. Some of this energy drives the motions which transfer heat towards the poles to balance the energy budget. These motions will have both zonal and meridional components and it has been argued elsewhere[5,9] that they are quite adequate to explain the difference in amplitude and phase between models of the thermospheric temperature distribution and deductions based on satellite drag. The lower thermosphere thus functions essentially like a heat engine. Is this surmounted by a refrigerator as is the case for the heat engines of the 0–10 km and 30–50 km layers? If so, the motions involved would be driven from the heat engine and there would be a tendency at the equinoxes for parcels moving toward the poles to subside and thus become warm and for parcels moving towards the equator to rise and cool. In fact, at the solstices one would anticipate a warm winter pole and a cold summer pole at heights of about 400 km if the temperature changes arising from the vertical components of the motion can offset the changes due to diabatic effects. Jacchia[13] has analysed the drag from a high-latitude perigee satellite which shows such a temperature distribution at 500 km although he feels there is an alternative explanation of the finding[14]. Thus, temperatures may increase with latitude at such altitudes in the winter and at the equinoxes. Such an increase has also been postulated from the composition measurements of the topside ionosphere[15,16].

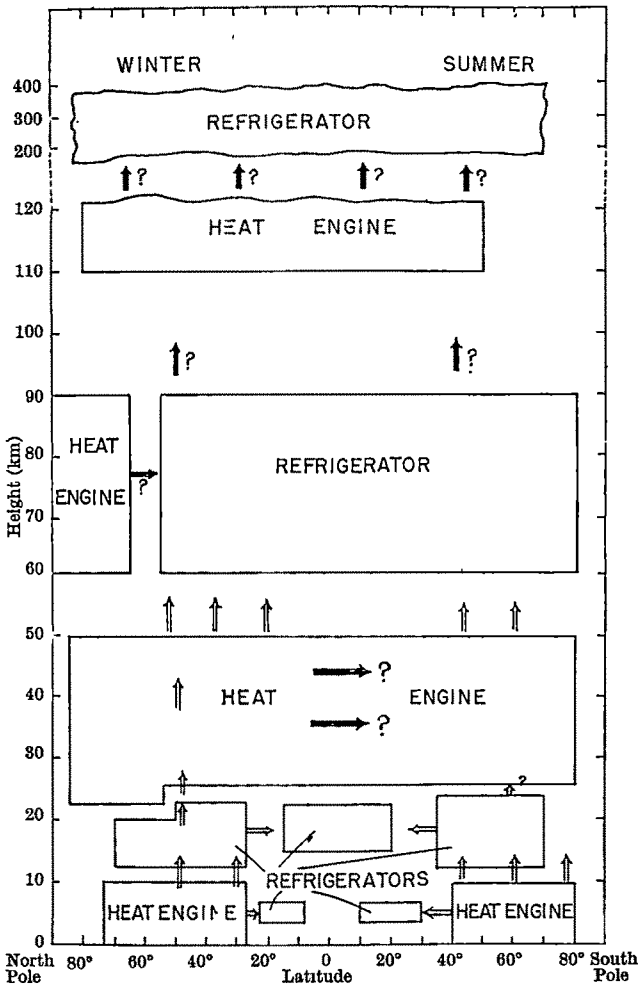


Fig. 1. Possible atmospheric energy flow diagram

Table 1. ZONAL AVAILABLE POTENTIAL ENERGY (A_z), ITS GENERATION (G_z), AND THE ZONAL KINETIC ENERGY (K_z), ALL AS DEFINED BY LORENZ. All energy contents and generation rates apply to layers 5 km thick

Height (km)	Winter G_z (ergs cm^{-2} sec^{-1})	Winter A_z (10^5 ergs cm^{-2})	Winter K_z (10^5 ergs cm^{-2})	Summer G_z (ergs cm^{-2} sec^{-1})	Summer A_z (10^5 ergs cm^{-2})	Summer K_z (10^5 ergs cm^{-2})
80	−0·07	0 4	1·3	−0·10	0·3	0·1
75	−0·15	0·6	3·4	−0·17	0·2	0 5
70	−0·47	0·6	7·3	−0·03	0 2	4·2
65	−0·59	0·2	13	−0·05	0 2	9·9
60	−0·16	0·1	11	0·11	0·3	11
55	1·1	1·9	20	0·12	0 0	16
50	2·2	7·6	33	0·19	0·4	22
45	3·7	15	44	0 12	1·2	29
40	5 1	23	60	0·38	3 6	36
35	2·8	33	74	0·79	5·7	47
30	6·4	51	63	−0·43	2·7	40
25	4·1	13	65	−13	94	100
20	−42	392	155	−11	299	156
15	−64	1,201	1,021	−65	1,894	293
10	−112	2,575	2,160	−9	1,099	1,059
5	276	18,675	1,452	534	7,388	637

Table 2. A_z, G_z AND K_z IN THE LOWER THERMOSPHERE

Height km	K_z	A_z (ergs cm^{-2})	G_z (10^{-5} ergs cm^{-2} sec^{-1}) 0800	1200	1600	2000
			Winter			
120	42	9	30	22	5	−5
115	85	5	25	27	7	−5
110	130	0	0	0	0	0
105	96	73	−113	−159	−57	−9
100	128	520	−264	−388	−171	−97
95	896	1,315	−357	−583	−227	−175
90	6,270	4,405	−295	−434	−225	−157
			Summer			
120	1	9	6	−5	11	8
115	14	72	18	−12	30	23
110	84	109	27	−4	44	32
105	363	84	21	0	51	24
100	1,020	54	18	−11	30	9
95	2,210	30	2	2	4	0
90	4,160	518	7	89	9	4

Energy Calculations

A summary of the various atmospheric regions is presented in Fig. 1. The arrows represent possible interchanges of energy and will be discussed more fully later. In Table 1 there is a summary of the mean values for summer and winter of zonal available potential energy, its generation by adiabatic processes and the kinetic energy content of the 0–30 km region. The heating rates and temperatures used in these calculations have been presented elsewhere[17]. The equations used were those of Lorenz[11]. The tabulated values refer to atmospheric layers 5 km thick and bounded by latitudes 5° and 75° N. In each, temperature, wind and heating rates were read from cross-sections drawn for winter and summer at intervals of 10° in latitude. It is difficult to choose appropriate values for the troposphere, and considerable generation in the lowest 5 km has probably been omitted by the technique. Generation rates for the troposphere as a whole[3] are probably close to 2,000 ergs cm^{-2} sec^{-1}. Some 100 ergs cm^{-2} sec^{-1} leaks upwards out of the troposphere and is used to drive the lower stratosphere. About the same amount of energy is consumed in the tropical troposphere refrigerator and this is provided by lateral

transfer from the middle latitude troposphere. The middle stratospheric heat engine generates its own energy, about 20 ergs cm^{-1} sec^{-1}, in winter and in summer generates a smaller amount and probably receives energy laterally from the winter hemisphere. In addition, there is a possible flux of several ergs cm^{-2} sec^{-1} into the stratospheric heat engine from below in winter[18]. About 1 erg cm^{-2} sec^{-1} has to be supplied upwards from the heat engine in the 25–50 km region to the refrigerator in the 60–80 km region in winter and somewhat less than this in summer. Table 2 presents similar estimates for the 80–120 km region based on the radiative heating rates of Mahoney and Lagos[9,10] and a temperature distribution modified from Murgatroyd[7]. The generation rates in this region vary during the day by large amounts and are presented for four times. The same temperature structure was used for each time and a single wind structure for K_z. The results in Table 2 give a typical generation rate of about 20×10^{-5} ergs cm^{-2} sec^{-1} per 5 km thick layer for the heat engine region above about 105 km. A typical content of zonal available potential energy is about 10 ergs cm^{-2} and the ratio A_z/G_z is about 14 h. This time is so short, as I emphasized earlier[5], that much of the kinetic energy derived from the available potential energy probably appears in short-period motions, namely, the tides. It is also noteworthy that above 100 km in winter the kinetic energy content is larger than that of zonal available potential energy, as occurs in the 25–60 km heat engine but not in the troposphere. Some of the mean kinetic energy evident in the lower thermosphere may be derived from tidal kinetic energy. In turn the tidal kinetic energy may be partly derived from differential heating *in situ* and partly from tidal forces acting on the atmosphere below[19].

No doubt these numbers will have to be revised as more is learned about the upper atmosphere, but they do provide a guide to further exploration. They show immediately, for example, that the effects of the lower on the upper atmosphere are liable to be much greater than the converse effects. A similar conclusion has emerged from purely theoretical studies[20]. Although this may seem a trivial point, it is often ignored by investigators studying solar–terrestrial relationships.

Implications for K_p and Aurorae

The thermosphere is characterized by a number of temperature changes. Two of these which are well marked are the diurnal temperature change and the 27 day temperature change, and these are thought to be due to changes in the incident extreme ultra-violet radiation. In fact, the 27-day cycle has been demonstrated experimentally by Bourdeau *et al.*[21], who monitored solar extreme ultra-violet from a satellite for 2 months and compared the results with thermospheric temperatures derived from satellite drag data. A preferred area of extreme ultra-violet emission on the Sun produced higher thermospheric temperatures when directed towards the Earth. In so far as solar radio noise is also related to extreme ultra-violet emission and varies over the solar sunspot cycle, it has been argued by Nicolet[22] and others that the change in thermospheric temperatures that occurs over the sunspot cycle also results basically from changes in solar extreme ultra-violet emission.

The additional hypothesis will be made here that changes in the thermospheric temperatures and wind structure can be brought about by absorption of energy supplied from below. The hypothesis is not new and has been discussed in various forms by several authors[23,24]. With its aid it may be possible to account for the increases in thermospheric temperature associated with large values of K_p, particularly noticeable at high latitudes[25], and the increase in the drift speed of ionospheric irregularities also associated with large K_p (ref. 26). K_p itself is a global measure of the degree of disturbance of the Earth's magnetic field as monitored at the Earth's surface[27]. The normal quiet day variation of the surface magnetic field is thought to arise from electrical currents in the E-region. These in turn are apparently driven by the large-scale atmospheric motions. Because the gyro-frequency for electrons becomes equal to the collision frequency with neutral constituents at about 90 km while the same criterion for positive ions is not reached until about 140 km, there is a layer in the E-region in which ions are strongly influenced by atmospheric motions while electrons are strongly influenced by the Earth's magnetic field[28]; it is thus possible for the atmospheric motions to produce differential motions of the electrons and ions, in other terms an electric current. The current is manifested as changes in the magnetic field at the Earth's surface. When the field departs strongly from its regular variation it seems reasonable to assume that the E-region currents are responsible. Thus changes in either atmospheric motions or conductivity are the ultimate cause for changes in K_p. There seems to be no difficulty in supplying the perturbation magnetic energy from the kinetic energy; for example, a field change of 200γ corresponds to a change in magnetic energy of $\sim 2 \times 10^{-7}$ ergs cm^{-3}. whereas a typical kinetic energy density at 120 km, for a speed of 50 m sec^{-1}, is 5×10^{-4} ergs cm^{-3}.

Many attempts to explain auroral phenomena on the basis of charged particle streams from the Sun do not appear to be correct now that the solar wind has turned out to contain only relatively low-energy particles. A search is therefore under way for acceleration mechanisms that can act on terrestrial ions. The details are not yet clear; in fact, the relationships between motions of neutral particles, motions of ions, pressure gradients, electrodynamic forces and viscosity are not as yet completely understood[29–33]. The possibility that the charged particle energy comes ultimately from the kinetic energy of large-scale motions is one which has only been investigated in a preliminary fashion[34]. Seasonal changes in preferred L shells[35,36] and indeed their very existence may be related to preferred regions of atmospheric acceleration. Further work may use considerations such as those applied to the solar atmosphere by Starr and Gilman[37] with diffusion of magnetic field lines included. Nevertheless, it is clear that the necessary energy is readily available. The energy deposited in a typical aurora is about 10^{17} ergs sec^{-1} or 0·02 ergs cm^{-2} sec^{-1} when averaged over the globe, which is a relatively small fraction of the atmospheric energy available (see Table 1). Even a large event only involves 0·2 ergs cm^{-2} sec^{-1} compared with 100 ergs cm^{-2} sec^{-1} which is constantly being supplied by the troposphere to the lower stratosphere. It should be noted that the generation rates for the thermosphere alone are much too small to provide the energy (Table 2).

Students of the ionosphere are aware of the long debate 25–30 years ago between Hulburt, Chapman and others concerning the ultimate cause of geomagnetic disturbances. The principal mechanisms proposed were extreme ultra-violet and solar corpuscular radiation; the debate is delightfully summarized in the book by Chapman and Bartels[38]. The present hypothesis is that two factors can alter the circulation of the thermosphere and thereby produce geomagnetic changes; these are extreme ultra-violet changes (with concomitant conductivity and motion changes) and energy received from below. A particularly difficult problem is to differentiate between cases involving one factor alone, or both jointly. As an example, suppose that the energy transmitted from below is most effective (because the transmission of the lower thermosphere is high) when the extreme ultra-violet flux is at a minimum in its 27-day cycle or at a particular level below the maximum. Then geomagnetic disturbances (as opposed to regular variations) might predominate. This simple suggestion may suffice to account for the claimed existence of M-regions on the Sun. These "magnetically effective regions", so named by Bartels[38], were

introduced to explain the origin of magnetically disturbed conditions on the Earth which were not accompanied by visible solar disturbances.

Changes in the horizontal pressure gradient forces in the thermosphere which can be brought about as a response to changes in the thermal structure below could change the kinetic energy in the lower thermosphere. It is supposed here that this action occurs by way of the large-scale wave motions of the atmosphere below 100 km. The energy is supposed transmitted by pressure-work boundary terms rather than by an actual vertical flux of kinetic energy. The formal mathematical development applicable to vertical transmission by atmospheric motions of both large- and small-scale waves has been summarized by Van Mieghem[39].

Several years ago, Charney and Drazin[20] posed the question, "Why doesn't the Earth's atmosphere have a corona ?". They answered it with a preliminary numerical computation of the transmission of the atmosphere below 100 km to planetary scale, zero frequency, wave energy, which showed that in general such energy was trapped in the lower layers. Strong westerly winds or easterly winds in the 50–60 km region were found to inhibit the vertical flux of energy; only near the equinoxes when the winds were very light westerly could transmission occur. It is quite likely that small amounts of energy can escape at other times also and, in fact, it appears that the atmosphere does have a semi-permanent corona as shown by the presence of radio aurora. Equinoctial peaks in K_p and radio aurora[40] could thus result from an increased vertical energy flux at these times. They do not appear on the same dates each year and it is evident that the dates of optimum transmission of the atmosphere below 100 km are likewise variable. Other variables are the vertical energy flux from troposphere to lower stratosphere and the generation of energy in the 25–50 km layer.

As is common to most postulates concerning geomagnetic disturbances, there are several items that do not easily fit into the scheme. For example, the frequent suddenness of onset of a geomagnetic perturbation is difficult to explain. When long records of the circulation of the lowest 100 km are available it may be possible to distinguish characteristics associated with sudden and gradual disturbances. Another objection that might be raised is that K_p was found to be related to the solar wind velocity by the *Mariner* probe experiment[41]. It is difficult to argue that the changes induced in the magnetosphere could have produced changes in direction of the solar wind at such large distances. Nevertheless, there are no energy difficulties with the postulate and it may well be invoked for those radio aurora and K_p events not obviously related to events on the Sun. A similar postulate is often used to account for the temperature of the Sun's corona although usually the small-scale motions are held responsible; large-scale photospheric motions provide an equally attractive energy source.

A final comment concerning solar–weather relationships is that most investigators have examined meteorological processes occurring after K_p changes (with the assumption that K_p reflected a solar change). It may be more profitable to examine meteorological processes in the lowest 100 km before K_p changes.

It is clear that the hypothesis presented here can be tested when extensive observations of items (*a*)–(*g*) in the 80–300 km region have been collected so that their variations with time of day, season, position in 27-day and sunspot cycle and change with K_p can be assessed. If the hypothesis is correct, then such observations would have obvious practical applications.

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THE REVERSIBLE OXYGEN ELECTRODE

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FOR an electrode process consisting of a series of consecutive steps to be truly reversible, not only must the overall electrode reaction be the same in both the anodic and cathodic directions, but also each individual step of the forward process must be reversed in the back reaction. When oxygen is reduced at a platinum cathode in oxygen-saturated acid solution, hydrogen peroxide is detected[1,2] in the electrolyte, but when oxygen is evolved

at such an anode, hydrogen peroxide is not detected[3]. This observation indicates that the mechanism for the reduction is different from that for the evolution of oxygen at a platinum electrode, as hydrogen peroxide is a stable intermediate in the one case but not in the other.

On the basis of steady state polarization curves, Hoar[4] was the first to show that the extrapolated Tafel lines for the cathodic reduction and the anodic evolution of oxygen cross at the theoretically calculated potential value of 1·23 V (ref. 5). These data have been confirmed[1,2] and support the conclusion that the overall electrode reaction is the same for platinum/oxygen anodes and cathodes. The electrode process is the four-electron oxygen/water reaction:

$$O_2 + 4H^+ + 4e \rightleftharpoons 2H_2O \quad (1)$$

In the anodic evolution of oxygen, it is generally agreed that the first step is the discharge of adsorbed water molecules to form adsorbed hydroxyl radicals:

$$(H_2O)_{ads} \rightarrow (OH)_{ads} + H^+ + e \quad (2)$$

What happens next is not clear, because steady state polarization measurements give information only about the rate-determining step which appears to be equation 2 (refs. 1 and 2). Probably a method using short pulses may yield evidence of the nature of the steps which follow the rate-determining step. However, a possible path which seems reasonable is as follows. The hydroxyl radicals may interact, producing an adsorbed oxygen atom and a water molecule:

$$2(OH)_{ads} \rightarrow O_{ads} + (H_2O)_{ads} \quad (3)$$

Finally, two adsorbed oxygen atoms may combine to form the adsorbed molecule of oxygen from which oxygen is desorbed:

$$2\,O_{ads} \rightarrow (O_2)_{ads} \quad (4)$$

The current goes into the formation of hydroxyl radicals, but oxygen is evolved from the chemical interaction of these adsorbed hydroxyl radicals. If this mechanism is accepted, it may be seen that the rate-determining step (equation 2) must occur four times to produce one oxygen molecule, so that the stoichiometric number is 4. Furthermore, hydrogen peroxide is not involved.

From investigation of the reduction of oxygen on carbon electrodes using oxygen-18 as label, Yeager *et al.*[6] found that the oxygen–oxygen bond is not broken and hydrogen peroxide is the final product. It is generally known that carbon is a poor catalyst for the decomposition of hydrogen peroxide. If a good catalyst for hydrogen peroxide decomposition such as platinum is used as the cathode material, water is the final product; but hydrogen peroxide builds up in solution to a steady-state concentration of about 10^{-6} M[1,2,7]. These results suggest that the reduction of oxygen on platinum proceeds through the stable intermediate, hydrogen peroxide. A possible mechanism is as follows. Oxygen is adsorbed on the electrode surface, and the rate-determining step is the discharge of the first electron[2]:

$$(O_2)_{ads} + e \rightarrow (O_2^-)_{ads} \quad (5)$$

This step is followed by the reaction with a hydrogen ion:

$$(O_2^-)_{ads} + H^+ \rightarrow (HO_2)_{ads} \quad (6)$$

After discharge of another electron and the reaction with another hydrogen ion, a molecule of adsorbed hydrogen peroxide is produced:

$$(HO_2)_{ad} + e \rightarrow (HO_2^-)_{ads} \quad (7)$$

and

$$(HO_2^-)_{ad} + H^+ \rightarrow (H_2O_2)_{ads} \quad (8)$$

The peroxide may desorb into solution or may undergo catalytic decomposition to oxygen and water:

$$2(H_2O_2)_{ads} \rightarrow (O_2)_{ads} + H_2O)_{ads} \quad (9)$$

The oxygen formed from the hydrogen peroxide may undergo the reactions shown in equations 5–8, and effectively four electrons will have been passed and oxygen will have been reduced to water. If the electrode surface has adsorbed oxygen on it, such as a partial layer of platinum–oxygen[8], produced by pre-anodization, the decomposition of hydrogen peroxide occurs through interaction with the platinum–oxygen layer as suggested by Bianchi *et al.*[7]:

$$H_2O_2 + Pt\text{–}O \rightarrow H_2O + O_2 + Pt \quad (10)$$

When the platinum–oxygen layer has been reduced, hydrogen peroxide is decomposed by a local cell mechanism[9] as first described by Gerischer and Gerischer[10]:

$$H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O \quad (11)$$

and

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e \quad (12)$$

In the cathodic case a stoichiometric number of 2 is expected. The stoichiometric number, ν, may be determined from the anodic (b_a) and cathodic (b_c) Tafel slopes by the relationship:

$$\nu = \frac{4F}{RT}\left(\frac{b_a b_c}{b_a + b_c}\right) \quad (13)$$

only if the activated complexes for the anodic and cathodic reactions are the same as pointed out by Parsons[11]. Because on platinum electrodes the activated complex involves a single-bonded oxygen as shown in equation 2 at the anode, but a double-bonded oxygen (equation 5) at the cathode, equation 13 cannot be used, and such calculations are meaningless.

However, Bockris and Huq[1] were able to observe experimentally the reversible oxygen potential, 1·23 V, for about 1 h on platinum electrodes under open-circuit conditions in a rigorously purified system. They suggested that these observations had not been made before because others had worked with impure systems.

When a clean platinum electrode was placed in a purified acid solution[8], the reversible potential was not observed; and the rest potential was explained in terms of a local cell mechanism composed of the oxygen/water reaction, equation 1, and the platinum/platinum–oxygen reaction:

$$Pt\text{–}O + 2H^+ + 2e \rightleftharpoons Pt + H_2O \quad (14)$$

Recently, it was found[12] that the reversible oxygen potential could be observed for extended periods of time on a platinum electrode which had been treated with concentrated nitric acid. This treatment with nitric acid, it was suggested, produces a complete electronically conducting layer of adsorbed oxygen. In this way, the mixed potential mechanism is suppressed, and the surface is both electronically conducting and inert to oxygen. Consequently, a potential of 1·23 V is observed.

It is to be noted that those electrodes on which Bockris and Huq observed 1·23 V at open circuit were heated at 500° C for 2 h just before they were plunged into the electrolyte which, it is contended, produced a complete layer of electronically conducting platinum–oxygen. Similarly, Watanabe and Devenathan[13] observed the 1·23 V only after exposing the clean electrode to pure oxygen for 48 h.

Therefore, another condition as well as the rigorous control of impurities maintained by Bockris[1] must be imposed on the platinum–oxygen acid system before the reversible oxygen potential can be observed. The platinum surface must be covered by a complete layer of platinum–oxygen.

When the nitric acid-treated platinum electrode, referred to as a platinum–oxygen–alloy electrode[14], is polarized at very low current densities, a plot of the potential as a function of current density gives a straight line (see Fig. 1 of ref. 15) which passes from anodic to cathodic polarization through a potential of 1·23 V without a change of slope. As long as this electrode is not polarized beyond about 50 or 60 μamp/cm^2, the

system is stable; and the polarization curve is reproducible. At high anodic or cathodic current densities, the complete film of platinum–oxygen is impaired; a mixed potential mechanism sets in; and the reversible electrode is destroyed. Peroxide is not detected when a platinum–oxygen–alloy electrode is cathodized[15]. As the rest potential is 1·23 V, the slope of the anodic and the cathodic branches of the voltage-current (E–i) curve is the same, and hydrogen peroxide is not detected with cathodic polarization, it is concluded that a platinum electrode completely covered by a conducting layer of platinum–oxygen produced either by exposure to nitric acid[12] or to pure oxygen[1,13] is a true reversible electrode. Therefore, the oxygen–oxygen bond must be broken at the cathode before an electron is transferred instead of afterwards as in the mechanism described by equations 5–9.

At the reversible platinum–oxygen electrode, the potential is much more noble (by 200–300 mV) than at an irreversible platinum/oxygen electrode, as local cell action is missing. Also, oxygen is adsorbed at the reversible electrode on a complete layer of platinum–oxygen symbolized by Pt–O(O_2). Under these circumstances of high potential, the electrode surface is capable of breaking the oxygen–oxygen bond before electron transfer takes place. A possible mechanism may be visualized as follows. The oxygen–oxygen bond is broken,

$$\text{Pt–O}(O_2) \rightarrow 2\text{Pt–O(O)} \qquad (15)$$

followed by the reaction with an adsorbed water to form adsorbed hydroxyl radicals:

$$\text{Pt–O(O)} + \text{Pt–O}(H_2O) \rightarrow 2\text{Pt–O(OH)} \qquad (16)$$

Finally, water is formed from the discharge of absorbed hydroxyl radicals:

$$\text{Pt–O(OH)} + H^+ + e \rightarrow \text{PT–O}(H_2O) \qquad (17)$$

It can be seen that the cathodic mechanism, equations 15–17, is the reverse of the anodic mechanism, equations 2–4, as originally suggested by Bockris and Huq[1].

These mechanisms, however, can occur only on clean platinum electrodes covered by a complete layer of platinum–oxygen at low current densities, because it was observed[15] that after high anodic or cathodic polarization the system did not return to its original state. With anodic polarization, current goes into converting platinum–oxygen sites into platinum dioxide sites (see Fig. 4 of ref. 15) which impairs the complete layer of platinum–oxygen, so that under open-circuit conditions the local cell mechanism takes place with a resulting loss of potential. With cathodic polarization, the platinum–oxygen layer is also partially reduced, destroying the conditions for a reversible electrode. As a result, any kinetic data, such as Tafel slopes and i_0 values, obtained from polarization data at relatively high current densities do not relate to the reversible but to the irreversible platinum/oxygen electrode where the anodic and cathodic mechanisms are not the same. Values of ν calculated from equation 13 are meaningless. Also ν values determined from low current density data using

$$\nu = \frac{4Fi_0}{RT}\left(\mathrm{d}\eta/\mathrm{d}i\right) \qquad (18)$$

(where i_0 is the exchange current and $\mathrm{d}\eta/\mathrm{d}i$ is the slope of the linear E–i curve, see Fig. 1 of ref. 15), are doubtful because i_0 as obtained from the Tafel slope is unreliable. Values of i_0 between 10^{-9} and 10^{-10} amp/cm^2 recorded in the literature[1,2,4,13] relate to the irreversible platinum/oxygen electrode. The i_0 for the reversible platinum/oxygen electrode is between 10^{-5} and 10^{-6} amp/cm^2 (see Fig. 3 of ref. 15).

In conclusion, one must distinguish between two types of platinum/oxygen electrodes. The irreversible platinum/oxygen electrode, which is the one normally prepared, is a polyelectrode[16]; the rest potential is a mixed potential[17]; the anodic mechanism, equations 2–4, is different from the cathodic, equations 5–9; and in the reduction of oxygen, electrons are transferred before the oxygen–oxygen bond is broken, producing the stable intermediate, hydrogen peroxide. The reversible platinum/oxygen electrode prepared only by special techniques[1,12,13] is a single electrode[16]; the rest potential is determined by the oxygen/water reaction, equation 1; the anodic, equations 2–4, and the cathodic, equations 15–17, mechanisms are the reverse of one another; in the reduction of oxygen, electrons are transferred after the oxygen–oxygen bond is broken; and the only intermediate involved is the unstable adsorbed hydroxyl radical.

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ELECTRICAL CONTROL OF BEHAVIOUR BY PROGRAMMED STIMULATION OF THE BRAIN

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The ability to control electrically the behaviour of animals would greatly aid physiologists and behavioural scientists in the investigation of the neural mechanisms of motor activity, and the normal behaviour patterns involved in purposive movement and social interaction. Quite a number of experiments have shown that such an approach is feasible. For example, Hess[1] has shown that a wide variety of movements of the head, forelegs, hindquarters, and the muscles of the face may be elicited by electrical stimulation of diencephalic structures. Hess, and others, have further demonstrated that a "normal" progression of behaviour in time such as feeding, attack, and avoidance, can be initiated by electrical stimulation in one site. von Holst and von St. Paul[2,3] were able to obtain both simple movements and complex barnyard behaviour patterns in chickens by stimulation of sites in the brain stem. These forms of behaviour included sitting, standing, preening, seeking and eating food and drinking water, escaping from predators, and the like. Delgado[4] found that long term stimulation of the red nucleus of

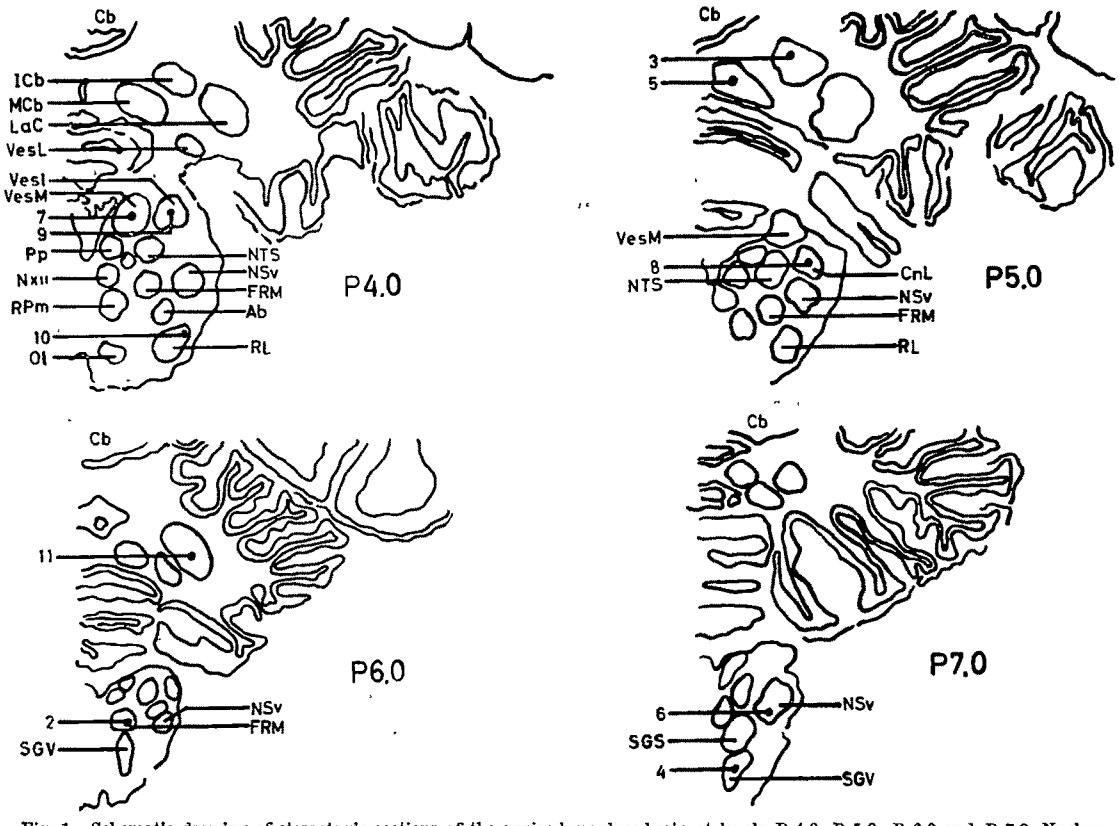


Fig. 1. Schematic drawing of stereotaxic sections of the squirrel monkey brain at levels *P* 4·0, *P* 5·0, *P* 6·0 and *P* 7·0. Nuclear classifications are according to ref. 8. Numbers refer to electrode positions on right side of animal that provided specific movements on stimulation (see Fig. 2). *Ab*, Nucleus ambiguus; *Cb*, cerebellum; *CnL*, nucleus cunneatus lateralis; *FRM*, formatio reticularis myelencephali; *ICb*, nucleus intermedius cerebelli; *LaC*, nucleus lateralis cerebelli; *MCb*, nucleus medialis cerebelli; *NSv*, nucleus tractus spinalis n. trigemini; *NTS*, nucleus tractus solitari; *Nxii*, nucleus n. hypoglossi,; *Ol*, nucleus olivaris inferior; *RL*, nucleus reticularis lateralis myelencephali; *RPm*, nucleus reticularis paramedianus myelencephali; *SGS*, substantia gelatinosa spinalis; *SGV*, nucleus substantiae griseae ventralis; *Ves I*, nucleus vestibularis inferior; *Ves L*, nucleus vestibularis lateralis; *Ves M*, nucleus vestibularis medialis

rhesus monkeys evoked a reliable sequence of behaviour such as bipedal locomotion, climbing, vocalization, and social interactions.

The difficulties with the results of these experiments are three. (1) In normal behaviour a given group of muscles are used in a wide variety of ways, but stimulation as in the experiments described does not allow for any variability in behaviour using the same electrodes. Rather, it appears that in most instances behaviour was initiated by stimulation of centres or pathways pre-existing at a high level of integration in the nervous system. (2) Unfortunately, histological analysis of the brain sites subserving the movements elicited in these experiments has not revealed a simple spatial separation into discrete centres, each of which might represent one specific function. (3) The type and complexity of the movements depended to a large extent on antecedent conditions, such as sleeping versus awake, reclining versus erect.

What is obviously needed, then, is a set of discrete brain centres which, whenever stimulated, always results in relatively simple movements (that is, at a relatively low level of integration), and the same movements each time the sites are stimulated. By combining or programming several different simple movements in time, one might produce a particular sequence of behaviour; by changing the programme, but still stimulating the same sites, a different sequence of behaviour would result.

A clue to this possible solution of the problem is suggested by experiments of Brodal, Pompeiano and others[5–7] on decerebrate rigidity. Their work showed that electrical stimulation of cerebellar nuclei, vestibular nuclei, and other nuclei in caudal brain stem produces unitary movements such as flexion or extension of a paw, foreleg or hip. Though simple, these elementary responses still require co-ordinated integration of synergistic and antagonistic systems, often involving as many as thirty different muscles. Nevertheless, the movements were quite discrete, and it was found that many of the nuclei were somatotopically organized, that a given locus of stimulation always produced the same kind of movement, and that some of the same movements could be produced by stimulation in more than one nucleus. To me, these results seemed admirably to answer the question of how to programme stimulation of the same sets of muscles in order to control many different behaviour sequences.

To this end, our first attempts have been directed toward control of the right arm and hand of a squirrel monkey (*Saimiri sciureus*) in the performance of various actions and to the determination of the level of organization of these movements in the brain. Preliminary experiments were carried out on five cats and six monkeys. A single concentric electrode was used stereotaxically[8] to explore various sites in animals anaesthetized with pentobarbital sodium (30 mg/kg in cats) and seconal (20 mg/kg in monkeys), in order to determine the type of movement elicited at each locus and the optimum electrical parameters required for the best results. Electrodes consisted of an outer stainless steel tube (0·5 mm outer diameter) insulated to within 1 mm of the end, and an inner stainless steel wire (0·1 mm outer diameter) insulated to its tip; electrode tip separation was 1·0 mm. Stimulation was provided with a Grass *S*-4 stimulator through an isolation unit, and the stimulating current monitored on a 'Tektronix 565' cathode-ray oscilloscope by measuring the *IR* drop across a 1,000 ohm resistance in series with the electrode. Exploration extended to 1 mm steps from stereotaxic co-ordinates *P* 3·0 to *P* 7·0 in the anterior–posterior plane, laterally from 0·5 to 3·5 on the right side, and from +1·0 to −13·0 in the horizontal plane.

Nuclei stimulated included the interpositus, fastigial, and dentate of the cerebellum, and the superior, medial, lateral, and inferior vestibular nuclei, as well as other brain stem nuclei (Fig. 1).

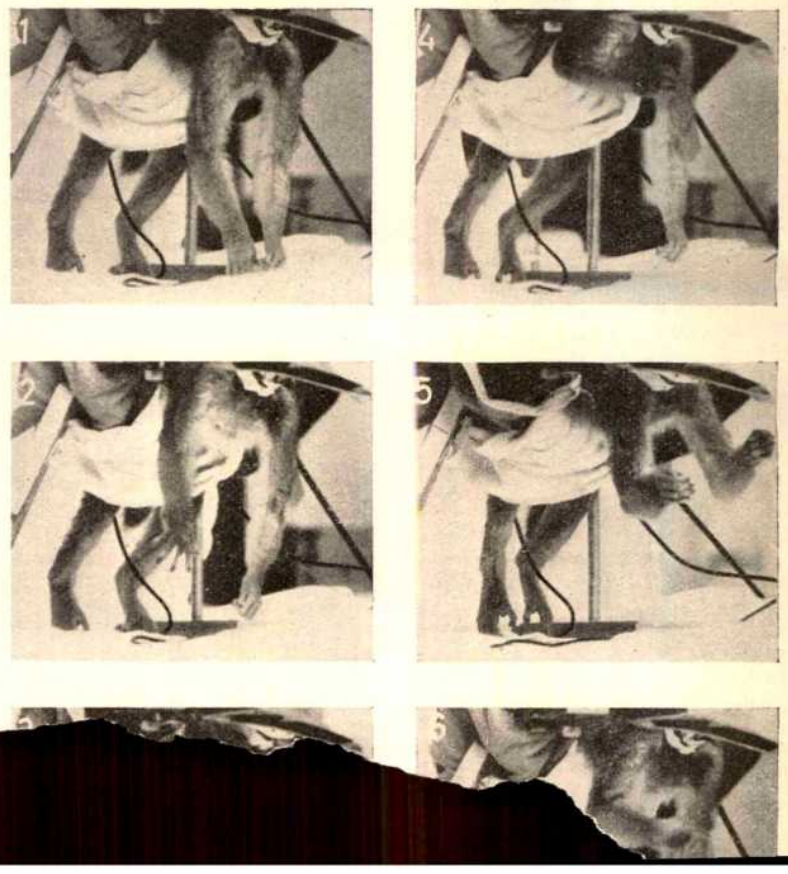
In each anterior–posterior–lateral stereotaxic locus, the stimulating electrode was moved vertically down 0·5 mm at a time, and pulsed current increased slowly from zero to 800 μamp, base-to-peak. Whenever a movement was elicited by a high current value, invariably the same movement was produced at a relatively low current value by simply lowering the electrode by 1·0–2·0 mm, or by placing the electrode laterally at the same depth. That locus where the lowest current produced the maximum movement effect was judged to be the site responsible for the movement, and the current used as the "threshold" current. Optimum electrical parameters at such threshold sites were usually found to be 200–300 rectangular pulses per second, 0·1–1·0 msec duration, and 50–200 μamp base-to-peak, monophasic with the inner electrode—the cathode. Each site was very specific, for if the electrode was moved up or down during threshold stimulation as little as 100μ the elicited movement ceased; it could be re-elicited by an increase in current or repositioning of the electrode at the former site. In all the locations examined, the mechanical effect of movement was proportional to current strength so that very finely graded movements could be obtained.

Some of the simple, non-programmed movements produced by this exploratory stimulation in an anaesthetized monkey are shown in Fig. 2; the position of the stimulating electrode in each case is indicated in Fig. 1. At any given locus an increase in current significantly above threshold (for example, up to 800 μamp in photographs 4, 5 and 6 of Fig. 2), produced more than one fragmentary response. Presumably this resulted from spread of current to adjacent sites, as movement of the electrode up, down or laterally produced each movement separately at low current values. In addition to the grasping and extension of the fingers, the extension and flexion of the limbs at the wrist, elbow, shoulder, ankle, knee, or hip, we also found sites for opening and closing the mouth as in mastication (site No. 10), movement of the tongue in or out as in licking (not shown), curling or sideways movement of the tail (not shown), and were able to control heart rate and rate and volume of respiration.

Programmed stimulation was achieved in three additional squirrel monkeys. Unfortunately, all three died as a result of oedema of the brain stem before motion picture camera was avail record their movements. Ho movements can be descri the technique. U trodes were chr

waveform and pulse generators) and additional stimulators to produce the desired effects. Most of the sequences chosen were based on the naturally occurring movements observed in the awake animal before implantation, and the particular simple movements we were able to elicit at each electrode site after implantation. Several arbitrary combinations were also used to explore other possible movements not previously observed in the monkeys. The elementary movements in the three monkeys were very similar when stimulated in homologous loci, and a given sequence of stimulation produced essentially the same sequence of behaviour in each animal. Abnormally appearing temporal sequences or "slow motion" behaviour could be obtained as well.

In one programme of stimulation (sequence 7 for 0·3 sec; 7 & 2, 0·1 sec; 7 & 8, 0·6 sec; 3, 0·2 sec; 3 & 9, 0·2 sec) the awake monkey would grasp an object (for example, monkey chow) with its right hand and rapidly and smoothly bring the object to its mouth. This sequence could be repeated as often as desired, and the movements could not be discriminated by observers to be different from that which occurred when the animal voluntarily performed the same act. Using the same set of electrodes, different programmes produced different sequences of movement. For example, sequence 9, 0·1 sec; 9 & 7, 0·5 sec; 9 & 7 &



2, 0·1 sec; 9 & 7 & 8, 0·3 sec, produced extension of the arm outward and upward as in reaching or climbing, while sequence 9, 0·1 sec; 9 & 11, 0·4 sec; 7, 0·4 sec; 9 & 3 & 11 & 2, 0·4 sec; etc., produced extension of the arm backward over the body with a back-and-forth movement (as in scratching) at the base of the tail. It appeared that an almost endless repertoire of sequential movements could be elicited in this limb by simply changing the programme and stimulus duration of the same set of electrodes. In all cases, the electrical stimulation over-rode and pre-empted any simultaneous voluntary movement of the animal, and a movement sequence could be elicited in its essential form whatever the position of the animal (provided the limb was free to move) and whether the monkey was asleep, awake, or under anaesthesia.

At the levels of the central nervous system where these stimulations were performed, it is most unlikely that individual nerve fibres were selectively excited and in the proper temporal sequence to produce such complex and organized movements. Rather it would seem that, as Delgado[9] has suggested, behaviour is composed of integration of "behavioural fragments", and each "behavioural fragment" has a specific neuronal organization pre-existing in an anatomical structure. The organized structures in this investigation were presumably triggered by the increase in tonic activity at the sites of electrical stimulation, giving pre-eminence to one or more functional motor responses to the exclusion of others.

In order to isolate the level of the organization of the elementary responses, various lesions were made in several animals anterior to the electrode sites. Destruction of the left motor cortex for the right hand and arm in one of the chronic squirrel monkeys produced paralysis to voluntary movement in that limb as it does in man. Yet, both single and programmed movements were produced after the lesion that did not differ in any way from those elicited before the monoplegia. In two of the acute monkeys, in which electrodes were located in the vestibular nuclei at the level of P 5·0, a complete section of the brain stem immediately anterior to the stimulating electrode did not alter the movements elicited by stimulation before the section was made. From histological investigations in another animal, it was found that the electrode producing integrated movement had actually been in the spinal cord at about C 2. Apparently, the organization of these elementary movements exists at the level of the spinal cord, and they are elicited directly by way of efferent pathways or reflexly by stimulation of afferent fibres.

Apart from the oedema produced in the chronic animals (which we are trying to correct by using smaller, multiple electrodes in a single chain), these results suggest that programmed stimulation of the brain can be a powerful research tool in initiating, modifying, and controlling behaviour. For example, we are at present mapping the entire neuraxis in order to determine the type, complexity, and extent of movement that may be elicited at each site and the electrical parameters required. Simultaneously, we are observing by means of cinematography the complex behaviour of primates in isolation and in social interrelations with other primates. By combining the two investigations with programmed stimulation in chronically implanted animals, we hope rigorously to control their movements in order to determine the fundamental nervous mechanisms governing purposive motor behaviour. Because the level of integrative organization of the elementary responses elicited in this investigation is in the caudal brain stem and spinal cord, it may even be possible in the future to stimulate the brains of human beings in a similar way afflicted with paralysis from cerebral strokes, Parkinsonism, epilepsy and other diseases of the central nervous system, in order to restore normal motor function and voluntary control of purposive movement.

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AN OPERATOR MODEL OF CROSSING-OVER

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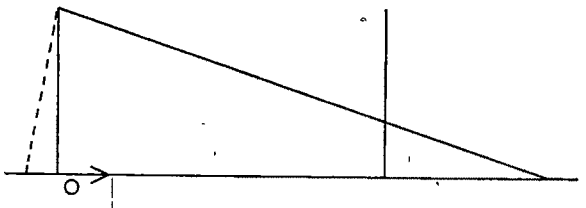


Fig. 1. Diagram to show the postulates of the operator model of crossing-over. The vertical lines show the ends of a gene, the unbroken sloping line the extent of formation of cross-over (and double cross-over) hybrid DNA originating from primary breaks at the gene end at the top of the slope, and the broken line shows the extent of double cross-over hybrid DNA in a reverse cross-over. The letter *O* and the arrow show the position of the operator and the direction of opening of the gene

bring this about has much shorter hybrid DNA segments. The process of reverse crossing-over would require dissociation of the nucleotide-chains, synthesis and hybrid DNA formation on the other side of the primary breakage-point ('opening-point') from which the original cross-over was initiated. These hybrid DNA segments would extend a short distance into the neighbouring gene on the other side. This model of crossing-over is shown diagrammatically in Fig. 1.

Hastings and I[4] pointed out the resemblances between the postulates of the polaron hybrid DNA model and the likely events in messenger RNA (*m*RNA) formation. It remains uncertain whether *m*RNA is synthesized along the intact duplex DNA of the gene (or group of genes forming an operon), or whether a single chain acts as the template after breakage of one chain at the end of the gene (or the operon) and dissociation of the chains over its length. In either event, it would seem that a recognition site, that is, an operator, must exist at one end of the gene (or the operon), where synthesis of the messenger is initiated. According to Jacob and Monod[6], the operator is not only the initiation site for the transcription of the messenger, but also the receiving site for a specific repressor produced by a regulator gene. Through the mediation of the operator, the repressor is thought to block the initiation of transcription.

Like transcription, the recombination mechanism proposed here requires recognition of one particular end of each gene. Moreover, there is evidence (given later) for a control mechanism for recombination similar to that postulated by Jacob and Monod[6] to control transcription. In view of these similarities, it is proposed to call this recombination mechanism an operator model of crossing-over. The possibility that the recombination and transcription operators are identical is discussed later.

Many of the predictions of the operator model resemble those of the polaron model. The chief differences concern the patterns of polarity in recombination within the gene, and the interrelations of neighbouring genes in recombination.

Polarity in Intragenic Recombination

Two primary patterns of intragenic polarity are predicted by the operator model, depending on the direction of dissociation of the DNA in the neighbouring gene beyond the terminus (non-operator) end of the first gene. If this neighbour showed the same direction of dissociation, the first gene would have all its cross-over hybrid DNA arising from the operator end, whereas if the neighbour showed the opposite direction, both genes would have cross-over hybrid DNA entering from both ends. Allowing also for the effects of reverse cross-overs, eight patterns of recombination within genes are predicted, as shown in Fig. 2. The alternatives depend on the orientation of the gene and of its neighbours on each side.

Hastings and I[4], from an investigation of published data, found two primary patterns of intragenic recombination such as this model predicts. In unipolar genes all the dissociation of nucleotide-chains leading to hybrid DNA formation and crossing-over appeared to originate from one end of the gene, while in bipolar genes hybrid DNA appeared to originate from primary breaks at (or beyond) either end of the gene, but with one end predominating. In Table 1, nine genes are classified in terms of these patterns of polarity in recombination, and also with respect to the predominant direction of the polarity relative to the position of the centromere, where this is known. It is evident that both unipolar and bipolar genes may be orientated in either direction relative to the centromere. As already mentioned, the two basic patterns of polarity within the gene correspond to the predictions of the operator model, genes 1, 2, 7 and 8 in Fig. 2 being unipolar and 3–6 bipolar. Moreover, the genes *ad*-9 (adenine-9) and *paba*-1 (*para*-aminobenzoic acid-1) in *Aspergillus nidulans* are thought to be neighbours, and their patterns of polarity are appropriate to a bipolar pair such as 3 and 4 (or 5 and 6) in Fig. 2. The model predicts that if the two ends of a gene are denoted by *O* (operator) and *T* (terminus), at the *T* end of a unipolar gene the neighbouring gene has its *O* end nearest, while the *T* neighbour of a bipolar gene has its *T* end nearest. Applied to *ad*-9 and *paba*-1, it is suggested that the *T* ends of these genes adjoin one another.

Table 1. Patterns of Intragenic Polarity in Recombination

Predominant direction of polarity with respect to the centromere	Pattern of polarity: Unipolar	Pattern of polarity: Bipolar
Proximal	*Saccharomyces cerevisiae* *hi*-1 (ref. 7)	*Aspergillus nidulans* *ad*-9 (ref. 8)
Distal	*Neurospora crassa* *me*-2 (ref. 9)	*Aspergillus nidulans* *ad*-8 (ref. 10); *paba*-1 (ref. 11); *Neurospora crassa* *cys* (ref. 12); *his*-5 (ref. 13)
Unknown	*Ascobolus immersus* series 46 (ref. 14)	*Ascobolus immersus* series 19 (ref. 14)

Genes are expected to vary in length depending on the length of the polypeptide for which they code. If the length of hybrid DNA arising from an opening-point were approximately constant in terms of nucleotide-pairs (ignoring the effects of mutations within the hybrid region), the pattern of recombination within a gene would be expected to depend to some extent on the length of the gene and of its immediate neighbours. The rather slight evidence of polarity in the gene *pan*-2 (pantothenic acid-2) in *Neurospora crassa* investigated by Case and Giles[15] would be accounted for if it and a neighbour formed a bipolar pair and if one or both of them were comparatively short. A similar explanation might apply to *hist*-3 (histidine-3) investigated by Webber[16]. By the same argument, more than two genes might sometimes be included in the hybrid DNA from an opening-point.

On the polaron hybrid DNA model, the chromosome was divided into segments or polarons defined by the opening-points and within which recombination was entirely by conversion. On the operator model, polarity is a consequence of the fixed direction of opening in each gene and the term polaron ceases to have a precise meaning,

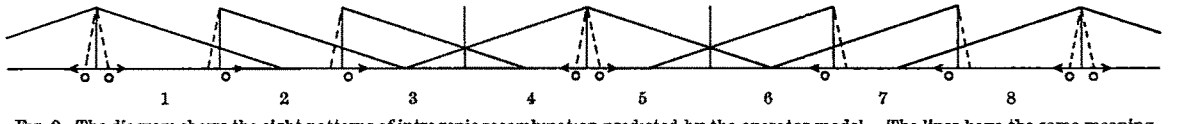


Fig. 2. The diagram shows the eight patterns of intragenic recombination predicted by the operator model. The lines have the same meaning as in Fig. 1

because hybrid DNA may extend through one gene into the next and so may pass another potential opening-point.

Reverse Cross-overs

If a sufficient number of allelic mutants were available in a recombinant selection experiment with outside markers, it is likely that some of the alleles would be close to the ends of the gene, and these might allow detection of reverse cross-overs, indicated by the broken lines in Fig. 2. The operator model predicts that an excess of parental combinations for outside markers, such as a reverse cross-over would give, is expected in unipolar genes either at the *T* end (genes 2 and 7 in Fig. 2) or at both ends (genes 1 and 8) and in bipolar genes either at the *O* end (genes 4 and 5) or at neither end (genes 3 and 6).

In the gene in which recombination has been investigated by prototroph selection using the largest number of mutant sites, namely, *me*-2 (methionine-2) in *Neurospora*, Murray[9] found an excess of parental arrangements for the outside markers when one of the methionine mutants was at the distal end of the gene map. This would be the *O* end on the operator model, since *me*-2 seems to be unipolar with distal polarity. There is also evidence from her data that these reverse cross-overs have shorter hybrid DNA segments than the primary cross-overs (see p. 80 of ref. 5). The model predicts reversed polarity at the other end of the gene (compare with gene 8 in Fig. 2), and this Murray[17] has now found but without the expected excess of parental combinations.

Fogel and Hurst[7] have found a pattern of recombination in *hi*-1 (histidine-1) in *Saccharomyces cerevisiae* similar to that in *me*-2 in *Neurospora*, except that *hi*-1 has proximal polarity. When the mutant at either end of the *hi*-1 map is crossed with an allele, prototroph selection gives a higher proportion of parental combinations of outside markers than in most other allelic crosses, indicating reverse cross-overs at each end. There is also evidence that at the proximal end of the gene these reverse cross-overs have shorter hybrid DNA segments than the primary cross-overs, and that at the distal end they have reversed polarity. These findings are expected if *hi*-1 is like gene 1 in Fig. 2.

Investigation of recombination either by prototroph selection with allelic nutritional mutants, or by aberrant ascus selection with allelic ascospore colour mutants, and outside markers present, would allow further tests of the predictions of the model regarding reverse cross-overs, provided mutants were available situated near the ends of the gene as well as in other positions.

Bipolar Genes

The operator model predicts that at the *T* end of a bipolar gene hybrid DNA will arise about equally often from proximal and from distal directions (compare Fig. 2, junction of genes 3 and 4, or of 5 and 6). Such equality is evident at one end of all the bipolar genes that have been investigated by prototroph selection with outside markers (see diagrams in ref. 5). A further example is provided by the work of Smith[13] with *his*-5 (histidine-5) in *Neurospora*. His data are plotted in Fig. 3 using the method adopted by Hastings and myself[5]. The sequence of the mutant sites is shown at the top without the recombination frequencies, which among the crosses shown ranged from 2·1 per 10^5 viable ascospores for $K155 \times K542$ to 37·4 per 10^5 for $K516 \times K548$. The proximal end of the gene (which is in the right arm of linkage group IV) is to the left. For each allelic cross (indicated by the horizontal lines on the left of Fig. 3) the figures show the number of histidine-independent progeny which were found to have a parental combination of the outside markers. Only those crosses which gave more than twenty such progeny are included. The explanation of the graph is as follows.

If M/m and N/n denote the outside markers on the proximal and distal sides, respectively, of the nutritional locus, which has alleles 1 and 2 with 1 nearer to M/m and 2 to N/n, then from the cross:

$$\frac{M\ 1\ +\ N}{m\ +\ 2\ n}$$

the prototrophs may be of four genotypes, namely:

$$\begin{aligned} A:&\ M\ +\ +\ N \\ B:&\ m\ +\ +\ n \\ C:&\ m\ +\ +\ N \\ D:&\ M\ +\ +\ n \end{aligned}$$

Those of class *A*, that is, with additional recombination between the proximal allele (+/1) and the proximal outside marker (M/m), may have arisen through hybrid DNA reaching the site of allele No. 1 alone, such as might occur if the opening-point was on the proximal side of the gene. Conversely, prototrophs of class *B* might have arisen through hybrid DNA reaching the site of the distal allele (No. 2) alone, as from an opening-point on its distal side. Both *A* and *B* classes of prototroph might also have arisen as a result of hybrid DNA extending to both sites (1 and 2) from an opening point on the proximal or on the distal side of them. Hybrid DNA extending from one

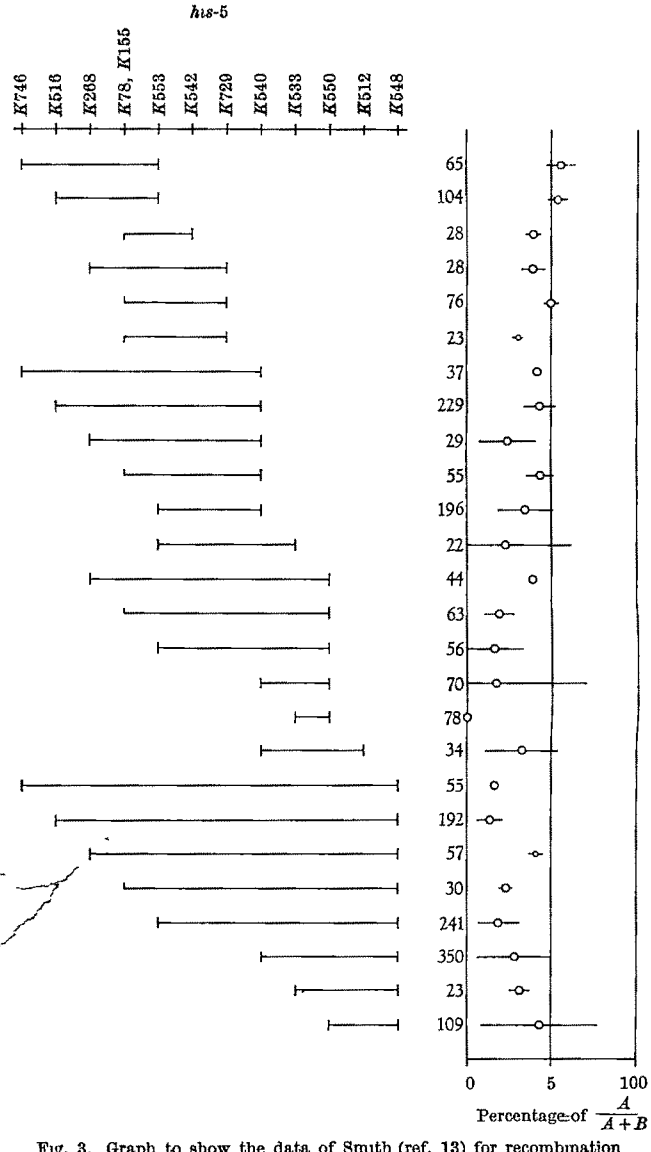


Fig. 3. Graph to show the data of Smith (ref. 13) for recombination within *his*-5 of *Neurospora crassa*. The diagram is explained in the text. Crosses involving *K*78 or *K*155 are distinguished by the horizontal line turning up or down, respectively

end to both sites is expected to contribute equally to the *A* and *B* classes. It is assumed that in the prototrophs any hybrid DNA entering the site underwent correction of mispairing to wild-type, as there was selection for this character. The points plotted in Fig. 3 show for different pairs of *his*-5 alleles the percentage frequency of class *A* prototrophs out of the total number of prototrophs with parental combinations of the outside markers ($A + B$).

Class *C* prototrophs could arise as a result of hybrid DNA reaching one site only (the proximal or the distal one) or both sites, while class *D* prototrophs require hybrid DNA to have reached both sites. Hybrid DNA at both sites is expected to give class *C* and class *D* with equal frequency. Hence $\frac{2D}{C+D}$ is a measure of the frequency of double-site hybrid DNA. The horizontal lines on each side of the points in Fig. 3 show for each cross the percentage frequency of class *D* prototrophs out of the total number of prototrophs with non-parental combinations of the outside markers ($C + D$). The total length of each line is $\frac{2D}{C+D}$ and is plotted on the same percentage scale as was used for the points in the graph, and with the half-length $\left(\frac{D}{C+D}\right)$ extending on each side of these points. In calculating $\frac{D}{C+D}$ a correction has been made for the distance of the outside marker genes, using the method proposed by Hastings and myself[5]. It has been assumed in making this correction that the proximal and distal markers (pyrimidine-3 and leucine-2) showed 1 per cent and 9 per cent recombination, respectively, with *his*-5. For most of the crosses the value of $C + D$ was approximately equal to that for $A + B$.

On the basis of these interpretations of the *A*–*D* classes, the horizontal lines on each side of the points in Fig. 3 show the contribution to the prototrophs due to hybrid DNA at both mutant sites. This hybrid DNA might have come from an opening point on the proximal or on the distal side of the sites. The interval between the left-hand end of each line and the left-hand axis $\left(\frac{A}{A+B} = 0\right)$ hybrid shows the frequency with which DNA has reached the proximal site alone, and similarly the right-hand interval shows the contribution from the distal site alone.

Smith's data suggest that hybrid DNA reaches the mutant sites within *his*-5 predominantly from the distal side of the gene, except near the proximal end, where the contributions of proximal and distal origin appear to be equal. As already indicated, this equality is expected on the operator model, for the *T* end of a bipolar gene.

Neighbouring Genes

The operator model makes different predictions from the polaron model concerning the behaviour of neighbouring genes in recombination. The polaron model predicted that *D* class prototrophs should not occur with two mutants in neighbouring genes, as the formation of hybrid DNA at both sites, such as the *D* class requires, would then need a double crossover from the opening-point, which would be between the sites, and this would give *A* or *B* class prototrophs, not *D*. On the operator model, however, *D* class prototrophs are expected with mutants in neighbouring genes, provided both mutants can be reached by hybrid DNA extending in one direction from an opening point. This is most likely to happen if the mutant site in one gene is near the end which adjoins the other gene. No *D* class would be expected when the neighbouring genes have adjoining operators, such as numbers 4 and 5 in Fig. 2.

A further prediction of the operator model is that the presence of a mutant in one gene may affect recombination in a neighbouring gene, as there is evidence that mutants interfere with the formation of hybrid DNA[3,5]. In particular, with a bipolar pair of genes such as 3 and 4 (or 5 and 6) in Fig. 2, the presence of a mutation in the proximal gene might be expected to weaken the proximal component of recombination in the distal gene, and conversely a mutant in the distal gene would be expected to reduce the distal component in the proximal gene.

Genetic Control of Recombination

Direct support for the operator model is given by the work of Jessop and Catcheside[18] with a gene *rec* (recombination) which affects recombination within the gene *his*-1 (histidine-1) in the right arm of linkage group V in *Neurospora*. In the presence of the recessive mutant *rec* in homozygous condition, the recombination frequency between *his*-1 mutants was more than ten times greater than when the dominant allele rec^+ was present. The recombination frequencies per 10^5 progeny with homozygous *rec* ranged from 3·61 for the allelic cross $K83 \times K627$ to 51·3 for $K83 \times K316$, and with rec^+ from 0·24 for $K566 \times K617$ to 3·96 for $K83 \times K616$. Moreover, the pattern of polarity in recombination within *his*-1 appears to be profoundly modified by *rec*. This is shown in Fig. 4, where the data are plotted in the same way as Smith's results for *his*-5 given in Fig. 3. Data relating to mutant *K*620 have been omitted as its map position is uncertain. In correcting for the distance of the outside markers (*amination* on the proximal side and *inositol* on the distal side), it has been assumed that they showed 3 per cent and 7 per cent recombination, respectively, with *his*-1. Where *spray* was used as proximal marker, its recombination frequency with *his*-1 was taken to be 7 per cent. The numbers in classes *C* and *D* taken together were usually about 40 per cent of the total prototrophs.

As with Fig. 3, the contribution to the prototrophs from each cross due to hybrid DNA at the proximal mutant site alone is indicated by the space between the left-hand end of each horizontal line and the vertical axis corresponding to $\frac{A}{A+B} = 0$. Similarly, the frequency of prototrophs due to hybrid DNA at the distal site alone is indicated by the space between the right-hand end of each line and the vertical line representing $\frac{A}{A+B} = 100$ per cent. The horizontal lines themselves show the contribution due to hybrid DNA at both sites, and this may be of proximal or distal origin. The simplest hypothesis is to assume that this double-site hybrid DNA shows the same proportion of proximal and distal origin as the single-site hybrid DNA.

It is evident that in the presence of homozygous *rec*, *his*-1 shows predominantly proximal polarity, while when rec^+ is present the recombination within *his*-1 appears to be entirely of distal origin. On the operator model this would be explained if rec^+ is a repressor for *his*-1 and if *his*-1 is opened at the proximal end. When rec^+ is present all the recombination within *his*-1 would then be due to hybrid DNA originating at an opening-point at the far end of the next gene to *his*-1 on the distal side, and extending through that gene to enter *his*-1 at the distal end. The recessive mutant *rec*, when homozygous, would prevent the *his*-1 repressor from functioning. This would explain both the effects of *rec*, that is, the much increased frequency of recombination within *his*-1 and the proximal polarity, assuming the *his*-1 operator is at the proximal end. Further data are needed to test whether the relatively little recombination within *his*-1 of distal origin is affected by *rec*.

Jessop and Catcheside[18] found that the recombination frequency between the outside markers *am* and *inos* was not lowered significantly when *rec* was replaced by rec^+.

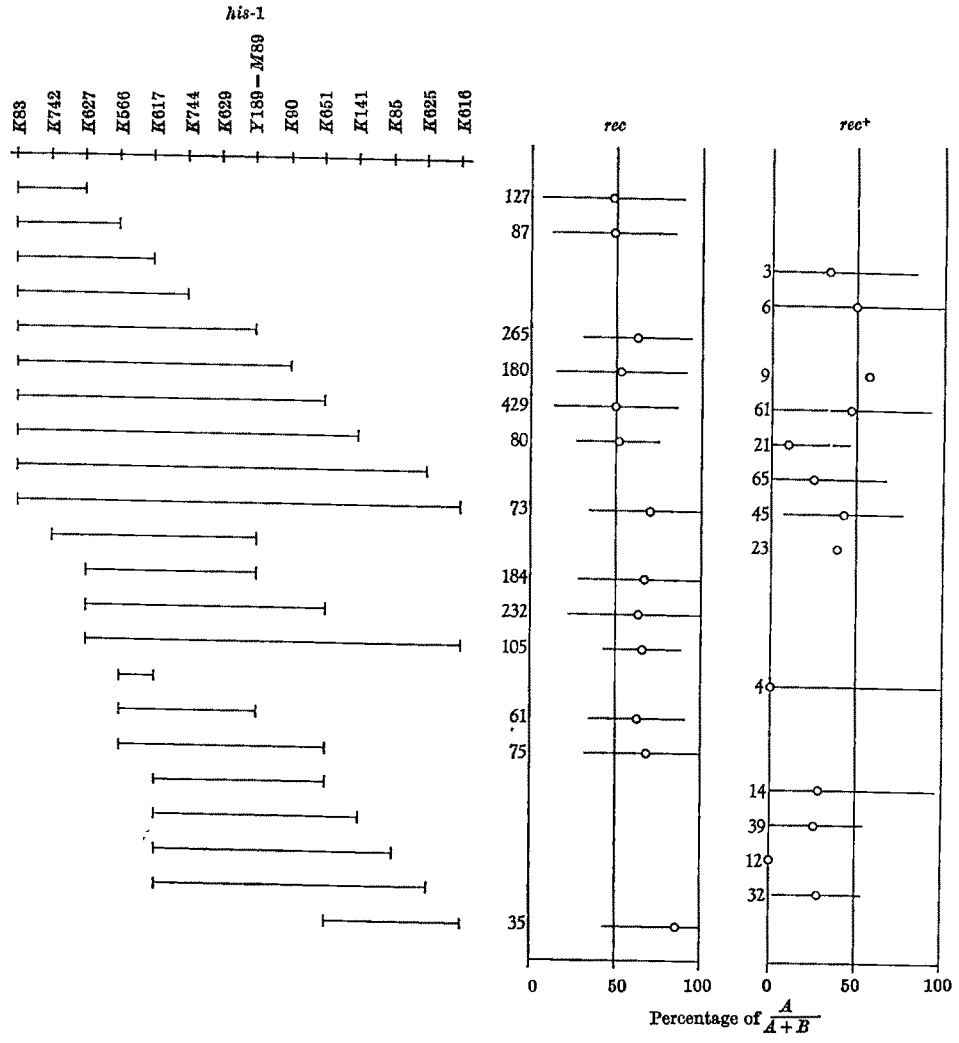


Fig. 4. Graph to show the data of Jessop and Catcheside (ref. 18) for recombination within *his*-1 of *Neurospora crassa*. The results with homozygous *rec* and in the presence of *rec*$^+$ are shown separately. The diagram is explained in the text

This might be accounted for if the effect of *rec* were masked by heterozygosity for repressors of recombination in other genes in the *am-inos* interval.

If the recombination and messenger operators were the same, *rec* would be expected to be a regulator for the functioning of *his*-1 as well as for its recombination. It is evident that study of genes such as *rec* may provide a direct approach to an understanding of the mechanism of control of gene activity, as well as to that of recombination. If for each gene there is a common operator for recombination and for transcription, the predominant direction of polarity in recombination (in the de-repressed gene) will coincide with the direction of messenger synthesis, since it has now been established that the messenger is synthesized from the operator end[19]. Furthermore, in an operon containing more than one gene, polarity in recombination is expected not to relate to the junctions between genes within the operon, but to the ends of the operon, and predominantly to one of them. These predictions provide a means of testing the hypothesis of a common operator for recombination and transcription.

It is significant that if dissociation of the nucleotide chains of DNA were to occur in the formation of *m*RNA, it would be necessary for the dissociation to extend beyond the end of the gene in order to expose the whole of its length for transcription. A similar extent of dissociation is postulated on the operator model of recombination, suggesting that if these mechanisms for transcription and recombination are true they may have had a common origin in evolution. It also appears significant that if crossing-over occurs by the opening of structural genes through their de-repression, this would explain the lack of crossing-over in heterochromatic regions of chromosomes[20] if, as evidence suggests, heterochromatin contains only regulator genes. Such genes appear to have other recombination mechanisms, which cause transposition to new locations[21] and specific factor transfer[22].

Summary

It is suggested that dissociation of nucleotide-chains, before the formation of hybrid DNA in crossing-over, occurs in a fixed direction from one specific end of each gene, and extends through it into the next gene. On this operator model, unipolar and bipolar genes are determined by the orientation of the neighbour. It is further postulated that recombination events within a gene, associated with a parental arrangement for marker genes outside, are due to a second or reverse cross-over, with short hybrid DNA segments extending in the opposite direction into the neighbouring gene on the other side. It is shown how this model can account for a number of previously unexplained features of recombination, including data on genetic control. Experimental tests of the model are

outlined. The possibility is discussed that for each structural gene (or group of genes forming an operon) there may be a common operator and a common regulator gene for both transcription and recombination.

I thank Dr. C. Bresch, Dr. F. Cooke, Dr. H. Gutz, Dr. P. J. Hastings and Dr. S. F. H. Threlkeld for invaluable discussion and criticism during the preparation of the manuscript, and Dr. S. Fogel, Dr. D. D. Hurst and Dr. N. E. Murray for permission to refer to their unpublished work. I thank Mr. G. J. Clark for preparing the diagrams.

Note added in proof. I am grateful to Dr. J. R. S. Fincham for directing my attention to the work of Luzzati[23], who has found evidence for a common regulation mechanism for recombination and transcription. Luzzati *et al.*[24] discovered that non-reciprocal mitotic recombination between alleles at the *ad*-3 locus in *Saccharomyces cerevisiae* is inhibited by histidine, which is an end-product of a biosynthetic pathway affected by this gene, since *ad*-3 mutants require adenine and histidine for growth. Luzzati[23] has now found, not only that this inhibition of conversion is specific to L-histidine and to the *ad*-3 gene, but also that a dominant mutant, *S*9, and a recessive mutant, *s*15, not linked to one another or to *ad*-3, suppress the requirement for histidine but not that for adenine in *ad*-3 mutants, and also inhibit recombination within *ad*-3, whether histidine is present or not. A dominant mutant, *S*24, apparently due to mutation within *ad*-3, suppresses the histidine requirement but does not affect the intragenic recombination, which is inhibited when histidine is added. Detailed interpretation of these results requires more information than is known at present about the function of the *ad*-3 and suppressor loci, but a relationship between recombination within a gene and its activity is manifest.

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HYPERPLOIDY EFFECT OF LYMPHOCYTE EXTRACT ON FIBROBLASTS *IN VITRO*

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A RELATIVELY high frequency of thyroid autoantibodies has recently been observed in patients with diseases characterized by chromosomal aberrations, such as gonadal dysgenesis[1–3] and Down's syndrome[4]. These observations together with the older reports of thyroid dysfunction and goitre in the mothers of children with Down's syndrome[5–7] led us to suggest that immunological aberrations in a parent may be related to the production of chromosomal abnormalities in the offspring[8]. Afterwards, more evidence along these lines was provided by the demonstration of a significantly increased frequency of thyroid autoantibodies in the mothers of children with Down's syndrome[9]. To examine further the possibility that immunological mechanisms predispose to chromosomal aberrations, an investigation of the hyperploidy effect of lymphocyte extracts on mitoses of fibroblasts in cell culture was undertaken.

Human lymphocyte-rich plasma was prepared under sterile conditions by allowing the red blood cells to sediment and the polymorphonuclear cells to adhere to glass[10]. The cells were washed three times in a phosphate isotonic buffer (*p*H 7·1), diluted to a concentration of 2 or 4 × 10^6 lymphocytes/ml., and crude extracts prepared by rapid freezing and thawing ten times. The extracts were stored frozen and retained their activity for at least 3 weeks. No relationship between the extent of contamination by polymorpho-nuclear or red blood cells and the degree of hyperploidy was evident. No effort was made to determine what, if any, part of the hyperploidy effect resulted from monocytes. The cell culture and cytological techniques used in these experiments have already been described[11,12].

Varying doses of extract were added directly to 80,000–100,000 freshly transferred fibroblasts growing in Waymouth's medium supplemented with 15 per cent foetal calf serum. After 3–5 days the cells were collected and scored for hyperploidy. To allow the screening of a large number of cells, slides were screened under low power and if a mitosis appeared to be hyperploid, the chromosomes it contained were counted under higher magnification. Cells with 55 or more chromosomes were scored as abnormal. Fifty-five was chosen as the dividing line since cells with that number of chromosomes usually appear hyperploid under low power whereas cells with fewer chromosomes can be missed. No effort was made to score for the type of hyperploidy (for example, triploidy or tetraploidy) except in the case of endoreduplication.

At the time of use the cultures varied in age from two to twenty-seven transfers and for each experiment duplicate cultures not treated with lymphocyte extract served as controls. For each of these, and for each dosage of extract, 500–1,000 metaphases were usually scored for hyperploidy. In order to get an estimate of the error involved in the culturing and scoring procedure, fourteen replicate pairs were sub-cultured and scored separately. The mean difference between pair members was 0·4 per cent with a standard deviation of 0·31 per cent. The range was from 0 to 1 per cent.

In untreated fibroblast cultures obtained from skin, the percentage of mitoses which were hyperploid by our criteria ranged from 2 to 17 but most cultures had 3–5 per cent hyperploidy. Fibroblasts obtained from muscle seemed to have higher control levels. Endo-

reduplicated mitoses were observed in seven of twelve untreated skin cultures and in three of four untreated muscle cultures.

Lymphocyte extracts from four patients with "autoimmune" diseases were tested on allogeneic fibroblasts and the results are given in Table 1. Subjects 1, 3 and 5 have Hashimoto's thyroiditis and subject 6 has "autosensitization" eczema. Subject 7 is also included in Table 1 although, as indicated later, her illness is not clearly defined. In each case significant increases were observed (greater than three times the standard deviation of the experimental error obtained from the replicate cultures as already mentioned). Moreover, there was a strong correlation with dosage of lymphocyte extract.

Increases in endoreduplication were observed in most experiments. These increases appeared to be correlated with the overall degree of hyperploidy and may not result from a separate effect.

In order to see whether the hyperploidy effect was specific for extract obtained from patients with "autoimmune" diseases, lymphocyte extracts from eleven "normal" subjects (for example, no known "autoimmune" or chromosomal disorders) were tested on allogeneic fibroblasts. Thirteen of fifteen allogeneic extract-fibroblast combinations produced significant responses, and again there was a strong correlation with dosage (Table 2). The degree of response elicited by the same dose was not the same for each extract, and also the control and induced frequencies of hyperploidy seemed to depend in part on the nature of the fibroblast cultures. One of the two extracts which did not induce a significant increase in hyperploidy was retested on a second recipient and appeared to give a mild response (subject 18). Extract from one subject was cytotoxic (subject 16).

In order to test the specificity of the reaction, autogeneic lymphocyte extracts were tested, and the results were compared with results obtained when the same extracts were tested simultaneously on allogeneic fibroblasts (Table 3). There were two of seven extracts from "normal" subjects that showed slight responses (subjects 10 and 14). However, none of the seven extracts tested showed an effect on autogeneic fibroblasts near the order

Table 1. HYPERPLOIDY EFFECT OF LYMPHOCYTE EXTRACT FROM PATIENTS WITH "AUTOIMMUNE" DISORDERS ON ALLOGENEIC FIBROBLASTS

Donor No.* (lymphocyte extract)	Recipient No. (fibroblast† culture)	No. of transfers at time of examination	Hyperploidy (per cent) Extract dose (×10⁶ lymphocytes) 0	1	2	4	Endoreduplication (per cent) Extract dose (×10⁶ lymphocytes) 0	1	2	4
1	2	27	2·2	5 2			0·0	0·8		
3	4	4	4·5	9·4	14·1		0·0	2·5	4·0	
5	2	6	4·6	9 4	14·0		0·0	0·2	2·2	3·4
5	2	22	2·5		7·2		0·0		1·0	
6	2	27	2 2	4·6	11·2	25·0	0·0	0 0	1·0	6 0
7	5	7	5·6	9·0	13·0	22·0	0·3	0 2	2·6	6·7

* The diagnoses are: subject 1, 46-year-old female with Hashimoto's thyroiditis (H.T.) and a child with Down's syndrome (D.S.); 3, 25-year-old female with H.T. and gonadal dysgenesis; 5, 18-year-old female with H.T. and *XO/XX* mosaicism; 6, 29-year-old male with "autosensitization eczema"; 7, see text.

† The fibroblasts were obtained from skin biopsies except for 1 gingival biopsy (subject 7).

Table 2. HYPERPLOIDY EFFECT OF LYMPHOCYTE EXTRACT FROM "NORMAL" SUBJECTS ON ALLOGENEIC FIBROBLASTS

Donor No.* (lymphocyte extract)	Recipient No. (fibroblast† culture)	No. of transfers at time of examination	Hyperploidy (per cent) Extract dose (×10⁶ lymphocytes) 0	1	2	4	Endoreduplication (per cent) Extract dose (×10⁶ lymphocytes) 0	1	2	4
8	9	5	4·6	6·4			0·2	0·2		
10	11	12	3·8	6 2	8·6	13·2	0·0	0·0	0·2	0 5
12	11	16	4·0	6·2			0·0	0·0		
2	7	5	3·5		6·0		0·0		0·0	
13	2	7	4·8		10·2		0·0		0 6	
13	14	7	7·1		18 0		0·4		6·7	
14	2	7	4·8		10·4		0·0		0·4	
14	13	7	4·8		9·2		0·0		0·0	
15	16	2	13·9		30·0		1·4		7·3	
16	15	9	12·3		Cytotoxic		0·5		Cytotoxic	
17	2	25	2·4		6·0	8·0	0·0		2·6	1 4
17	2	26	2·4		5·2		0·0		4·4	
17	2	27	2·2	4·2	5·2		0·0	0·8	4·2	
18	2	26	2·4		2·0	2·4	0·0		0·0	0·0
18	19	4	2·0		2·8	4·0	0·4		0·4	1·0
20	2	26	2·4		2 0		0·0		0·0	
21	2	26	2·4		5·2		0·0		1·2	
21	17	4	11·0		16·0	26 2	0·0		1·2	3·2

* The diagnoses are: subject 8, 29-year-old female; 9, 28-year-old female with keloid; 10, 37-year-old female with benign colon polyp; 11, 2-year-old female with clitoromegaly; 12, 60-year-old female with diabetes; 2, 37-year-old normal male (brother of D.S. patient); 13, 8-year-old male with muscular dystrophy (MD); 14, 6-year-old normal male (brother of patient No. 13); 15, 43-year-old female (mother of patient No. 13); 16, 9-year-old male with MD; 17, 37-year-old normal male; 18, 20 year-old female with secondary amenorrhea; 19, 37-year-old female (mother of D.S. patient); 20, 46-year-old male (father of D.S. patient); 21, 30-year-old normal male.

† The fibroblasts were obtained from skin biopsies except for 1 gingival (subject 7) and 4 muscle biopsies (subjects 13–16).

Table 3. HYPERPLOIDY EFFECT OF LYMPHOCYTE EXTRACT ON AUTOGENEIC AS COMPARED WITH ALLOGENEIC FIBROBLASTS

Extracts from 7 "normal" and 2 "abnormal" (5 and 7) subjects

Donor No.* (lymphocyte extract)	Recipient No. (fibroblast† culture)	No. of transfers at time of examination	Hyperploidy (per cent) Extract dose (×10⁶ lymphocytes) 0	1	2	4	Endoreduplication (per cent) Extract dose (×10⁶ lymphocytes) 0	1	2	4
2	2	5	3·3		3·4		0 0		0 0	
2	7	5	3·5		6·0		0 0		0 0	
10	10	2	4·2	4·0	5 4	6·4	0 0	0·0	0·0	0·6
10	11	12	3·8	6·2	8·6	13·2	0 0	0·0	0 2	0 5
12	12	17	2·3	2·1			0·0	0 0		
12	11	16	4·0	6·2			0·0	0 0		
13	13	7	4·8		5·0		0·0		0 0	
13	2	7	4·8		10·2		0 0		0·6	
13	14	7	7·1		18·0		0·4		6·7	
14	14	7	7·1		8·8		0·4		0·4	
14	2	7	4·8		10·4		0 0		0·4	
14	13	7	4·8		9·2		0·0		0·0	
15	15	9	12·3		11·6		0 5		0·2	
15	16	2	13·9		30·0		1·4		7·3	
17	17	4	11·0	11·0			0·0	0·2		
17	2	27	2·2	4·2			0·0	0·8		
5	5	7	5·6		9·0	18 8	0·3		5·3	6 6
5	2	6	4·6	9·4	14·0	18·8	0·0	0·2	2·2	3·4
7	7	5	3·5	5·6	9·0		0·0	0·0	0·0	
7	5	7	5·6	9·0	13·0		0·0	0·0	0·0	

* See Tables 1 and 2 for diagnoses.

† See Table 2 for source of biopsies.

Table 4. HYPERPLOIDY EFFECT OF FIBROBLAST EXTRACT ON ALLOGENEIC AND AUTOGENEIC FIBROBLASTS

Donor No.* (fibroblast extract)	Recipient No. (fibroblast† culture)	No. of transfers at time of examination	Hyperploidy (per cent) Extract dose (× 10⁶ fibroblasts) 0	1	2	4	Endoreduplication (per cent) Extract dose (× 10⁶ fibroblasts) 0	1	2	4
2	2	10	3·5	4·2	4·0		0·0	0·0	0·4	
2	2	16	2 5	3 3	3·4	3 5	0 0	0·0	0·0	0 2
2	22	4	7 2	11·0	10·2		2 6	3·2	3·0	
2	23	7	2 0		1·6	1·4	0 8		0 2	0 6
2	17	8	4·2			6 3	0·4			0·4
2	24	22	17·2	16·6	16 2		0 8	0 0	0 0	
22	22	4	7 2	9 0	11·0		2 6	1·6	4 0	
22	2	16	2 5	4 5	4·0	3 9	0 0	0 4	0 4	0·3
25	2	13	2·0	2·1	4 3	2·1	0·0	0 0	0 0	0 0
26	2	14	2 6	2·7	3·6		0 0	0 0	0 8	
27	17	8	4 2			5·8	0 4			0 0

* Diagnoses: 2 and 17, see Tables 1 and 2; 22 and 27, normal male infants; 23, 37-year-old female with H.T. (mother of D.S. patient) 3 months after thyroidectomy; 24, adult female with breast tumour; 25 and 26, normal adult males.
† All fibroblasts obtained from skin biopsies.

of magnitude seen when the same extract was tested on allogeneic fibroblasts. The means of the distributions of the two groups are significantly different at the 0·01 level (Wilcoxin two-sample test; see ref. 13). There were two other autogeneic cases that did, however, give substantial increases (on an order of magnitude similar to the allogeneic experiments), but both of these were "abnormal". One was an *XO/XX* mosaic with Hashimoto's thyroiditis (subject 5) and the other was a patient with a history of chronic intermittent undefined severe dermatitis and extensive autogeneic skin grafting (subject 7). The fibroblasts in this one patient were obtained from gingival tissue while the patient was undergoing therapy for periodontal disease.

Finally, as a control, the effect of extracts prepared from fibroblasts was investigated (Table 4). Although these extracts may induce slight responses, the effect of fibroblast extracts from "normal" donors on fibroblast mitoses is smaller than that induced by lymphocyte extracts and there does not seem to be a clear cut dose response. The means of the distributions of the lymphocyte extract results and fibroblast extract results are significantly different at the 0·05 level[13].

These results demonstrate that lymphocyte extracts can produce significant, dose related, increases in the number of hyperploid and endoreduplicated mitoses in allogeneic fibroblasts *in vitro*. The failure to obtain similar results when fibroblast as opposed to lymphocyte extracts were tested suggests that the effect may be specific for certain cells such as the lymphocyte. Moreover, the results obtained when lymphocyte extracts were tested autogeneically were significantly different from the results obtained allogeneically, suggesting that the reaction may at least in part involve self-recognizing mechanisms.

The mechanism producing the hyperploidy is unknown. Polyploidy and endoreduplication have been observed under a variety of conditions[14] including the addition of β-mercaptopyruvate to lymphocytes in culture[15] and ageing of long-term tissue culture[16]. However, no matter what the precipitating event may be, the actual mechanism probably involves either fusion of cells or errors in chromosomal distribution. It is of interest that Ohno has recently demonstrated tetraploidy arising from fusion of coisogenic cells when lymphocytes from animals reciprocally sensitized against each other by skin grafting are mixed together in culture[17]. Cell fusion may also be induced *in vitro* by animal viruses[18]. However, even if the hyperploidy we observed resulted entirely from cell fusion, it is not unreasonable to suggest that the resultant tetraploidy would set the stage for the recovery of a whole range of aneuploid cells. Apart from the observed effect on hyperploidy, there is also a clear-cut effect of allogeneic extract on endoreduplication. This effect indicates that at least some of the hyperploidy may result from errors in chromosomal distribution.

Discussions of the relevance of these results to the clinical chromosomal abnormalities are highly speculative. Before the *in vitro* data can be related to this problem, the active principle will need to be identified through cell fractionization procedures and perhaps also through the application of immunological techniques.

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DEOXYRIBONUCLEIC ACID CONTENT OF MITOTIC NUCLEI OF TWO MOUSE TUMOURS

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CYTOPHOTOMETRIC estimation of the deoxyribonucleic acid content of various cell systems has generally revealed a common coefficient of variation of about 15 per cent for diploid (2*N*) nuclei[1], but with few exceptions it has been technically difficult to estimate the DNA content of mitotic (4*N*) nuclei because of the gross inhomogeneity of the DNA. It has been usual to attribute the variability in DNA content found in studies of diploid DNA contents to instrumental and technical errors, and to assume that the content of cellular DNA is constant in

accordance with the generally accepted hypothesis chromosome complement of a nucleus is identified uniform and constant amount of DNA. Recently we ha reported that mitotic nuclei of dog erythroid bone marrow have a coefficient of variation again in the region of 15 per cent[2]. In addition, we have found DNA contents in excess of $4N$ for some non-mitotic nuclei of this presumably non-hyperdiploid cell system. Such observations prompt one to question the validity of the measurement systems.

A new cytophotometric instrument for two wave-length spectrophotometry of cells has recently been developed[3]. This photometer, using the model dye droplet system suggested by Pollister and Ornstein[4], can perform measurements such that the standard error of estimate of droplet dye content derived from the regression of dye content against droplet volume is less than 1 per cent (0·8 per cent). Because the development of the two wave-length method[5,6] and the improvement of electronic and optical devices have permitted refinements not previously available, we have attempted to define the limits of reliability of our system, and at the same time provide basic information on the DNA content of mitotic nuclei. Here we are reporting results obtained with this instrument on two mouse tumour systems, one an ascitic tumour and the other an *in vitro* culture of a leukaemic cell line.

Table 1. RELATIVE DNA CONTENT OF MITOTIC NUCLEI IN TUMOUR *BP*-8 FEULGEN STAIN

Nuclei measured	*N*	Relative DNA content	Standard deviation	Coefficient of variation (per cent)
Prophase to anaphase	45	125·0	6·4	5·1
Telophase (measured together)	20	124·9	6·5	5·2
Telophase (sum of daughters)	20	122·0	7·1	5·8
1/2 Telophase	40	61·1	4·1	6·7
			Mean	5·7

Table 2. RELATIVE DNA CONTENT OF NUCLEI OF CELLS *L*5178*Y* FEULGEN STAIN

Nuclei measured	*N*	Relative DNA content	Standard deviation	Coefficient of variation (per cent)
Prophase to anaphase	110	125·1	7·5	6·0
Telophase (measured together)	22	124·1	7·5	6·0
Telophase (sum of daughters)	19	125·1	8·6	6·9
1/2 Telophase	38	62·7	4·6	7·3
"2*N* cells"	67	61·2	3·6	6·0
			Mean	6·5

The mouse ascitic tumour cell, *BP*-8, was collected from a 7-day old transfer, and a thin smear prepared, air dried, and fixed in formalin-ethanol. The *in vitro* culture of leukaemic cells, *L*5178*Y*, was collected from a log-phase culture 48 h after transfer, and a thin smear prepared and fixed in the same fashion as the *BP*-8. All slides were stained by a modified Feulgen procedure[7]. A standard hydrolysis time of 12 min was used. Each stained slide was mounted on the microspectrophotometer with high viscosity immersion oil ($N_D^{25°C} = 1{\cdot}5150$) on both objective and condenser. Measurements were made by the standard two wave-length method and optical densities converted to relative DNA content from a ready reference table machine-computed and printed from Mendelsohn's[8] adaptations of Patau's[6] formulation. Each nucleus or mitotic figure was measured in duplicate, and all measurements were made on a single slide preparation.

In Tables 1 and 2 are shown the results for measurement of mitotic nuclei of both cell lines. Analysis of variance indicated no systematic difference among prophase, metaphase, anaphase or telophase nuclei for either cell-line. The measurements for the first three of these morphological groups were then pooled. However, values for telophase nuclei were retained in a separate grouping as their characteristics made it possible to measure their relative content of DNA by two independent methods. On one hand, the pair of daughter nuclei may be measured as a single unit to provide an estimate of $4N$ relative DNA content. On the other hand, the two daughter nuclei may be measured separately. In the latter case, the $4N$ value can be obtained by summing the values for pairs of nuclei ("sum of daughters"), or by computing the mean $2N$ value from the individual values and doubling the result.

The mean values for the relative DNA content of mitotic nuclei by the three independent measures, that is, prophase to anaphase nuclei, telophase nuclei, and sums of parts of telophase nuclei, differ by less than 1 per cent with the exception of the "sum of daughters" telophase measurements in tumour *BP*-8. This mean is significantly different ($P < 0{\cdot}01$) from the mean for whole mitotic nuclei, but we feel the discrepancy may be due to failure to measure small chromatin bridges that occasionally exist in the telophase pairs. When the cell line *L*5178*Y* was measured, great care was taken to exclude such cells, and no significant difference among means was discovered. In addition to mitotic measurements, we have estimated the content of DNA in *L*5178*Y* cells judged to be $2N$ by morphological criteria (Table 2). It is obviously difficult to choose by morphology alone those cells which have not yet commenced DNA synthesis. However, plotting a distribution histogram of several hundred non-mitotic cells reveals a modal group at the same relative DNA content. The frequency histogram for "$2N$ cells" and for mitoses of *L*5179*Y* is shown in Fig. 1. The mean of the $2N$ group is 61·2 relative DNA units, slightly less than the $2N$ amount estimated by measurement of mitotic cells (−1 per cent), but this small difference cannot be assigned any real significance because of the selection procedures for the cells.

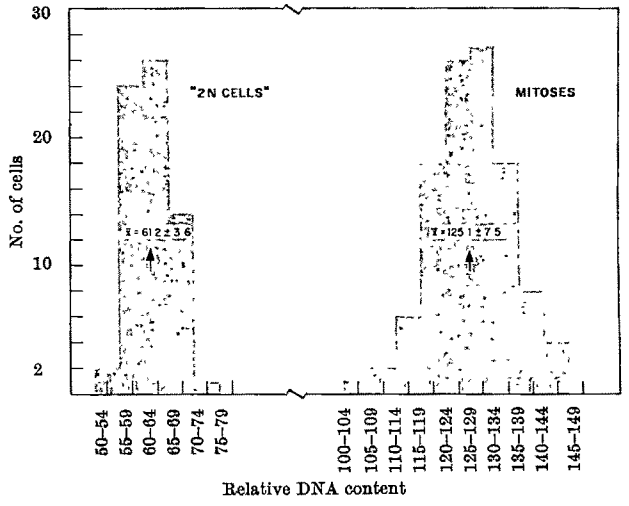


Fig. 1. Frequency histogram for cells of *L*5178*Y*

These measurements simultaneously provided a very rigorous test of the adequacy of the two wave-length correction for chromophore inhomogeneity and of the constancy of DNA content of mitotic nuclei in these cell lines. The coefficient of variation for the mitotic nuclei measured here is about 6·0 per cent. Duplicate measurements of the same cell consistently gave values differing by no more than 1 per cent—a value consistent with that found for error that could be assigned to instrumental causes as determined in tests with the dye droplet model system previously mentioned[4]. Two independent estimates of the telophase content of DNA made by measurement of telophase nuclei as a whole and also as the sum of two parts enable us to estimate

Table 3. ANALYSIS OF RELATIVE DNA CONTENT FOR TELOPHASE NUCLEI COMPARISON OF PAIRED OBSERVATIONS

Cell line	No. of pairs	Mean of paired differences (relative DNA content)	Coefficient of variation (per cent)
BP-8	20	−1·9*	5·9
L5178Y	19	+0·4	4·2

* Significantly different from zero ($P < 0.01$).

those errors attributable to instrumentation and those resulting from the failure of the two wave-length computational method to correct for grossly inhomogeneous distribution of chromophore. The data for the two independent measurements are analysed (Table 3) by computing the paired differences for each pair of nuclei measured by the two methods. The coefficients of variation of the differences for the two cell lines are 4·2 per cent and 5·9 per cent. Because the overall coefficient of variation is of the same order of magnitude (5·7–6·5 per cent), it is possible to assign most of the variability seen to instrumental and mathematical errors inherent in two wave-length microspectrophotometry.

Analysis of goodness of fit to a normal or log-normal distribution for DNA content of mitotic figures in both cell lines demonstrated that the normal distribution best described the data rather than the log-normal distribution as suggested by Garcia[1].

The "constancy" hypothesis has been most clearly restated by Walker and Richards[9]. They stipulate that constancy is applicable at the chromosome level, that is to say, the content of DNA is constant in an individual chromosome and thus also constant in a chromosome set providing that DNA synthesis is complete and that there is no variation in chromosome number. Departure from the constancy hypothesis such as that reported by Lison and Valerie[10] must be supported by evidence that the data are not the result of significant methodological errors. As we have also reported findings[2] of variability in DNA which are not consistent with the constancy hypothesis, albeit in a population of erythroid cells where variability might be more expected, it is incumbent on us to demonstrate the validity of our methodology. For this purpose, we have chosen mitotic cells of two tumours and have shown that the Feulgen method combined with two-wave-length spectrometry is capable of measuring nuclear DNA with an overall coefficient of variation of 5·6 per cent. Furthermore, there was no evidence for alteration in the content of DNA with increasing progress through the stages of mitosis, as analysis of variance of the content of DNA for the morphologically identifiable mitotic stages indicates no systematic variation.

Of equal significance are the results which we obtained for measurement of telophase nuclei by two independent methods: measurement of the whole telophase pair, and measurement of the two daughter nuclei independently. By analysis of these data we have shown that the sum of errors assignable to the instrumental technique plus failure of the two wave-length method to correct completely for chromophore inhomogeneity is of the order of 4–6 per cent. The value of 4 per cent probably represents the best estimates of these errors, as improvements in our manipulative techniques successfully removed a constant negative bias seen when the two measurements were made on telophase nuclei of *BP*-8.

In the light of these findings, it is possible to reaffirm that the stoichiometric relationship of the Feulgen method for measurement of the DNA content of nuclei of at least these two cell lines has a precision of less than 1 per cent. Certainly, these data indicate that the DNA content of mitotic nuclei of these two cell lines rigorously meet the criteria of constancy as set forth by Walker and Richards[9]. The data have also been shown to be best described by the usual normal distribution rather than the log-normal distribution suggested by Garcia[1] for human blood cells.

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DEOXYRIBONUCLEIC ACID SYNTHESIS TIME OF ERYTHROPOIETIC AND GRANULOPOIETIC CELLS IN HUMAN BEINGS

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THE DNA synthesis time of canine myelocytes has been reported to be about 7 h[1]. This estimate was used to calculate the relative rates of production of granulocytic precursors at different levels of maturation[2,3]. From these calculations, Patt *et al.*[3] proposed that a "myelocyte sink" exists and that in part granulocyte production may be "death controlled". This question was considered by Cronkite[4] in respect of human erythropoiesis and granulopoiesis. When the 7-h canine DNA synthesis time was used to calculate the relative production rate of red and white cells in human bone marrow, it appeared that a "myelocyte sink" and ineffective erythropoiesis was in operation. However, if one used the DNA synthesis time of about 13 h which has been determined experimentally for the human gastrointestinal tract by Lipkin *et al.*[5], the "myelocyte sink" and ineffective erythropoiesis were not evident. Clearly it became necessary to estimate the DNA synthesis time of human haemopoietic precursors in order to resolve this dilemma. Since the experimental determination of DNA synthesis time of haemopoietic precursors necessitates the administration of tritiated thymidine (^{3}HTdr), a substance which may produce genetic and late effects by radiation injury, only patients incapable of procreation and/or with limited expectancies of life have been investigated. In this article the evidence for a DNA synthesis time of about 13–14 h in human haemopoietic precursors will be presented.

Tritiated thymidine (specific activity 1·9 c./mmole) was given intravenously in a dose of 0·1 μc./g of body weight. Bone marrow was aspirated and blood samples taken at intermittent intervals after injection for 10–15 days. Smears of the marrow were made, dried, fixed in absolute methanol, dipped in Eastman '*NTB*-2' liquid emulsion or covered with 'British Kodak *AR*-10' stripping film, and exposed for 30–120 days in a dry atmosphere at 4° C. They were developed in the standard manner and stained by Giemsa's method. The marrow preparations obtained during the first 48 h are the subject of this

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article. Granulocytic and erythrocytic mitotic figures were identified, and the progression of cells (whose DNA was labelled) through the mitotic window and the silver grains overlying these mitoses were observed. Corrections for background fog were made by a modification of Stilstrom's technique[6].

Five patients were investigated. One (SUL) had a brain tumour but was haematologically normal. Another, haematologically normal, was a comatose quadriplegic. One (TOW) had polycythaemia vera (PCV) in a compensated steady state and the other two patients had myeloid metaplasia (ST and TO). Bone marrow could not be obtained in the last two patients, but erythrocytic and myelocytic precursors and mitotic figures were present in the blood. In patient TO, 12–73 erythrocytic mitoses and 6–24 granulopoietic mitoses were observed in each sample. In patient ST, 10–62 basophilic erythroblastic mitoses and 25–86 polychromatic erythroblastic mitoses were seen in each sample.

The principles involved in deducing the length of various phases of the generative cycle from the progression through mitosis have already been published[7].

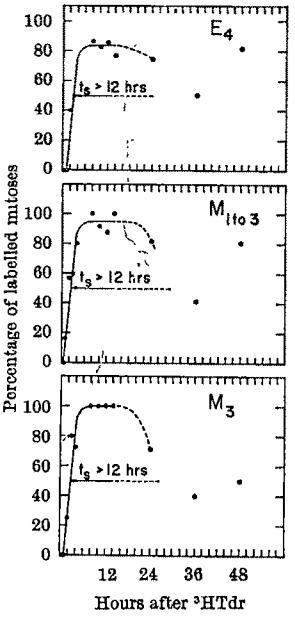


Fig. 1. Progression of labelled erythro- and granulo-poietic precursor cells through mitosis in the bone marrow of a patient with normal haemopoiesis

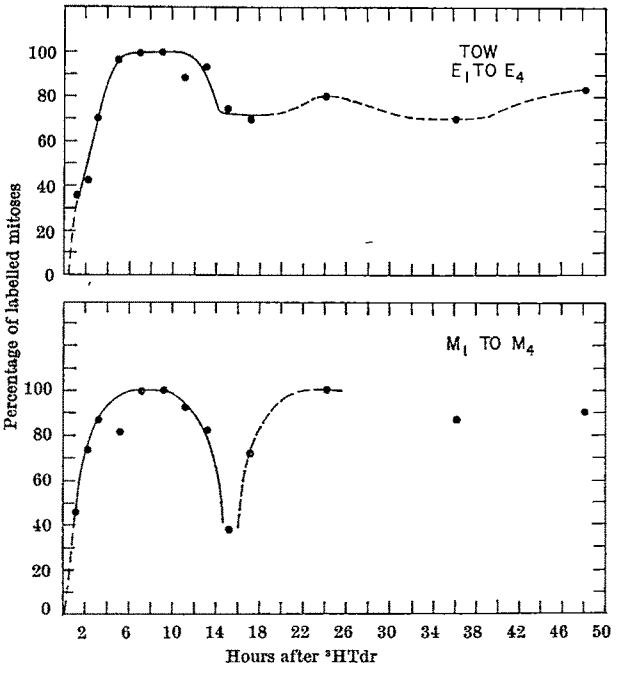


Fig. 2. Progression of labelled erythro- and granulo-poietic precursor cells through mitosis in the bone marrow of a patient with mild polycythaemia vera

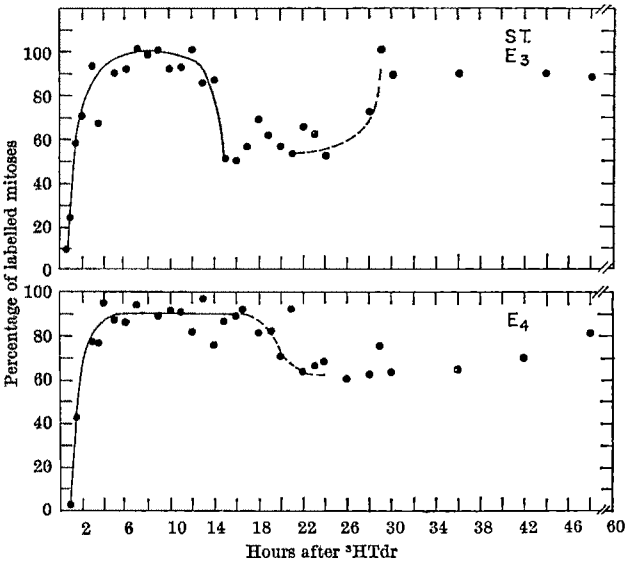


Fig. 3. Progression of labelled erythropoietic precursors through mitosis in the peripheral blood of a patient with myeloid metaplasia

Figs. 1–4 illustrate the progression of the labelled cells through mitosis following the injection of ³HTdr. In Figs. 1 and 2 erythrocytic and granulocytic mitoses are plotted separately. In Fig. 1 (SUL) at the top, progression of the label through mitosis for E_4 (polychromatic normoblasts) is shown. Labelling does not rise above 85 per cent and is maintained for at least 12 h. In the middle, data pooled for M_1–M_3 (myeloblasts through myelocytes) are plotted. Labelling attains about 100 per cent and is maintained for longer than 12 h. At the bottom of Fig. 1, data for only M_3 (large myelocytes) are plotted. Labelling rises to 100 per cent and is maintained for 14 h. Since there were no samples in this patient between 14 and 24 h, a more accurate definition of average DNA synthesis time other than that it is greater than 12 h cannot be made. The progression of labelled erythropoietic precursors through mitosis in the quadriplegic patient indicated an average DNA synthesis time of about 13 h in the pooled erythroblasts. In Fig. 2, progression of labelled cells through mitosis in pooled erythrocytic and granulocytic precursors is shown for a patient with PCV. The minimum synthesis time is about 11 h and the average is about 13 h. At the bottom of Fig. 2, a similar curve is shown for pooled granulocytic precursors. A second wave of labelled mitotic figures is also evident in this case. In Fig. 3, at the top, the progression of labelled basophilic erythroblasts (E_3) through mitosis in the peripheral blood is shown. Bone marrow was not obtainable in this individual because of extensive myelofibrosis. In this patient the minimum time of synthesis is about 12 h and the average about 14 h. In the bottom part of Fig. 3, the progression of labelled polychromatophilic normoblasts (E_4) through mitosis is shown for the same patient. The DNA synthesis time appears longer but cannot be clearly defined. In this instance 100 per cent labelling of E_4 was not observed. Since this may have been due to a failure to detect lightly labelled cells because of inadequate exposure time, a set

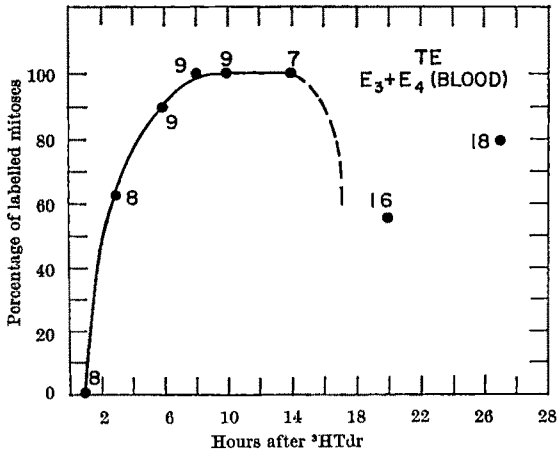


Fig. 4. Progression of labelled erythropoietic precursor cells through mitosis in the peripheral blood of a patient with myeloid metaplasia

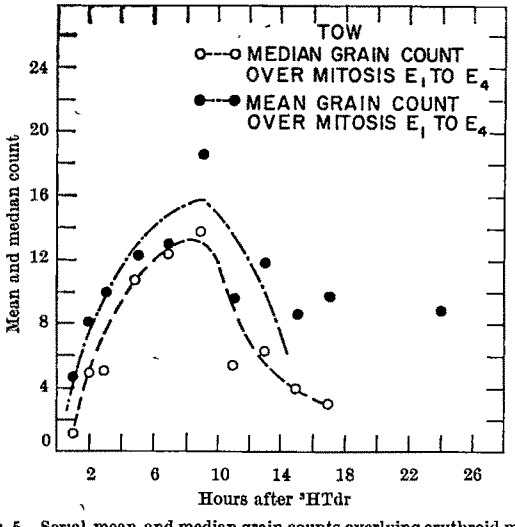


Fig. 5. Serial mean and median grain counts overlying erythroid mitotic figures in the bone marrow of a patient with mild polycythaemia vera after single injection of ^{3}HTdr intravenously

of slides was exposed for 100 days but there was no further increase in the fraction of E_4 labelled. In Fig. 4, another curve of labelled mitoses is shown. This is also based on observations in the peripheral blood. Since mitoses were scarce, the number seen is recorded above each point. DNA synthesis time has a minimum of about 13 h. Data are inadequate to estimate the average synthesis time. In Figs. 5 and 6, the mean and median grain counts overlying mitotic figures are plotted. The variation with time parallels the curves for labelled mitotic figures. The grain count begins with low values, increases to a maximum and subsequently diminishes. The low intensity of labelling over the mitotic figures early and later corresponds to the cells that were just finishing or beginning DNA synthesis respectively during the short time of availability of the ^{3}HTdr. Since they would be in synthesis only for a fraction of the time of availability of the ^{3}HTdr, one would expect lesser incorporation of the label into DNA. Fig. 5 is based on a patient with polycythemia vera. It appears that DNA synthesis time is between the two minima mentioned earlier or about 12–14 h. In Fig. 6, the time between the initial and later minimum suggests a DNA synthesis time between 16 and 18 h for E_4.

In another patient with myeloid metaplasia (St), the labelled mitosis curve for E_4 remained at approximately 100 per cent for more than 20 h without any decrease.

Table 1. PROGRESSION OF LABELLED CELLS FROM E_4 TO E_5 AND THE RELATION BETWEEN NUMBER OF CELLS AND MEAN GRAIN IN E_4 AND E_5 AT SPECIFIED TIME INTERVALS

Time after ^{3}HTdr	Type of cells	No. of cells	No. of grains	No. of labelled cells	Mean grain count
1 h	E_4	125	1,375	51	27
1 h	E_5	360	0	0	0
6 h	E_5	371	362	18	20
12 h	E_5	375	800	49	16·3
18 h	E_5	397	1,433	109	13·1

Table 2. ESTIMATES FOR DNA SYNTHESIS TIME

Patient	Cells	DNA synthesis time Minimum	Average
Normal blood 1	E_4	11	> 12
	M_1–M_3	13	> 13
	M_2	13	> 13
Normal blood 2	E_1–E_4	11	13
Polycythaemia vera	E_1–E_4	11	13
	M_1–M_4	11	13
Myeloid metaplasia	E_3	12	14
	E_4	~16	?
Myeloid metaplasia	E_3 E_4	13	?

Thus an estimate of DNA synthesis time by this method was not practical. However, in Table 1 an analysis of the progression of labelled cells from the dividing E_4 to the non-dividing E_5 (orthochromatic normoblasts) was made as follows. One hour after the intravenous injection of ^{3}HTdr, the labelling index was 51 per cent in E_4 with 1,375 grains overlying 125 E_4 cells. While counting 125 E_4 cells, 360 E_5 were observed which were not labelled. Six and 12 h after ^{3}HTdr injection, the number of grains over the E_5 had increased significantly but had not reached the number found in E_4 at 1 h. However, at 18 h, the number of grains found overlying E_5 equalled the number found over E_4 at 1 h. At 18 h, the number of labelled cells in E_5 was twice that found in E_4 at 1 h and the mean grain count was approximately half. This suggests that the sum of premitotic rest, DNA synthesis, mitosis for

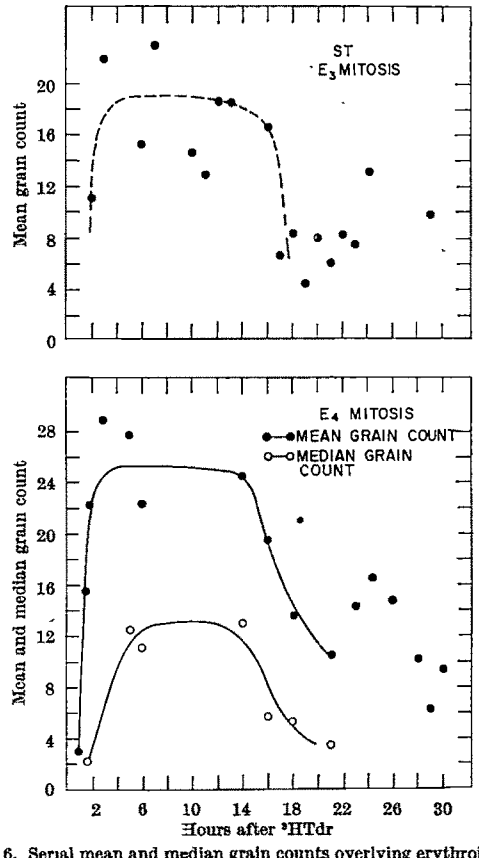


Fig. 6. Serial mean and median grain counts overlying erythroid mitotic figures in the peripheral blood of a patient with myeloid metaplasia

E_4 and maturation to E_5^- is of the order of 18 h in this patient. In Table 2, minimum and average estimates of DNA synthesis are tabulated.

The percentage of labelled mitoses in erythropoiesis did not reach 100 per cent in all instances. Since the increased exposure time of the emulsion did not increase the fraction of mitoses labelled, inadequate exposure time is probably not the cause. Possibly a long premitotic rest (G_2) or maturation of unlabelled cells in parallel with labelled cells to the cellular compartment under investigation are responsible. The increase in the intensity of label over mitotic figures, the plateau and the subsequent decline is probably due to a small fraction of cells at the end and beginning of DNA synthesis which were synthesizing DNA only for a small part of the time when ^{3}HTdr was available and hence are labelled less intensely. Accordingly we believe the time between these minima is approximate to the DNA synthesis time. The computations based on the progression of labelled cells from E_4 to E_5 in patient ST are valid only if E_5 does not lose any label by maturation during the period of observation. Fortunately, the oldest E_5, with partially extruded nuclei, become slightly labelled only 15 h after injection of ^{3}HTdr. This analytical approach shows that the sum of DNA synthesis time, premitotic rest, mitosis and maturation to E_5 is of the order of 18 h. Furthermore, it also suggests that there is only one mitosis of the labelled E_4 cells before they resemble E_5 cells. The apparent generation time of erythroblasts in man is approximately 24 h and cells observed at the beginning of the experiment should thus have divided within 24 h. The cells that were labelled at 1 h should all have divided after an interval equivalent to the sum of DNA synthesis time, premitotic rest and mitosis. In Table 3, the distribution of 437 interphase erythroblasts 1 h after injection are divided into different highly labelled categories. The number of erythroblastic mitoses in the interphase population was recorded during the following 24 h. The distribution of the highly labelled cells is similar in interphase and mitotic cells. This indicates that the most highly labelled cells divided and suggests that there is no radiation inhibition of mitosis by the tritium. The observations have been made on two persons with normal haemopoiesis and three persons with aberrations of haemopoiesis (polycythaemia and myeloid metaplasia) and one cannot be sure that these estimates represent the average for normal human beings. However, the estimates of about 13–14 h for DNA synthesis time in erythropoietic and granulopoietic precursors found in marrow (one normal, one polycythaemic) and in the blood of two patients with myeloid metaplasia are strikingly close to each other and to the estimates of DNA synthesis time in the normal mucosa of the gastrointestinal tract of patients with cancer of the bowel published by Lipkin *et al.*[5]. Furthermore, three different approaches in our investigation (labelled mitosis curves, time between minima in grain counts over mitotic figures, and transport of label from E_4 to E_5) were internally consistent. As it is rare to see a patient with normal haemopoiesis on whom it would be permissible to experiment in this way and who will agree to the multiple bone marrow punctures, it may be a very long time indeed before we will produce more data in our laboratory. However, others are pursuing similar investigations, and the publication of data, scanty as it may be from any one laboratory, appears desirable in order to make it possible ultimately to determine the average values for DNA synthesis on larger numbers of haematologically normal individuals. Clearly DNA synthesis time of erythropoietic and granulopoietic precursors of man in the patients investigated is longer than that of 7 h for canine myelocytes[1–3]. We believe that, until further data are made available, 13–14 h is the best approximation for average DNA synthesis time of erythro- and granulopoietic precursors in man.

Table 3. DISTRIBUTION OF LABELLED INTERPHASE ERYTHROBLASTS 1 H AFTER INJECTION AND OF LABELLED ERYTHROBLASTIC MITOSIS DURING THE ENSUING 24 H

Patient with polycythaemia vera	Number of cells in each grain category				
	> 10G	> 20G	> 30G	> 40G	> 50G
448 mitotic figures the first 24 h after ^{3}HTdr (E_1–E_4)	96	36	6	4	1
437 interphase cells at 1 h after ^{3}HTdr (E_1–E_4)	81	35	5	2	1

To sum up, the DNA synthesis time of erythro- and granulo-poietic precursors of five patients was estimated by observing the movement of labelled cells through mitosis, the time between minima in the grain count over labelled mitotic figures and from an analysis of the movement and intensity of label from a proliferating (E_4) to a non-proliferating (E_5) compartment. We propose that the best present estimate for DNA synthesis time in erythro- and granulo-poietic precursors of man is of the order of 13–14 h.

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CHEMICAL TREATMENTS OF STAPHYLOCOCCAL INFECTIONS IN MICE

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PREVIOUS publications by this group of investigators have indicated low values for glucose, protein and cholesterol in the carcases of mice dying of *Staphylococcus aureus* infection. Raised values for inorganic phosphorus and transaminase were also found[1]. Examination of the blood[2] and individual organs[3] of dying mice indicated a low blood sodium and a high alkaline phosphatase. Many, but not all, these derangements occurred in the liver. Unpublished experiments have shown that the blood carbon dioxide combining power in normal animals is 23 μmoles/l. At 1 h, 2 h and 3 h after infection, and at death, it averages 22, 19, 22 and 18 μmoles/l., respectively.

It is important to know if these chemical derangements can provide a clue to the mechanism of death from infection with *Staphylococcus aureus* in mice. We decided to test the importance of these chemical findings by trying to ameliorate or to worsen the derangement by addition or withdrawal of the affected chemicals before or soon after challenge with staphylococci. The results demonstrate that some of these derangements can be altered by subcutaneous treatment with appropriate chemicals to alter significantly the length of life after staphylococcal challenge.

We used the haemolytic coagulase-positive Smith strain of *Staphylococcus aureus*. This strain, originally phage-type 44*A*/42*E* (ref. 4), was isolated from a patient with osteomyelitis. Using the standard phages, however, it is now non-typable. It has been maintained in our

laboratory by regular *in vitro* transfer. Our investigations have shown a reasonably constant number of staphylococci in the bodies of moribund mice regardless of the inoculation route. This work was therefore confined to the use of heavy-dose (0·1 c.c. = 10^9 organisms) intraperitoneal infections. Control infected mice live 270 ± 20 min. The methods have been described previously[5]. The mice used were white Swiss Webster male mice. The animals weighed 20–26 g. Chemicals used for treatment were all given subcutaneously 1 h after challenge unless otherwise stated. All reagents were chemically pure or of U.S.P. standard. Control treatments of equal volume with saline or sucrose were used initially. As they never differed from animals challenged intraperitoneally, they were later abandoned in favour of animals challenged intraperitoneally without subcutaneous treatment as controls.

The analysis of the data was made by comparing the time from challenge until death for ten control animals with the same measurement for ten animals treated. A statistical analysis was also made by the chi-square method. The level of significance used was $P \lesssim 0{\cdot}05$. For the sake of clarity of presentation the tabular material shows the difference in minutes between the control and treated 50 per cent lethal times (*L.T.* 50) with significantly different values starred in the tables.

Glucose given subcutaneously significantly prolongs the life of groups of ten infected mice when compared with groups of ten simultaneously infected control animals. Fasting for 16 h enhances the effect (Table 1). Adrenaline, although it markedly elevated blood sugar, had no effect in six experiments. Insulin was found to have a significantly deleterious effect in most experiments (Table 2). Tolbutamide was tried in twelve experiments, but it rarely had any deleterious effect.

Table 1. GLUCOSE TREATMENT OF STAPHYLOCOCCAL INFECTION
(10^9 cells intraperitoneally)

200 mg glucose subcutaneously.

Time from challenge (h)	Difference of $L.T._{50}$ from control (min)† Fed			Fasted		
−2	+7	+39	+11	+6	+69*	+28*
−1	+17	+56*	+6	+80*	+25*	+37*
0	+66*	+25	+55*	+59*	+49*	+74*
+1	+61*	+20	+46*	+59*	+57*	+69*
+2	+51	+10	+50*	+34*	+61*	+88*

* Statistically significant difference in survival time: see text.
† Controls live 254 ± 27·7 min.

Table 2. INSULIN TREATMENT OF STAPHYLOCOCCAL INFECTIONS
(10^9 cells intraperitoneally)

0·002 U. regular insulin subcutaneously:

Time from challenge (h)	Difference of $L.T._{50}$ from control (min) Fed			Fasted		
−2	−7	−11	−1	+14	+14	+1
−1	−18	−27	+26	−5	+8	+50
+1	−28*	−23*	+3	+11	−19*	−2
+2	−29*	−14*	−24*	+17	−47*	−17*

* Blood sugar of normal animals was 189 mg per cent.

Mouse protein (serum, spleen or muscle) or other proteins (human serum or albumin) were without effect in lengthening the life in infected animals.

Injection of crystalline cholesterol failed to raise mouse blood cholesterol, but 1 c.c. of egg yolk daily intraperitoneally for 3 days raised the blood cholesterol from 139 to 1,040 mg per cent, but it failed to delay death from infection.

Phosphate administered as Sorenson's buffer (1/15 M KH_2PO_4 and 1/15 M $Na_2HPO_4.2H_2O$) was a consistently beneficial treatment which prolonged the life of infected mice (Table 3). The components of the buffer were less effective in seventy-two experiments than the combined solution.

Transaminase derived from horse serum (supplied by Sigma Laboratories, St. Louis) was without effect. Sodium given as 2 ml. of normal saline was ineffective and attempts to deplete the animals of sodium with chlorothiazide or mercuhydrin were equally unsuccessful. Alkaline phosphatase from calf intestine or inhibition of alkaline phosphatase by ethylenediamine tetraacetic acid (EDTA) were ineffective treatments.

An investigation of lipid metabolism in mice dying of staphylococcal infection is at present being made. Treatments with castor oil or 'Lipomul' (cotton seed oil with 4 per cent dextrose) were without effect in eighteen experiments, but olive oil (Table 4) probably had some effect.

Table 3. PHOSPHORUS TREATMENT OF STAPHYLOCOCCAL INFECTION
(10^9 cells intraperitoneally)

2 ml. subcutaneously:

Time from challenge (h)	Difference of $L.T._{50}$ from control (min) Fed				Fasted		
Buffer (18·4 mg)							
−2	0	−150*	+19		+37*	+37*	−30
−1	+11	+8	+25*	+37*	+46*	+12	−34
0	+31*	+55*	+25*	+14	+44*	+81*	+25*
+1	−18	+39*	+21*	+42*	−34	+32*	+31*
+2	−24	+17	+23*	−14	−20	+19*	+17
				+5			
				+10			
				+20*			

Table 4. LIPID TREATMENT OF STAPHYLOCOCCAL INFECTIONS
(10^9 cells intraperitoneally)

1 ml. olive oil subcutaneously:

Time from challenge (h)	Difference of $L.T._{50}$ from control (min) Fed	
−1	0	+23
0	+33*	+49*
+1	+35*	+36*
		+28
		+11
		+7

Table 5. ACID-BASE TREATMENT OF STAPHYLOCOCCAL INFECTIONS
(10^9 cells intraperitoneally)

2·0 ml. 1/6 M sodium lactate subcutaneously:

Time from challenge (h)	Difference of $L.T._{50}$ from control (min) Fed			Fasted			
−2	+10	+55*	+13	+24*	+29*	+24*	
−1	+24	+61*	+35*	+18	+29*	+42*	
0	+20	+22	+83*	+34*	+47*	+59*	
+1	+45*	+83*	+65*	+40*	+29*	+54*	+59*
+2	+50*	+74*	+13	+78*	+55*	+67*	

1·0 ml. sodium bicarbonate subcutaneously:

Time from challenge (h)	Difference of $L.T._{50}$ from control (min) Fed			Fasted			
−2	−4	−4	+2	+38*	+44*	+20*	
−1	+115*	+60*	+9	+29*	+62*	+37*	
0	+92*	+46*	+51*	+17	+25	+40*	+39*
+1	+31*	−101*	+46*	0	−12	+49*	

Treatment with 1/6 M sodium lactate or sodium bicarbonate was consistently beneficial in lengthening the survival time of challenged animals when compared with controls infected simultaneously (Table 5).

The treatment of shock gave confusing results. The mice are profoundly hypothermic (rectal temperature falls from 100° to 65° F before death), but we have been unable to measure blood pressure or cardiac output in these small animals. Urine output ceases soon after challenge. While cortisol in normal doses (5 mg/kg) has no effect on the outcome, if given up to 3 h after challenge in very large doses it will prolong life significantly (Table 6 and ref. 6). Aramine and noradrenaline are without effect or may be deleterious if given 1 h after infection. Wyamine and vasoxyl were also without effect. Warming to 37° C for the duration of the infection is significantly deleterious. Cooling to 4° C was beneficial[7].

Table 6. ANTISHOCK TREATMENT OF STAPHYLOCOCCAL INFECTION
(10^9 cells intraperitoneally)

10 mg hydrocortisone subcutaneously:

Time from challenge (h)	Difference of $L.T._{50}$ from control (min) Fed			Fasted			
+1	+80*	+47*	+102*	+45*	+45*	+45*	+31*

Because the mice die after a period of over-activity ending with clonic convulsion[5], it was decided to try to control this over-activity. To this end, treatment with librium and dilantin was used with surprisingly good effects (Table 7). We used amphetamine in an attempt

Table 7. HYPERKINETIC, HYPOKINETIC AND ANTICONVULSANT TREATMENTS OF STAPHYLOCOCCAL INFECTION
(10^9 cells intraperitoneally)

Time from challenge (h)	Difference of $L.T_{50}$ from control (min) Fed		
5 mg librium subcutaneously:			
−1	+68*	+35*	+103*
0	+24*	+27	65*
+1	+119*	+37*	0
2 5 mg thorazine subcutaneously			
Time from challenge (h)			
−1	+29*	+48*	+54*
0	−14	−16	+14
+1	−72*	−65*	−41*
5 mg dilantin subcutaneously:			
Time from challenge (h)			
−1	+97*	+84*	+101*
0	+60*	+70*	+94*
+1	+70*	+86*	+88*
10 per cent oxygen, 90 per cent nitrogen with 0·6 atm. pressure for duration of infection	+215*	+241*	+178*

to reverse this effect. This produced well marked excitability in the animals. However, this produced no change in the period of survival. Thorazine was helpful when given before challenge, but deleterious afterwards. The convulsions might either be hypoglycaemic, hypocalcaemic or anoxic. Glucose, as already mentioned, was beneficial, but oxygen and calcium were not. In contrast, low concentration oxygen (10 per cent) or low pressure (0·6 atm.) or both were markedly beneficial (Table 7 and ref. 8).

To sum up, sodium, mercuhydrin, alkaline phosphatase, cholesterol, protein, transaminase, pressor amines, amphetamine and calcium salts had no effect on the time of survival of groups of treated animals compared with untreated control groups when both were challenged with intraperitoneal staphylococci. Insulin had a deleterious effect. Both 10,000 mg/kg doses of glucose and 920 mg/kg doses of phosphate resulted in a 25–35 per cent lengthening in the mean survival time of animals challenged intraperitoneally with *Staphylococcus aureus*. Cortisol in a dose of 250 mg/kg (but not in lower doses), cooling (but not warming) and oxygen deprivation (but not 50 or 100 per cent oxygen treatment) resulted in 16–84 per cent lengthening in the mean survival time of infected animals. Other treatments of value were olive oil and anticonvulsant therapy. This type of approach provides clues to the chemical nature of the death process from *Staphylococcus aureus* in mice.

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INHIBITORY ACTION OF THE TESTOSTERONE AND LIVER EXTRACT ON STAPHYLOCOCCAL INFECTIONS IN MICE

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THE biochemistry of death from *Staphylococcus aureus* infection has recently been under intensive study[1]. Various chemical constituents of the carcass vary markedly compared with normal values in animals dying of infection. One explanation for these changes could be an exhaustion of normally occurring enzymes or trace elements which could be obtained from suitably treated extracts of normal healthy mouse tissues. In order to explore this possibility we have tested various aqueous extracts of mouse organs and used them for the treatment of severe intraperitoneal infections with staphylococci.

The mice, the staphylococci and the methods used for inoculation and treatment have been described[2]. Organ extracts were made as 1 or 10 per cent w/v aqueous extracts of grinding normal organs with 'Teflon' grinders in suitable volumes of sterile distilled water. The extracts were given subcutaneously 1 h before or 1 h after intraperitoneal challenge with 10^9 *Staphylococcus aureus* cells. All cultures made on these extracts with the exception of 48 h cultures on certain extracts of liver were sterile. With the liver extracts *Escherichia coli* grew in a titre of 10^3 in the 60 per cent suspension within a 24 h period and even later. *E. coli* used in titres of 10^2–10^9 as subcutaneous treatment had no effect on longevity after *S. aureus* challenge. We were unable to detect any deleterious effects of the organ suspensions in normal animals. Controls with normal animals were always run with each experimental test. Liver microsomes were obtained by differential centrifugation in a refrigerated centrifuge using the method of Hogboom, Schneider and Ballade[3]. We consider that microsomes were present in the supernatant and mitochondria in the sediment.

Our results are summarized in Table 1. None of the extracts regularly gave any striking benefit. Liver and testicular extracts had some intermittent beneficial effects but it was only on rare occasions that these results could be confirmed statistically. Adrenal extracts were occasionally helpful.

Table 1. EFFECTS OF ORGAN SUSPENSIONS AS TREATMENT OF STAPHYLOCOCCAL CHALLENGE
(Dose 1–2 c.c. subcutaneously)

	−1 h				+1 h			
	1 per cent		10 per cent		10 per cent		10 per cent	
	No. expt.	Av. min	No. expt.	Av. min	No. expt.	Av. min	No. expt.	Av. min
Liver	3	+10	3	+11	3	+8	3	−11
Spleen	2	−22	1	−16	1	+4	1	+5
Kidney	3	+8	6	−18	3	−7	3	−35
Heart	2	0	2	−7	1	+1	1	−4
Lung	2	−6	1	−9	1	−33	1	+8
Brain	1	−8	3	+4	2	+1	1	−9
Adrenal 0·1%	1	+17	—	—	1	−52	—	—
Muscle	1	−33	3	+1	2	+11	1	−14
Intestine (small)	—	—	2	+4	—	—	6	−6
Intestine (large)	—	—	2	0	—	—	2	−27
Stomach	—	—	2	+7	—	—	2	−33
Testicle	3	−18	1	+26	—	—	6	−7

We have tried various commercial liver extracts with an extension of the $L.T._{50}$ of from 10–30 min, but again it is not clear that this was not due to the effects of chance. Concentration of the extract (Table 2) produced somewhat better results. There was some evidence that the liver microsomes were more beneficial than the mitochondria (Table 3).

Table 2. EFFECT OF LIVER HOMOGENATE INJECTED SUBCUTANEOUSLY ON TIME OF DEATH AFTER INTRAPERITONEAL *Staphylococcus*

Per cent	−1 h (min)			+1 h (min)		
1	+34*	−6	−14	+17	+1	−50*
10	+55*	+35*	+33*	+5	−27	−42*
20	+39*	+22*	+7	+11	−4	−4
30	+33*	+10	+6	+15	+8	−20*
40	+23*	+17	+1	+15	+11	−22*
60	+69*	+8	−15	0	−39*	−44*

* Statistically significantly different from concurrent control.

Table 3. SUBCUTANEOUS TREATMENT WITH LIVER FRACTIONS 1 H BEFORE INTRAPERITONEAL STAPHYLOCOCCAL CHALLENGE

3,400*g*				13,000*g*			
Supernatant		Sediment		Supernatant		Sediment	
No. of expt.	Av. min	No. of expt.	Av. min	No. of expt.	Av. min	No. of expt.	Av. min
2	−7	2	−46*	2	+46*	1	+17

* Statistically significantly different from concurrent control.

Table 4. EFFECT OF HYALURONIDASE AND TESTOSTERONE

1 mg testosterone subcutaneously: Time from challenge (h)	Difference in $L.T._{.50}$ from control		
−1	+32	+30	+119*
0	+22	+40*	+5
+1	+10	+114*	+109*
7·5 U. hyaluronidase subcutaneously: Time from challenge (h)			
−1	−7	−69*	−23
0	−10	+32	−11
+1	+84*	−30	−23

We have separately tried hyaluronidase and testosterone and we consider these should be the main constituents of testicular extracts. In general, the results with hyaluronidase have not been encouraging. Testosterone, however, has been beneficial (Table 4).

Our results with adrenal extracts led us to a re-examination treatment with cortisone and to raise the dose from 5 mg/kg to 300 mg/kg with excellent results. This investigation will be reported separately[4]. Previously, whatever results have been obtained with organ extracts have been attributed to the fever and leucocytosis which they produce. In the short period available with our animals (about 200 min) we think that this does not occur. We know that in the untreated infection there is a profound hypothermia and leucopenia[1].

Non-specific shock can be induced by protein injections, and this might have some additional effect, but it should be detrimental. Leucocytes, injected subcutaneously as such, have no effect on longevity. If mice are warmed to 37° C they perform poorly after challenge. An environment of 4° C is helpful, and it seems unlikely that fever could be the explanation of the effects of organ extracts[1].

The available information on organ extracts is meagre[5] and it is hard to find it in various published indexes. Weinstein has investigated a variety of compounds of organ extract type in the subcutaneous treatment of an experimental subcutaneous anthrax infection of mice[6]. These include insulin, pineal extract, thymus extract, testosterone, progesterone, thyroxine, pituitary extract, parathyroid extract, gonadotropic hormone, adrenal cortical extract, thyrotropic hormone, growth hormone and prolactin. Certain substances exerted a marked prophylactic effect on infection with anthrax bacilli in mice. These were parathyroid and various extracts of the pituitary gland. Testosterone propionate was moderately protective. Extracts of thymus and pineal gland thyroxine and extracts of the posterior pituitary were also moderately protective. High doses of testosterone were less beneficial than low ones. Doses of 0·12 mg testosterone propionate per mouse per day for 14 days were better than doses of 1·25 mg for the same period.

Nutini and Lynch[7-9] have used extracts of beef brain, spleen, heart and kidney precipitated with 80 per cent alcohol to change an *aureus* virulent form of *staphylococcus* to an *albus* avirulent form. Infections by the subcutaneous, intraperitoneal or intravenous routes in mice could be treated with oral or subcutaneous brain extracts. These extracts, when used prophylactically or therapeutically, reduced the fatality rate in mice from between 76 and 90 per cent to 2 per cent. Spleen was also active, but less markedly so than brain. Heart and kidney had little effect, but liver was deleterious because of its toxicity. We have carried out some experiments with alcoholic extracts of liver and we can report similar toxic responses. Comparisons between their brain extract treatment and penicillin showed the extract to be more active than 2,000 u. penicillin *G* per day per mouse in subcutaneous infections with twenty-four penicillin *G*-resistant strains. It was thought that the extract had a direct effect on the organism, changing it from a virulent to an avirulent form.

Further investigations by Berger *et al.*[10] characterized these extracts as chiefly comprised of aspartic acid, glutamic acid, glycine, alanine, arginine, histidine, amino butyric acid and peptides. The ninhydrin-positive materials were not thought to be responsible for the activity of the extract. Ether extracts of acid fractions of crude brain extract at *p*H 4·5 were very active and therapeutic principle was thought to be the result of unidentified acid substances.

Kroenberg *et al.*[11] have used the coagulate from fresh beef blood. A deproteinized extract from the supernatant of a centrifugation of this coagulate was successfully used to treat a subcutaneous infection of mice with 10^9 *Staphylococcus aureus* or an intraperitoneal infection with 10^8 cells. A 62 per cent protection was found, compared with 21 per cent protection using a crude 80 per cent alcoholic extract of beef blood. They compare this with the effects of an antibiotic called erythrin which was isolated from the blood of various mammals by Zil'ber and Yakobson[12] and was effective both *in vivo* and *in vitro*.

It has been demonstrated that protein synthesis in the *C3H* milk-factor virus mammary tumour is capable of being inhibited by testosterone *in vitro*[13]. It was not effective *in vivo* and it appeared that this could be explained by the rapid metabolism of testosterone by the tumour. Parsons believes that the locus of inhibition of protein synthesis may be at the level of RNA synthesis. This finding may help in the further analysis of our results in the treatment of *Staphylococcus aureus* infections.

It is concluded from these results that a factor or factors may be present in extracts of normal mouse liver adrenal and testicle which is effective in partially protecting mice from the deleterious effects of severe *Staphylococcus aureus* infection. The factor in testicle is probably testosterone. More investigations in this area are needed.

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LETTERS TO THE EDITOR

ASTRONOMY

Counts of Radiosources in the *Revised 3 C Catalogue*

IT has been shown that the log N/log S curve for radiosources of high galactic latitude is a straight line with a slope of $-1{\cdot}85 \pm 0{\cdot}05$—at least for fluxes greater than 9×10^{-26} W m^{-2} (c/s)$^{-1}$ at 178 Mc/s (ref. 1). (N is the number of sources brighter than the flux S.)

These radiosources are known to be extra-galactic and to belong to two main classes of objects: the radiogalaxies and the quasi-stellar radiosources (quasars). The radiogalaxies are elliptical giant galaxies; the quasars are stellar objects with very large red-shifts[2-7]. The theoretical log N/log S curves for all the models of the universe, without any evolutionary effect, have a slope of $-1{\cdot}50$ for the larger fluxes and this slope decreases numerically to zero with decreasing flux. Thus the observed slope of $-1{\cdot}85$ for radiosources of high galactic latitude seems to show that there is really some evolutionary effect; if this is so, then the effect must be larger for the quasars than for the radiogalaxies, because on the average the quasars have red-shifts larger than those of the radiogalaxies. To verify this assumption, it is necessary to construct separate log N/log S curves for radiogalaxies and for quasars; to accomplish this a very large number of identifications is required.

A year ago, the number of identifications that had been published was very small, but since then many very accurate radio positions have become available[8-11]. Using these positions, Wyndham[12] and I have made a systematic search to identify any objects in the field of the radiosources in the *Revised 3 C Catalogue*[13] on the *Palomar Sky Survey* prints and plates. We were able to make many positive and tentative new identifications.

The *Revised 3 C Catalogue* provides an extensive survey of the sky north of $\delta = -5°$, for fluxes larger than 9×10^{-26} W m^{-2} (c/s)$^{-1}$, at $\nu = 178$ Mc/s, and it is thus suitable for statistical analysis.

The *Catalogue* deals with 328 sources: (*a*) 32 can be classified as galactic or possibly galactic sources on the basis of positive identifications (Crab Nebula = 3*C* 144, *IC* 434 (H II region) = 3 *C* 147·1, etc.), thermal spectrum, large angular size, radio structure and small galactic latitude; (*b*) 42 sources are probably extragalactic but in absorbed fields near the galactic plane and thus identification is impossible or extremely difficult; (*c*) 254 sources occur in clear fields.

The sources in group *c* were sub-divided into six classes: 100 certain radiogalaxies; 44 possible radiogalaxies; 39 certain quasars; 21 possible quasars; 36 empty fields; 14 non-identified sources. Identification with a galaxy was considered to be 'certain' when there was a very good agreement between optical and radio positions, or when a spectroscopic confirmation had been obtained. It was considered 'possible' when the agreement was not so good. Identification with a quasar was considered 'possible' when a blue star (blue on the *Palomar Sky Survey* prints) was present at the radio position, and 'certain' when spectroscopic or photoelectric confirmation had been obtained[7,14,15]. An 'empty field' was considered to be one where nothing was visible at the limit of the *Palomar Sky Survey* plates ($v \sim 21$) at the radio position. A source was classified as 'not identified' when the radio position was not sufficiently accurate to classify it in any of the previous classes.

It was now possible to construct log N/log S curves for the radiogalaxies and for the quasars. Fig. 1 shows these curves. (*a*) For all the extragalactic sources in the *Revised 3 C Catalogue*, we found the already known slope of $-1{\cdot}85 \pm 0{\cdot}05$. (*b*) For 'certain' and 'possible' radiogalaxies, we found a slope of $-1{\cdot}55 \pm 0{\cdot}05$: this value is normal for objects as near as these galaxies. (*c*) For 'certain' and 'possible' quasars, we found the very large slope of $-2{\cdot}2 \pm 0{\cdot}1$. (*d*) For the quasars, the empty fields and the non-identified sources, we found the same slope, $-2{\cdot}2$.

These findings would seem to indicate that the peculiar slope of $-1{\cdot}85$ found for all the radiosources is due only to the quasars and that almost all the empty fields and 'non-identified' sources are quasars and not radiogalaxies.

Fig. 2 shows the curves for log N/V for 'certain' and 'possible' radiogalaxies and for the 'certain' quasars. (The visual magnitudes (v) of the quasars were measured by Sandage and those of the radiogalaxies either measured by Sandage or estimated on the *Palomar Sky Survey* prints[14,15].) The slope of the log N/V curve for the radiogalaxies at the limiting magnitude $V \sim 21$ is very small and this indicates that the number of galaxies associated with radiosources in the *3 C* revised catalogue, with magnitude greater than 21, is very small. The slope of the log N/V curve for the quasars is much larger and an extrapolation of this curve indicates that a number of quasars has still to be identified. This confirms my suggestion that almost all the 'empty field' and 'non-identified' objects are quasars rather than radiogalaxies.

I thank R. L. Adgie, B. G. Clark, I. I. K. Pauliny-Toth, C. M. Wade, D. S. Heeschen, A. R. Sandage and J. D. Wyndham for enabling me to see their results before publication.

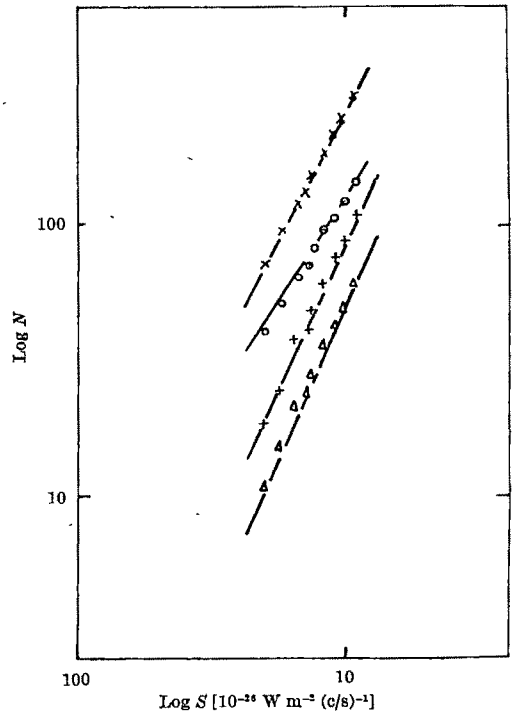


Fig. 1. Log N/log S curves for: (*a*) all the extragalactic radiosources in the *Revised 3 C Catalogue* (×); (*b*) radiogalaxies (○); (*c*) quasars (△), (*d*) the quasars, the empty fields and the 'non-identified' sources (+)

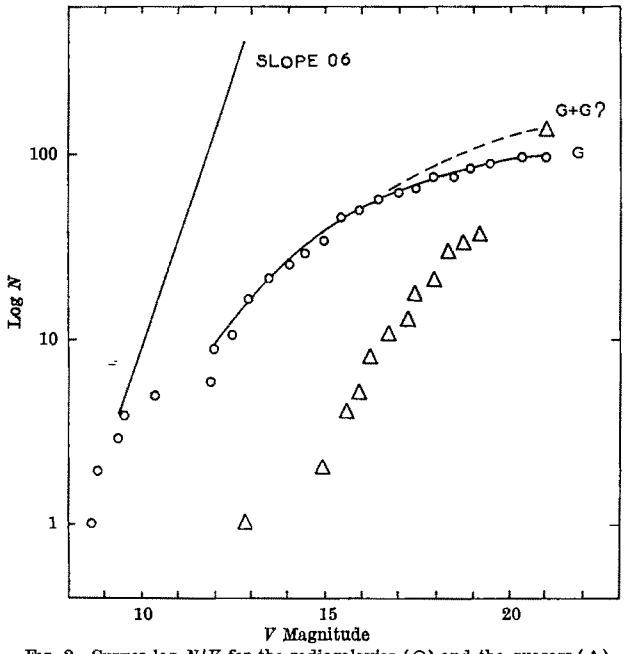


Fig. 2. Curves log N/V for the radiogalaxies (○) and the quasars (△) in the *Revised 3 C Catalogue*

This work was undertaken while I was a research assistant at the Mount Wilson Observatory.

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PLANETARY SCIENCE

Potassium–Argon Age of Plutonic Intrusives on Cape Colville Peninsula and Cuvier Island, New Zealand

THIS communication reports the results of potassium–argon age determinations of biotite and hornblende concentrates from each of two quartz diorite samples of the Paritu Pluton (36° 32′ S., 175° 20′ E.), and of plagioclase and biotite from the hornfels on Cuvier Island (36° 26′ S., 175° 46′ E.) from which we were able to obtain an estimate of the age of the Cuvier Plutonics (Table 1).

The field evidence for the age of these intrusions has not been conclusive. The Paritu Tonalite[1] intrudes a basement sedimentary sequence which may have begun in the Permian[1] and extended through to possible Jurassic[2]. Fossil control is poor. These rocks are overlain by terrestrial volcanics which have been subdivided into three successions. Published age estimates range, for the First Period andesites, from Eocene–Miocene[2] to Pliocene–Miocene[3]; for the Second Period (Beeson's Island Volcanics), Miocene[2], Upper Miocene–Pliocene[1], Miocene–Pliocene[3]; for the Third Period, Pliocene[1–3]. Dykes of porphyritic Tertiary andesite are commonly found which cut the Paritu Pluton, which has been classified as pre-Jurassic, but with some Tertiary components, by Frazer and Adams (ref. 2, p. 89); Jurassic to possibly Miocene by Thompson[1]; and Tertiary by Williams (ref. 3, map facing p. 98). D. Skinner, in an unpublished thesis (1962), concluded that the Paritu Pluton was penecontemporaneous with the First and Second Period andesites, which he adjudged to have been erupted during one continuous volcanic episode, ranging from low Pareora (basal Miocene) to low Wanganui (basal Pliocene) in age.

Table 1

Sample No. *GA*	Locality	Mineral	K (per cent)	^{40}Ar (atm. per cent)	^{40}Ar*/^{40}K	Age (m.y.)
1497	Cuvier Island	Biotite	6·70	34·7	0 000958	16 3
		Plagioclase	0·803	60 6	0 000913	15 6
1498	Paritu Quarry	Biotite	7·26	27·2	0 000947	16·1
		Hornblende	0 371	80 3	0·000941	16·0
1499	Darkie Stream	Biotite	5·76	30 3	0 000936	16 0
		Hornblende	0·398	71·3	0 001005	17·1
				79 8	0 001005	17 1

Thompson[1] made the first record of plutonic rocks on Cuvier Island. He suggested that these Cuvier Plutonics are Jurassic to possibly Miocene in age, and that they can probably be correlated with the Paritu tonalite. The occurrence of tourmaline on Cuvier Island and its possible genetic significance have been discussed by Reed[4].

Our Paritu samples come from the type area in the Paritu Quarry[1], 40 ft. from the contact (*GA* 1498), and from Darkie Stream, 500 yd. south-east of Paritu and about 100 yd. from the contact (*GA* 1499). They are essentially fresh quartz-diorite samples, although they both show some signs of uralitization of the hornblende (*GA* 1499, more than 50 per cent; *GA* 1498, somewhat less so), with *GA* 1499 showing sericitization of some feldspars, and presence of epidote. The rock at Paritu is very rich in xenoliths; at Darkie Stream they are much less abundant. The Cuvier Island hornfels sample (*GA* 1497) comes from within 2 ft. of the intrusive contact, sixteen chains south-east of the type area, Scotts Monument. Some sericitization was observed in the plagioclase. Despite this, essentially clean concentrates were obtained for all six minerals analysed

Argon was measured by isotope dilution, using a Reynolds-type mass-spectrometer and a Berkeley-type argon extraction system[5]. Potassium was measured by means of a flame photometer[6,7]. Non-geological sources of error should make up less than 3 per cent of the reported figure (that is, ±0·5 m.y.).

The age concordance between biotite and hornblende from Paritu Quarry suggests that the time of original crystallization has been recorded, for the reason that a later event causing partial argon loss usually results in a hornblende age significantly greater than that of the less-retentive biotite[8]. Biotites from the other two samples suggest that these also are of the same age. The slightly younger age of the Cuvier Island plagioclase relative to the cogenetic biotite can be regarded as confirmatory, as plagioclase feldspars, which often yield reasonable ages, will nevertheless tend to be somewhat less retentive of argon than biotite. The reproducible argon result on the Darkie Stream hornblende suggests that its apparent difference from the biotite result could be real. This could result from: (1) a wrong potassium value (which we doubt, for the potassium replicates (not reported) show their usual "better than 1 per cent" agreement); (2) differential losses of argon from the minerals (the *GA* 1499 hornblende concentrate crystals appear more translucent —that is, have less inclusions—than those of the *GA* 1498 concentrate); (3) the inclusion in the hornblende of a small amount of "initial argon" during crystallization. We cannot distinguish between these alternatives. However, the discrepancy is small, and the samples show no perceptible indications of any alteration which could not be described as deuteric. Thus it seems not unreasonable to conclude that all six ages refer to the same

event, which occurred in mid-Miocene time, probably between 17·1 m.y. and the average, 16·2 m.y., ago. The correlations of Evernden and others[9] suggest that this is equivalent to Late Hemingfordian–early Barstovian on the North American land-mammal sequence. They equate this to the lower Vindobonian of the European sequence, which seems to approximate[10] to the Waiauan Stage in the New Zealand Tertiary succession (Upper Southland Series).

Thus the result confirms the correlation by Thompson[1] of the Cuvier Island Plutonics with the Paritu Tonalite, and also his tentative suggestion that they may be Miocene. On the basis of the allocation of the First and Second Period andesites to the Miocene, our result also lends support to Skinner's suggestion of a close relationship between the plutonic activity and these earlier periods of major andesitic volcanicity.

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Determination of Trace Amounts of Cobalt in Sea-water after Enrichment with Solid Manganese Dioxide

FEW results are available on the occurrence of trace amounts of cobalt in sea-water. Noddack and Noddack[1] and Black and Mitchell[2] reported obtaining the values 0·1 μg cobalt/kg and less than 0·3 μg cobalt/l., using emission spectrometry. Ishibashi[3] obtained a value of 0·4–0·7 μg cobalt/l. by spectrophotometry. With improved spectrophotometry, Weiss and Reed[4] and Thompson and Laevastu[5] found values of 0·038 μg cobalt/l. and 0·2–0·3 μg cobalt/l. respectively.

Since a fairly specific and sensitive reagent, nitroso *R* salt, has been widely used for the spectrophotometric estimation of cobalt, it seems that the key to the analysis of trace amounts of cobalt in sea-water is to enrich that element from its sea-water concentration to a concentration suited to the sensitivity of nitroso *R* salt reagent. The method of co-precipitation of cobalt with ferric hydroxide, followed by dithizon extraction used by Ishibashi[3] is inadequate for the recovery of cobalt in sea-water within the range of *p*H in which the bulk of the precipitation of magnesium carbonate and hydroxide can be avoided. The procedure used by Weiss and Reed[4], in which cobalt was directly co-crystallized with α-nitroso-β-naphthol from sea-water, is not practical because of the long after-treatment, involving the decomposition of relatively large quantities of organic reagent, ion-exchange purification and other steps. Thompson and Laevastu[5] utilized carbonate precipitation of naturally occurring magnesium for the recovery of cobalt, but this results in a troublesome filtration procedure. It seems that none of these procedures is practicable for use as a routine method. On the other hand, solid manganese dioxide was used successfully by Yamagata and Iwashima[6] to enrich in a sea-water medium various radionuclides which occur in reactor coolants. They obtained almost complete recovery of manganese-54, cobalt-60 and zinc-65, among others. However, they used 70 g granular manganese dioxide for 20 l. of sea-water, that is, 3·5 g/l. of water. This quantity of manganese dioxide is certainly too great for the purpose of trace analysis of cobalt.

We have attempted to apply the principle of the procedure proposed by Yamagata and Iwashima to the trace analysis of cobalt in sea-water, reducing the quantity of manganese dioxide to minimize the possible contamination and interference caused by excessive amounts of that substance. Experiments using cobalt-60 as tracer showed that as little as 10 mg/l. of manganese dioxide in sea-water can effectively recover trace quantities of cobalt (compare Table 1). The cobalt recovered on solid manganese dioxide can be eluted by less than 50 ml. of 8 M nitric acid. By filtering the elutant through a 'Millipore' filter, a clear solution for cobalt analysis can easily be obtained. This solution can be subjected to the normal procedures for trace analysis of cobalt, that is, the extraction of the α-nitroso-β-naphthol complex and spectrophotometry with nitroso *R* salt[7].

Table 1. RECOVERY AND ANALYSIS OF COBALT DELIBERATELY ADDED TO SEA-WATER

Medium	Cobalt added (μg)	Manganese dioxide (mg)	Cobalt recovered with manganese dioxide (per cent)	Cobalt found* (μg)	Error (per cent)
1 0 l. sea-water	1·0	10·0	90·8	0 92	− 8
,,	2 0	10 5	88·8	2·19	+10
,,	3 0	9·9	91 0	2·54	−15
,,	4 0	10·9	94·3	3·56	−11
,,	5·0	10 0	81·7	4 27	−15
,,	10 0	10 2	84·1	9·31	− 7
,,	Blank	9 8	–	0 46†	–

* Corrected for blank and recovery.
† Reagent blank plus cobalt content of sea-water used.

One possible disadvantage of this enrichment procedure is that small amounts of manganese dioxide might dissolve in the course of the acid treatment. We showed that the presence of 1 mg of manganous ion had a negligible effect on the spectrophotometry of cobalt, while the presence of 10 mg had a definite effect. However, it was shown through tracer experiments with manganese-54 that the quantity of dissolved manganese found after treatment with acid was less than 1 per cent of the total added, and after extraction with α-nitroso-β-naphthol, the contamination of manganese in the final solution was less than 0·5 per cent of the dissolved manganese, a harmless amount.

The main advantages of this enrichment procedure are its speed and simplicity, which make it suitable for use on board ship with the minimum possible contamination. Manganese dioxide, practically free from cobalt, can be prepared by the oxidation of manganous ions in strong nitric acid with potassium bromate. The main source of the reagent blank in the spectrophotometric procedure was the concentrated nitric acid used, which contained up to 0·3 μg cobalt/100 ml.

Table 1 shows the results of experiments carried out on a standard series of water samples, to which known amounts of cobalt had been added, to ascertain the recovery of added cobalt on manganese dioxide and the accuracy of cobalt analyses. In order to measure the percentage recovery 0·1–0·2 μc. of cobalt-60 (~0·001 μg cobalt) was added to each sample. As can be seen from Table 1, the percentage recovery of cobalt ranges from 80 to 95 per cent in these conditions. The error in the determination of cobalt in the 1–10 μg range is expected to be less than ± 20 per cent, although the values obtained tend to be lower than 100 per cent. Table 2 shows the results of the determination of cobalt in a sea-water sample taken from a station located 2 km

Table 2. ANALYSIS OF COBALT IN SEA-WATER

Sample	Chlorinity (per cent)	Manganese dioxide added (mg)	Overall recovery (per cent)	Cobalt found (μg)	Cobalt content (μg/l.)
20 l. filtered sea-water	20 87	200	79 3	1·82	0 091
Analysis of the 'Millipore' filter used for filtration of 20 l. sea-water	–	–	88 8	0 06	0 003

south-west of Monaco in June 1965. In this case also 0·2 μc. of cobalt-60 was added immediately after filtration through an *HA*-type 'Millipore' filter. After the addition of manganese dioxide the sea-water was left to stand for 15 h with stirring.

From these results it is estimated that the content of soluble cobalt in sea-water is of the order of 0·1 μg cobalt/l. and that of particulate cobalt of the order of less than 0·01 μg/l., although the percentage error of the latter was estimated to be much higher than that of the former. Details of the procedures and the results obtained will be presented in greater detail elsewhere.

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Oxygen Isotopic Composition of Phosphate from Shells of some Living Crustaceans

RECENTLY one of us[1] reported some results of measurements of the oxygen isotopic composition of phosphate from shells of living marine organisms. Four of the specimens measured were crabs and from the results obtained it was inferred that biological fractionation effects are involved in the process of precipitation of the calcium phosphate of crab shells. It was important to have better information of the behaviour of crabs in this regard in view of the possible utilization of fossil specimens for palaeotemperature measurements on phosphates. For this reason, some crabs were grown under controlled temperature conditions. Some ten specimens of *Carcinides maenas* Rath. (*Carcinus maenas*) were maintained in a thermostatic bath with a continuous inflow of sea water. The reasons for this were: (*a*) to maintain the oxygen isotopic composition of the water at a constant value it is essential to reduce the effects of evaporation; (*b*) that it is necessary to provide a continuous supply of sea water just in case crabs precipitate (at least partially) their calcium phosphate directly from sea water.

The oxygen isotopic composition of the sea water was measured weekly on average samples obtained from daily collections of about 20 c.c. The results covering the period from 1 month before exuviation to 1 month after exuviation give an average oxygen isotopic composition of + 0·6 parts per mil versus standard mean ocean water (SMOW). One standard deviation equals ± 0·1 per mil.

The temperature of the water bath was 25° C and over the whole period it did not vary by more than ± 0·5° C. Such a fluctuation can be considered to be negligible considering the continuous inflow of sea water and the fact that the standard error of the measurements of phosphate δ's, calculated on about 150 measurements, was ± 0·18 per mil which corresponds to about ± 1° C.

The crabs were kept at 25° C some months in advance of moulting in order to ascertain that all the biochemical processes preceding exuviation (principally the major portion of skeletal resorption[2]) occurred at such a temperature.

Because the time which is necessary for the mineralization of the newly formed exoskeleton is not well known, the crabs were kept for various periods in the thermostatic bath after their moulting. In the case of two specimens measured about 2 weeks after exuviation, only traces of calcium phosphate could be detected. Three specimens measured about 5 weeks after exuviation had a phosphate (III) ion content of about 2·0 per cent.

The oxygen isotopic composition of the phosphate of these three specimens was measured according to an earlier technique[1]. The results obtained are expressed in the δ terminology as deviations in per mil of the ratio of oxygen-18 : oxygen-16 in the sample from that in the standard:

$$\delta = 1{,}000 \left(\frac{^{18}O/^{16}O \text{ sample} - ^{18}O/^{16}O \text{ standard}}{^{18}O/^{16}O \text{ standard}} \right)$$

values of δ are given in Table 1.

Table 1

	$\delta^{18}O$ (PO_4^{3-}) vs. CO_2-*PDB*-1	$\delta^{18}O$ (PO_4^{3-}) vs. SMOW
Carcinides maenas No 1	−20 39	+19 68
Carcinides maenas No 2	−20·21	+19·87
Carcinides maenas No 3	−20 50	+19 57

The tentative equation calculated for the relationship between temperature and phosphate δ (ref. 3) is:

$$t = -90{\cdot}0 - 4{\cdot}8\,(\delta_p - \delta_w) \qquad (1)$$

where t is the growth temperature, δ_p is the oxygen isotopic composition of the phosphate and δ_w is the oxygen isotopic composition of carbon dioxide equilibrated isotopically at 25·2° C with the environmental water both measured against the same mass spectrometer standard gas. From this equation the phosphate δ corresponding to a temperature of 25° C with a water δ of + 0·6 (versus SMOW—all the δ's are given here versus SMOW unless specified otherwise) is + 16·60. The average δ from these results is + 19·70. The value is much more positive than would be expected; it can be concluded that, at least in the case considered of the species *Carcinides maenas*, there must be some biological fractionation effect. This effect could be determined, for example, by the processes of partial resorption of the shell material before exuviation and the following redeposition in the newly formed shell.

On the other hand, it seems probable that biological fractionation effects also occur in other crustaceans. In fact we have also obtained measurements for two Mediterranean specimens of *Squilla mantis* and one specimen of *Munida* sp. from deep waters off Florida. In *Squilla mantis* the temperature range is about 13°–23° C and the corresponding phosphate δ's from equation 1 should be (water δ = 0·0) between + 18·7 and + 16·4. In *Munida* sp. (depth about 570 m, growth temperature 7·1 ± 0·1° C) the phosphate δ should be about + 19·9 (water δ = 0·0). The results obtained are given in Table 2.

Assuming a δ value of + 1·0 for Mediterranean water (measured) and a δ value of + 0·6 for *Munida* environmental water (calculated from salinity data from the area) the phosphate δ's are still more positive than they should be. The difference between the two specimens of *Squilla* could be ascribed to different periods of moulting. Also if the number of samples measured is quite small one could conclude that: (*a*) the oxygen isotopic composition of the calcium phosphate of the shell of *Carcinides maenas* (and probably other crabs) is more positive than would be expected on the basis of the equation calculated

Table 2

	$\delta^{18}O$ (PO_4^{3-}) versus CO_2-*PDB*-1	$\delta^{18}O$ (PO_4^{3-}) versus SMOW
Squilla mantis No. 1	−17·50	+22·69
Squilla mantis No. 2	−19 10	+21·02
Munida sp.	−17·44	+22 75

from the results obtained from various marine animals; (*b*) this is also the case for other crustaceans—there seems to be a general tendency toward positive values determined (with some evidence) by biological fractionation effects.

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Fossil Ice Wedges in Hampshire

THREE large fossil ice wedges have been discovered in a coastal section exposed at Highcliffe in Hampshire (*M.R.* 215931). They are to be found within the Plateau Gravel in the uppermost part of the cliff at about 100′ *O.D.* Here 2·4 m of gravels rest on Barton Beds, at this point consisting of an impermeable glauconitic clay. On top of the gravels is an uneven layer of brown loam up to 80 cm thick.

The gravels are made up of sub-angular pebbles and smaller chips and flakes of flint; some derived Eocene material, particularly rounded and blackened small pebbles; and quartz and silica sand. There is some differentiation within the gravels, with a lower horizon of coarser stratified material which stands out slightly from the cliff, and thin seams and lenses of finer material incorporated into the gravels above. Two samples taken from the coarser gravel had median grain sizes of 7 mm and 9 mm; they were not well sorted and were strongly skewed towards the finer sediments. A sample from a pinched out lense of finer material, which was some 3 m long with a maximum depth of 20 cm, had a median grain size of 0·35 mm, and consisted largely of medium and coarse sand with patinated chips of flint.

The loam is a structureless deposit, strong brown in colour (Munsell colour 7·5 *YR* 5/6, dry). A layer about 20 cm deep at the surface is weathered to a dark brown (7·5 *YR* 3/2). Four samples taken from the loam had median grain sizes of 14–57μ, but while this falls within the size range characteristic of loess deposits, the samples were not, in fact, well sorted (Trask sorting coefficients from 0·16–0·33), and more closely resembled flood loams. Small pebbles are incorporated into the deposit. Analogous loams may also be found within several New Forest valleys adjacent to the streams along the valley bottoms.

The wedges appear as 'V'-shaped forms of disturbed gravel, often with pebbles inclined vertically, which extend down into the horizontally bedded gravels, and in one case to the top of the clay which is slightly arched up. There is no surface indication of the features on the ground, possibly because the area has been developed as a car park, although there may well have been none. The pattern of the wedges in plan cannot be established; however, they do extend as wedges back into the cliff. They are particularly noticeable because the material within them is partially bleached to a pale brown (10 *YR* 6/3) as compared with the reddish or strong brown of the local material (7·5 *YR* 5/6 or 6/8), although this, too, is also bleached in parts. This is probably because percolating water has found an easy passage down the disturbed gravel[1].

Two of the wedges are almost vertical; the other is inclined with a disturbed area near where it disappears behind scree at about 2 m. The deepest wedge reaches 3 m to the Barton Clay; all three vary from 0·9 m to 1·2 m in width near the top, tapering downwards.

The material fill at first glance appears to be no different from the local gravel, except for the pebble alignment and colour. However, mechanical analysis of samples from one of the wedges reveals that it has a higher proportion of both coarser and finer fragments than the local gravels; median grain size is 14 mm; but 7 per cent is finer than 2 mm (compared with less than 1 per cent), and 43 per cent is more than 16 mm (compared with about 25 per cent). There is no distinctive element from the overlying loam within the wedge. The most likely explanation for all this is that the wedge was an open feature filled from above by fine dust and larger pebbles before the deposition of the loam. Some form of surface depression still existed at that time, however, because the loam thickens immediately over the wedge where the coarser infill does not quite rise to the level of the gravel on either side. Thus, the former existence of an open crack is shown by the nature of the infill, the aligned pebbles and by the thickening of the loam. In two of the wedges there is distortion of the bedded gravels on either side, suggesting ice growth. However, this is not generally marked.

These features are consistent with those of true ice wedges[2], and in size they are closely comparable with those found near Cambridge[3] and in Yorkshire[1]. A number of opinions have been put forward concerning the climatic requirements for the formation of wedges. Corbel[4] suggested that in Alaska they formed under cold continental conditions with little plant cover and low annual precipitation, with a summer maximum, and no considerable snow cover or moisture in winter. He postulated a mean temperature in the warmest month of 10° C, a January–February temperature of −10° to −20° C; Péwé[6] put forward a mean annual temperature of −6° to −8° C persistent for several years.

In the face of these opinions, it may at least be concluded that the fossil ice wedges were formed under a cold continental climate. Temperatures were low enough for permafrost. The wedges may date from the last (Weichsel) glaciation, or conceivably from the Gipping glaciation, as the ground is almost level and may have suffered little disturbance since then. An earlier date is unlikely because the gravels which here form a terrace feature can be correlated with the Goodwood–Slindon raised beach[7], itself assigned to the previous interglacial.

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An Unusually Radioactive Fossil Fish from Thurso, Scotland

THE results obtained by Diggle and Saxon[1] on a *Homosteus* plate from Clardon Haven, Thurso, do not, as they suggest, differ in any significant way from those published previously by Atkin and myself[2]. We showed that "whereas both uranium and thorium form organic complexes or compounds with hydrocarbon, only uranium is fixed by bone apatite". This means that a radioactive fish plate without much associated hydrocarbon would have a higher ratio of uranium : thorium than one with abundant hydrocarbon. Thus if the plate studied by Diggle and Saxon was mainly composed of bone apatite

the bulk of the radioactivity would be attributable to uranium.

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PHYSICS

Quarks and Cosmology

RECENTLY, Zeldovic *et al.*[1] have described several important astrophysical aspects of quarks. In this communication I shall make some suggestions as to the part that quarks may play in cosmology. Although these ideas are now necessarily speculative, their potential importance seems to warrant at least a brief discussion at this time. A particularly attractive possibility is that a low observed abundance[2,3] of quarks may impose much more stringent limits on the anisotropy of the universe than can be obtained from investigations of radio and quasi-stellar objects.

It seems reasonable to assume that at cosmical densities greater than nuclear densities, and temperatures greater than the quark mass (in units where $c = k = 1$), quarks would be in equilibrium with baryons and consequently would be the dominant form of matter. From these initial conditions, we suppose that the universe expands and two-body reactions between quarks and diquarks produce baryons and mesons. If the baryon mass defect with respect to its component quarks is large, it is possible to ascribe the primeval radiation field to quark combination. If this is not so, then it appears necessary to assume an initial universe comprised of quarks and of radiation in equilibrium with pair-produced particles.

If quark combination is responsible for the radiation field, the required quark mass may be estimated. For example, in the simplest case in which all the cold primordial quarks combine yielding an equilibrium temperature T_q, the present total baryon number density, N_0, and the present radiation temperature T_0, the quark mass would be about $1{\cdot}5 \times 10^{-12}\ T_0^2 T_q / N_0$ GeV. Recent observations[4–8] tend to support a present background radiation temperature[9–11] of about 3 °K. The present total baryon number density is thought to be $N_0 = 10^{-6}$ cm^{-3} (ref. 12) with an uncertainty of about an order of magnitude. For these values and $T_q = 10^{15}$ °K*, the necessary mass is $M_q \approx 4{\cdot}5 \times 10^{10}$ GeV. Although this mass, of the order of that of the most energetic cosmic rays, is very much greater than what is usually considered reasonable for quarks, there is no evidence against the existence of such massive particles.

If quarks did not produce the primeval radiation, Zeldovic *et al.* have shown that their fusion in a homogeneous, isotropic Friedman model would give a present quark concentration in the range $10^{-19} < C_q < 10^{-10}$. Though the uncertainties are large, it is important that even the upper limit implies $C_q \ll 1$, in agreement with available observations. In a homogeneous, anisotropic universe which initially expands from a two-surface[12], it is possible to carry out a similar simple calculation. If such a universe is radiation dominated, we have $T \sim t^{-1/3}$ for the relation between temperature and time, as contrasted with $T \sim t^{-1/2}$ for the isotropic expansion. We assume the total number of primordial quarks to be three times the present number of baryons, and assume, following Zeldovic *et al.*, that quarks combine by way of two-body collisions with an effective cross-section σ (where, for the temperature region of interest, $\langle \sigma v \rangle \approx$ constant). Then the condition for the time-scale of quark combination to be less than or equal to the time-scale for expansion and cooling requires that:

$$\langle \sigma v \rangle \geq 2 \times 10^{-30} \frac{T_0^3 \sqrt{t_a}}{N_0} \text{ cm}^3 \text{ sec}^{-1} \qquad (1)$$

Here v is the quark velocity, and t_a is the time for which the universe preserves its anisotropy. If the primordial quarks also combine to form mesons, the numerical factor in equation 1 could be decreased somewhat. The most uncertain quantity in equation 1 is $\langle \sigma v \rangle$, which eventually should be measured or estimated theoretically. The relation between $\langle \sigma v \rangle$ and the time-scale of anisotropy is plotted in Fig. 1 for several values of T_0^3/N_0 corresponding to $T_0 = 3{\cdot}2$ °K; $N_0 = 10^{-5}$, 10^{-6} and 10^{-7} cm^{-3}. Knowledge of the effective cross-section and velocity, $\langle \sigma v \rangle$, N_0 and T_0 then provides an upper limit on the anisotropy time-scale t_a. If the time-scale of anisotropy were longer than this limit, the rate of cooling would be so rapid that quarks would not have time to combine; their present concentration would be $C_q \approx 1$.

The cross-section for quark production from proton–proton scattering in the statistical model is estimated to be 3×10^{-28} cm^2 for 3 GeV quarks[14]. It decreases very rapidly with increasing quark mass. If this value is typical of the range of cross-sections for quark interactions and is taken as an approximate upper limit to σ for quark combination, then for $v \approx c$, $\langle \sigma v \rangle \approx 10^{-17}$ cm^3 sec^{-1}. This may be compared with $\langle \sigma v \rangle = 7 \times 10^{-20}$ cm^3 sec^{-1} for the formation of helium[13]. From Fig. 1, this puts a very approximate upper limit of 10^{10} sec on t_a. Even allowing for the considerable uncertainty of $\langle \sigma v \rangle$, this suggests that t_a is many times less than the present age ($\approx 4{\cdot}5 \times 10^{17}$ sec) of the universe.

If the universe has always been isotropic during its expansion, a similar calculation shows that $\langle \sigma v \rangle_{\text{isotropic}} \geq 3 \times 10^{-21}\ T_0^3 / N_0 T_q$. For $T_0 = 3{\cdot}2$ °K, $T_q = 10^{13}$ °K, and $N_0 = 10^{-6}$ cm^{-3}, this gives $\langle \sigma v \rangle_{\text{isotropic}} \geq 10^{-26}$ cm^3 sec^{-1}.

The effect of anisotropy (and indeed the effect of any mechanism, such as a scalar field[10], which increases the early rate of expansion) is complementary to its later effect on the evolution of a hydrogen universe. Hawking and Tayler[13] have pointed out that a moderate amount of anisotropy substantially decreases the primeval helium concentration below that predicted by the α–β–γ theory[11]. However, observations do not appear inconsistent with a primeval helium concentration of zero[15]. At present, therefore, the helium calculation can only provide a lower limit to the anisotropy.

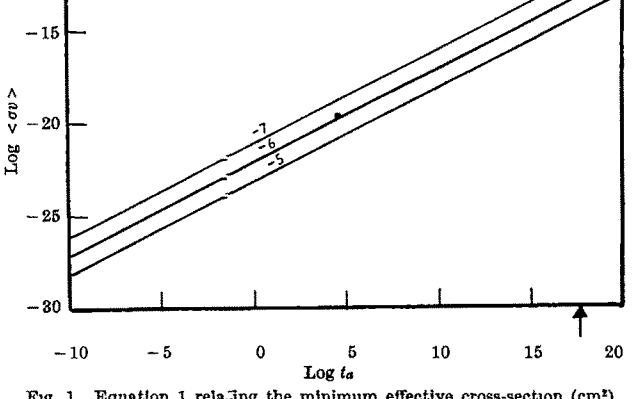


Fig. 1. Equation 1 relating the minimum effective cross-section (cm^2) and velocity (cm sec^{-1}) for quark combination to occur to the time-scale, t_a (sec), for anisotropy. The lines −7, −6, and −5 are for $T_0 = 3{\cdot}2$ °K and for densities $N_0 = 10^{-7}$, 10^{-6} and 10^{-5}. An arrow indicates the approximate present age of the universe

* This temperature is obtained by assuming that the quarks combine when the cosmic density becomes less than the nuclear density of 10^{38} cm^{-3}. Whether the reaction time scale is sufficiently short for this actually to occur depends on the totally unknown cross-sections for extremely massive particles.

It is also important to note another aspect of this model. At the very high temperatures, $T \gtrsim 10^{13}$ °K, at which these reactions occur, pair production of baryons may increase the density substantially. Under these conditions the Fermi temperature $T_f \approx 10^{-13} T^2 (M_n/M_b)$, where M_n is the neutron and M_b the baryon mass. Thus neutrons will not become degenerate until $T \approx 10^{13}$ °K. If quarks are to combine at this or higher temperatures, the quark-baryon mass defect would have to be sufficiently large to release a Fermi energy of about $10^{-26} T^2 (M_n/M_b)$ GeV. Another effect which may become important at high densities is induced if the effective quark radius is much smaller than that of the baryon. In order to create a space Δv for the newly formed baryon, an amount of energy approximately equal to the work $p\Delta v$ must be liberated, where p is the local pressure. Were the mass defect unable to supply the required energy, quark combination would have to be balanced by processes which reduced the local pressure. For quark masses of the order of a few GeV, this also becomes important at temperatures of about 10^{13} °K and such quarks would not be expected to combine at earlier phases of the expansion, except possibly to form a statistical equilibrium with baryons. At these high temperatures there may also be pair production and annihilation of quarks. Such considerations provide relations between quark mass and temperature, and the resultant time-scales for evolution will also be affected by anisotropy and inhomogeneities.

If quark combination from an initially cold universe is responsible for the radiation field, the effects of degeneracy and pair production are less important. In an oscillating universe, the role of quarks may be to exchange energy between the matter and the radiation field during dense phases. Such an exchange, if irreversible, could lead to a damping of the oscillations. The discovery of quarks and their properties may well provide further constraints on theories of the early stages of an expanding universe.

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THE SOLID STATE

Simultaneous Magneto-optic Recording of Hysteresis Loops from Both Surfaces of a Magnetic Film

MAGNETIZATION reversal processes in thin magnetic films have been extensively investigated in recent years. The usual techniques adopted for the display of hysteresis loops have been based on the use of a pick-up coil to detect dM/dt, as described in detail by Crittenden *et al.*[1,2].

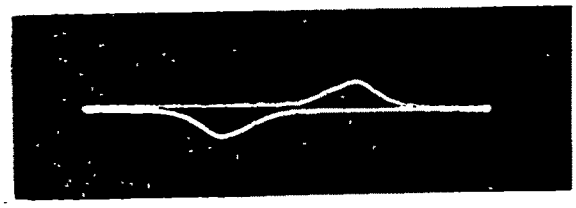


Fig. 1

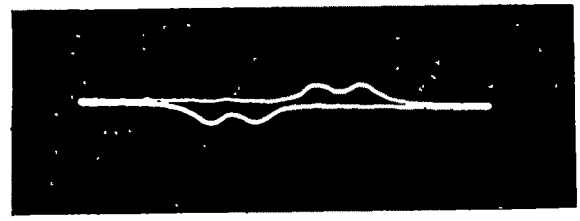


Fig. 2

More recently, use has been made of the magneto-optic effects and, in particular, Green and Thomas[3] have developed a simple technique in which hysteresis loops are obtained using the transverse Kerr effect in the infra-red. With a 50 c/s drive field reversing the magnetization, the intensity changes in the reflected beam are detected by a phototransistor and then amplified.

This technique is being used in investigations of cobalt films in the thickness range 500–4000 Å produced by evaporation on to glass substrates (10–20 Å/sec) and some of the results have already been reported elsewhere[4]. The simplicity of the method has now permitted simultaneous investigations of the magnetization changes occurring at both surfaces of a single film.

In the case of the thinner films (< 2000 Å) the signals detected from each side appear identical. For the thicker films, however, differences are apparent in the signals obtained from each side. This effect first becomes noticeable at a thickness of about 2000 Å and is very pronounced at about 4000 Å. Figs. 1 and 2 show the signals of dM/dt versus drive field taken from the free surface and the substrate surface respectively of a 4000 Å film. It is clear that the reversal process is different at each surface and, furthermore, preliminary results indicate that the effect is dependent on the orientation of the film with respect to the drive field.

Simultaneous recording in this fashion should find useful application in investigations of multiple films.

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Synthetic Single Crystals of Graphite

THE formation of Kish graphite in iron/carbon is well known[1], and reports have appeared of the growth of usable single crystals of graphite at 3,200° C in these systems[2]. Such crystals have not had the benefit of formation at a high temperature, so that in order to produce

long-range order they must be annealed. Accordingly, attempts have been made to crystallize graphite from higher melting solvents such as refractory carbides. No graphite was formed when tungsten monocarbide (m.p. 2,785° C) was used as the solvent, but an abundance of large single crystals of graphite was obtained from tantalum monocarbide (m.p. ~3,820° C).

About 40 g of hot pressed (3,100° C; 100 kg cm^{-2}) tantalum monocarbide having a bulk density of 86–91 per cent was placed in a polycrystalline graphite crucible (Morganite *EY9A*) and sealed with a tight-fitting graphite plug. The crucible was insulated with wrappings of graphite felt (National Carbon Co., grade *WDF*) and assembled in a susceptor for use in a developed version of the high-temperature induction furnace described by Moore *et al.*[3]. After outgassing *in vacuo* at 2,000° C and further degassing treatments in 1·6 atm. pressure of argon at temperatures up to 2,900° C, the furnace was filled with fresh argon and the sample rapidly heated to about 3,900° C, at which temperature it was maintained for 9 min. This required a power input of 19–21 kW. At such temperatures the graphite vapour pressure is so high (~0·9 atm.) that more than 60 per cent of the crucible/susceptor evaporated evenly from the outer surface, but of course the molten tantalum carbide was exposed to the same carbon vapour pressure in a closed system.

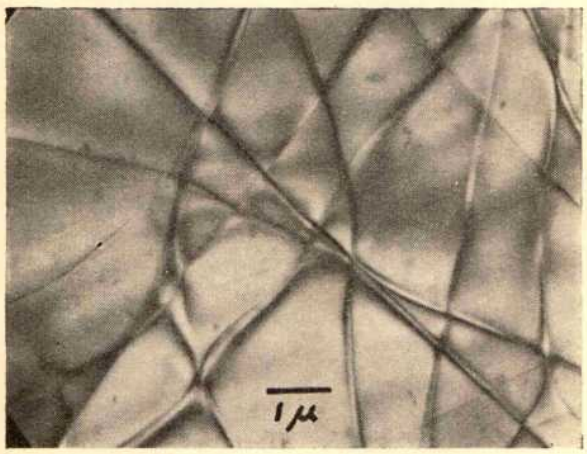


Fig. 1. Electron micrograph of basal plane dislocations in a graphite single crystal grown from solution in molten tantalum carbide. The specks on the plate are artefacts of sample preparation

On examination of the cooled sample, it was found that the molten tantalum carbide had eroded the interior of the crucible at the tantalum carbide/graphite interface only, and that the carbon so removed had reformed as lustrous black flakes up to 8 mm across and 0·3 mm thick, had nucleated at the base of the melt and had grown wholly within it. The interstices were filled with a friable polycrystalline tantalum carbide with the composition $TaC_{0·99}$; this was inferred from the characteristic golden colour and the lattice parameter (4·455 Å)[4]. We therefore conclude that the molten tantalum carbide acted as a transporting solvent.

Examination of the graphite flakes by optical and electron microscopy confirmed that they were single crystals of high quality. Thus characteristic twins contributing equilateral networks of 1·5–2 mm period or basal surfaces were observed to be straight over distanc of 5–8 mm, and specimens taken for electron microsc investigation gave single crystal patterns. The dislocr content, as shown in Fig. 1, is not unduly high. In the extent to which the partial dislocations are dis at nodes implies that this structure is not pinned.

We thank Mr. J. R. White for the electron micrograph.

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A Simple Method of Producing Extremely Pure Crystals of α-Silicon Carbide

THIS communication describes a method of preparing colourless, extremely pure crystals of α-silicon carbide by a modified Lely process in a high-vacuum, high-temperature furnace from silicon of a purity sufficient for semi-conductors and pure graphite. The temperature at which the main reaction occurs, in the course of which the crystals are formed, is 2,800° C. The energy gap, determined according to the method devised by Macfarlane and Roberts, is 2·96 eV. Crystal wafers 100–200μ thick with fresh surfaces exhibited a transmission factor of 80–85 per cent at the characteristic wave-length of 2–4μ.

Silicon carbide is an interesting semi-conductor material, not only from the point of view of its physical properties but also because of its use in high-temperature components and possibly also for its applications in lasers. Silicon carbide has been used in the manufacture of non-linear resistors for many years, although the mechanism that gives it the non-linear current-voltage characteristic has hitherto not been fully explained. However, until now it has not been possible commercially to obtain pure, colourless silicon carbide for a satisfactory investigation of its physical properties. For this reason, we set out to produce hexagonal crystals of silicon carbide of high purity; success was finally achieved by means of a modification of the Lely process[1], which will be described here.

From our experience the following are the most important points for any method which is to yield a pure product: (1) Only silicon carbide can be used as the starting material; however, it must be considerably purer than that which can be obtained commercially (but it should be possible to produce it in fairly large quantities without undue expense). (2) The distribution coefficient for impurities varies appreciably with temperature. The higher the temperature of formation in the range investigated (up to 2,900° C), the higher the purity of the final silicon carbide monocrystals for the same starting material. The main difficulty is to attain the high partial pressures for carbon and silicon required to produce the crystals.

To begin with, fine crystalline yellow silicon carbide rting material; however, the production of this by the well known silicon method is a relatively protracted devised another method which ting material with adequate ner. The heating element as a system of chambers pure graphite is used. or quality is placed in heated for a long time lting point of silicon for a brief period gradient between

the directly heated graphite chamber and the silicon, as a result of which molten silicon penetrates the porous graphite where it reacts to form silicon carbide before it can evaporate. Thus the silicon is converted quantitatively into silicon carbide. The entire process takes place in an inert atmosphere. After the excess graphite has been disposed of by combustion, sintered, yellow microcrystalline silicon carbide remains and can be pulverized and used as the starting material for the subsequent process.

As already stated, various experiments indicated that the distribution coefficient of impurities varies appreciably with the temperature, and for this reason it is necessary for the recrystallization of the silicon carbide to occur at as high a temperature as possible. Obviously, because of the high partial pressures required, the ideal would be to use a totally enclosed hollow body of silicon carbide. We endeavoured to realize this by means of the following process. In the first stage a hollow cylinder of graphite is filled with yellow silicon carbide, produced by the foregoing method, and a graphite rod is pushed into the powdered silicon carbide so that it is surrounded by a uniform layer of the material (Fig. 2). If this arrangement is now heated in a high-vacuum furnace to 2,200° C for about 30 min and the graphite is allowed to burn away in air at 800°–900° C for a fairly long period, we are left with a mechanically strong, still slightly porous hollow cylinder of silicon carbide. On account of its porosity, the graphite core can be completely burnt away. The hollow cylinder of silicon carbide is now placed inside another hollow cylinder of graphite and the reaction temperature is increased to about 2,800° C, so that as a result of dissociation and recrystallization the hollow cylinder of silicon carbide becomes much denser. In the hollow space, plane-parallel, colourless crystals—some hexagonal—begin to grow (their dimensions depend on the geometry of the active space). In our experiments the dimensions were a few millimetres in either direction; the thickness varied between 100 and 200μ.

Naturally it is essential to ensure that there is no contamination in any of these processes. The inert gas used

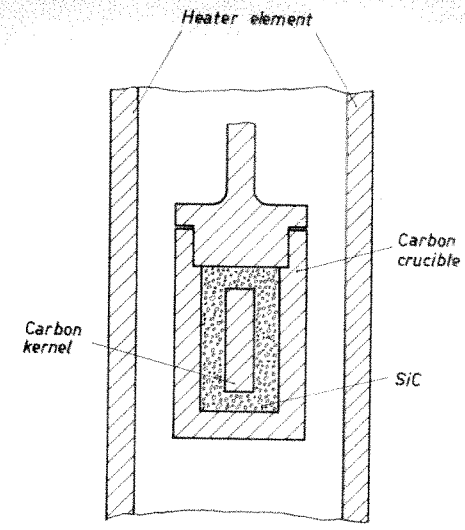


Fig. 2. Arrangement for the production of a cylinder of silicon carbide

was helium—any traces of nitrogen were removed by a calcium trap operated at 700° C, besides the normal methods of purification.

According to Lipson[2], the transmission of light in the range 2–5μ is characteristic of the purity of the material. Our optical measurements confirmed this. For the best wafers the transmission factor in this range amounted to 80–85 per cent for a thickness of 150μ and an untreated surface.

As a means of estimating the purity, we also used the phosphorus content, which can be determined quantitatively with relative ease by measuring the artificial radioactivity (activation analysis) following decomposition of the silicon carbide in a stream of chlorine, or in a sodium carbonate melt. Unfortunately, a number of interesting impurities cannot be considered with this method as their half-lives are extremely short. Although phosphorus is a very specific impurity, the content of this element would seem to offer an appropriate way of determining the general degree of impurity—at least so long as no special measures are taken which affect its content in the compound.

The following are some of the values determined:

Silicon carbide:

(i) commercial, black	1×10^{-2} per cent phosphorus
(ii) commercial, green	3×10^{-4} per cent phosphorus
(iii) starting material, yellow	8×10^{-3} per cent phosphorus
(iv) crystals produced by the method described in this communication	$< 10^{-5}$ per cent phosphorus

Absorption measurements carried out on colourless crystals yielded limits at 3·22–3·25 eV. If these measurements are evaluated with respect to reflexion, according to the methods devised by Macfarlane and Roberts[4], a minimum energy gap of 2·96 eV is obtained. The accuracy of this quantity largely depends on the extent to which the obviously existing long-wave tail can be correctly estimated.

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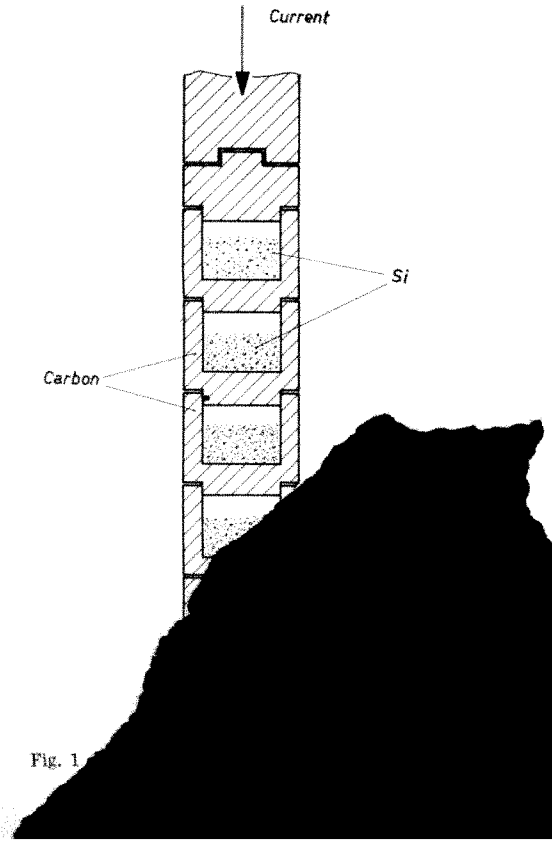


Fig. 1

Internal Friction Effects in Tempered Martensite

It is generally accepted that the high temperature internal friction effects in cold-worked iron containing interstitial atoms and the corresponding effects in martensite are analogous. Both high temperature peaks appear when dislocations and interstitial atoms are present and it is therefore difficult to envisage separate models, although the evidence is mainly comparative and not in any way conclusive.

In the case of the cold-work peak, the dependence on dislocations and interstitial content is easily demonstrated by the fact that the peak is not present when dislocations or carbon or nitrogen atoms are present alone. It is difficult to form martensite with martensitic steels without carbon being present. The most favourable evidence for the similarity between the two effects is to be found in the work of Beaulieu *et al.*[1], who discovered a Koster effect in freshly quenched steels. The Koster effect[2] is found in freshly worked metals where a high internal friction is obtained immediately after working which slowly decays with time. This effect has been attributed to a change in dislocation density and loop-length, although Nowick[3] has suggested that dislocation pinning could also account for this effect. Beaulieu has concluded that the decay of internal friction in martensite arises from the oscillating dislocations collecting point defects and becoming locked. These workers also suggest that the internal friction should pass through a maximum value where the dislocations are moving with the defects.

Gladman and Pickering[4] attempted to investigate the effect of working on the peak height in 0·1 per cent carbon martensitic steel. The martensitic steel was cold-worked and no increase in peak height was obtained. This was attributed to cold-working having little effect on the high dislocation density present in 0·1 per cent carbon martensite. However, the effect of cold-working on the peak height in tempered martensite can be easily investigated. It can be seen in Fig. 1 that tempering reduces the peak height and that a considerable increase in peak damping can be obtained for a very small amount

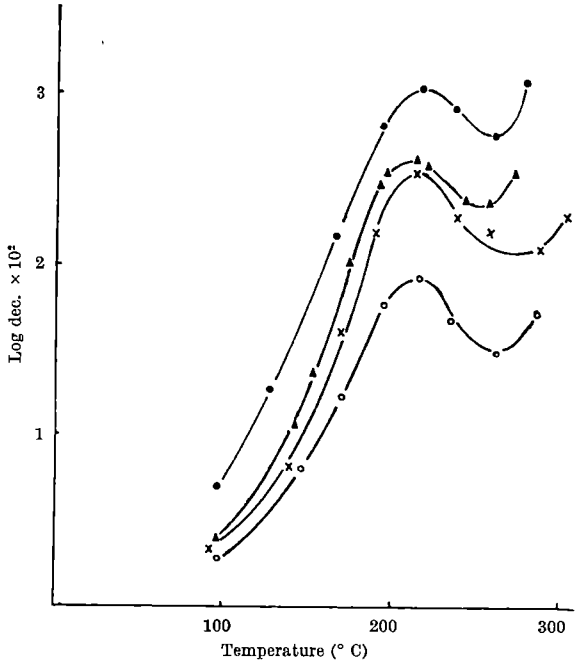


Fig. 1. Effect of tempering and straining 0 4 per cent in tension on the height of the internal friction peak in martensite. Testing frequency, 0 5 c/s. ●, Martensite; ▲, tempered 1 h at 300° C; ×, tempered 1 h at 400° C strained 0 4 per cent; ○, tempered 1 h at 400° C

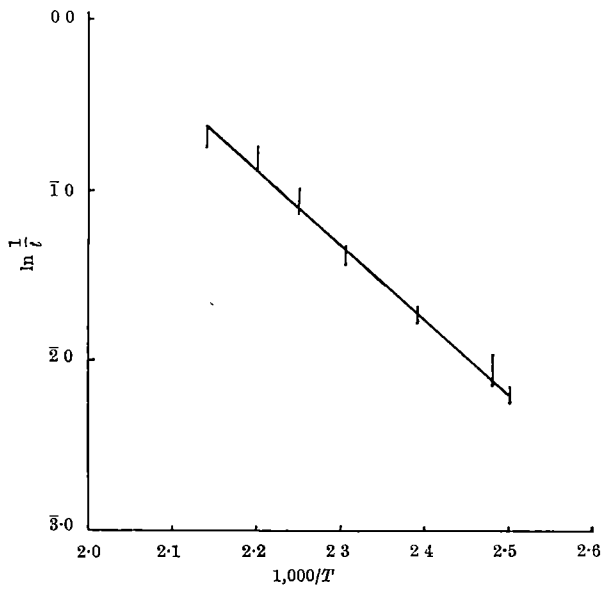


Fig. 2. Arrhenius plot of the natural logarithm of the reciprocal time for complete decay against the reciprocal temperature

of plastic strain. Cottrell[5] has shown that a strain of 10^{-2} can increase the density of mobile dislocations 10^6 times; the strong dependence on cold-working is therefore not surprising.

The connexion between peak height and dislocation population in tempered martensite suggests that, like the cold-worked peak, dislocations are required. The part played by carbon is not easy to establish but some indication can be obtained by performing a Koster-type experiment at elevated temperatures. A 0·4 per cent carbon martensite was prepared by quenching, under cover of argon, from 850° C into brine. Tempering was carried out at 300° C for 1 h before mounting in a torsional pendulum with an optical lever of 3 m. The testing temperature was maintained for 30 min in order to allow temperature gradients within the furnace to be removed before testing. The specimens were deformed *in situ* to a torsional strain of 0·006 radians and the internal friction changes with time followed by determination of the logarithmic decrement over the amplitude range 5 cm down to 1 cm. An Arrhenius plot for the time required for complete decay at various temperatures is shown in Fig. 2, from which an activation energy of $20{\cdot}2 \pm 2$ kcal/mole was obtained. This value is close to the accepted figure of 20·1 kcal/mole for diffusion of carbon in α-iron, and therefore suggests that carbon atoms as well as dislocations play an important part in the high temperature internal friction behaviour of martensite.

These results are interpreted as follows. Immediately on straining, fresh mobile dislocations are formed which are free to bow between their pinning points under the action of the applied stress. The moving dislocation does work against the lattice friction stress. The value of the lattice friction stress controls the extent to which a dislocation will bow, and as a result the damping will decay smoothly with time until the dislocation becomes saturated. Extrapolation of the decay time curves shown in Fig. 2 to zero time, when the dislocation will be moving at the same speed as the carbon atoms, gives a critical temperature of 217° C which agrees with the peak temperature of 215° C. In addition, extrapolation to room temperature shows that the time for complete decay is about 40,000 min. Beaulieu's results show that the damping decayed up to and beyond 20,000 min and an estimate by use of a French curve gives a value for complete decay of 30,000 min. The agreement seems reasonable in view of the difficulty of obtaining the 'end point' experimentally and the inaccuracies inherent in extrapolation. This

indicates that the activation energy for the decay at room temperature is the same as that determined by the present work, and is therefore additional evidence in favour of the pinning model suggested by Beaulieu. However, in my opinion, the high initial internal friction arises from the large area swept out by the moving dislocation. As carbon diffuses to the dislocation and increases the number of pinning points, the total area swept out and thus energy absorbed are smaller than immediately after straining. As a result the damping decreases with time and no maximum on the decay time curve is to be expected, in contrast to that suggested by Beaulieu.

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Fatigue Hardening in Face-centred Cubic Metals

A SOFT copper crystal, cycled between constant alternating total strain limits, shows an initially rapid increase in the required alternating peak stresses, $\pm \sigma_P$. At low amplitudes (lives greater than 10^6 cycles) the increase in peak stress becomes very slow after ~ 1 per cent of the expected life. A crystal in this state is said to be fatigue hardened to saturation. Similar behaviour is observed for other face-centred cubic metals of high stacking-fault energy, for example, aluminium or nickel (for a review see ref. 1).

Electron microscopy of thin films from such specimens reveals a high density of edge dislocation dipoles, and few "free" dislocations[2]. This has led Feltner[3] to propose that at saturation the prescribed strain amplitude can be accounted for by flipping of the dipoles between the two stable equilibrium positions for infinite parallel opposite edge dislocations, with much smaller contributions from bowing of segments of free dislocation and of the dipole legs. He also suggests that the shape of the mechanical hysteresis loop at saturation can be accounted for by the observed distribution of dipole widths; narrow dipoles require greater stresses to flip than wide ones.

The following experiment was designed to test Feltner's theory. A 99·994 per cent copper crystal of orientation [112] was cycled in push-pull to saturation with a modified Instron machine reaching an alternating shear stress of $\pm$ 2·76 kg/mm^2. Then a set of hysteresis loops was plotted with a Moseley 'Autograf *XY*' recorder, using an Instron strain gauge extensometer attached to the gauge length. Special precautions ensured that buckling did not affect the readings. Loops were plotted at the saturation stress, and at increasingly lower stress amplitudes down to the limit of the resolution of the system. This procedure did not affect the hysteretic behaviour on returning to saturation stress.

A typical hysteresis loop taken at the saturation stress is shown as *ABCD* in Fig. 1 (the strain axis is horizontal). When the stress amplitude is lowered, the loop shrinks rapidly, as to *EFGH*. Now, from Feltner's theory, it should be possible to construct the loop *EFGH* from *ABCD* as follows. The dipoles which begin to flip on passing from tension to compression at *C* on the curve *BCD* should be the same dipoles that flip on passing *G* on the curve *FGH*. The basic assumption here is that the flipping of these, the widest dipoles, is not influenced by the state of all the other dipoles and whether they have flipped back or not. Thus by simply translating *BCD* bodily to the left, we obtain *B'GH'*; similarly, carrying *DAB* to the right, we obtain *D'EF'*. The 'Feltnerian' loop so constructed, *EF'GH'*, is obviously too soft compared with the observed loop *EFGH*. This means that there must exist internal 'back' stresses at the end of the stroke *DAB* which assist reverse plastic flow during the stroke *BCD*. These back stresses are evidently present to a lesser degree after the stroke *HEF*, so that the curve *FGH* is obtained rather than the predicted *F'GH'*. The origin of the back stresses is not described by the Feltner theory in its present form. However, they may be measured by noting that at *B*, $\sigma_P = \sigma_F + \sigma_B$, where σ_F is a frictional stress opposing flow in either sense, and σ_B is the back stress. Reverse plastic flow on the curve *BCD* begins at $\sigma_R = \sigma_F - \sigma_B$. σ_R may be taken at some arbitrary (small) offset, in order to obtain relative measurements of $\sigma_B = (\sigma_P - \sigma_R)/2$ (see Fig. 1). In this way, a set of values for σ_B can be obtained for a set of immediately preceding peak stresses. Thus the difference between the back stress at *C* after the stroke *DAB* and the back stress at *G* after the stroke *HEF* can be measured. This difference can now be applied to the curve *B'GH'* as a translation downwards along the elastic line *B'G*. If this is done, it is found that *B'GH'* usually fits reasonably well along *FGH*, within experimental error. Similarly, *D'EF'* is moved upwards, and fits along *HEF*. The fact that this construction is satisfactory suggests that there is, approximately at least, a cyclic state which is a function of amplitude, but the metal must be taken around the hysteresis loop at the chosen amplitude a few times in order to wipe out the previous internal stress history. It seems difficult to reconcile this behaviour with a pure dipole flipping model, as the stress field associated with a dipole does not extend much further than one dipole width away. In general, the dipoles appear to be separated more widely than this; further, a rather special grouping of dipoles would be required.

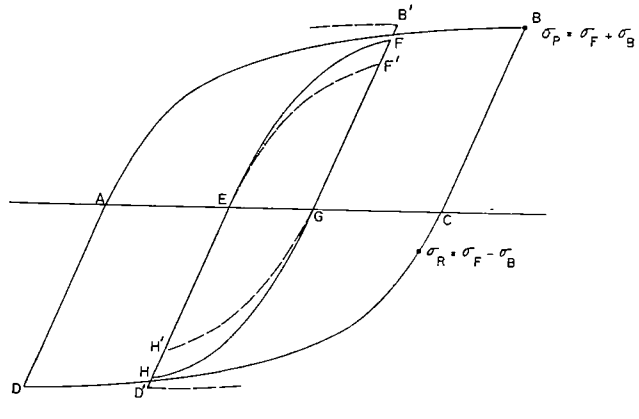


Fig. 1

We suggest that a substantial part of the prescribed strain amplitude does not arise from dipole flipping, but is a result of flow in the soft[4] persistent slip bands which form at saturation[1]. They are associated with a cell structure of dislocation[5]. At high amplitudes (lives less than 10^4 cycles), a similar cell structure fills the crystal, and high internal stresses are observed in corresponding hysteresis loops[6]. This structure appears able to account for the present observations. Dislocations shuttling back and forth between cell walls contribute to the observed strain amplitude; as they pile up at one side σ_B increases; σ_F arises from their tangling with the walls. At σ_R the first dislocations are disentangled from the walls and move back; as they do so, σ_B is reduced.

The problem now is to assess the relative contributions of dipole flipping and cell shuttling to the total strain.

The present experiments are being extended to higher and lower saturation stresses, and correlated with observations of slip. The effects of orientation and temperature have also been measured.

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ENGINEERING

Wind Forces and the Proximity of Cooling Towers to Each Other

Gunn and Malik have recently suggested[1] that their results showing the effect of spacing on the drag of an array of spheres in a closed duct provide an explanation for the collapse of the Ferrybridge cooling towers. It is well known that the drag of a grid of bars or a screen in a wind tunnel increases rapidly as the solidity (that is, the blocked area over the total area) increases. The air passes through the grid as a series of jets and, crudely, it can be argued that the velocity in these jets varies as the solidity. Because aerodynamic forces increase as the square of velocity the drag of the grid will go up as the square of the solidity. For an array of spheres the solidity is $\frac{\pi}{4}(1+a)^{-2}$, where a is the gap in diameters between adjacent spheres; Gunn and Malik present results for $a = 1{\cdot}0$, $0{\cdot}5$ and 0. Using this crude theory, over this range one might expect an increase in drag coefficient based on upstream velocity of some sixteen times.

However, Baines and Peterson[2] devised a more exact theory for the drag of grids. They equated $\frac{R}{\rho U^2}$, where R is the force on unit projected area, to $\frac{S}{2(1-S)^2}$, where S is the solidity. The shape of the bars and any Reynolds number effects are ignored. At high Reynolds numbers these effects are likely to be small in comparison with the effect of the wind tunnel blockage. The array of spheres tested by Gunn and Malik was twenty layers deep but because the spacing was small it will be considered as a single grid. Table 1 gives a comparison of the measurements of Gunn and Malik, at their highest Reynolds number of 2×10^3, with those predicted by the method of Baines and Peterson

Table 1

a	S	$\frac{R}{\rho U^2}$ measured	$\frac{S}{2(1-S)^2}$ predicted
0	0·785	7·3	8 5
0 5	0·349	0 25	0 41
1 0	0 197	0 14	0 15

Thus, although Gunn and Malik's results are very interesting, they were to be expected. However, they bear little relation to the forces experienced on an array of cooling towers in an unconstrained airflow: indeed, if their results were relevant to groups of cooling towers, they would also be applicable to building complexes generally, and would imply wind loadings large enough to have caused failure of a great many of our major buildings and structures.

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CHEMISTRY

Food from Coal-derived Materials by Microbial Synthesis

THE explosive increase in the world's population has been accompanied by an overall food shortage. New and increased supplies of food, especially high quality protein, are urgently needed. Earlier investigations have described the growth of micro-organisms on paraffins, synthetic liquid fuel ('Kogasin'), and petroleum fractions[1–3]. Recently, it has been found that micro-organisms convert petroleum or petroleum fractions to protein, vitamins, or amino-acids[4–14]. Because coal is a cheap fossil fuel and by far the most abundant source of readily available, fixed carbon, investigations were initiated to determine whether coal could serve as a source of high protein food.

This communication reports some results of an evaluation of a variety of coal-derived materials as growth substrates for micro-organisms. Emphasis was placed on liquid coal-derived fractions because of the possibility of extending the utility of fuels and chemicals from coal conversion processes. In general, we found that yields of growth on several coal-derived materials compared favourably with yields on pure hydrocarbons and on a petroleum-derived fraction.

The yeasts *Candida lipolytica* (strains 409, 409*A*, 409*B*), *C. tropicalis* 410, and six unidentified species of bacteria isolated from soil by enrichment culture were used as test organisms. The basal growth medium (*p*H 7·0) contained 5·0 g ammonium nitrate, 2·5 g potassium monohydrogen phosphate, 1·0 g $MgSO_4 \cdot 7H_2O$, 0·1 g yeast extract per litre of tap water and to this was added, as the major source of carbon and energy, a weighed quantity of one of the substrates described here. Triplicate cultures (50 ml. of medium in 300 ml. Erlenmeyer flasks) were incubated at 30° C for 4–6 days (bacteria) or 6 days (yeasts) on a rotary shaker. The resultant growth was collected on tared solvent resistant membrane filters, washed free of residual substrate with acetone followed by *n*-hexane, and then dried and weighed. Yields were corrected for growth in triplicate controls without added substrate.

The following substrates were tested as major sources of carbon and energy:

Fischer-Tropsch synthetic liquid fuel fractions: FTL (boiling 0°–204° C), FTD (204°–316° C), and FTW (> 316° C) were Bureau of Mines products (iron catalyst); fraction SASOL (C_{11}–C_{20}, mainly C_{12}–C_{18}: 64 per cent paraffins, 33 per cent olefines; both mostly normal) was a product of the South African Coal, Oil, and Gas Corporation.

Low temperature tar fractions: HSF (a "hexane solubles" forerun with about 7 per cent phenols) and HSD (a "hexane solubles" distillate with about 12 per cent phenols) were obtained from the Texas Power and Light Company. These fractions constituted 7 and 46 per cent, respectively, of the primary tar from Rockdale lignite. Passage of fractions HSF and HSD through alumina provided hydroxyl-free fractions HSFφ and HSDφ (infra-red analysis). Paraffin-rich fraction CTP (88 per cent *n*-paraffins-C_8–C_{15}; 12 per cent normal α-olefines, C_{10}–C_{14}) and paraffin,

olefine fraction CTPO (44 per cent *n*-paraffins, C_9–C_{18}; 56 per cent normal α-olefines, C_9–C_{18}) were derived from the neutral oil of low temperature tar by the Bureau of Mines.

Petroleum-derived fraction PET, obtained from an industrial company, consisted of C_9–C_{14} *n*-paraffins. Other substrates tested were three coal acid mixtures (one from anthracite, two from bituminous coal) and an artificial mixture of polynuclear aromatic hydrocarbons found in high temperature coal tar.

The highest yields of growth with yeasts were obtained with *n*-hexadecane as substrate; yields on 1-octadecene were decreased by about one-half (Table 1). Normal paraffin fraction PET (from petroleum) supported growth yields ranging from a low of 244 mg dry weight (*C. tropicalis* 410) to a high of 430 mg dry weight (*C. lipolytica* 409*B*). Fischer–Tropsch fractions FTD (*C. lipolytica* 409*B*), FTW and SASOL (all *Candida* strains) as well as low temperature tar fraction CTPO (*C. lipolytica* 409, 409*A*, 409*B*) supported growth yields within the broad range obtained on fraction PET. The yield of *C. lipolytica* 409*B* on low temperature tar fraction CTPO approached the lower limit of the yield on fraction PET. The crude protein content of *Candida lipolytica* 409*B* grown on Fischer–Tropsch fraction SASOL was 41·1 per cent, which compares favourably with values reported for micro-organisms grown on petroleum hydrocarbons[10–14].

Table 1. GROWTH YIELDS OF YEASTS ON PURE HYDROCARBONS AND FRACTIONS DERIVED FROM COAL OR PETROLEUM

Substrate (carbon source) Fischer–Tropsch	Growth yield (mg/g substrate)[a] *C. lipolytica* 409	*C. lipolytica* 409*A*	*C. lipolytica* 409*B*	*C. tropicalis* 410
FTL	0(1)	0(1)	0(1)	0(1)
FTD	114–134(2)	115–150(2)	243–280(2)	0(1)
FTW	287–306(2)	127–167(2)	329–344(2)	161–200(2)
SASOL	322–326(2)	341–347(2)	348–451(3)	224–261(2)
Low temperature tar				
HSF	0(1)	0(1)	0(1)	0(1)
HSFφ	0(1)	0(1)	0(1)	0(1)
HSD	14(1)	6(1)	0(1)	0(1)
HSDφ[b]	73*–83†(2)	43*–72†(2)	0*–106†(2)	62*–102†(2)
CTPO	113(1)	124(1)	233(1)	205(1)
CTP	259–272(2)	235–276(2)	287–294(2)	208–232(2)
Petroleum				
PET	280–342(3)	306–338(3)	374–430(3)	244–290(3)
Pure hydrocarbons				
1-Octadecene	357(1)	295(1)	244(1)	344(1)
n-Hexadecane	719(1)	628(1)	433(1)	453(1)

[a] Growth yield (mean of growth in triplicate culture vessels) refers to mg of dry cell material synthesized per gram of substrate added. Mean low and high growth yields are given for experiments which were repeated (number of experiments in parentheses).

[b] Growth yield on HSDφ freed of phenols by two (*) or three (†) passes on alumina.

The coal acids and the mixture of polynuclear aromatic hydrocarbons proved inferior nutrients for bacteria; yields of growth in all cases were less than 100 mg dry weight. No significant growth of yeasts could be obtained on the coal acids and the polynuclear aromatics.

These results suggest that, in general, higher yields of growth of yeasts are favoured by substrates (carbon and energy sources) which (*a*) are higher boiling, and thus of higher molecular weight (as shown by yields on FTL, FTD, and FTW); (*b*) contain a high ratio of normal paraffins to olefines (compare yields on *n*-hexadecane with 1-octadecene; PET, CTP, and SASOL with CTPO); and (*c*) are low in phenols or other toxic compounds removed by alumina (compare yields on HSD with HSDφ).

We conclude that paraffin-rich, higher molecular weight fractions derived from low temperature coal tar and from Fischer–Tropsch synthetic liquid fuel compare favourably with a petroleum-derived fraction as substrates for microbial production of food. Coal, therefore, may join petroleum as a potential source of high protein food.

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Chemical Synthesis of the Tryptophan Path Intermediate 1-(*o*-Carboxyphenylamino)-1-deoxy-D-ribulose 5-Phosphate

THE Amadori compound 1-(*o*-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate has been shown to be an intermediate of tryptophan biosynthesis in *Aerobacter aerogenes*[1,2] and *Escherichia coli*[3]. In the course of an attempt to synthesize chemically the suggested intermediate, *N*-(5′-phosphoribosyl) anthranilic acid, I found that a compound with the properties and spectral characteristics of the Amadori rearrangement product was formed[4]. The method of synthesis of this compound has not been previously described, as until recently I have not used it in biological systems. The product (synthesized in 1960) has recently been used as a substrate in investigations of indoleglycerolphosphate synthetase in extracts of different micro-organisms. Since 1960 the intermediate, in the form of a sodium salt and another sample in the form of a barium salt, has survived a trip round the world and storage under refrigeration at 4° and −15° C.

The method of synthesis described here relates to the particular sample for which spectra are given and which we used as an enzyme substrate. Smith and Yanofsky[5] have described a synthesis of the intermediate, but in their method a crystalline product was not obtained. The present method is only one of several procedures successfully tried, but no attempt has been made to develop the synthesis fully.

Ribose 5-phosphate barium salt (1 mmole) was dissolved in 1 ml. water. Sodium sulphate (1·1 mmoles) was added and the precipitate of barium sulphate filtered off and washed with 0·25 ml. water. The combined filtrate and washings were added to 2 ml. ethanol with stirring. This resulted in a slight cloudiness. Anthranilic acid (2 mmoles) in 2 ml. ethanol was added and resulted in a rapid clarification of the reaction mixture and a tendency to turn yellow. After stirring for 3 h 10 min the reaction mixture was placed in the refrigerator. The supernatant was decanted and the residual oil triturated with ethanol. The resulting yellow solid was filtered, dried under vacuum. The yield was 150 mg (41 per cent based on ribose 5-phosphate). The product was very hygroscopic. A similar

Table 1. PROPERTIES OF REACTION PRODUCT COMPARED WITH THOSE OF ANTHRANILIC ACID AS DETERMINED ON PAPER CHROMATOGRAMS

Reagent	Reaction product	Anthranilic acid
R_F	0·63 (tailing)	0·86
Ultra-violet light	not fluorescent	blue fluorescence
Alkaline triphenyltetrazolium chloride	Red (positive)	nil
Ammonium molybdate (75°, 15 min)	Blue-green	nil
Phloroglucinol (90°, 2·5 min)	Purple (weak reaction)	nil, yellow on keeping

The reaction with phloroglucinol is somewhat different from that of the non-phosphorylated Amadori compound which gives a green-purple reaction (ref. 2).

product melted at 177–181° C (with violent decomposition).

A sample of the crude product was dissolved in water, then acidified to a *p*H below 2 and chromatographed on Whatman No. 1 paper (solvent, methanol : butanol : benzene : water, 2 : 1 : 1 : 1 by volume) using a descending technique. A comparison between the properties of the product and anthranilic acid is given in Table 1. These properties are as expected for the phosphorylated Amadori intermediate. A comparison with the mobility of the non-phosphorylated form, as found in earlier work[2], indicates a slightly lower mobility for the phosphate. It should be noted that the phosphorylated intermediate does not fluoresce in ultra-violet light. This is in contrast with the non-phosphorylated form which fluoresces blue in a similar manner to anthranilic acid.

Ultra-violet spectral analyses carried out at the time of synthesis have been given previously[4]. The crude product was assumed to be 1-(*o*-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate disodium salt. Weight, spectra and conversion with alkali to anthranilic acid[3] indicated a purity of 60 per cent, with an impurity of 8 per cent anthranilic acid, on molar calculation. Previous experience with glycosylamine derivatives of anthranilic acid has indicated that they can have considerable water, or solvent, of crystallization[2].

For enzyme experiments, a solution of the disodium salt, synthesized in 1960, was made in water. This was acidified with hydrochloric acid to a *p*H below 2·5 and extracted with 2 × 4 ml. ether in order to remove anthranilic acid and the dephosphorylated Amadori compound. The aqueous layer was separated and the remaining traces of ether evaporated off by bubbling a stream of argon through the solution. The *p*H was then adjusted to 5·2. Conversion to anthranilic acid with alkali (*p*H greater than 10, 1·5 h at room temperature) and measurement of the absorption at 307 mμ allowed the concentration of the Amadori compound to be calculated[3]. The spectral characteristics of 1-(*o*-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate have now been calculated (Fig. 1). 0·1 M *tris*-hydrochloric acid buffer, *p*H 7·4, $\varepsilon_{249m\mu}$ 8,100, $\varepsilon_{326m\mu}$ 3,250; *p*H adjusted to less than 2·5 with hydrochloric acid, $\varepsilon_{249m\mu}$ 6,600, $\varepsilon_{343m\mu}$ 3,800 (measured immediately, later falling to extinction similar to *p*H 7·4, because of the keto-enol equilibrium). In water adjusted to *p*H 5·2, $\varepsilon_{248\cdot5m\mu}$ 7,200, $\varepsilon_{336m\mu}$ 3,500.

Because of the cost of ribose 5-phosphate and the suitability of the product for enzyme work, no further chemical investigations were carried out. In an early preparation a barium salt was precipitated with barium acetate. Smith and Yanofsky[5] comment that their attempts at purification, using ion exchange resins, failed. However, taking into account the method of preparation and the likely impurities, the following purification procedure is suggested.

Prepare the sodium salt by the method given here. The likely impurities are anthranilic acid and the non-phosphorylated Amadori compound. These can be removed by acidification and extraction with ether. The aqueous layer will probably contain some ribose 5-phosphate and ribose. If barium acetate is added, the Amadori compound will be precipitated as the barium salt, while barium ribose 5-phosphate and ribose remain soluble. A preliminary preparation indicated that a barium salt of at least 95 per cent purity was obtained. This is not very soluble, but may readily be converted to the soluble sodium salt with sodium sulphate, or presumably with an appropriate ion exchange resin.

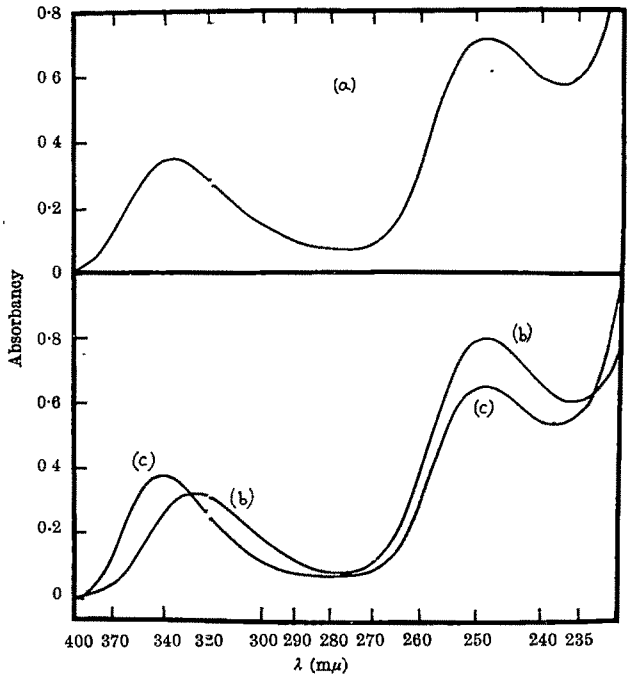


Fig. 1. Spectra of 1-(*o*-carboxyphenylamino)-1-deoxy-D-ribulose 5-phosphate at 10^{-4} M (*a*) in water at *p*H 5·2. (*b*) in 0·1 M *tris*-hydrochloric acid buffer, *p*H 7·4, (*c*) as (*b*) but *p*H adjusted to less than 2·5 with hydrochloric acid and spectrum recorded immediately. The spectral data given by Smith and Yanofsky (ref. 5) suggest that their sample was contaminated with anthranilic acid, but no extinctions or actual spectra were given. The extinctions indicated by the spectra of Doy (ref. 4) were lower than the present ones because that sample on a weight basis was only 55–60 per cent pure with some contamination with anthranilic acid (probably 8 per cent on a molar basis)

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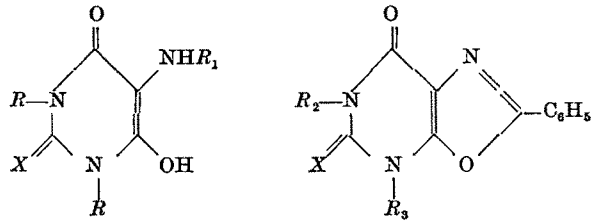
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Transacylation of Acetyluramil or Acetylthiouramil; formation of Oxazolo(5,4-*d*)pyrimidines

CONDENSED pyrimidine ring compounds have been widely investigated, but relatively little is known of oxazolo-(5,4-*d*)pyrimidines, which have so far been prepared by the cyclization of uramil with acetic anhydride or benzoyl chloride[1,2], of 5-acyl- or 5-aroyl-amino-4-hydroxypyrimidines with phosphorus oxychloride[3–5], or by the Curtius reaction of 4-aminopyrimidine-5-carbohydrazides[6].

In order to obtain 2-methyloxazolo(5,4-*d*)pyrimidines, unsuccessful attempts were made to cyclize acetyluramil (I), m.p. 270–3° C (dec) (acetic acid) (found: carbon, 38·63 per cent; hydrogen, 3·79 per cent. The formula $C_6H_7N_3O_4$ requires: carbon, 38·92 per cent; hydrogen, 3·81 per cent), or its 2-thio analogue (II), m.p. > 300° C (dissolved in 10 per cent sodium hydroxide afterwards acidified with hydrochloric acid. Found: carbon, 33·38 per cent hydrogen, 3·97 per cent. $C_6H_7N_3O_3S{\cdot}H_2O$ requires: carbon, 32·87 per cent; hydrogen, 4·14 per cent), obtainable by the condensation of ethyl acetaminomalonate with urea or thiourea in the presence of sodium ethoxide, with acetyl chloride, acetic anhydride, or phosphorus oxychloride.

On the other hand, the treatment of these compounds with benzoyl chloride gave the cyclized products. Heating (I) in refluxing benzoyl chloride with subsequent dissolution of the reaction mixture in ethanol gave 2-phenyloxazolo (5,4-*d*)pyrimidine-5,7-dione (III), m.p. 282–3° C (dec) (methanol) (found: carbon, 57·49 per cent; hydrogen, 3·00 per cent; nitrogen, 18·36 per cent. $C_{11}H_7N_3O_3$ requires: carbon, 57·64 per cent; hydrogen, 3·08 per cent; nitrogen, 18·34 per cent), λ_{max} 321 mμ (log ε 4·10) in ethanol. Its major infra-red absorptions ('Nujol') were at 1689(*s*), 1639(*w*), 1597(*vw*), 1550(*w*), 1316 (*w*), 1261 (*w*), 1227(*w*) and 1136(*w*) cm^{-1}. However, with large excess of this cyclization reagent, 2-phenyloxazolo(5,4-*d*)-pyrimidine-4,6-dibenzoyl-5,7-dione (IV), m.p. 188–9° (ethyl acetate) (found: carbon, 68·32 per cent; hydrogen, 3·40 per cent. $C_{25}H_{15}N_3O_5$ requires: carbon, 68·65 per cent; hydrogen, 3·64 per cent), λ_{max} 301 mμ (log ε 4·40) with an inflexion at 293 mμ (log ε 4·38) in chloroform was isolated, the major infra-red absorptions ('Nujol') of which were at 1767(*s*), 1631(*s*), 1608(*m*), 1587(*w*), 1550(*w*), 1486(*m*), 1418(*m*), 1325(*m*), 1266(*s*), 1242(*s*), 1181(*m*), 1099(*s*), 1080(*s*), 1060(*s*), 1037(*s*), 1031(*s*), 1015(*s*), 1002(*m*) and 986(*s*) cm^{-1}. Hydrolysis of (IV) with aqueous sodium hydroxide in acetone afforded (III), with m.p. 282° C (dec) (found: carbon, 57·29 per cent; hydrogen, 3·07 per cent).



(I) R=H; R_1=Ac; X=O
(II) R=H; R_1=Ac; X=S
(VIII) R=C_6H_5; R_1=H; X=S
(III) R_2=R_3=H; X=O
(IV) R_2=R_3=C_6H_5CO; X=O
(V) R_2=H, R_3=C_6H_5CO; X=S
(VI) R_2=C_6H_5CO; R_3=H, X=S
(VII) R_2=R_3=H; X=S
(IX) R_2=R_3=C_6H_5; X=S

The ease of formation of (IV) indicates the presence of (III) predominantly as a lactam form. This was confirmed by infra-red spectrum analysis ($\nu_{C=O}$ 1,689 cm^{-1}).

Analogous treatment of (II) with large excess of refluxing benzoyl chloride gave monobenzoylated compound, m.p. 228–9° C (dioxane-*n*-hexane) (found: carbon, 61·48 per cent; hydrogen, 3·09 per cent; nitrogen, 11·86 per cent. $C_{18}H_{11}N_3O_3S$ requires: carbon, 61·89 per cent; hydrogen, 3·15 per cent; nitrogen, 12·03 per cent), λ_{max} 319 mμ (log ε 3·97) with an inflexion at 332 mμ (log 3·87) in dioxane, showing major infra-red absorptions ('Nujol') at 1695(*s*), 1647(*sh*), 1582(*w*), 1553(*w*), 1515(*w*), 1351(*m*), 1318(*w*), 1267(*w*), 1221(*w*), 1205(*m*), and 1176(*w*) cm^{-1}. No absorption was found at about 2,500 cm^{-1}, and this suggested a thiolactam structure for this compound.

Hydrolysis of this compound with aqueous sodium hydroxide in acetone afforded 2-phenyloxazolo(5,4-*d*)-pyrimidine-5-thione-7-one (VII), m.p. 248–9° C (dec) (acetone) (found: carbon, 53·79 per cent; hydrogen, 3·04 per cent. $C_{11}H_7N_3O_2S$ requires: carbon, 53·87 per cent; hydrogen, 2·87 per cent), showing major infra-red frequencies ('Nujol') at 1695(*s*), 1667(*sh*), 1634(*m*), 1613(*w*), 1600(*sh*), 1575(*m*), 1351(*m*), 1230(*s*), and 1106(*s*) cm^{-1}.

These results indicate the occurrence of transacylation of (I) or (II) with a benzoyl group before cyclization to oxazolo(5,4-*d*)pyrimidine ring. This would be caused by the hydrolytic cleavage of an acetyl group, with the subsequent benzoylation of uramil or thiouramil. The labile nature of an acetyl group in acetyluramil was noted by Piloty *et al.*[7].

Nitrosation of 1,3-diphenyl-2-thiobarbituric acid gave its 5-nitroso compound, m.p. 226–8° C (dec) (methanol) (found: carbon, 58·72 per cent; hydrogen, 3·53 per cent. $C_6H_{11}N_3O_3S$ requires: carbon, 59·07 per cent; hydrogen, 3·41 per cent), which was reduced with ammonium sulphide to its 5-amino derivative (VIII), m.p. 190–2° C (dec) (dissolved in 10 per cent sodium hydroxide with subsequent acidification with hydrochloric acid) (found: carbon, 54·98 per cent; hydrogen, 4·94 per cent. $C_{16}H_{13}N_3O_2S{\cdot}2H_2O$ requires: carbon, 55·32 per cent; hydrogen, 4·93 per cent). (VIII) was cyclized with benzoyl chloride to 2,4,6-triphenyloxazolo(5,4-*d*)pyrimidine-5,7-dione (IX), m.p. 264–5° C (dec) (toluene) (found: carbon, 69·46 per cent; hydrogen, 3·78 per cent; sulphur, 8·48 per cent. $C_{23}H_{15}N_3O_2S$ requires: carbon, 69·50 per cent; hydrogen, 3·80 per cent; sulphur, 8·07 per cent), λ_{max} 329 mμ (log ε 4·41) with an inflexion at 341 mμ (log ε 4·22) in chloroform, which showed major infra-red absorption ('Nujol') at 1727(*s*), 1645(*m*), 1597(*w*), 1493(*m*), 1326(*m*), 1297(*w*), and 1250(*s*) cm^{-1}.

Carbonyl stretching vibrations in the cyclization product of (II), and that of (VII), were at the same value (1,695 cm^{-1}). The lower value of this frequency, compared with that in (IX) (1,727 cm^{-1}), are probably due to intermolecular association.

However, no conclusion could be drawn from these and other available data on the structure of the cyclization product of (II), which should be (V) or (VI).

A noteworthy feature of the infra-red spectra of (III), (IV), (V or VI), (VII) and (IX) is the appearance of an absorption, the intensity of which is variable, in the region 1,650–1,630 cm^{-1}. This may be associated with the C=C or C=N valency vibration of the oxazole ring, if the pyrimidine moiety takes the lactam form. An absorption with medium intensity in the region 1,600–1,490 cm^{-1} ((II), 1,567; (VII), 1,575; (IX), 1,493 cm^{-1}) can be attributed to a vibration of the N—C=S group. The shift of this absorption to the lower value in (IX) would be caused by the increased conjugation of nitrogen atoms with phenyl groups, but for (V or VI) no frequency due to this group can be seen in this region. An absorption at about 1,200 cm^{-1} ((II), 1,224; (V or VI), 1,205; (VII), 1,230; (IX), 1,250 cm^{-1}) was correlated with $\nu_{C=S}$.

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Proton Microanalysis

A NUMBER of papers have been published[1-5] which demonstrate the use of nuclear reactions induced by charged particles as a tool for the measurement of light element concentrations. This "nuclear analysis" technique differs from conventional activation analysis in that suitable detectors are used to measure promptly the products (particles or radiation) of the reaction rather than the decay products present after the completion of the irradiation. Despite its many advantages, the technique has not become widely known or used. We show here that it is particularly suitable for application to the microanalysis of light elements in a manner which complements the electron probe microanalyser. The use of a small diameter proton beam (for example, 0·010 in. or less) to induce nuclear reactions permits non-destructive measurements to be made, particularly for light elements, which are often difficult to detect by other methods.

The specific object of the particular experiments was the determination of the degree of homogeneity of thin foils of αTi-O alloys prepared for an investigation of the

solid solution region (less than 33 atom per cent oxygen) by electron diffraction methods. A suitable nuclear reaction for the investigation of oxygen concentration is the $^{18}O(p,\alpha)^{15}N$ reaction. Using protons of 0·86 MeV, a dominant resonance is observed in the alpha-particle yield, and the cross-section is sufficiently high to offset the low natural abundance of oxygen-18 (0·2 per cent).

The experiment was carried out using a proton beam from the 3-MeV accelerator at the Physics Division of the Australian Atomic Energy Commission Research Establishment. A simple chamber was used, with the titanium oxide samples mounted on a movable arm controlled from outside the vacuum system. A silicon semi-conductor counter was mounted on a second arm to receive α-particles emitted at 90° to the incident proton beam. A 2·5 mg/cm² aluminium foil in front of the counter absorbed scattered protons but allowed the 3·86 MeV α-particles to penetrate to the detector. The pulse height spectrum observed with a multi-channel analyser is shown in the inset to Fig. 1. A broad peak is observed owing to energy loss by some α-particles as they emerge from the surface of the sample. Most of the α-particles are produced within a distance of 2μ from the surface but a small number are produced at depths up to 5–10μ, and lose considerable energy in the sample. Repeated measurements of the pulse height spectrum for a fixed number of microcoulombs of protons incident on the sample were made to investigate the accuracy and reproducibility of the results. No effects were observed from drifts in the accelerator or measuring equipment or from build-up of contamination on the surface of the sample. However, when a sample of rutile (TiO_2) was used, a slow drop in oxygen concentration with time of bombardment was observed for a current density of 1 m.amp cm⁻². No such change was observed for the other titanium oxides used—a result which is consistent with the differences in thermal conductivity. An inspection of the rutile sample after bombardment showed that it had melted in the region of the beam spot.

For microanalysis, an aperture 0·010 in. in diameter was used to restrict the size of the beam striking the sample, and the total number of counts in the peak region of the alpha spectrum was used as a measure of the oxygen concentration in the region of the beam spot. The results are shown in Fig. 1, plotted against position

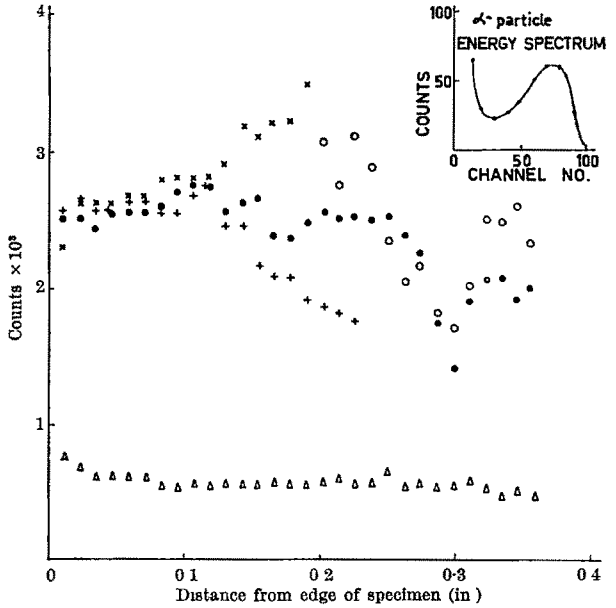


Fig. 1. Oxygen distribution in titanium oxides. 1*a* (●), 15 per cent oxygen by weight with scanning lines 0·15 in. apart; and 1*b* (○), 15 per cent oxygen by weight with scanning lines 0·15 in. apart; 2*a* (×), 12·4 per cent oxygen by weight with scanning line perpendicular to edge; 2*b* (+), 12·4 per cent oxygen by weight with scanning line parallel to edge; 3 (△), 3 per cent oxygen by weight

on the sample for a number of specimens. Using a beam current of 0·2 μamp and a counting time of about 3 min (33 μC), a statistical accuracy of from 2 per cent to 5 per cent was obtained depending on the oxygen concentration. Repeated results obtained by returning to a particular spot on the sample always gave results which agreed within the statistical errors.

Curves 1*a* and 1*b* in Fig. 1 are for two traverses, 0·015 in. apart, across one sample. The dip observed in both traverses occurs for a particular grain which shows an oxygen concentration 30 per cent lower than that of the surrounding material. Curves 2*a* and 2*b* show a relatively smooth change in oxygen concentration and this can be correlated with an observed change in average grain size. Curve 3 shows a uniform oxygen concentration across the surface of the sample.

Some measurements have been made with a 0·004 in. diameter beam spot and it should be possible to reduce this to 0·001 in. Special equipment would be needed to allow measurements for much smaller spot sizes, but such work would be feasible provided care were taken to avoid damage to the sample by high-beam densities.

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Proton Nuclear Magnetic Relaxation Investigations of Water

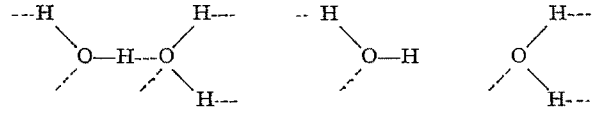
A STRUCTURAL model for water represents the liquid as a mobile, imperfect framework with molecular water or waters at defect sites[1]. The framework is connected by hydrogen bonds while the molecules held interstitially are hydrogen bonded neither to each other nor to framework molecules. This communication reports experimental results consistent with this model as provided by an investigation of aqueous proton nuclear magnetic relaxation times.

Relaxation measurements[2] were made on solutions in the *p*H ranges 2–5 and 9–12. T_1 was found to be neither *p*H- nor frequency-dependent and the values at room[3] and elevated temperatures[4] agreed with those reported by others for oxygen-free water. At 26° C and in the *p*H ranges indicated, T_2 was dependent on frequency but not on *p*H. At 60 Mc/s, T_2 was found to be less than T_1, while both were equal at 4·3 Mc/s. Representative measurements at various temperatures in the acidic range and at 60 and 4·3 Mc/s are shown in Table 1. Further data on the *p*H and frequency dependence can be found elsewhere[2].

Table 1. T_1 AND T_2 FOR ACIDIC WATER SOLUTIONS AT VARIOUS TEMPERATURES AND FREQUENCIES

*p*H 2·5, 60 Mc/s			*p*H 1·8, 4·3 Mc/s		
T° C	T_1	T_2	T° C	T_1	T_2
27·7	3·70	3·42	25·5	3·56	3·55
31·1	4·10	3·76	30·5	3·98	—
34·9	4·50	4·13	31·6	—	4·07
41·0	5·22	4·72	37·7	4·60	4·55
46·5	5·72	5·27	46·8	—	5·54
55·5	6·60	6·10	48·0	5·60	—
63·6	7·50	6·90	53·0	6·07	6·10
			60·5	6·85	6·87

In treating the data, we assume that the frequency dependence of T_2 can be traced to effects associated with a chemical shift, δ. The shift is ascribed to the difference in resonance frequency between that for a hydrogen bonded proton and one without this specific interaction. The mean diffusion lifetime[5] for water, however, renders it unlikely that the frequency dependence observed by us could be related to the reorientation of molecules partially involved in hydrogen bonding as represented below:



With this structure, fluctuations result in the breaking of one hydrogen bond (dotted lines) while others are left intact. We require a process, for example, whereby a water molecule is transferred from one state with hydrogen bonds to another without hydrogen bonds. This can best be described as the kinetic process, in the model, for transfer of a water molecule from the framework to an interstitial site. Furthermore, due to the *p*H independence of T_1 and T_2 in the *p*H ranges indicated, water molecules rather than protons are transferred from state to state.

In the limit of fast exchange, T_1 and T_2 can be related to a mean lifetime, τ, by equation 1 (ref. 6).

$$\frac{1}{T_2} - \frac{1}{T_1} = P_A P_B \delta^2 \tau; \tau = 1/(1/\tau_A + 1/\tau_B) \qquad (1)$$

In (1), P_A and P_B are the mole fractions of water in the framework state, A, and the interstitial state, B. Further, $\tau_A/\tau_B = P_A/P_B = K$, for the reaction $B \rightleftarrows A$.

The X-ray investigations of Danford and Levy[7] suggest that P_A is 0·80 and P_B is 0·20 at room temperature. If P_A is taken as 0·68 and P_B as 0·32 at 65° C[1,8], the experimental change in heat capacity can be realized after making a reasonable choice for the heat of vaporization of A, 11 kcal. The chemical shift is not required in evaluating thermo-dynamic constants, but only in order to obtain τ_A and τ_B. δ has been chosen as 10 p.p.m., a factor of two higher than that estimated by Hindman[9]. Values for τ_A and τ_B and enthalpic and enthropic rate and equilibrium constants are shown in Table 2.

Table 2. SUMMARY OF KINETIC AND THERMODYNAMIC CONSTANTS

P_A	0 80†	0 68‡	τ_A†	$5{\cdot}8 \times 10^{-8}$ sec	$\Delta S^+_{A\to B}$†	0 eu
P_B	0 20†	0·32‡	τ_B†	$1{\cdot}4 \times 10^{-8}$ sec	$\Delta S^+_{B\to A}$†	−7 7 eu
ΔS^*	−7·7 eu		$\Delta H^+_{A\to B}$ 7·7 kcal			
ΔH^*	−3·1 kcal		$\Delta H^+_{B\to A}$ 4 6 kcal			

* For the equilibrium, $B \rightleftarrows A$. † At 25°C. ‡ At 65° C.

The process $A \rightleftarrows B$ is a type of self-diffusion in water. The forward rate constant, however, is more than three orders of magnitude slower than that for the self-diffusion process postulated as $A \to A$. Applying first-order rate theory to both processes, the enthalpic term is found to reduce the rate $A \to B$ as compared to $A \to A$[4,5] by somewhat more than two orders of magnitude. The rate $A \to A$ is associated with a positive enthropic term[5], while the enthropic term for $A \to B$ is approximately zero. The latter corresponds to the usual enthropic variation for diffusion processes[10]. The indicated difference in rate constant between that for the processes $A \to A$ and $A \to B$ seems consistent, therefore, with that expected for a self-diffusion process.

The rate constant for $B \to A$ shows a similar consistency. The enthalpic terms for $A \to A$ and $B \to A$ are essentially identical, but the enthropic term for the latter reaction is at least 13 eu more negative than that for the former and indicates the expected restrictions associated with a water molecule joining the framework. Nothing can be said, at present, concerning the process $B \to B$.

Further investigations in this area promise additional information about the effects of solutes and mixed solvents on the properties of water. Temperature investigations, above and below the range presently examined, should also provide interesting data on structural variations.

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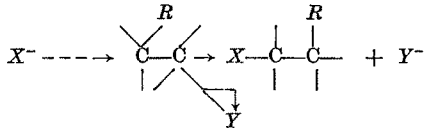
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Criterion of Mechanism based on Optical Rotation and Rearrangement in Alkyl Groups during Formation of Alkyl Halides

THE geometrical and electronic requirements of replacement reactions designated S_N2, S_N1 and S_Ni were described by Ingold and Hughes on the basis of kinetics and changes in optical rotation[1]. Recent work[2,3], however, has shown the need for caution in the practice which afterwards became fashionable of assigning these classifications on the basis of the optical result, unsupported by kinetic data, in other types of reaction, such as replacement of hydroxyl by halogen. The only unequivocal decision in such a reaction would seem to be the assignment of the exclusive S_N2 process to account for the isolation of, for example, optically pure (−)-2-chlorooctane from (+)-2-octanol. Isolation of the optically pure (+)-halide, retained configuration, does not necessarily point to the S_Ni, internal, mechanism, since this result could be due to a double S_N2 process, the first one being independent of halogen. Predominant inversion but with some loss in optical purity could be due to (S_N2 + S_N1 + S_Ni) or (S_N2 + S_N1) or (S_N2 + S_Ni), whereas predominant retention could be due to (S_N2 + S_Ni + S_N1) or (S_N1 + S_Ni) or (S_N2 + S_Ni), bearing in mind that the effect ascribed to S_Ni could be due entirely or partly to the double S_N2.

Alkyl rearrangement is usually taken as being indicative of a carbonium cation mechanism, although possibility of a bimolecular rearrangement process must be acknowledged.



(R = H or CH_3).

As a kinetic and optical result attributable to the S_N1 process need not lead to rearrangement in the alkyl group[2], absence of rearrangement does not necessarily exclude the S_N1 process, even with a group such as 2-octyl, which under various circumstances rearranges readily to 3- and 4-octyl.

Complications in interpretation can also arise because of loss of rotatory power and/or rearrangement after the initial formation of the halide, or from the lack of experi-

mental precision in analysing the halide product, that is, assessment of isomeric constitution.

The mechanism itself may change in the period during which the product is being formed, so that two or more mechanisms may contribute. In the alcohol–phosphorus trihalide systems, for example, the alkyl halide is produced by three consecutive reactions comprising the removal of the three alkyl groups of the trialkyl phosphite by hydrogen halide, the first two presumably being clean S_N2 processes, while the third involves rearrangement and loss in rotatory power for a secondary alkyl group[2]. The formation of isomerically pure *s*-alkyl halides by the first dealkylation led us to examine the related system in which an alcohol is treated with triphenyl phosphite and hydrogen or alkyl halide[4].

The main sequence of reactions appears to be as follows, although other sequences may contribute:

$$(PhO)_3P + ROH \rightarrow PhOH + (PhO)_2POR \xrightarrow[(R' = H, Me, etc.)]{R'X}$$

$$X^- \xrightarrow{S_N2} R\text{—}\overset{+}{O}PR'(OPh)_2 \rightarrow RX + R'PO(OPh)_2$$

In the original work[4] various optical results were obtained, for example, with $R'X$ = HCl, 2-octanol ($\alpha_D^{20\cdot4}$ − 7·83°, l = 1) gave 2-chloro-octane ($\alpha_D^{22\cdot2}$ + 18·8°, l = 1), thus showing a 40 per cent loss in optical purity, while with $R'X$ = $C_6H_5CH_2Cl$, an 18 per cent loss in activity was observed. These results indicate a mixture of mechanisms, with contributions from S_N2 and S_N1 modes according to conditions; although for certain reasons the original authors suggested the S_N2 mechanism.

We find, however, that at room temperature (or just above) this system ($R'X$ = HCl) gives 2-chloro-octane (α_D^{18} + 30·15° from ROH, α_D^{18} − 7·96°, l = 1) isolated by detailed fractionation of the primary product. This represents inversion without loss in rotatory power, and therefore presumably the clean S_N2 mechanism as originally suggested. It also points to the non-intervention of the possible ROH + HCl reaction[5], confirmed by the preparation of isomerically pure 2- and 3-chloropentanes in the triphenyl phosphite system. When $R'X$ = HBr or HI, some rearrangement occurred, namely, 3-bromopentane (25 per cent) and 3-iodopentane (10 per cent) from 2-pentanol. This effect could be due to the quicker interaction between the HBr or HI and the alcohol itself. *n*-Alcohols, however, still gave isomerically pure halides.

In the reaction of methyl bromide or methyl iodide with triphenyl phosphite and a secondary alcohol we appear to have an example in which the mechanism of formation of the halide changes with time, that is, as the reaction medium changes. From both 2- and 3-pentanol the highest yields (60–65 per cent) were obtained by heating the reactants at 100° C for 20 h (iodide, under reflux) or 42 h (bromide, in sealed tube), and all products contained about 5 per cent of the rearranged isomer. By restricting the yields to 40–50 per cent by a shorter period of heating (8 h) isomerically pure products were obtained. The possibility of isomerization after formation is discarded, because when the 2-halogenopentanes were heated in the presence of *n*-butanol, triphenyl phosphite, and methyl halide, as already mentioned, they were recovered unchanged. From the *n*-butanol isomerically pure halides resulted.

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Resolution of Oxalato*bis*(ethylenediamine)-cobalt(III) Bromide by a Preferential Induced Crystallization

The resolution of oxalato*bis*(ethylenediamine)cobalt(III) bromide by way of the preferential crystallization of one optical enantiomorph was first demonstrated by Werner[1]. Seed crystals of optically active oxalato*bis*(ethylenediamine)cobalt(III) were added to a solution containing 2 g of the racemic compound. The subsequent addition of alcohol gave 0·2 g of dextro bromide in 95 per cent optical purity (sodium *D*-line rotations). A marked improvement of this resolution is reported here in which optically active *tris*(ethylenediamine cobalt(III) acetate is used.

Oxalato*bis*(ethylenediamine)cobalt(III) bromide (8·5 g) was shaken vigorously with silver acetate (about 4 g) suspended in hot water (73 ml., 70° C). The silver bromide was filtered off and to the filtrate was added a solution of D-*tris*(ethylenediamine)cobalt(III) acetate prepared as follows. D-*tris*(Ethylenediamine)cobalt(III) iodide (12·9 g)[2] was shaken vigorously with a suspension of silver acetate (about 10 g) in hot water (80 ml., 70° C). The silver iodide was filtered off and the filtrate evaporated to a volume of 18 ml. To the mixed acetate solutions a solution of potassium bromide (20 per cent w/v) was added. Table 1 shows the yields of optically active bromide obtained.

Table 1

Fraction	Potassium bromide added (ml.)	[Co ox(en)$_2$]Br (g)	$[\alpha]_D$*
1	5	1·3	−796
2	3	0·8	−796
3	5	1·6	+492
4	4	0·3	+236
5	5	1·0	+428

Fractions 1 and 2 were combined and recrystallized from hot water (10 ml.) containing potassium bromide (0·5 g) and gave 1·7 g of pure oxalato*bis*(ethylenediamine)-cobalt(III) bromide-1 hydrate (40 per cent of the theoretical yield). The analysis was as follows: calculated for $[Co(C_2O_4)(C_2H_8N_2)_2]Br.H_2O$; carbon, 19·73; hydrogen, 4·97; bromine, 21·9 per cent; found: carbon, 19·83; hydrogen, 4·99; bromine, 21·89 per cent. A 0·25 per cent aqueous solution in a 10-cm tube gave $[\alpha]_D$ −812.

Solutions of ammonium *d*-tartrate or ammonium *d*-camphor-10-sulphonate (10 per cent w/v) may be used instead of *tris*(ethylenediamine)cobalt(III) but lower yields of active oxalato*bis*(ethylenediamine)cobalt(III) were obtained (20 per cent compared to 40 per cent). In addition, the least-soluble fractions were now dextro-rotatory in the sodium *D*-line.

An attempt to resolve *cis*-dinitro*bis*(ethylenediamine)-cobalt(III) acetate by a method analogous to that described here using either optically active *tris*(ethylenediamine)cobalt(III) acetate or ammonium *d*-camphor-10-sulphonate gave only a 6 per cent yield of optically pure *cis*-dinitro*bis*(ethylenediamine)cobalt(III).

For the method to be generally applicable it is necessary that the optical forms of the complex be less soluble than the racemic form. The success of the resolution with oxalato*bis*(ethylenediamine)cobalt(III) is due no doubt to the large difference in solubilities of the active (1·05 g/100 ml. at 17° C) and inactive (1·85 g/100 ml. at 17° C) forms[1] together with the fact that the seed crystals are generated "homogeneously" throughout the solution, thus greatly improving the efficiency of the method.

Mason and Norman[3] recently reported circular dichroism produced in the region of the $^1A_{1g} \rightarrow {}^1T_{1g}$ transition of the hexamminecobalt(III) cation by *d*-tartrate ion or *d*-camphor-sulphonic acid. The mode of the outer sphere interaction between these anions and the metal complex was tentatively suggested to involve chelate hydrogen bonding. The overall dipolar nature of the oxalato*bis*-(ethylenediamine)cobalt(III) cation would undoubtedly augment any hydrogen bonding in the interaction with the *tris*(ethylenediamine)cobalt(III) ion. However, other

* All rotations in this letter refer to the sodium *D* line.

factors must be involved since specific effects between the *tris*(ethylenediamine)cobalt(III) cation and the *tris*(2,2′-bipyridine)osmium(II) and (III) cations have been reported[4], where chelate hydrogen-bonding is not possible.

It appears probable that all these phenomena are but manifestations of configurational activity as originally proposed by Dwyer[5]. Therefore those optically inactive complexes for which activity effects have been observed should also display circular dichroism when in the presence of the optically active agent.

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Relation between Thermodynamic Property Change and Vibrational Frequency Shift in Ethyl Halides

RELATIONS can be found by plotting the difference of the ideal gas state thermodynamic properties $\Delta S°$ and $\Delta C_p°$; formed between the thermodynamic property values of ethane, C_2H_6, and its homologues, C_2H_5X (X = F, Cl, Br, I), against the corresponding C–X bond stretching and C–C–X bond bending frequencies in ethyl halide series (Fig. 1). The results are presented in Table 1.

As the shifts in C–X bond stretching frequency with change in substituent in ethyl halide series correlate nearly linearly with the respective thermodynamic property increments in entropy, $\Delta S°$, and heat-capacity, $\Delta C_p°$, it seems plausible to believe that these stretching frequencies do not appreciably interact with other modes. The C–C–X bond bending frequencies although also yielding smooth curves (Fig. 1), however, have a greater degree of possible interaction.

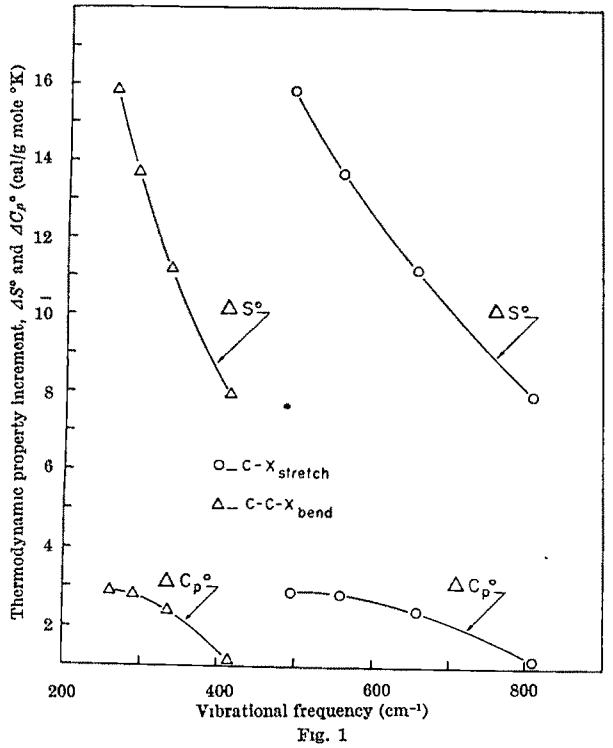


Fig. 1

Table 1

Compound	Thermodynamic property difference (at 298 16° K) (cal /g mole °K) $\Delta C_p°$	$\Delta S°$	Vibrational frequency (cm⁻¹) C–X stretch	C–C–X bend
Ethane C_2H_6	0 (ref. 1)	0 (ref. 1)	—	—
Ethyl fluoride C_2H_5F	1·14 (ref. 2)	7·93 (ref. 2)	810 (ref. 4)	415 (ref. 4)]
Ethyl chloride C_2H_5Cl	2·42 (ref. 3)	11·14 (ref. 3)	658 (ref. 5)	336 (ref. 6)
Ethyl bromide C_2H_5Br	2·83 (ref. 3)	13·65 (ref. 3)	560 (ref. 6)	292 (ref. 3)
Ethyl iodide C_2H_5I	2·88 (ref. 2)	15·80 (ref. 2)	497 (ref. 6)	262 (ref. 7)

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BIOCHEMISTRY

Identification of the Yellow Lupin Growth Inhibitor as (+)-Abscisin II ((+)-Dormin)

THE presence of an abscission-accelerating substance in the pods of yellow lupin (*Lupinus luteus* var. Weiko II) was deduced by Van Steveninck[1]. He was able to show that the whorls of fertilized pods at the base of the inflorescence induced the abscission of younger, distal pods. Plants infected with pea mosaic virus underwent a different pattern of abscission suggesting that the hormonal balance was disturbed[2]. Paper chromatography of the acids extracted from lupin pods coupled with a wheat coleoptile extension assay revealed an inhibitory band which appeared to contain the factor controlling pod abscission[3].

A highly active and partly crystalline concentrate of the inhibitory material obtained from 700 kg of yellow lupin pods by Rothwell and Wain[4] has now been examined by a spectropolarimetric method recently described[5]. The sample showed a positive Cotton effect with extrema at 287 and 245 mμ and a profile identical with that of (+)-abscisin II. The ultra-violet absorption spectrum in alkaline and acidic ethanol was consistent with that of abscisin II (ref. 6) together with a superimposed, weak absorption peak at 237 mμ which could be attributed to an impurity. Based on absorbance at 262 mμ the molar amplitude of the Cotton effect was ~240,000°, which is in good agreement with that of (+)-abscisin II (ref. 5).

The inhibitory activity of the sample in the wheat embryo assay[6] was slightly less than twice that of synthetic racemic abscisin II used for comparison. Since we have previously shown[7] that (+)-abscisin II has about twice the inhibitory activity of the racemic compound, the spectropolarimetric assay is confirmed; the sample is largely (+)-abscisin II.

The identification of sycamore dormin as abscisin II extended the known range of this compound's physiological effects. Recently the dormancy inducing inhibitor in birch leaves[8] and the germination inhibitor in rosehips[9] have also been identified as (+)-abscisin II (ref. 5) so that the role of this compound in controlling dormancy is now well established. The identification of the lupin factor places its abscission-inducing role[10] on a firmer basis,

and shows also that abscisin II concentrations within the plant may be affected by a virus infection[2].

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A Simple Bioassay for Gibberellic Acid*

IN his review of 1965, Paleg[1] outlined the many biological tests that have been used as quantitative assays for gibberellins. The lettuce hypocotyl test is perhaps the simplest to carry out, but the lowest concentration that can be reliably assayed is only 0·1 mg/l. The most sensitive assay is the barley endosperm test which detects concentrations as low as 0·0001 mg/l., but this method has the disadvantage that the procedure is both involved and laborious.

A method for detection of kinins has been reported by Osborne and McCalla[2] based on retardation of senescence in leaf disks of *Xanthium*. Fletcher and Osborne[3,4] more recently showed that senescence in leaf cells of *Taraxacum officinale* Weber is retarded by supplying gibberellic acid, GA_3 (GA). This effect was observed with concentrations as low as 0·001 mg/l. and has been made the basis of a simple bioassay for GA. This communication describes the method.

A continuous supply of *Taraxacum* leaves can be ensured by keeping potted plants in the greenhouse under natural light conditions. The oldest mature leaves from these plants or from those growing out of doors are suitable for use in this test. The leaves are detached, and if fully expanded and sufficiently mature they can be used immediately. If they are not sufficiently mature, the leaves are "aged" under a bell jar with their petioles in water until the glossy green colour fades to a uniformly pale green; this indicates that an appropriate stage of maturity has been reached.

Disks of a convenient diameter are punched from the interveinal portions of the leaf using a cork borer. Duplicate sets of disks are extracted at once with hot 80 per cent ethanol and the extracts stored in the dark for subsequent determination of the original chlorophyll contents. Other matched sets of disks are placed in Petri dishes on filter papers moistened with either water or one of a graduated series of GA concentrations. The abaxial surface of the leaf tissue is in contact with the moist filter paper. Care should be taken not to moisten the filter paper too much for this results in waterlogging and blackening of the disks. A satisfactory amount of liquid for a 7 cm diameter filter paper is 0·8 ml. The covers of the Petri dishes are lined with moist filter paper and the dishes are then placed in a plastic box to maintain high humidity, and stored in darkness at 25° C.

With properly "aged" starting material the disks in the water controls are uniformly chlorotic after 4 days, and the retention of chlorophyll in the treated disks is visibly greater with increasing concentrations of GA.

Each set of disks is then dropped into test-tubes containing hot 80 per cent ethanol and boiled gently. The test-tubes are then stoppered and transferred to an incubator at 37° C overnight to complete the extraction of chlorophyll. An alternative and quicker procedure is to boil the tubes gently in a water bath until all the chlorophyll has been extracted from the disks. The chlorophyll extracts are brought to volume with 80 per cent ethanol and the absorbancy of each solution and those of the initial controls are measured in a suitable colorimeter (in this instance, a Spectronic 20) at 665 mμ.

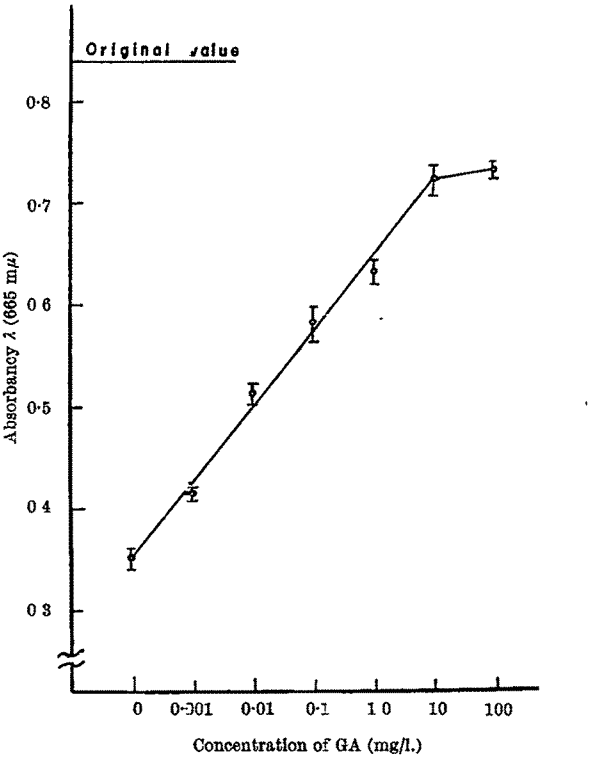


Fig. 1. Retardation of chlorophyll degradation in leaf disks of *Taraxacum officinale* by gibberellin (GA). Chlorophyll content expressed as absorbancy λ, 665 mμ of 80 per cent ethanol extracts at 0 and 4 days

When the absorbancies are plotted against concentrations of GA (Fig 1) there is a linear relationship from 0·001 to 10 mg/l. At higher concentrations this relationship is not maintained because a maximum value for chlorophyll retention is reached. Our earlier work[3] showed that at concentrations from 10 to 100 mg/l. the values lie on a plateau.

In order to determine the specificity of this bioassay, other growth substances, including indolyl-3-acetic acid (IAA), 6-furfurylaminopurine (kinetin), 2,4-dichlorophenoxyacetic acid (2,4-D) and α-naphthylacetic acid (NAA), were investigated. It was found (Table 1) that IAA and NAA did not retard senescence. Kinetin and 2,4-D produced effects somewhat similar to those produced by low levels of GA, but the concentrations required were several orders of magnitude greater than for GA.

This assay can be conveniently used for the quantitative determination of GA concentrations from 0·001 mg/l. to 10 mg/l. and for the qualitative detection of concentrations up to 100 mg/l. It does not require the use of elaborate

* This article was received in the office of *Nature* on June 20, 1966, five days before the appearance of an article by Whyte and Luckwill describing a similar method of assay applied to a different plant—Editor, *Nature*.

Table 1. CHLOROPHYLL CONTENT IN LEAF DISKS OF *Taraxacum officinale* TREATED WITH FIVE GROWTH SUBSTANCES

Sample	Treatment (mg/l.)	Absorbancy λ (665 mμ) GA	K	IAA	2,4-D	NAA
0 day	–	84	84	84	84	84
4 days	0	35	35	35	35	35
	0·001	42	37	34	37	37
	0·01	51	36	36	33	39
	0·1	58	35	34	37	31
	1·0	63	41	33	36	36
	10·0	72	50	33	42	39
	50 0	–	65	–	–	–
	100·0	73	–	36	49	37

Disks were maintained for 4 days on filter papers moistened with either gibberellin (GA); kinetin (K); indolyl-3-acetic acid (IAA); 2,4-dichlorophenoxyacetic acid (2,4-D); or α-naphthylacetic acid (NAA). Chlorophyll content expressed as absorbancy λ, 665 mμ in 80 per cent ethanol. The values are a mean of two experiments carried out in duplicate. The *S.E.* did not exceed ± 3·4.

equipment, complex media or sterile conditions. The greatest advantage lies in its simplicity and its specificity for low concentrations of GA.

This work was started at the Department of Agriculture, University of Oxford, and completed in the University of Guelph. We thank Prof. G. E. Blackman for his interest and encouragement. This investigation was supported by a grant from the National Research Council of Canada.

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Biological Detection of Phytochrome in Callus Tissue of *Nicotiana tabacum* L.

DURING recent years, considerable information has been gained concerning the role of light in germination, etiolation, and control of flowering. The discovery of phytochrome has greatly aided the understanding of these processes. Briggs and Siegelman[1] found that seedling plants had highest phytochrome concentrations in regions of active cell division. We wished to know whether or not phytochrome had any function in actively dividing cells cultured from undifferentiated stem pith. This communication reports evidence that the phytochrome system was involved in growth of callus tissue cultured *in vitro* from undifferentiated pith from stems of *Nicotiana tabacum* L.

Sections of pith obtained from stems of normal plants of three varieties of *N. tabacum* were cultured and subcultured on a chemically defined nutrient medium. Callus tissue from all three of the varieties responded similarly and data are composited in the tables presented in this report. The basic medium (modified from Murashige and Skoog[2]) consisted of: sucrose, 20 g; ammonium nitrate, 1·65 g; potassium nitrate, 1·9 g; potassium dihydrogen phosphate, 0·17 g; $MgSO_4.7H_2O$, 0·37 g; $CaCl_2.2H_2O$, 0·44 g; $MnSO_4.H_2O$, 22·3 mg; $ZnSO_4.7H_2O$, 8·6 mg; *ortho* boric acid, 6·2 mg; $Na_2MoO_4.2H_2O$, 0·25 mg; potassium iodide, 0·83 mg; $CuSO_4.5H_2O$, 0·025 mg; $CoCl_2.7H_2O$, 0·025 mg; ethylenediamine tetraacetic acid, 22·4 mg; ferric sulphate, 10·67 mg; thiamine hydrochloride, 0·1 mg; nicotinic acid, 0·5 mg; pyridoxine hydrochloride, 0·5 mg; glycine, 2·0 mg; *myo*-inositol, 100 mg; *N*-acetyl-L-aspartic acid, 0·1 mg; kinetin, 0·2 mg; and agar, 6 g in 1 l. of distilled water. The *p*H of the solution was 6·0 before autoclaving at 15 lb. for 20 min. Successive subcultures were started at 2 or 4 week intervals with tissue pieces weighing about 40 mg. The tissues were cultured in standard 100 × 20-mm Petri dishes to enhance uniform illumination during treatments. Five uniform-sized pieces of tissue were distributed on the surface of 40 ml. of nutrient medium in each dish. Transparent tape was used to secure the covers, which were left on during entire experiments including time of illumination, to reduce airborne contamination.

Growth of tissues under light or in complete darkness was compared. The illuminated lots were subjected to 16-h periods of low intensity (20–30 ft.-candles) light from standard cool-white fluorescent lamps. All tissues were maintained at 26° ± 1° C. In a typical experiment (Table 1) the lighted lots were significantly heavier than those maintained in darkness. The higher average weight per piece of tissue was interpreted as an increased rate of growth in the lighted tissues.

Table 1. WEIGHTS OF *Nicotiana tabacum* CALLUS TISSUE CULTURED FOR 30 DAYS IN COMPLETE DARKNESS OR IN LIGHT AT 26° ± 1° C

Condition when weighed	Mean weight (mg) per tissue piece grown in: Darkness	Light
Wet*	711	883
Oven dried*	49	60

Mean wet weight per tissue piece at start of experiments was about 40 mg. Data are means of 60 pieces of tissue.
* Analysis of variance showed that differences between dark- and light-grown tissues were significant at $P = 0·01$. Variations between tissues within treatments were not significant.

Several experiments were carried out to determine whether the additional growth of the illuminated tissues was related to function of the phytochrome system. This was tested by moving tissues to darkness at 25° ± 0·5° C immediately after sub-culturing. Some tissues were kept continuously in darkness; others were given daily 5-min irradiations with red (2 μW/cm²/mμ at 660 mμ) or far-red (1·3 μW/cm²/mμ at 730 mμ) light to produce phytochrome in the far-red-absorbing (P_{fr}) or red-absorbing (P_r) form, respectively. As a test of photoreversibility, some tissues were given 5 min of red, to convert phytochrome to P_{fr}, followed immediately by 5 min of far-red, to revert it to P_r, each day from the previously described sources. After the brief exposures to red and/or far-red light, all the tissues were returned to darkness in the same 25° C incubator. All treatments were repeated daily for the duration of each experiment. Brief irradiation periods were used because prolonged exposure to far-red often causes the same response as that obtained from exposure to red light. This has been attributed to the weak overlapping of P_r absorption into the far-red region of the spectrum[3]. Duncan's multiple range test[4] was used to determine whether differences between treatments were statistically significant. Results of a typical experiment (Table 2) clearly show a promotion of growth in tissues irradiated with red light. The red-irradiated tissues were significantly heavier than the dark-controls and those that received far-red only. These data indicate that P_{fr} functioned in the growth of callus tissue sub-cultured from normal plants of *N. tabacum*. The fact that tissues irradiated with red, followed immediately by far-red, grew less than those irradiated with red alone shows a reversal of the effect of red light

Table 2. WEIGHTS OF *Nicotiana tabacum* CALLUS TISSUE CULTURED FOR 14 DAYS IN COMPLETE DARKNESS AT 25° C, OR WITH RED (*R*) AND/OR FAR-RED (*FR*) TREATMENTS

	Mean wet weight per tissue piece Dark	*FR*	*R*+*FR*	*R*
Mg/piece	223	229	249	282
M.R. test*	*a*	*a*	*ab*	*b*

Irradiations with *R* and *FR* were for 5 min at 24-h intervals for the duration of the experiment. Mean wet weight per tissue piece at start of experiment was about 40 mg.
* M.R. test, multiple range test. The difference between red-irradiated and dark-control was significant at $P = 0·01$. Means with a common letter did not differ at $P = 0·05$. Variation between tissues within treatments were not significant.

and is further evidence that the phytochrome system was involved.

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Enzymatic Degradation of L-Cystine by Cytoplasmic Particles from Cabbage Leaves

THE degradation of cystine by cystathionase preparations from liver[1] and *Neurospora*[2] has been reported. A highly purified cystathionase from *Neurospora* catalysed the cleavage of cystine to pyruvate, ammonia and thiocysteine[3]. L-Cystine was utilized at only 17 per cent of the rate of cystathionine. A second cystathionine cleavage enzyme which utilizes L-cystine at the same rate as cystathionine has recently been found in *Neurospora*[3]. We have found that homogenates of cabbage leaves can be fractionated into particulate and soluble cytoplasmic fractions, both possessing a heat-sensitive ability to degrade L-cystine to pyruvate. The two enzymatic activities appear to have different characteristics. The present communication briefly reports on some of the properties of the particulate enzyme.

Cabbage leaves were homogenized in a solution of 0·25 M sucrose which was 0·01 M with respect to sodium phosphate at *p*H 7·2. One ml. of homogenizing medium was used for each g of tissue. All operations were carried out at 4° C. The homogenate was passed through cheesecloth and then centrifuged at 1,000*g* for 10 min. The precipitate was discarded, and the homogenate then centrifuged for 1 h at 70,000*g*. The supernatant solution, which contained the soluble enzyme, was decanted. The sedimented particles were washed by being resuspended in the sucrose–phosphate buffer and recentrifuged. The particles were then suspended again in fresh sucrose–phosphate solution and dialysed overnight against the same medium. The final suspension contained the particles from 10 g of leaves per ml. of suspension. The protein concentration varied from 30 to 60 mg per ml.

It was found that the *p*H optimum of the reaction was a broad peak between *p*H 8·5 and 8·9. The customary reaction mixture consisted of the following in a final volume of 1 ml.: 200 μmoles *tris* buffer, *p*H 8·7, 10 μmoles L-cystine, and 0·1 ml. of the dialysed, washed particle suspension. The reaction was stopped by the addition of 2 ml. of 10 per cent trichloroacetic acid, and the denatured protein removed by centrifugation. The formation of pyruvate was followed by colorimetric assay of its 2,4-dinitrophenylhydrazone[4].

The evidence that the particulate and soluble enzymes are different consists of the following: (*a*) continued washing and re-sedimentation of the particles does not release further soluble enzyme; (*b*) the activity of the particulate enzyme is stimulated from 40 to 100 per cent by the addition of 5×10^{-5} M pyridoxal-5′-phosphate, whereas the soluble enzyme shows essentially no stimulation; (*c*) sonic oscillation of the particles yielded a soluble fraction which retained the property of having a large stimulation of activity on the addition of pyridoxal-5′-phosphate. Pyridoxal could not replace pyridoxal phosphate. The substrate specificity of the particulate enzyme is shown in Table 1.

There appears to be a small activity with D-cystine and L-cysteine. Both may be reflexions of a small contamination of L-cystine in the substrate. Significantly, L-cystathionine is completely inert as a substrate. This distinguishes this enzyme completely from the liver and *Neurospora* cystathionases. Also of interest is the fact that L-cysteine-S-SO_3, which has been suggested as an intermediate in sulphate reduction in moulds[5], is an even better substrate than L-cystine. This may be of some physiological significance.

The inhibition of the reaction by carbonyl reagents is shown in Table 2. Pyridoxal phosphate-requiring enzymes are very strongly inhibited by such reagents. The inhibition by hydroxylamine is very large even at a concentration as low as 1×10^{-5} M. We have found that L-cysteine is an excellent inhibitor of the reaction with L-cystine. At equimolar concentration it inhibits the reaction almost completely, and at 2×10^{-5} M it inhibits it by 70 per cent. D,L-Homocysteine is also very effective as an inhibitor, but D,L-homocystine is almost inactive. Reduced glutathione is intermediate in action, inhibiting 42 per cent at equimolar concentration with the L-cystine. Ethylenediamine tetraacetate gave only a 15 per cent inhibition at a concentration of 10^{-3} M.

The proof that pyruvate is a product was obtained by use of an isotope dilution procedure. A reaction mixture containing 0·3 μmoles of D,L-cystine-1-^{14}C which had 2 μc. of activity, 0·05 μmoles of pyridoxal phosphate, 200 μmoles *tris* buffer of *p*H 8·7 and dialysed washed particles was incubated for 2 h and then stopped and the particles removed as usual. Two mmoles (220 mg) of sodium pyruvate were added to the reaction mixture and followed by excess 2,4-dinitrophenylhydrazine. The precipitate was collected, dissolved in a chloroform–ethanol (20 : 80) solution and the hydrazones extracted into 1 N sodium carbonate. The sodium carbonate solution was acidified and the hydrazones extracted back into the chloroform–ethanol solution. The latter was brought to dryness *in vacuo*, and then the residue recrystallized three times, first from water, followed by ethyl acetate, and 70 per cent ethanol. Weighed samples were dissolved on planchets and dried and then counted in a gas flow counter. Table 3 summarizes the results. There appears to be little doubt that pyruvate is formed during the reaction. Tests for ammonia formation were positive, but no sulphydryl groups could be detected. The rate of formation of pyruvate was not constant, and the reaction

Table 1. SUBSTRATE SPECIFICITY OF THE PARTICULATE ENZYME

Substrate	Final concentration (M)	Relative activity
L-cystine	1×10^{-3}	100
L-cystathionine	1×10^{-3}	0
D-cystine	1×10^{-3}	11
L-cysteine	2×10^{-3}	10
S-methyl-L-cysteine	2×10^{-3}	0
D,L-homocysteine	2×10^{-3}	0
D,L-homocystine	1×10^{-3}	10
L-cysteine-S-SO_3	1×10^{-3}	152

Reaction system was as described in the text except the substrate was as shown above. Incubation time was 60 min.

Table 2. INHIBITION BY SODIUM CYANIDE AND HYDROXYLAMINE

Inhibitor	Final concentration (M)	Relative activity
None	—	100
Sodium cyanide	1×10^{-3}	8
	1×10^{-4}	70
Hydroxylamine	1×10^{-3}	0
	1×10^{-4}	0·5
	1×10^{-5}	5

Reaction mixture was as described in the text but including the inhibitor as shown.

Table 3. SPECIFIC ACTIVITY OF THE RECRYSTALLIZED PYRUVIC ACID 2,4-DINITROPHENYLHYDRAZONE

Sample	Specific activity (c.p.m./mg)
Chloroform–ethanol residue	393
First recrystallization from water	399
Second recrystallization from ethyl acetate	385
Third recrystallization from 70 per cent ethanol	380

stopped when 15–20 per cent of the cystine had disappeared. Whether this is due to product inhibition has not been ascertained, since all the products, particularly the sulphur-containing ones, have not been identified.

We are at present purifying the soluble enzyme and investigating its properties in detail. These results will be reported elsewhere. The present work was supported by a grant from the National Institutes of Health, U.S. Public Health Service.

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Purification of Insulin labelled with Iodine-131

THE procedure described by Hunter and Greenwood[1] with some minor modifications has been used in this laboratory for small-scale preparation of iodine-131-labelled insulin. About 1·2 mc. iodine-131 from Kjeller is used in a volume of less than 0·025 ml. for the labelling of 5 μg of purified insulin. The iodination efficiency is usually in the region of 60–80 per cent, giving a specific activity of about 150 μc. iodine-131 per μg insulin.

Various purification techniques for iodine-131-labelled insulin have been reported[2–4]. In some experiments we used the method devised by Banerjee and Gibson and found it possible to purify the reaction mixture in one operation only, thus omitting the dialysing step for removal of free iodine-131.

0·2 ml. of human serum albumin solution (20 mg albumin per ml. buffer) was added to the reaction mixture prepared according to Hunter *et al.* A column of 1 g 'Sephadex *G*–50' gel (fine) was prepared, and 0·07 M sodium barbitone buffer (*p*H 8·6) was used as the elution medium. The column was about 600 mm long and had an inner diameter of 6 mm. After slow elution, the various fractions were examined for radioactivity and analysed by paper chromatography and paper electrophoresis.

The elution pattern obtained from three experiments was as follows: (1) Altered or damaged insulin which came out together with the serum protein and some unaltered insulin in the first 6 ml. eluate showed 5–25 per cent of the total activity. (2) Purified insulin which held 35–57 per cent of the total activity and 2–4 per cent impurities was contained in the next 2 ml. portion. The impurity is assumed to be insulin which has been altered when the material passes through the column. This fraction was collected and diluted in 0·5 per cent serum albumin solution immediately after elution and then subjected to deep freezing. The last portion (8–25 ml.) mainly contained free iodine-131. Usually less than 10 per cent of the total activity is absorbed to the column.

Determination of pure and altered insulin was performed using ascending paper chromatography and two different solvents (barbital buffer, *p*H 8·6 (ref. 4), and a mixture of *n*-butanol, acetic acid and water[5]). Free iodine-131 was determined by paper electrophoresis in which 0·1 N sodium hydroxide was used as electrolyte[6].

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Isolation of the Scent-trail Pheromone of an Australian Termite

THE trail-laying capabilities of termites have been known for many years and, quite recently, this activity has been shown[1,2] to be based on pheromones produced by the sternal gland. Both primitive and advanced termites possess such a gland, which consists of a rather diffuse structure beneath the fifth abdominal sternite[3]. In two species, namely *Zootermopsis nevadensis* (Hagen) (Hodotermitidae)[1,2] and *Nasutitermes corniger* (Mots.) (Termitidae)[3], extracts of the appropriate region, or indeed of the whole insects, could be used to lay artificial trails that were readily followed by the parent termites. However, no clear indication of the chemical nature of the pheromones involved has yet been published.

In the present work, a scent-trail pheromone common to several Australian species of *Nasutitermes* has been isolated and characterized as an unsaturated diterpenoid hydrocarbon, $C_{20}H_{32}$. The mainstay of the investigation has been the southern, ground-nesting termite *Nasutitermes exitiosus* (Hill). This species has the advantage that it is available in large quantities and it shows a comparatively "docile" disposition in culture which is admirably suited to the requirements of a reliable biological test. In the absence of repellent substances, both soldiers and workers readily follow artificial trails traced on paper from ethereal pheromone, over the range of concentrations about 10^{-3}–10^{-5} g/ml., although above and below these limits there is little or no response. Thus, to test a preparation for trail-laying activity, it is necessary to use several successive tenfold dilutions in order to cover the possibility that pheromone may originally be present at too high a concentration.

In such a test, the *exitiosus* pheromone was shown to be extractable from homogenized termites by light petroleum, and to be stable to alkaline hydrolysis. The unsaponifiable fraction of the extract in light petroleum was refined by means of chromatography on a dry column of alumina (activity I). Under these conditions, inactive saturated hydrocarbons were the first to emerge, followed by colourless unsaturated hydrocarbons and later by bright orange–yellow β-carotene (identified by its characteristic absorption between 300 and 550 mμ). Trail-laying activity was confined to the colourless unsaturated fractions, and vacuum distillation of these, to which a little *n*-eicosane had been added as a carrier, afforded an active, volatile fraction, b.p. 120°–220° (bath temperature), at 25 min of mercury.

The active fraction was next subjected to thin-layer chromatography on silica gel, with light petroleum as the mobile phase. Treatment of a longitudinal section of the chromatogram with cold concentrated sulphuric acid revealed five major bands as purplish spots, fading to pale brown, with approximate R_F values of 0·0, 0·15, 0·4, 0·6 and 0·85. Saturated materials travelled with the solvent front. Trail-laying activity was associated with the R_F 0·4 zone, and elution of this separated portion of the chromatogram with light petroleum gave a few milligrams of a highly-active, colourless oil. Gas chromatography at 175° (on a Pye 'Argon' instrument, with a 120-cm by 4-mm column, packed with 10 per cent silicone oil on 'Celite', and a gas flow of 75 ml./min) showed that this material was made up of two components with retention times of 2·7 and 14·5 min respectively, and suggested molecular complexities of C_{15} and C_{20}. Resolution of the binary mixture was achieved either by preparative gas chromatography at 225° (120-cm by 1-cm column, packed with 10 per cent 'Apiezon-*L*' on 'Celite', and a gas flow of 100 ml./min), or by short-path, fractional distillation at 25 mm. Progress in either case was monitored on the analytical gas chromatograph. The former method gave products of high purity, but recoveries were low and the latter was preferred for most purposes. Only the higher boiling substance was active in the trail-laying test and

the amounts isolated from active insects indicated a natural content of about one part in four million. However, with hibernating termites, the pheromone titre fell considerably.

The molecular weight of the pheromone was determined by mass spectrometry as 272, thereby confirming a diterpenoid hydrocarbon constitution. The ultra-violet spectrum (in *cyclo*hexane) showed only end absorption above 220 mμ and the infra-red spectrum (liquid film) included strong bands at 3,070, 1,640 and 880 cm^{-1}, which suggested one or more α-substituted vinyl groups. On catalytic hydrogenation, the pheromone gave an inactive octahydro-derivative (molecular weight 280, by mass spectrometry) and it presumably has a monocyclic structure, with four isolated double bonds.

An apparently identical pheromone has been isolated in small amounts from the arboreal termites, *Nasutitermes walkeri* (Hill) and *N. graveolus* (Hill), using *N. exitiosus* as the test animal. However, the pure pheromone shows no activity with the more distantly related *Coptotermes lacteus* (Froggatt) and it may therefore prove to be genus specific. Screening tests (with *exitiosus*) of various essential oils have also revealed activity in one plant species, namely the West Australian sandalwood, *Eucarya spicata* (R. Br.) Sprag. and Summ. Preliminary isolation experiments with this material indicate that activity is definitely associated with the small diterpenoid hydrocarbon fraction of the oil, and probably with a specific component which parallels closely, in thin-layer and gas chromatography, the behaviour of the true pheromone. Attempts are now being made to obtain this plant constituent in a state of purity.

Final confirmation of the nature of the pheromone must await an unambiguous synthesis. However, the possibility, always present in this type of work, that the substance isolated merely represents a carrier for an even more potent pheromone seems in the present instance rather remote.

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A Biochemical Factor in the Zonation of Marine Molluscs

THE trochids *Monodonta turbinata* and *Gibbula divaricata* are midlittoral and sublittoral animals respectively. On the rocky shores of Malta, *M. turbinata* is found semi-exposed in the very narrow "intertidal" zone. *G. divaricata* is usually found submerged in shallow water. It was considered that *G. divaricata* might be adapted for prolonged submersion by having among other factors a higher capacity to incur an oxygen debt than *M. turbinata*. The experiments described here were carried out to test this hypothesis.

The snails were removed from the shells, weighed and finely minced with scissors. The operculum was discarded. The minced tissue was homogenized with sea water containing 10 mM sodium fluoride. The homogenate contained 10 per cent snail tissue. 1 ml. homogenate was incubated for 30 min at 25° C with 0·8 μmoles pyruvate and 1·3 μmoles nicotinamide-adenine dinucleotide (NADH) in a final volume of 2 ml. In one set of experiments the amount of pyruvate consumed by the reaction mixture was determined. The pyruvate was estimated as the dinitrophenylhydrazone. The oxygen consumption of the reaction mixture was measured with a Warburg respirometer in another set of experiments. The gas phase was air. The results were expressed in μmoles pyruvate or oxygen consumed per minute per gram dry weight of homogenate. The dry weight determinations were made on the material precipitated from the homogenate by trichloroacetic acid; this material was washed twice with distilled water and dried at 110° C for 24 h.

The mean rates of pyruvate and oxygen consumption observed are given in Table 1.

Table 1

	Pyruvate consumption (μmoles/min/g dry wt.) (Mean ± S.E.)
M. turbinata	1·54 ± 0·08 ($n = 5$)
G. divaricata	1·94 ± 0·06 ($n = 5$)
	Oxygen consumption (μmoles/min/g dry wt.) (Mean ± S.E.)
M. turbinata	3·04 ± 0·10 ($n = 3$)
G. divaricata	2·26 ± 0·04 ($n = 3$)

The difference between the mean rates of pyruvate consumption is significant ($0·002 < P < 0·01$). The mean rates of oxygen consumption also differ significantly ($P = 0·002$). Because 1 mole of pyruvate requires 2·5 moles of oxygen for its complete oxidation, it will be seen that the pyruvate consumed by the homogenates was not completely oxidized. The pyruvate which was consumed but not oxidized may be regarded as an oxygen debt as NADH was added to the reaction mixtures to favour the reduction of pyruvate to lactate. The homogenized tissues of *G. divaricata* consumed pyruvate at a higher rate and oxygen at a lower rate, and showed a greater oxygen debt as defined here, than the homogenized tissues of *M. turbinata*. This property may enable *G. divaricata* to withstand longer periods of submersion than *M. turbinata*.

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Paralytic Shellfish Poison: Serological Assay by Passive Haemagglutination and Bentonite Flocculations

PARALYTIC shellfish poison (PSP) is a potent toxin, of small molecular weight ($C_{10}H_{17}N_7O_4.2HCl$), which is produced by the dinoflagellate *Gonyaulax catenella* and which is concentrated, with no ill effects, in bivalves that use the *Gonyaulax* as a food source[1]. Human ingestion of PSP results in paralytic poisoning and occasionally death[2]. Mouse bioassay is nowadays used for the detection of PSP in contaminated bivalves. This test, however, is not specific, as other marine toxins, such as puffer fish poison (tetradotoxin), produce similar paralytic symptoms in mice[3]. Recently, PSP was shown to be haptenic when conjugated to proteins by formaldehyde condensation[4].

This report compares passive haemagglutination and bentonite flocculation with mouse bioassay for the specific detection of PSP in contaminated shellfish. The approach used here for the sensitive and specific detection of PSP in bivalves should be applicable to the serological study of other marine toxins.

Details of the source of purified PSP, preparation of PSP-protein conjugates, production of antisera to PSP in rabbits, passive haemagglutination (HA), and haemagglutination-inhibition (HI) have previously been described[4]. Non-toxic butter clams (*Saxidomus giganteus*)

Table 1. INHIBITION AND RECOVERY STUDIES WITH PSP THAT HAD BEEN ADDED TO NON-TOXIC CLAMS USING PASSIVE HAEMAGGLUTINATION, BENTONITE FLOCCULATION, AND MOUSE BIOASSAY

PSP added per 100 g of clams (μg)	PSP recovered per 100 g of clams as measured by mouse bioassay (μg)	Minimal amount of PSP per ml. completely inhibiting a 4+ haemagglutinating dose of anti-PSP (μg)		Minimal amount of PSP per ml. completely inhibiting a 2+ bentonite flocculating dose of anti-PSP (μg)	
		Based on amount added to clams	Based on amount recovered by mouse bioassay	Based on amount added to clams	Based on amount recovered by mouse bioassay
200	50 2	0 032	0·008	0 25	0 063
600	331 2	0·048	0·026	0 375	0 2
1,000	621	0 04	0·025	0 31	0 19
1,400	1,045·8	0 052	0·039	0 22	0 16

were obtained from A. K. Sparks, University of Washington, Seattle. Varying amounts of a solution of purified PSP (5,000 μg per ml.) in acidified distilled water (*p*H 3·2) were added to constant amounts of the ground clams. The concentration of PSP in the clams was expressed in μg PSP per 100 g of clams. Saline extracts were made of the clams[5], and portions of these were quantitatively assayed for PSP by the mouse bioassay technique[6], while other portions were heated at 56° C for 30 min. The precipitate formed by heating was removed by centrifugation, and the supernate was used in HI and bentonite flocculation inhibitions. Bentonite flocculations (BF) were performed as previously described[7], using 1 per cent bovine serum albumin as the stabilizing agent. Bentonite flocculation inhibition (BFI) was carried out by serial dilution of the inhibitor in 0·1 ml. volumes in physiological saline in 10 by 75 mm tubes. To each dilution of inhibitor was added 0·1 ml. of anti-PSP serum in phosphate-buffered saline, *p*H 7·2. The serum had been diluted to contain a 2+ agglutinating dose of anti-PSP in 0·1 ml. after it was mixed with the inhibitor. After incubating at room temperature for 20 min, 0·1 ml. of each dilution of the mixture of inhibitor and anti-PSP was added to microflocculation slides, and bentonite flocculations were carried out.

Passive haemagglutination titres of PSP antisera varied from 1 : 16 to 1 : 1,024, 2 to 32 times greater than the BF titres. The antigen for HA, however, had to be prepared daily, whereas the bentonite antigen was stable for 4 days when stored at 4° C.

Data on HI and BFI with PSP that had been added to non-toxic clams are presented in Table 1. Minimal concentrations of PSP that completely inhibited anti-PSP are given on the basis of both the amount of PSP added to 100 g of clams and the amount of PSP recovered from 100 g of clams, as indicated by mouse bioassay. The term "recovered" is used here to indicate the amount of PSP detected by bioassay when known amounts had been added to non-toxic clams. Regardless of the amount of poison added to the clams (200, 600, 1,000 and 1,400 μg), the minimal concentrations that consistently inhibited anti-PSP were relatively similar for the individual tests—0·032, 0·048, 0·04 and 0·052 μg PSP per ml. for HI; and 0·25, 0·375, 0·31 and 0·22 μg PSP per ml. for BFI. The percentage of recovery of PSP by mouse bioassay increased with an increase in the amount of PSP added to the clams. The percentages of recovery for the 200, 600, 1,000 and 1,400 μg PSP clams were, respectively, 25·1, 55·2, 62·1 and 74·7 per cent. On the basis of the amount of PSP recovered by mouse bioassay, the clams that received 200 μg PSP, therefore, appeared to be most sensitive in inhibiting anti-PSP in both HA and BF (0·008 μg PSP per ml. for HI and 0·063 μg PSP per ml. for BFI). Obviously, the PSP that remained undetected by mouse bioassay was still active serologically. The specificity of the reaction is indicated by the fact that non-toxic butter clams (*Saxidomus giganteus*) and puffer fish poison did not inhibit anti-PSP in either test. HI was the more sensitive of the two serologic tests for the detection of PSP, but BFI involved the more stable antigen. Mouse bioassay detects a minimum of 0·18 μg PSP per ml. It follows that HI is considerably more sensitive in the detection of PSP than bioassay, while BFI is similar in sensitivity to the mouse test.

The data presented here indicate that HI and BFI are applicable to the serological detection of PSP in contaminated bivalves.

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Use of Liquid Ion Exchange Chromatography for the Separation of Organic Compounds

SEVERAL investigators have recently effectively separated metals by means of paper or thin-layer chromatographic systems using liquid ion exchangers[1–6]. With these materials, this technique can be considered as a form of reverse phase chromatography whereby the liquid stationary phase is an acid or a base with very low solubility in the aqueous mobile phase. It seems that the interaction between ionized groups of the solutes and the stationary phase may play a significant part in this separation process.

Although solvent extraction with liquid ion exchangers was used successfully in the separation of organic compounds[7], the potentialities of liquid ion exchange chromatography have yet to be explored in this field.

During the course of development of a liquid chromatographic system which provided both close control of operating parameters and a degree of versatility characteristic of gas chromatography, it was found that liquid ion exchangers proved to be useful stationary phases for the separation of certain non-volatile organic materials.

A block diagram of the instrument used in this investigation is depicted in Fig. 1. The system consists of the micro packed column, those parts necessary for maintaining a controlled continuous flow of the liquid mobile phase, a temperature controller, a sampling device, a detector and recorder. For the continuous detection of nucleic acid constituents as well as other ultra-violet absorbing solutes a Perkin–Elmer Hitachi '*UVIS*-139' spectrophotometer was modified to accommodate a micro flow cell having an effective cylindrical bore 5 mm long and 1 mm in diameter. The cell was kept under pressure by restricting the flow at the outlet in order to avoid the generation of bubbles during operation. The detector signal was monitored with a 1-mV recorder provided with a base line compensator. Full-scale deviation of the recorder pen corresponded to 0·045 absorbance unit.

The sample was introduced by a high pressure sampling valve equipped with a slide for 10-μl. sample. The valve

was filled with the same solution by means of a hypodermic syringe before the sample was introduced into the apparatus. All parts of the instrument which had contact with the mobile phase were made of stainless steel, nylon or 'Teflon'. The detector cell was made of quartz. Special care was taken to minimize dead volumes between the sampling valve and the detector outlet. 'Swagelok' fittings were used for all connexions.

Columns were made of siliconized nylon tubing (1·6 mm outer diameter and 0·85 mm internal diameter), dry packed with the column material and plugged with porous 'Teflon' disks at both ends. A device ('precolumn'), prepared in a similar manner to the separation column, was placed upstream to the sampling valve for conditioning the mobile phase.

The chromatograms in Figs. 2 and 3 show separations of thyroid compounds and nucleic acid constituents. These were obtained by means of liquid ion exchange columns. The stationary phase was 'Aliquat 336' (General Mills Co.), a mixture of quaternary salts of long-chain tertiary amines, and mainly tricaprylyl methyl ammonium chloride in 75 per cent aqueous isopropanol solution. 'Chromosorb *P*' mesh fractions 230–270 and 270–325 (Johns-Manville Co.) were used as the solid support.

The support was soaked with a solution of 'Aliquat 336' in diethylether and the solvent was evaporated. The coated support was then boiled with the mobile phase for 10 min and after cooling the liquid was decanted. The column material was dried in air for several hours and then placed in the nylon tube by the usual vibration technique.

Before commencing the analysis, the columns were conditioned overnight in a stream of the mobile phase at 50° C and 15 atm. inlet pressure. During this time the columns became deaerated and some entrainment of the liquid stationary phase and compression of the packing occurred. A few centimetres of the inlet end of the columns was then removed and a stable base line obtained. The columns were used continuously over a period of several days and did not show any alteration in their resolving power. The operating conditions of the column are described in the captions. On several occasions a programmed increase of the flow rate during a chromatographic run, as shown for example in Fig. 3, resulted in a decrease in analysis time.

Other liquid ion exchangers, such as 'Amberlite *LA*-1', 'Amberlite *LA*-2' (Rohm and Haas Co.) and di-2-ethylhexyl-phosphoric acid also were used on 'Chromosorb *P*' support for preparing stable columns with suitable flow characteristics. Preliminary results indicate that they too efficiently separate organic compounds of distinct acidic or basic character.

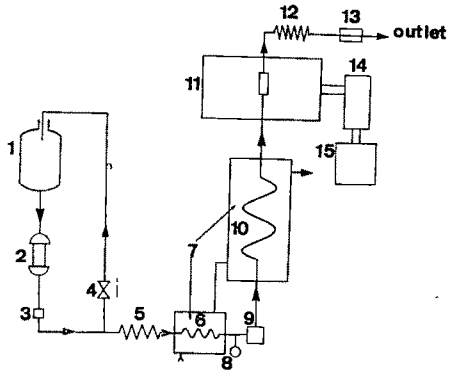


Fig. 1. Liquid chromatographic system. 1, Reservoir for mobile phase, 2, minipump (Milton Roy, Philadelphia); 3, filter; 4, needle valve for flow splitting; 5, capillary tubing, 0·1 mm internal diameter, by 5 m long for flow dampening; 6, precolumn; 7, thermostated compartment; 8, pressure gauge; 9, sampling valve; 10, column; 11, *UVIS*-139 spectrophotometer with 5 μl. flow cell (Perkin–Elmer); 12, flow restrictor; 13, capillary flow meter; 14, baseline compensator; 15, recorder (0–1 mV)

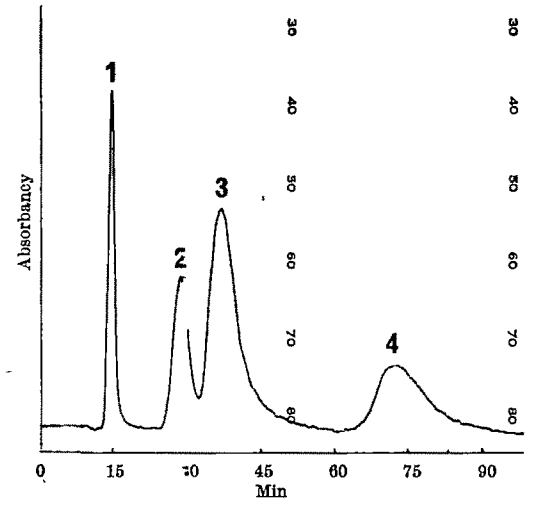


Fig. 2. Separation of thyroid compounds. 1, Tyrosine; 2, 3-monoiodotyrosine; 3, 3,5-diiodotyrosine; 4, thyronine. Sample size, 16 nanomoles of each component in 10 μl. of mobile phase; column, siliconized nylon tubing 235 cm × 0·85 mm internal diameter, packed with 30 w/w per cent 'Aliquat 336' on Chromosorb *P*' (270–325 mesh); mobile phase, 0·2 M potassium chloride in 0·04 N hydrochloric acid, saturated with 'Aliquat 336' at 30° C. Temperature, 70° C; flow rate, 7·5 ml./h; inlet pressure, 13·6 atm.; detector wave-length, 230 nm; detector slit 2·6 mm

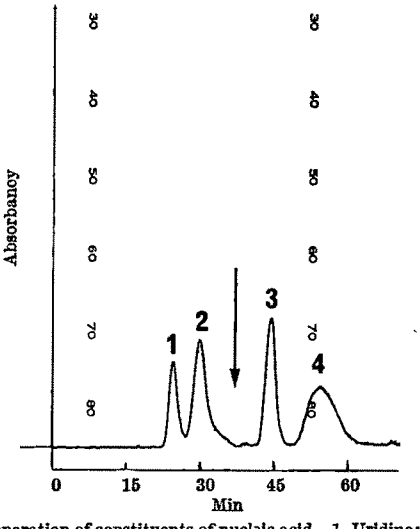


Fig. 3. Separation of constituents of nucleic acid. 1, Uridine; 2, adenosine; 3, uridine 3'(2') phosphoric acid; 4, adenosine 3'(2') phosphoric acid. Sample size, 1 nanomole of components 1 and 2, 10 nanomoles of components 3 and 4 in 10 μl. of the mobile phase. Column, siliconized nylon tubing, 157 cm × 0·85 mm internal diameter, packed with 30 w/w per cent 'Aliquat 336' on 'Chromosorb *P*' (230–270 mesh); mobile phase, 309·8 ml. of 0·5 M potassium dihydrogen phosphate plus 22·4 ml. 1 N sodium hydroxide diluted to 1 l. and saturated with 'Aliquat 336' at 30° C; temperature, 50° C; flow rate to arrow, 2·0 ml./h, beyond arrow 14·4 ml./h; initial inlet pressure less than 6 atm., final inlet pressure 30 atm.; detector wave-length, 260 nm; detector slit, 2·6 mm

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PHARMACOLOGY

Cholinergic Mechanism of Depressor Response to Small Doses of Adrenaline

MOORE and Puriton[1] in an early report in the literature state that small doses of ox adrenal extract reduce the blood pressure of the dog, but large doses increase it. The same observations have been reported by Cannon and Lyman[2] and by Dale and Richards[3]. This depressor response is not to be confused with that noted after the injection of larger doses of adrenaline or splanchnic nerve stimulation in the presence of α-adrenergic blocking agents such as dibenamine[4]. The latter observation is now explained on the basis of the unopposed stimulation of β-adrenergic receptors based on the receptor classification of Ahlquist[5].

Experiments in our laboratory have demonstrated a depressor response associated with the injection of small doses of adrenaline which appears to be cholinergic[6]. Under special circumstances, this can also be shown with noradrenaline and isoproterenol. Experiments have been carried out to elucidate the mechanism of this depressor response.

All experiments were carried out on anaesthetized dogs and cats without regard for sex or weight. The dogs were anaesthetized by intravenous injection of pentobarbitol sodium, 35 mg/kg, and the cats were anaesthetized by the intraperitoneal administration of chloralose dissolved in polyethylene glycol 200 at a dose of 80 mg/kg. All drugs were injected through a cannula placed in the femoral vein. The blood pressure was recorded from a carotid artery cannula by using a 'Sanborn' differential transformer-transducer coupled to an Offner 'Dynograph'. All drug doses were expressed in terms of the following salts: *l*-adrenaline bitartrate, *dl*-noradrenaline bitartrate, *l*-isoproterenol sulphate, phenoxybenzamine hydrochloride, dichloroisoproterenol hydrochloride (DCI), acetylcholine bromide and atropine sulphate.

Adrenaline administered to an animal at a dose of 1 μg/kg elicits a depressor response of approximately 20–35 mm of mercury as shown in Fig. 1. Repeated administrations of this dose at intervals yield smaller depressor responses and finally a pressor response or no response at all. This state of tachyphylaxis can be abolished by the administration of 10 μg/kg of acetylcholine, following which 1 μg doses of adrenaline again produce the depressor responses in the blood pressure.

Fig. 2 shows the interaction of various sympathomimetic amines with acetylcholine. The uppermost tracing shows a pressor response to a dose of adrenaline. After acetylcholine administration, adrenaline produces a depressor response on the first trial and somewhat less response on the second trial. Another dose of acetylcholine is administered which ordinarily would have re-established the depressor response to adrenaline. The administration of atropine, however, blocks the depressor response and a pressor response is obtained. The middle tracing shows the response to noradrenaline of an animal which had been treated with the α-blocking agent, phenoxybenzamine. The same sequence of events is shown as in the experiment with adrenaline. The bottom tracing shows the use of isoproterenol in the presence of the β-blocking agent DCI.

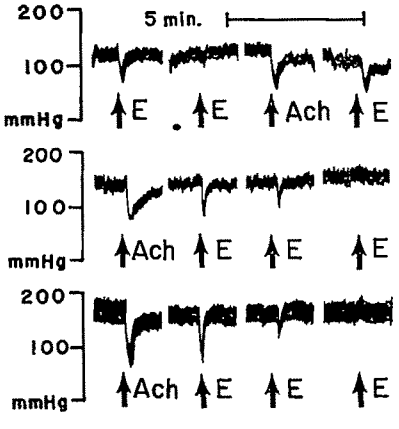


Fig. 1. Selected segments taken from a continuous record of the carotid blood pressure of an anaesthetized dog. E is *l*-adrenaline bitartrate (1 μg/kg) and Ach is acetylcholine bromide (10 μg/kg)

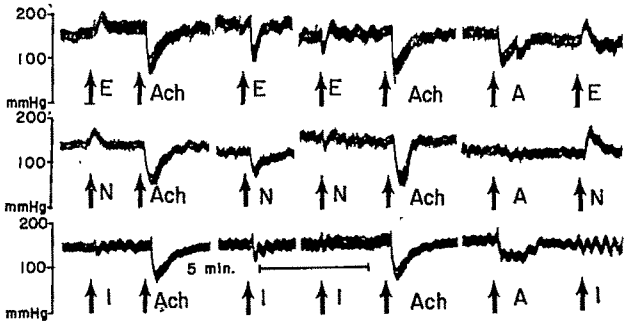


Fig. 2. Selected segments of records of the carotid blood pressure of three anaesthetized cats. E is *l*-adrenaline bitartrate (1 μg/kg), N is *dl*-noradrenaline bitartrate (1 μg/kg), I is *l*-isoproterenol sulphate (0 1 μg/kg), Ach is acetylcholine bromide (10 μg/kg) and A is atropine sulphate (1 mg/kg). See text

The tachyphylaxis to the small doses of adrenaline suggested that this phenomenon might be associated with a release of some agent which caused the depressor response. The elimination of the tachyphylaxis by injection of acetylcholine is taken as evidence that acetylcholine is liberated by a very small dose of adrenaline and that a dose of 10 μg/kg is sufficient to replete the stores. The blockade of the depressor response under consideration by a dose of atropine is taken as evidence that the depressor response is due to a muscarinic action. The production of depressor responses by noradrenaline in the presence of phenoxybenzamine and isoproterenol in the presence of DCI rules out the participation of α- and β-adrenergic receptors in this observation.

Many examples of the release of adrenergic mediators by cholinergic mechanisms exist at the present time[7]. The literature yields relatively few references to the release of acetylcholine by adrenaline[8,9].

Krayer and Verney[10] reported an increase in the acetylcholine content of the coronary sinus elicited by the administration of adrenaline. This was due to a reflex stimulation of the vagus and was eliminated by vagal section. Boatman[11] has observed a vasodilation in the hind limb of the newborn dog which is elicited by nerve stimulation and eliminated by atropine. When animals reach an age of 2 weeks, nerve stimulation produces only vasoconstriction. A recent publication by Kulkarni *et al.*[12] has shown that adrenaline produces depressor responses in animals infused with acetylcholine.

It is not possible to demonstrate the interaction between acetylcholine and the sympathomimetic amines in all animals. The effect was best demonstrated in animals during autumn and the susceptibility decreased during the winter months. In late winter only one animal in twenty-two was found to give the desired response. This may be the reason why such observations have not been reported previously. No correlation with age or sex of the animal could be made in our laboratory.

The site at which the acetylcholine is released by adrenaline to yield a depressor response has not been determined. Until it has been determined which vascular bed is being affected, the physiological significance is uncertain. In view of the small doses involved, the

release of acetylcholine by adrenaline could occur under physiological conditions.

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Modification of the Auditory Flutter Fusion Threshold by Centrally Acting Drugs in Man

PERCEPTUAL fusion of intermittent random noise stimuli at critical frequencies in man was first reported by Miller[1] and Miller and Taylor[2], and confirmed by Symmes, Chapman and Halstead[3]. The process is analogous to visual flicker fusion. The critical interruption rate at which fusion occurs is termed the "auditory flutter fusion threshold".

In view of the sensitivity of visual flicker fusion thresholds to the actions of psychotropic drugs, it is perhaps surprising that the psychopharmacology of auditory flutter fusion threshold has received little attention. Only Eysenck and Easterbrook[4] appear to have explored this field and they were unable to demonstrate any effects of sodium amylobarbitone (90 mg), dexamphetamine (5 mg) or meprobamate (100 mg) on auditory flutter fusion threshold. However, the interruption rates of their auditory signal varied in the relatively large steps of ten interruptions per second (i.p.s.) so that small changes in the threshold might have escaped detection.

We have found that the auditory flutter fusion threshold was highly sensitive to centrally-acting drugs, and this communication reports the actions of amylobarbitone, chlorpromazine and perphenazine.

The technique was a modification of that used by Miller and Taylor[2]. A random noise signal, which could be interrupted at rates of between 1·5 and 275 i.p.s. (on–off ratio 9 : 1), was used at an intensity of 55 db (re. 0·0002 dynes/cm²). The interruptions were automatically switched on and off at intervals of 1 sec so that the signal, presented binaurally to the subject, consisted of alternating 1-sec periods of interrupted or continuous noise. For each test the starting interruption rate was 100 i.p.s. and then the signal was presented in a sequence of decreasing interruption rates. The critical descending auditory flutter fusion threshold was taken as the fastest interruption rate at which auditory flutter (that is, the interruptions) could be distinguished and below which consistent positive flutter responses were reported by the experimental subject.

Six normal healthy male subjects (aged 21–30 yr.) participated in each of the double blind cross-over trials. Subjects were allowed a light breakfast. The active compound (amylobarbitone—100 mg, or chlorpromazine—25 and 50 mg, or perphenazine—2 and 4 mg), or its placebo control, was administered by mouth between 9 and 10 a.m. at 7-day intervals. On each treatment day, three threshold determinations were made, at 5-min intervals, before treatment was administered and at 90 and 180 min later. Changes in the mean thresholds from the control values on the active and placebo treatments were compared using the method of analysis of variance for factorial designs and Student's two-tailed '*t*' test (Snedecor[5]).

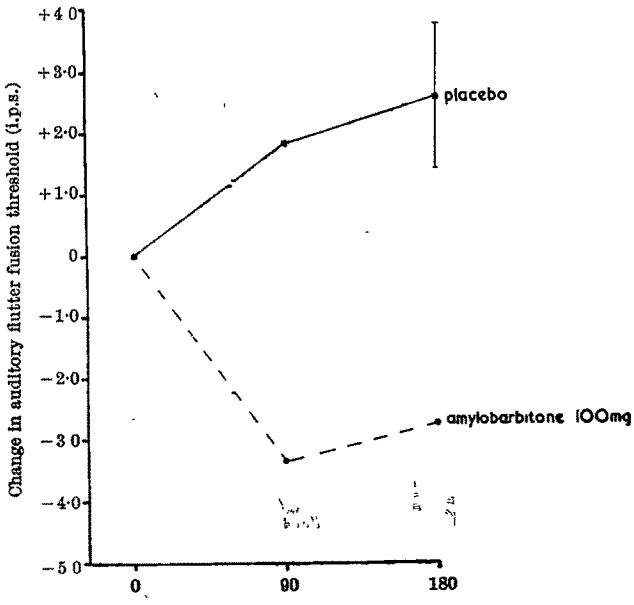


Fig. 1. The effect of amylobarbitone (100 mg) and placebo treatment on the descending auditory flutter fusion threshold. The changes in the mean thresholds are from those recorded before treatment (six subjects). ⊤ ■ ⊥, Standard error of the mean

The changes following amylobarbitone (100 mg) are illustrated in Fig. 1. The pretreatment mean thresholds were 42·93 i.p.s. for the placebo and 43·10 i.p.s. for the amylobarbitone treatment. The usual rise in the auditory flutter fusion threshold over the 180-min period on the placebo is seen (continuous line). After amylobarbitone (broken line), the threshold was lower than after the placebo both at 90 min (by 5·17 i.p.s.) and at 180 min (by 5·27 i.p.s.), and analysis showed that this depression was significant:

For the overall difference between placebo and amylobarbitone treatments effects, $F_{1,15} = 20{\cdot}03$, $P < 0{\cdot}001$.

For the difference between placebo and amylobarbitone treatment effects, at

90 min, $t_{15} = 3{\cdot}13$, $P < 0{\cdot}01$
and at 180 min, $t_{15} = 3{\cdot}19$, $P < 0{\cdot}01$.

The amylobarbitone induced depression thus developed within 90 min of oral administration and was maintained at about the same level for at least 180 min.

Chlorpromazine (25 and 50 mg) also produced statistically significant depression of the auditory flutter fusion threshold which was greater at 180 min than at 90 min. However, neither 2 mg nor 4 mg of perphenazine altered the auditory flutter fusion threshold, thus distinguishing between the actions of aliphatic and piperazine phenothiazine derivatives.

We thank Dr. P. Gorman and Mr. M. C. Martin of the Royal National Institute for the Deaf and Mr. P. M. G. Bell for their help.

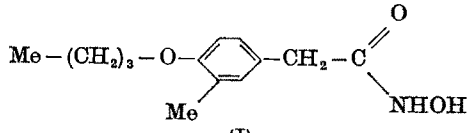
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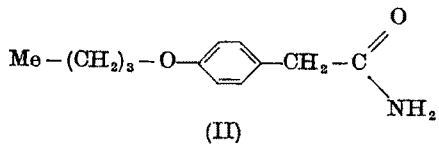
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Arylacethydroxamic Acids: a New Class of Potent Non-steroid Anti-inflammatory and Analgesic Substances

In recent years much effort has been directed to the search for non-steroid anti-inflammatory substances which can be used in the therapy of rheumatic diseases and related conditions[1]. This communication reports the finding that a chemical family hitherto unexplored in this respect, namely, the arylacethydroxamic acids, includes several substances endowed with considerable anti-inflammatory and analgesic properties, and also a remarkably low degree of toxicity. A particularly interesting compound in this series was 4-butoxy-3-methylphenylacethydroxamic acid (I). In a comparative investigation with known antiphlogistic compounds, using the Benitz and Hall carrageenin-induced abscess test in rats[2], compound (I), in exactly the same dose, proved only slightly less active (*c.* half) than phenylbutazone (1,2-diphenyl-3,5-diketo-4-butylpyrazolidine), definitely more active than 'Ibufenac' (*p*-isobutylphenylacetic acid), and considerably more so than aspirin. The activity shown by compound (I) at the high dosage of 1 g/kg was as intense as that of the maximum tolerated dose of phenylbutazone (400 mg/kg) and considerably more so than that (10 mg/kg) of 'Indomethacin' (1-*p*-chlorobenzoyl-5-methoxyindolyl-3-acetic acid), which is the most efficient non-steroid antiphlogistic substance known so far. Compound (I) was also strongly active in the carrageenin-induced granuloma test in rats, whereas phenylbutazone and aspirin are known to behave poorly in this type of assay[3].



(I)



(II)

Analgesic activity, determined in mice by means of Blake's test involving an electric stimulus[4], indicated that compound (I) was considerably more effective than the non-morphinic reference analgesics: the 50 per cent effective doses, measured after 90 min, were:

Compound (I)	18 mg/kg
'Ibufenac'	76 mg/kg
Acetophenetidine	103 mg/kg
Aminopyrine	109 mg/kg
Antipyrine	115 mg/kg
Aspirin	160 mg/kg

Comparative determination of acute toxicity in mice[5] with known anti-inflammatory agents gave the following figures for the 50 per cent lethal dose:

Compound (I)	1,800 mg/kg
Aspirin	1,100 mg/kg
Phenylbutazone	290 mg/kg
'Indomethacin'	26 mg/kg

Chronic oral toxicity tests performed in compound (I) in both rats and dogs produced no signs of toxicity at 125 mg/kg and per day.

The relationships between chemical structure and pharmacodynamic activity are evidenced in the data given in Table 1 for compounds chosen from among several hundred investigated.

Table 1

	Activity	
	Anti-inflammatory	Analgesic
4-Butoxy-3-methylphenylacethydroxamic acid (I)	++++	++++
4-Butoxyphenylacethydroxamic acid	++++	++++
4-Butoxy-2,3-dimethylphenylacethydroxamic acid	−	
4-Butoxy-5-isopropyl-2-methylphenylacethydroxamic acid	−	
4-Propoxyphenylacethydroxamic acid	+++	+++
4-Isopropoxyphenylacethydroxamic acid	+	
4-Amyloxyphenylacethydroxamic acid	±	
4-*Cyclo*pentyloxyphenylacethydroxamic acid	−	++
4-*Cyclo*hexyloxyphenylacethydroxamic acid	+	++
4-Propylphenylacethydroxamic acid	−	
4-Isobutylphenylacethydroxamic acid	++	
4-Butylphenylacethydroxamic acid	±	
4-Amylphenylacethydroxamic acid	−	
4-*Cyclo*hexylphenylacethydroxamic acid	−	+
4-Butoxyphenylacetic acid	−	−
4-Isobutoxyphenylacetic acid	−	−
4-Butoxyphenylacetamide (II)	−	++++
4-Butoxy-3-methylphenylacetamide	−	++++

The most interesting features to emerge from Table 1 are: (*a*) the complete disappearance of anti-inflammatory activity encountered in passing from 4-butoxyphenylacethydroxamic acid to 4-butoxyphenylacetic acid itself, thus pointing to a specific influence on the part of the hydroxamic acid function; (*b*) the discrepancy observed between the anti-inflammatory and analgesic activities—an extreme example being 4-butoxyphenylacetamide (II), the total inactivity of which as an anti-inflammatory agent contrasts strikingly with its remarkable analgesic potency, which is roughly the same as that of compound (I) (its 50 per cent effective dose being 17 mg/kg); 4-butoxyphenylacetamide, and 4-butoxyphenylacethydroxamic acid, also show antipyretic effects (in rabbits in a state of hyperthermia induced by injection of antityphoid vaccine TAB) comparable with that of aspirin; (*c*) the close dependence of anti-inflammatory activity on the number, nature, and site of the substituents on the aryl nucleus, a striking example being the absence of activity of 4-butoxy-2,3-dimethylphenylacethydroxamic acid, as compared with the outstanding activity of its lower homologue, compound (I). Further replacement of the nuclear methyl group in (I) by bromine, chlorine or fluorine results in comparably active compounds.

It is doubtless relevant to the mechanism of the anti-inflammatory action of compound (I) and its homologues that, on one hand, adrenalectomy inhibits their effects and, on the other hand, investigations made in rats with carbon-14-labelled 4-butoxyphenylacethydroxamic acid showed high levels of radioactivity in the adrenals.

Compounds (I) and (II) are both undergoing clinical trial.

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PHYSIOLOGY

Athletic Performance at High Altitude

OWING to the lower partial pressure of oxygen the maximum oxygen intake of acclimatized adults at high altitude is less than the sea-level value. The reduction in the maximum oxygen intake at the 7,500 ft. altitude of Mexico City, where the 1968 Olympic Games are to be held, is expected to be 8 per cent following the trend of experiments made in the Himalayas[1]. From this result it has been predicted that the times for the running events at Mexico City would be 8 per cent longer than the expected sea-level times[2] except for the shorter events where the oxygen requirement could be covered anaerobically.

Pugh[2] compared the results of the 1955, Mexico City, Pan-American Games, with the results of the 1956 Melbourne Olympic Games and found a linear relationship between the increase in time and the logarithm of the distance of the race. The increase varied from 2·6 per cent for the 800 m event to 14·9 per cent for the 10,000 m event. This comparison, however, does not take into account the generally lower standard of performance of the athletes who were eligible to compete in the Pan-American games when compared with the standard of the athletes who competed in the Olympic Games.

Table 1. COMPARISON OF HIGH-ALTITUDE AND SEA-LEVEL PERFORMANCES

Event and athlete	Mexico City time	Best sea-level time (1954–1955)	Percentage increase in time
400 m			
Jones	45 4	46·4	−2·2
Lea	45 6	46·3	−1·5
Mashburn	46·3	46·1	+0 4
800 m			
Sowell	1 : 49·7	1 : 48·3	1·3
Spurrier	1 : 50 0	1 : 46·8*	3·0
Sandoval	1 : 52·4	1 : 50·9	1·4
1,500 m			
Miranda	3 : 53·2	3 : 53·8	−0·3
Santee	3 : 53·2	3 : 42·8	4·7
Dwyer	3 : 55·8	3 : 45·4*	4·6
5,000 m			
Suarez	15 : 30·6	14 : 26·2	7·4
Ashenfelter	15 : 31·4	14 : 42·2*	5·6
Correa	15 : 39 2	14 : 42·2	6·5
10,000 m			
Suarez	32 : 42·6	30 : 30·0	7·2
Sanchez	33 : 00 4	Not available	–
Correa	33 : 42·6	31 : 41·0	6·4

* Time adjusted from the equivalent "English" distance.

A better method of checking the decrement in performance at Mexico City is to compare the times of the athletes placed in the 1955 Pan-American Games events[3] with their best sea-level times for the years 1954[4] and 1955[3] (Table 1). The percentage difference in time for each athlete was calculated and the mean for each event plotted against the distance of the race (Fig. 1). The 100 m and 200 m events were excluded as wind conditions can considerably affect the best recorded times of the athletes concerned.

Table 2. DECREMENT IN PERFORMANCES

Distance	Observed mean percentage decrement	Calculated percentage decrement from equation
400 m	−1·1	–
800 m	1·9	1·95
1,500 m	3·0	3·20
3,000 m	–	4·96
5,000 m	6 5	6·14
10,000 m	6·8	6·97

The increase in the times for the distance events are less than those calculated previously and range from 1·9 per cent for the 800 m to 6·8 per cent for the 10,000 m (Table 2). The 400 m runners at Mexico City seem to have been assisted by the low aerodynamic drag at the high altitude; their times were reduced by an average of 1·1 per cent.

An exponential curve was fitted to the points from 800 m to 10,000 m by minimizing the squares of the differences between the observed mean decrements and the calculated decrements. The equation for this curve was

$$P = 7{\cdot}1\,(1 - e^{-0{\cdot}00040D})$$

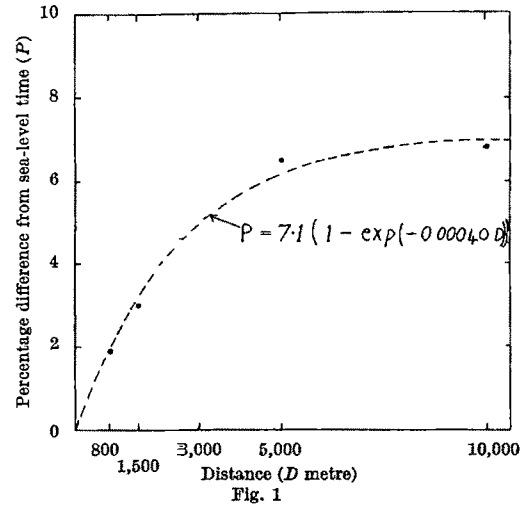


Fig. 1

where P is the percentage increase in time taken to run the distance in metres, D, at 7,500 ft. altitude. This equation indicates that the increase in time taken to run an event approaches the value of 7·1 per cent for the very long races. This is in excellent agreement with Pugh's estimate of 8 per cent based on maximum exercise tests as some assistance is probably gained from the lower aerodynamic drag on an athlete at an altitude of 7,500 ft.

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Tolerance of Chickens to Thiabendazole

THIABENDAZOLE, a broad-spectrum anthelmintic[1,2], is well tolerated by mice, rats and rabbits, with LD_{50} values exceeding 3·1 g/kg in oral acute toxicity experiments[3]. Furthermore, rats and dogs tolerated the drug quite well in chronic toxicity experiments[3].

Recent unpublished experiments with adult chickens fed thiabendazole continuously for 3 weeks revealed that a level of 0·1 per cent in the diet has no effect on reproduction, egg production or feed intake, but that levels exceeding 0·2 per cent produced lower values for all three criteria. In addition, the birds fed these high levels of thiabendazole appeared anaemic and about 7 per cent mortality occurred at the 0·2 per cent level with progressively higher mortality at higher drug levels. Post-mortem examination of the hens revealed haemorrhaging throughout most internal organs, but particularly at the ovary. Haematological data were obtained in a series of experiments in which thiabendazole was fed for 21 days to

Table 1. HAEMATOLOGICAL DATA ON ADULT FEMALE CHICKENS FED THIABENDAZOLE FOR THREE WEEKS OR THIABENDAZOLE WITH B_{12}, ASCORBIC ACID AND MENADIONE

	Control	0·2% Thiabendazole	0·4% Thiabendazole	0·4% Thiabendazole + 1 mg B_{12}/kg + 100 mg ascorbic acid/kg + 20 mg menadione/kg
No. birds	8	4	3	4
Haemoglobin (g %)	8·4 ± 0·3*	5·4 ± 0·7	4·4 ± 0·4	8·1 ± 0·2
Haematocrit (vol. %)	30·1 ± 0·8	20·9 ± 2·8	17·8 ± 1·4	27·5 ± 0·8
Sedimentation rate (mm/h)	0·5 ± 0·0	1·3 ± 0·3	1·3 ± 0·3	0·6 ± 0 2
Prothrombin clotting time (sec)	15·9 ± 0·9	17 4 ± 1·6	15 2 ± 0·9	14·0 ± 0·7
Whole-blood clotting time (min)	30 ± 3	24 ± 5	39 ± 11	29 ± 6
General appearance of birds	Normal	Pale skin Pale combs Partial moult	Pale skin Pale combs Partial moult	Normal

* Mean ± standard error of the mean.

Table 2. HAEMATOLOGICAL DATA ON ADULT FEMALE CHICKENS FED THIABENDAZOLE FOR THREE WEEKS OR THIABENDAZOLE WITH B_{12}, ASCORBIC ACID OR MENADIONE

	Control	0·2% Thiabendazole	0·2% Thiabendazole +1 mg B_{12}/kg	0·2% Thiabendazole +100 mg C/kg	0·2% Thiabendazole +20 mg K/kg	0·2% Thiabendazole +B_{12}+C+K
No. birds	4	4	4	3	4	4
Haemoglobin (g %)	9·0±0·2*	5·2±1·2	7·0±1·0	3·0±0·4	6·3±1·1	9·0±0·3
Haematocrit (vol. %)	33·8±1·9	19·1±1·6	26·8±4·7	12·8±2·3	23·0±3·5	33·0±1·0
Sedimentation rate (mm/h)	0·3±0·2	4·6±3·4	0·6±0·3	2·0±0·6	1·4±0·4	0·4±0·1
Whole-blood clotting time (min)	20±5	35±5	20±5	29±7	17±5	42±8

* Mean ± standard error of the mean.

hens. The data show that haemoglobin and haematocrit values were markedly below normal in hens treated with 0·2 or 0·4 per cent thiabendazole, and this correlated with their pale appearance (Table 1). Sedimentation rates were elevated, but whole-blood clotting time and prothrombin clotting time showed no consistent change from the norm. However, it should be noted that whole-blood clotting time assays are subject to wide variability and many values are required to show a trend. Also, it should be pointed out that two birds sampled at the end of the second week on 0·4 per cent thiabendazole had clotting times exceeding 60 min, the cut-off time for the assay, and that one of the four birds sampled at the end of the third week had a whole-blood clotting time exceeding 60 min. When these data are considered with the mortality values and the post-mortem findings, there is an indication that blood clotting time may be influenced provided the drug level is 0·4 per cent or higher.

Most interesting was that the addition of 1 mg vitamin B_{12} per kg, 100 mg ascorbic acid per kg, and 20 mg menadione per kg diet containing 0·4 per cent thiabendazole counteracted the adverse effect of the drug on the haematological values (Table 1) of the hens. These birds at necropsy did not have the haemorrhages that were observed in those receiving the drug alone. As shown in Table 2, B_{12} and menadione, and not ascorbic acid, counteracted the adverse effect of thiabendazole on haemoglobin and haematocrit levels. Each vitamin alone, partially reversed the effect at the levels fed, and the addition of both vitamins to the diet completely overcame the adverse effect of 0·2 per cent (Table 2), as well as 0·4 per cent thiabendazole (Table 1).

In comparison to other species of animals in which thiabendazole is well tolerated, the adult female chicken appears to be more sensitive to the drug. Hens fed a diet containing 0·2 per cent thiabendazole consumed 80 mg thiabendazole per kg body-weight daily. This dose is about 0·5–0·33 of the amount required to produce a moderate anaemia in dogs[3]. Single oral doses of 50 mg/kg have proved highly efficacious in roundworm infections in those mammals for which the drug is recommended, so there is an adequate safety margin for thiabendazole. We have investigated the effect of thiabendazole when fed to chickens to 6 weeks of age and find that they tolerate (for growth) about 0·1 per cent of the drug in the diet and have normal haemoglobin levels.

Investigations in progress are designed to reveal the maximum tolerated level of thiabendazole in the hens' diet without effect on their haematology, which is estimated, from the data in Tables 1 and 2, to be in the range of 0·05–0·1 per cent thiabendazole in the diet. In addition, investigations are at present in progress to reveal the mode of action between thiabendazole and vitamins B_{12} and K, and the possible interaction of the endocrines on the drug–vitamin relationship.

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Effect of Removing the Neocortex on the Response to Repeated Sensory Stimulation of Neurones in the Mid-brain

THE response of a high proportion of cells in the superior colliculus and subjacent structures (tecto-tegmental region) of the rabbit mid-brain gradually wanes when a sensory stimulus is repeatedly presented at rates between 1 per sec and 1/4 per sec[1,2]. The number of stimuli required to bring about attenuation of response (habituation) varies from cell to cell and, for a given cell, from stimulus to stimulus, but lies between two and fifty presentations. The response may be restored by withholding the stimulus for some 20 sec or more and presenting it again (recovery by time lapse). In the experiments on which these observations are based, the animals were anaesthetized with urethane-pentobarbitone and the recording micro-electrodes directed to the mid-brain by a hole drilled in the skull. Thus apart from the tracks made by the electrodes the cerebral cortex was intact.

The tecto-tegmental region of mammals receives an extensive innervation from the neo-cortex[3–5] and a question that remains is whether this structure must be present for habituation to occur in the tecto-tegmental cells. This question is answered in the experiments described here.

Five rabbits (weights 2·5–3·5 kg) were used. The solution and dosage of anaesthetic were the same as in our previous experiments[1,2]. Each animal was mounted in a stereotaxic frame and the roof of the cranial cavity removed. The neo-cortex lateral to the rhinal fissure was removed by aspiration, gelatin sponge being used to control haemorrhage. Care was taken to avoid damage to the optic nerves and tracts. When bleeding stopped, a 4 per cent aqueous solution of agar was poured over the brain to reduce cardiovascular and respiratory pulsations. The pupil of the right eye was then dilated and the eye refracted as before. The quadrigeminal bodies could be seen clearly through the solidified agar and the electrode was lowered into the left superior colliculus under direct vision. All penetrations were taken to a depth of 10 mm below the surface of the colliculus. At the end of an experiment the brain was fixed *in situ* by removing the head and immersing it in 10 per cent formol saline. The brain was afterwards removed, examined macroscopically to ensure that the optic nerves, chiasma and tracts were intact, and then sectioned and stained for histological examination.

The photic stimuli consisted of stationary and moving spots of light projected on to a screen. Acoustic stimuli consisted of tones and clicks and the tactile stimulus was air blown through a rubber tube over restricted regions of the skin. During the experiment the intensity of background illumination varied from 0·6 to 1·0 ft.-L.

Spontaneous impulse activity was usually brisk some 6–8 h after decortication. Before this, the mid-brain was relatively quiet and little spontaneous or evoked activity could be detected.

One hundred and ten units were studied of which 74 responded to at least one sensory stimulus. Functional lamination of the units was more marked in these preparations than in those with the cerebral cortex intact, the majority of units responding to photic stimuli lying between the surface and a depth of 2·3 mm (that is, within stratum griseum superficiale and stratum opticum)

and the majority of those responding to acoustic and to tactile stimuli lying between 3·0 and 10·0 mm below the surface of the tectum. Between 2·3 and 3·0 mm there was a transitional zone in which a few cells (four) could be activated by both photic and acoustic stimuli.

We used the same criteria for habituation as before, namely, that the response should wane within fifty presentations of a stimulus delivered at a rate of between 1 per sec and 1 per 4 sec. We were able to hold for the necessary length of time only fifty-one of the units which responded to sensory stimulation. Habituation was observed in nineteen of them and these units were not restricted to any particular depth below the surface of the tectum. The time-course of this process was not different from that observed previously in intact animals. Recovery by time-lapse could be demonstrated in all units whose response to a stimulus waned and is illustrated in Fig. 1. This unit responded with a burst of impulses when a spot of light was turned on in the middle of its receptive field. The stimulus (1 sec duration) was presented at the rate of 1 per 2 sec. Record 1 shows the response (five spikes) to the first stimulus of the series which was begun after a period of 60 sec, during which no stimuli were presented. Only one spike was evoked by the fourth stimulus (record 4) and there was no response to the next six stimuli. After the tenth stimulus presentation (record 10) the stimulus was withheld for 40 sec following which it elicited a burst (five spikes) of activity (record 11).

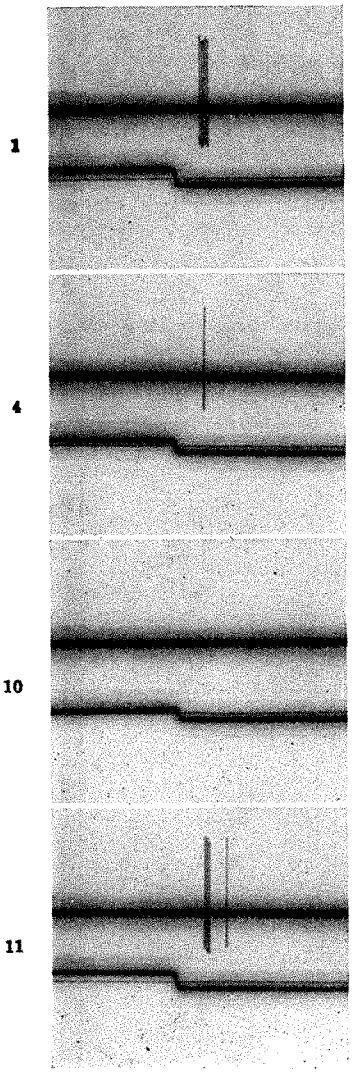


Fig. 1. Attenuation of response to repeated presentation of a spot of light at the centre of a unit's receptive field. When spikes are present they are shown on the upper trace of each record, upward deflexion being positive. A photocell placed in front of the light source was used to signal light onset, which is indicated by a downward displacement of the lower trace. Read records from left to right. Stimulus (intensity 15 ft.-*L*, 2° diameter, duration 1 sec) presented at rate of 1 per 2 sec. Record 1 shows the response (five spikes) to the first stimulus of the series presented after an inter-stimulus interval of 60 sec. Responses to fourth (record 4) and tenth stimuli (record 10) of the series are shown. An interval of 40 sec intervened between stimuli 10 and 11 and the response to the eleventh stimulus (five spikes) is shown (record 11). This unit was recorded at a depth of 1·3 mm below surface of the left superior colliculus. Scale 0·5 sec

At post-mortem it was found that the neo-cortex had been removed bilaterally in all animals and a varying amount of pyriform cortex, medial to the rhinal fissure, remained.

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Effect of Urgocyton on Capillary Permeability in Rabbits

IT has been reported that urgocyton, a non-coagulable and fluorescent protein (polypeptide) prepared from ox liver, markedly inhibits the bradykinin-destroying activity of haemolysed blood cells and also, to some extent, of plasma[1].

It is known that the intradermal injection of bradykinin in a guinea-pig with circulating blue dye regularly increases the passage of dye through the blood vessels and produces skin lesions of an intense blue colour. This response can also be elicited in rabbit skin[2].

We have investigated the effect of intradermal injections of urgocyton in rabbit skin, bearing in mind that inhibition of kininase-activity *in loco* might liberate bradykinin, which could then be demonstrated by the blue skin lesion produced in rabbits with circulating blue dye.

White land rabbits, weighing 1·8–2·5 kg, were used. The dorsal and, in some animals, also the abdominal skin was shaved with an electric razor 24 h before the tests. All materials to be tested were dissolved in saline and injected intradermally in amounts of 0·1 ml. Injection of saline (0·1 ml.) was used in the control animals. Crystalline bovine albumin and crystalline soy bean inhibitor were used to test the unspecific response to proteins.

Ten to 30 min after the intradermal injection, 1 ml./kg of 1 per cent Evans blue was injected intravenously.

Urgocyton from two different batches prepared as described previously[1] was administered in doses ranging from 0·5 to 0·005 mg.

For comparison, synthetic bradykinin or kallidin (kindly supplied by Sandoz A.G., Basle) was used and injected in amounts of 0·001 or 0·002 mg. Haemolysate prepared as described previously[1] and carboxypeptidase were used as sources of bradykinin-destroying enzymes. Crystalline carboxypeptidase *B* (Sigma Chemical Company) was tested for its bradykinin-destroying property using the rat uterus method[1]. It was found that 0·004 mg of this enzyme degraded bradykinin as fast as 1 ml. of the haemolysate.

Two to 3 min after the injection of Evans blue, visible blueing appeared at the sites where urgocyton had been

injected and increased in size in the next 45–60 min. The response given is the mean of two perpendicular diameters of the blue area after 30 min. In twenty-five of twenty-seven animals tested, intradermal injection of urgocyton provoked the extrusion of blue colour at the site of the injection. The two failures probably resulted from faulty technique. No difference in the response with the two different batches of urgocyton has been seen. The response to 0·5 mg urgocyton varied in different animals from 4 to 15 mm and corresponded to that of 0·002 mg bradykinin or kallidin (the response to these synthetic kinins was identical). The size of the response to 0·5 mg albumin, 0·5 mg soy bean trypsin inhibitor, and saline never exceeded 3 mm. There was little difference between the dorsal and abdominal regions of the skin in the reaction to intradermal injections of urgocyton or bradykinin. The smallest dose of urgocyton (in four animals) which gave a blue spot greater than that of saline was 0·005 mg.

The effect of urgocyton could be prevented by treatment of the animal beforehand with haemolysed blood injected intravenously. To demonstrate this, 3–5 ml. of blood was removed by cardiac puncture from each animal 24 h before the skin tests. The blood was kept at −20° C and became haemolysed when warmed to room temperature immediately before the injections. Intradermal injections of urgocyton, and of bradykinin or kallidin, were then made as described previously, on the dorsal skin of the rabbits. Ten min later, Evans blue was injected intravenously and blueing appeared at the sites of the intradermal injections. Thirty min after the injection of the dye, haemolysed blood was injected intravenously (each animal was given haemolysate from its own blood). Ten min after this injection, intradermal injections of urgocyton, bradykinin or kallidin were made on the abdominal skin of the animal.

Two ml. of haemolysed blood were given to each of six rabbits. In four of these, the reactions of the abdominal skin were identical with those on the dorsal skin. In two animals, however, no reactions appeared on the abdomen. When 4 ml. of haemolysed blood was injected intravenously, complete inhibition of the reaction due to intradermal injection of 0·5 mg urgocyton and 0·002 mg of bradykinin or kallidin on the abdominal skin in five of the five test animals was seen. In another eight animals, previous injection of 4 ml. of haemolysate completely prevented the reaction to intradermal injection in the dorsal skin of 0·5 mg urgocyton and of 0·002 mg of bradykinin, respectively (no abdominal injections were made in these animals).

No similar effect, however, could be accomplished by injecting carboxypeptidase *B* intravenously in eight animals in amounts corresponding to 4 ml. of haemolysate.

The demonstration that urgocyton provoked increased capillary permeability in rabbit skin by intradermal administration is interesting. The abolition of this effect by intravenous injection of haemolysate containing kininase might suggest that urgocyton works through a bradykinin effect elicited by inhibition of local kininases.

The failure of carboxypeptidase *B* to imitate the inhibitory effect of haemolysate is surprising, but it may be explained by one of the following possibilities: (1) The effect of urgocyton on the capillary permeability is not due to the neutralization of kininase. (2) Carboxypeptidase *B* is not capable of penetrating the capillaries. (3) The haemolysate contains other kininase(s) than carboxypeptidase *B*.

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Reduced Diphosphopyridine Nucleotide-dependent Diaphorase in Foetal, Newborn and Adult Cattle

ABORTIONS in cattle have been reported to occur as a result of nitrate poisoning[1]. Although the exact cause of death has not been elucidated, anoxia due to methaemoglobinaemia is believed to be responsible. Recently a transient deficiency of reduced diphosphopyridine nucleotide (DPNH)-dependent diaphorase in infants has been demonstrated, and it has been suggested that this may be a major cause of the increased susceptibility of newly born babies and animals to acquired methaemoglobinaemia[2]. It was also suggested that even lower enzyme activity would be encountered in foetal erythrocytes[2]. The present investigation was undertaken to determine whether the abortions occurring in nitrate poisoning of cattle could be related to foetal erythrocyte diaphorase levels.

Samples were taken from Holstein-Fresian cattle during slaughter, from foetal calves by cardiac puncture and newborn animals by venipuncture into acid-citrate-dextrose (ACD) to which 0·25 per cent sodium chloride had been added to increase the osmotic strength to a level more nearly isotonic with erythrocytes. DPNH-dependent diaphorase was measured by the method of Scott[3] incorporating the more prolonged incubation with sodium nitrite recommended by Ross[2]. All assays were carried out in a Gilford model 2,000 multiple absorbance recorder at room temperature and only the initial reaction rates were considered. The unit of activity has been defined by Scott[3].

The results are summarized in Fig. 1. The mean erythrocyte diaphorase level of thirty-nine adult cows was $22{\cdot}0 \pm 4{\cdot}6$ units, while that of foetuses was $169 \pm 25{\cdot}8$ units, and of eight newborn animals was 177 ± 51 units.

It is apparent that there are marked differences in the levels of methaemoglobin diaphorase in the newborn calf and the newborn baby. Average enzyme activity of the newborn baby is significantly lower than that of the red cells of an adult human being[2]; that of the unborn and newborn calf is spectacularly higher than that of adult cattle. Nitrite is a normal intermediate in the metabolism of nitrates by the bacterial flora of the rumen of cattle[4]. It is known that bovine foetal haemoglobin is much more susceptible to oxidation to methaemoglobin by nitrite than is bovine adult haemoglobin[5]. For these reasons the bovine foetus is an especially high risk with regard to the development of serious methaemoglobinaemia. An increase in the DPNH-dependent methaemoglobin diaphorase may represent an adaptation on the part of cattle to these special circumstances.

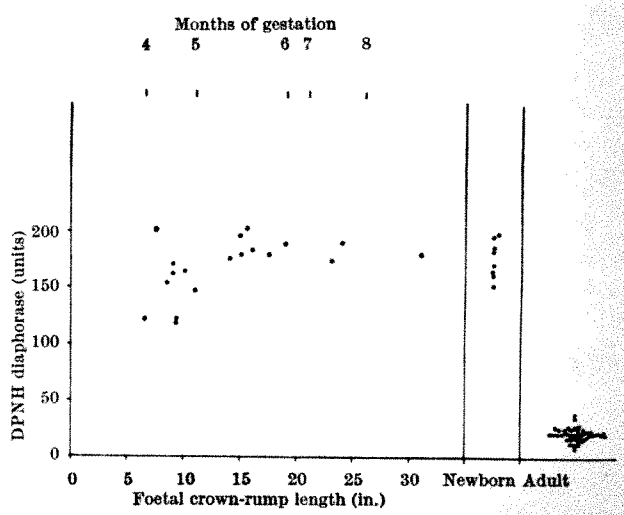


Fig. 1. DPNH-dependent diaphorase of foetal, newborn and adult cattle

It seems that the abortions resulting from nitrate poisoning in cattle are not related to a decrease in the level of DPNH-dependent diaphorase which is common to the entire species. However, we have not been able to examine individual foetuses, which were aborted as a result of nitrate poisoning, to rule out the possibility of a genetic polymorphism.

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BIOPHYSICS

Relationship of Ortho-cortex to the Crimp Wave in a Double-crimped Wool

THE cortex of a crimped Merino wool fibre comprises two hemi-cylinders, which differ in both chemical and physical properties. The form of the crimp wave is related to alternations in the positions of the two cortical components within the fibre—the ortho- and the para-cortex[1-4]. The ortho-cortex tends to lie on the convex aspect of the crimp wave and the para-cortex on the concave aspect.

The ortho-cortex can be demonstrated *inter alia* by dyeing with cupric ion in acidic nitrite solution[5].

Wool staples which have two clearly discernible staple crimp forms, one of short wave-length and the other of longer wave-length, occur in certain Merino wools from time to time. The question arises as to the alternations of the ortho- relative to the para-cortex, when the situation is complicated by the presence of two staple crimps.

An excellent example of this type of wool was received from the Western District of Victoria. The primary crimped form had 20–22 crimps/in. and the secondary had 6 crimps/in. The terms primary and secondary are used arbitrarily and purely for reference. A staple was dyed with cupric ions in acidic nitrite solution (1 per cent cupric sulphate in 1·5 per cent w/w aqueous sodium nitrite in 3 per cent acetic acid with a wool/liquor ratio of 1 : 120 or 1 : 150 for 10 min at 65–75° C). Ten fibres were withdrawn, of mean diameters ranging from 15μ to 27μ. These were thus removed from the staple environment and its forces, and cannot be assumed to have retained their intra-staple forms. However, the primary and secondary crimp forms were still clearly evident, and to allow examination by the microscope, the untensioned fibre was laid on a glass slide and a glass coverslip gently lowered on to it to hold the fibre in a single plane. The camera lucida image of the fibre with the position of the ortho-cortex inserted was traced by hand. Magnified lengths were correct, but the width was doubled for convenience in marking.

The alternations of the ortho-cortex showed two phases. In the first of these, the wave-length of the alternations was constant and in general was associated with a small-amplitude fibre crimp form. In the second, the wave-length was longer and variable and in general associated with abrupt bends in the fibre, which appeared to tally with the secondary crimp. Sometimes the abrupt form changed immediately back to alternations of the first phase; sometimes the abrupt form persisted for a much longer length, before the recovery to the first phase. Fig. 1 indicates two typical cases.

It therefore appears that whatever controls the regular alternation of the ortho-cortex may, by the interruption of its action, lead to the formation of the secondary type of crimp. The alternative may be that the periodic rate of alternation is constant, but the length-rate of growth shows spasmodic changes. Since the fibre axis of wool fibres tends to twist to and fro along its length with a period equal to the wave-length of the crimp form[6,7], it is possible that the abrupt bends may be related to peculiar effects of the axial twist. Unfortunately, measurement of axial twist is extremely difficult to carry out in wool fibres, because these are so sensitive to tensile and torsional forces and to relaxation of cohesive set, when the moisture content changes[7,8].

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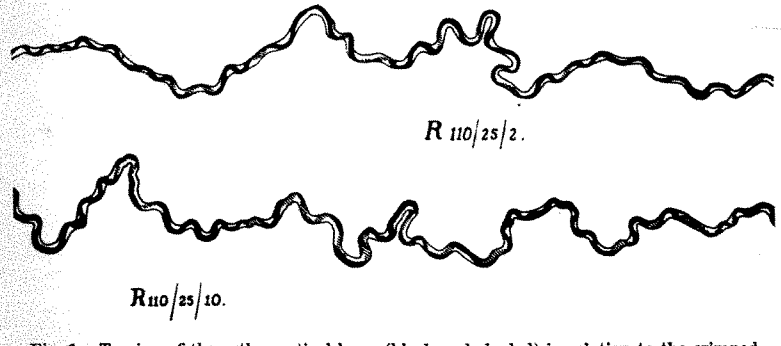


Fig. 1. Tracing of the ortho-cortical layer (black and shaded) in relation to the crimped form of two Merino wool fibres, withdrawn from a staple showing a short wave-length primary crimp and a longer wave-length secondary crimp. The relaxed fibre has been laid gently between two glass slides to maintain it in a single plane for microscopic examination

Crimp Form: a New Factor in Wool Science

WE have endeavoured to estimate the spatial configurations of single wool fibres by means of static and dynamic experiments in the decrimping region[1]. While some fleeces predominantly consist of helical shaped fibres, other fleeces are made up of almost perfectly sinusoidal fibres. Randomized assemblies prepared from the two extreme crimp types reveal strikingly different properties. One of the most outstanding results concerns the unique felting behaviour of wool fibres.

Under the influence of an external impressed force, assemblies of wool fibres in aqueous solution become highly entangled to form a dense mass or "felt". Although this property primarily arises from the unique surface structure of the fibre, no satisfactory explanation has been offered for the large variations that occur among different wools in their ability to felt. The factors to which these variations have most commonly been attributed include diameter, length and crimping. It can be demonstrated however, that these parameters are of little significance compared with crimp form.

Table 1

	Sample	Crimp configuration	Diameter of felt ball (cm)	Fibre diameter (μ)	Fibre length (cm)	No. of crimps (per cm)
A	Merino	Sinusoidal	2·15	22·1	11·4	4·5
B	Lincoln	Sinusoidal	2·24	40·0	20·3	0·35
C	South Down	Helix	3·42	30·0	12·8	3·6
D	Suffolk Down	Helix	3·74	30·4	12·0	4·4

Table 1 gives the various characteristics of four samples of wool, together with crimp form and felting results[2] (a small diameter for the felt ball indicates a high degree of felting). There are no marked differences in the frictional properties of the two sets of samples and it is clear that large variations in diameter, length and numbers of crimps have little influence on the felting results. The only consistent parameter is crimp form. Indeed, the felting of the helical fibres is so poor that individual fibres can be readily withdrawn from the large irregular balls (Fig. 1).

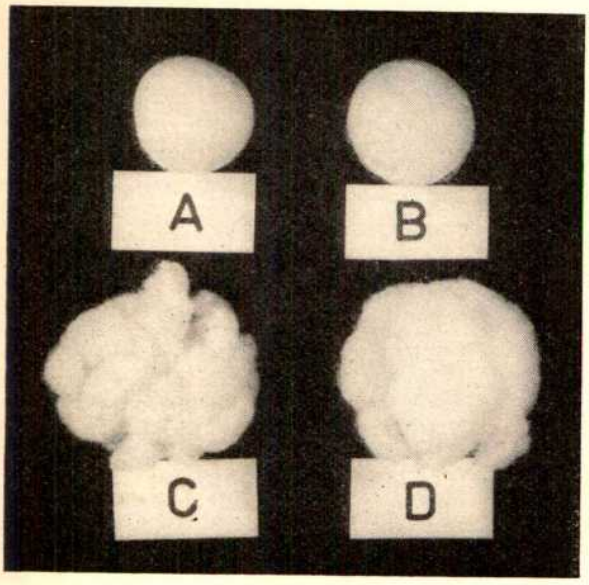


Fig. 1

The results cast considerable doubt on the traditional belief that fine fibres felt at a greater rate than coarse fibres. On the other hand, the results not only confirm some observations of Crewther and Dowling[3], who varied the rate of felting by modifying crimp form, but also offer a new explanation for the large variations in the felting properties of different wool types[4].

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BIOLOGY

Uncoupling of Auxin-induced Protein Synthesis and Water Uptake by Kinetin

IT is generally recognized that water uptake, synthesis of protein, and respiration are intimately related activities by which the growth of cells and tissues are controlled. Protein synthesis has been implicated in the mechanism of water uptake, and it has been shown that substances which inhibit protein synthesis also inhibit water uptake[1]. Evidence has also been presented that auxin-induced increases in growth and water uptake are not only accompanied by a net increase in protein content[2,3], but are also dependent on the continual synthesis of new protein[4]. Kinetin has been shown to inhibit increases in longitudinal growth[5] and in water uptake[6] caused by auxin in etiolated pea stem sections; however, it causes marked thickening of the sections. This report presents evidence to support the thesis that protein synthesis is even more intense during this kinetin inhibition of auxin-induced growth.

Pea (*Pisum sativum* var. 'Alderman') seedlings were grown in the dark for 8–9 days at 25° C, during which time they were subjected to various periods of red light. 10-mm sections cut from just below the "crook" region were used for the experiments and five such sections were made of each sample. The isolated sections were floated on 6 ml. of sucrose solution in Petri dishes with or without added auxin and/or kinetin, and were incubated in the dark for 24 h. The growth was assessed at regular intervals and the nitrogen content estimated by the routine micro-kjeldahl method[7].

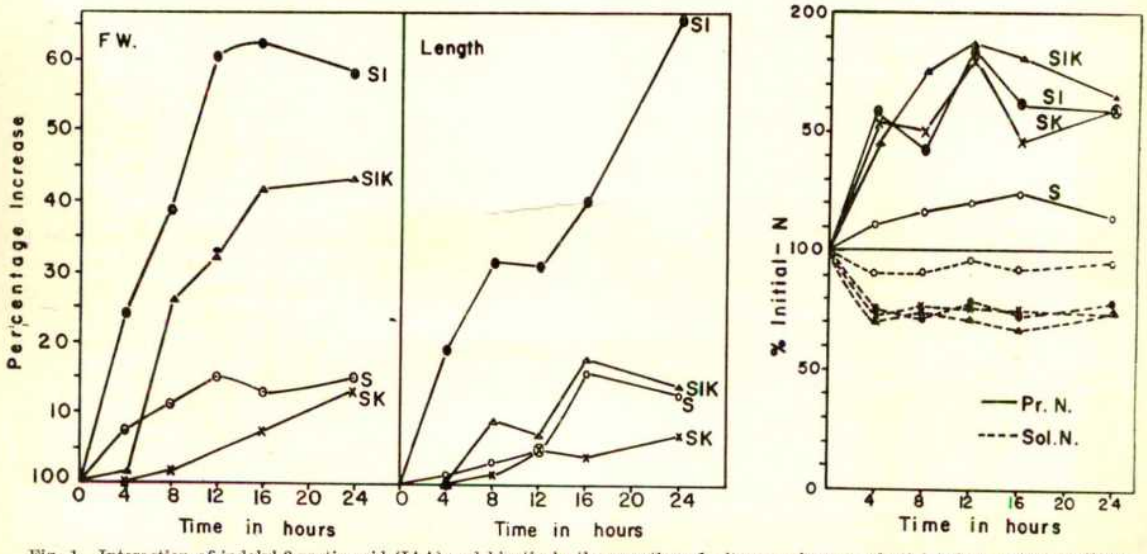


Fig. 1. Interaction of indolyl-3-acetic acid (IAA) and kinetin in the growth and nitrogen changes of etiolated pea stem sections. *S*, Sucrose 2 per cent; *SI*, 2 per cent sucrose + 1 p.p.m. IAA; *SK*, 2 per cent sucrose + 10 p.p.m. kinetin; *SIK*, 2 per cent sucrose + 1 p.p.m. IAA + 10 p.p.m. kinetin

Our findings confirm those of Katsumi[6] in that we observed that kinetin inhibits longitudinal growth and uptake of water in sections floated on sucrose alone and, furthermore, suppresses indolyl-3-acetic acid-promoted increases in length and green weight. The inhibitory effect on the former was found to be more marked than on the latter. Exogenous indolyl-3-acetic acid and kinetin, supplied individually, brought about almost identical changes in protein synthesis and further increase was noticed in sections which received both indolyl-3-acetic acid and kinetin. An optimum protein content was reached after about 12 h of growth in each case. Protein changes in indolyl-3-acetic acid-treated sections closely paralleled changes in longitudinal growth and green weight. It will be seen, however, that although the increase in length induced by indolyl-3-acetic acid was almost quantitatively reversed by kinetin and, moreover, the growth of control sections kept on sucrose was inhibited, the increase in protein content was far greater.

We conclude from these results that there may be no direct relationship between the observed enhancement of protein synthesis and the growth effects of auxin. Kinetin seems to act as an uncoupler of auxin-induced protein synthesis and water uptake. This suggests that auxin-dependent water uptake may be related to some metabolic activity other than to the synthesis of protein, but still associated with growth. The correlation usually made between protein synthesis and water uptake is most surprising. In theory, one molecule of water is released for each peptide bond formed during the synthesis of the protein molecule. Furthermore, coiling of the peptide chain would lock up various hydration sites[8] with the result that the osmotic concentration of the cell sap would decline. Indeed, there are examples on record which indicate that water uptake may occur in tissues showing a net decline in protein synthesis[9].

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Chromosome Number of a Sub-Antarctic *Rubus*

Rubus geoides from the southern hemisphere is a creeping form of bramble with simple, glossy leaves and hermaphrodite flowers. It occurs in Patagonia and in the Falkland Islands, where it is harvested locally for the red raspberry-like fruits. This species can be considered to be the southern equivalent of the northern hemisphere circumpolar species of creeping diploid *Rubus arcticus*. *R. geoides* was considered of possible interest for raspberry and blackberry breeding in Scotland because of its hardiness, lack of thorns, and its ability to produce fruits under windy and extreme climatic conditions. Plants raised from seeds received from Patagonia have grown well both indoors and outdoors in the east of Scotland, although its leaves are subject to mildew when kept in a poorly ventilated glasshouse and its flowering has been sparse.

The somatic chromosome number, previously unknown, has now been determined from root-tips of two plants[1]. Both these were tetraploid ($2n = 28$) and within the complement the contracted chromosomes vary slightly in size (Fig. 1). The complement includes only a single satellited chromosome, whereas in tetraploid species of the true blackberries (*Eubati*) from Scotland there may

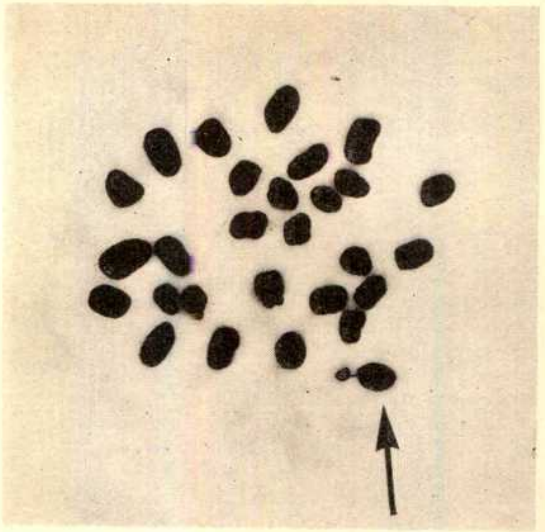


Fig. 1. Somatic chromosomes of *Rubus geoides* ($2n = 28$). (× 6,552). Note the occurrence of only one satellited chromosome (arrowed) in the tetraploid complement

be as many as five satellites present or two as in the diploid raspberry (*R. idaeus*)[3]. We have postulated[2] that in *Rubus* an increase in satellite number may have an adaptive function in relation to climate, especially in association with cooler growing conditions; but this does not appear to be so for *R. geoides*. Furthermore, the occurrence of only one satellited chromosome in a tetraploid species suggests amphiplasty, which has already been found by Heslop-Harrison[3] in *Rubus* and confirmed by Tun[4] for ribes species hybrids; thus *R. geoides* may be a true breeding species hybrid. In support of this, more flavonoid components are found to be present than in the two primary northern European diploid species of *Rubus*.

That *R. geoides* is a tetraploid has advantages for the breeder for it will probably readily hybridize through its diploid gametes. Furthermore, owing to wide geographical isolation from *R. idaeus* it is possible that such hybrids between these two species will show strong heterosis.

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Phosphatidyl Carnitine: a Possible Intermediate in the Biosynthesis of Phosphatidyl β-Methylcholine in *Phormia regina* (Meigen)

CARNITINE can replace the dietary choline, otherwise essential, when *Phormia regina* larvae are reared on a chemically defined diet[1,2]. When this substitution is made, β-methylcholine almost completely replaces choline in the lecithin of the larva[3,4]. Similar results have been obtained with the house fly, *Musca domestica* Linnaeus[5]. γ-Butyrobetaine can also substitute for choline in the diet[2], giving rise to phosphatidyl β-methylcholine[6]. Recent investigations by Hodgson and Dauterman[7] have demonstrated that trimethyl-(3-hydroxypropyl) ammonium acetate (TMAA) inhibits the growth of *P. regina* larvae when the

choline of the diet is replaced by carnitine or γ-butyrobetaine but does not when choline is present. Thus, it would appear to inhibit one of the metabolic reactions between carnitine or γ-butyrobetaine and the β-methylcholine moiety of phosphatidyl β-methylcholine.

The carnitine, labelled with carbon-14 in the methyl groups, used in the present investigation was synthesized according to the method of Mazzetti *et al.*[8], and the purity established by co-chromatography with authentic carnitine. The melting point of the synthesized material was 142° C. β-Methylcholine was prepared by the hydrolysis of acetyl β-methylcholine and recrystallization from *n*-butanol–ether.

P. regina larvae were grown in axenic culture on a chemically defined diet according to McGinnis *et al.*[9], as modified by Hodgson *et al.*[2]. In each experiment, carbon-14-D,L-carnitine was included in one set of flasks at a concentration of 0·2 mg/ml. of diet and carbon-14-D,L-carnitine and TMAA at concentrations of 0·2 and 0·17 mg in another. Third instar larvae were removed on the seventh day after inoculation, homogenized and extracted twice with 10 per cent trichloroacetic acid. The residue was extracted three times with chloroform–methanol (2 : 1, v/v). Trichloroacetic acid was removed from the water by washing with ether. Water-soluble contaminants were removed from the lipid layer by washing with 50 per cent methanol until no further radioactivity appeared in the upper layer. After hydrolysis in 6 N hydrochloric acid for 12 h at 110° C, β-methylcholine and carnitine were separated by paper chromatography and the radioactive spots detected by scanning in a 'Vanguard 4 pi' strip scanner. Spots were identified by co-chromatography in the following solvents: ethanol–ammonia, 95 : 5; *n*-butanol–water–ethanol–ammonia, 8 : 3 : 2 : 1; *n*-propanol–ammonia–water, 85 : 5 : 10.

Chromatograms of the hydrolysate of the whole lipid extract of larvae fed carbon-14-carnitine showed only one radioactive spot corresponding to β-methylcholine. This confirms the findings of Bieber *et al.*[3,4]. When carbon-14-carnitine and TMAA are fed, however, two radioactive compounds, β-methylcholine and carnitine, can be found in the lipid hydrolysate (Fig. 1).

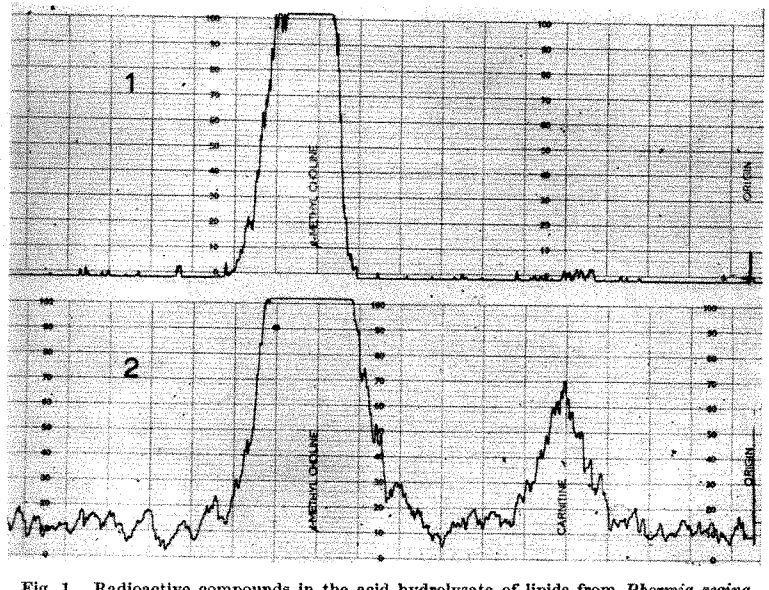


Fig. 1. Radioactive compounds in the acid hydrolysate of lipids from *Phormia regina* larvae fed methyl-carbon-14-carnitine. Hydrolysis products were separated by ascending paper chromatography in ethanol–ammonia and radioactive areas located by scanning in a 'Vanguard 4 pi' strip scanner. 1 is derived from larvae fed methyl-carbon-14-carnitine and 2 from larvae fed methyl-(3-hydroxypropyl) ammonium acetate

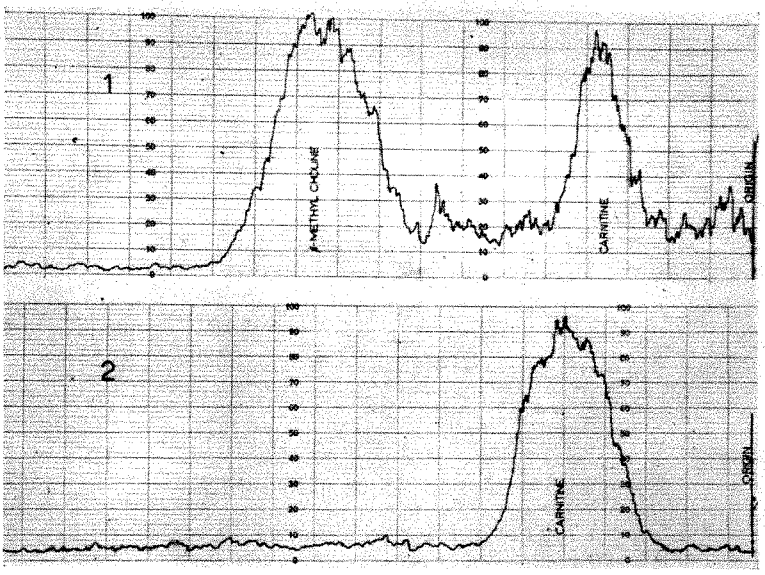


Fig. 2. Radioactive compounds in the acid hydrolysates of phospholipids from *Phormia regina* larvae fed methyl-carbon-14-carnitine and trimethyl-(3-hydroxypropyl) ammonium acetate. Hydrolysis products were separated by ascending paper chromatography in ethanol–ammonia and radioactive areas located by scanning in a 'Vanguard 4 pi' strip scanner. 1 is derived from the first radioactive peak from a silicic acid–'Hyflo Super-Cel' column. 2 is derived from the phospholipid eluted from DEAE-cellulose columns with chloroform–methanol (7 : 1) when the first peak from silicic acid–'Hyflo Super-Cel' columns is rechromatographed

Phospholipids were separated on silicic acid–'Hyflo Super-Cel' columns by methods previously used with this organism[10]. Three radioactive peaks were eluted from the lipids of larvae fed carbon-14-carnitine and TMAA, the first and largest peak containing, on hydrolysis, both carnitine and β-methylcholine while the last two contained only β-methylcholine. The first peak was rechromatographed on DEAE-cellulose according to the method of Rouser *et al.*[11], and a peak, eluted with chloroform–methanol (7 : 1, v/v), proved, on hydrolysis, to contain only carnitine (Fig. 2).

Similar experiments using carbon-14-carnitine without TMAA yielded β-methylcholine as the sole radioactive hydrolysis product in all three peaks separated on silicic acid–'Hyflo Super-Cel' columns.

The water layers, both with and without the inhibitor, were identical, four radioactive peaks being separated by paper chromatography. The hydrolysed water layer showed two radioactive compounds, carnitine and β-methylcholine, in a ratio of approximately 95 to 5.

The results reported here indicate that carnitine is incorporated into a lipid soluble compound before decarboxylation to the β-methylcholine of phosphatidyl β-methylcholine. The available evidence supports the hypothesis that this compound is phosphatidyl carnitine. The inhibitor, TMAA, previously implicated[6] on nutritional grounds in the decarboxylation of carnitine, causes this lipid soluble carnitine derivative to accumulate to the point where detection is possible.

The suggestion was considered that contamination of phospholipid fractions with an acyl carnitine had occurred. Palmitylcarnitine synthesized by the method of Bremer[12] was compared by thin-layer chromatography with the lipid extract obtained after feeding carbon-14-carnitine and TMAA

and was shown not to correspond to any of the radioactive spots. Short chain acyl carnitines would not be expected to contaminate washed lipid extracts.

The observation that TMAA is incorporated into a phospholipid may be of importance in elucidation of the mechanism of its inhibitory action. Thin-layer chromatography on silica gel *G* using chloroform–methanol–acetic acid–water (25 : 15 : 4 : 2) indicates that several of the unusual phospholipids formed when dietary choline is replaced by other compounds can be separated from each other. Phosphatidylcholine, phosphatidyl β-methylcholine and phosphatidyldimethylaminoethanol have R_F values of 0·33, 0·47 and 0·67, respectively. The lipid formed from TMAA has an R_F of 0·19. Furthermore, all these compounds can be clearly separated from each other after being mixed together. In addition to demonstrating that TMAA is incorporated into a lipid soluble derivative, this supports the observation of Bieber and Newburgh[13] that dimethylaminoethanol is incorporated into the phospholipid fraction and is not methylated to form phosphatidylcholine after incorporation.

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MICROBIOLOGY

Recoveries of Bacteria after Drying *in vacuo* at a Bath Temperature of 100° C

FOR the purposes of preservation, micro-organisms may be dried either from frozen or liquid suspensions and the recoveries are governed by a number of factors, in particular by the nature of the suspending medium[1]. So far, bath temperatures of no higher than about 30° C appear to have been used to maintain suspensions in the liquid state during drying *in vacuo*.

Recent investigations of the resistance of bacteria to heat in the dried state[2,3] prompted an attempt to determine what fraction, if any, of bacteria would survive the actual drying process when it was carried out *in vacuo* and when the ampoules were held at a bath temperature of 100° C. Under these conditions, drying could be expected to proceed very rapidly.

Suspensions of the test organism, *Salmonella ndolo* (N.C.T.C. 8700), were prepared in 20 per cent sodium glutamate[2] and 0·1 ml. volumes of it were dispensed into ampoules (Edwards, 36/*H*/0800). The ampoules were connected to a horizontal manifold[4] charged with phosphorus pentoxide and the assembly was connected to a 'Speedivac *SSC*20' vacuum pump. Three drying treatments were studied; the pump was switched on (*A*) 5 sec before the ampoules were immersed in boiling water, (*B*) at the same time as the immersion, or (*C*) 5 sec after immersion. As the suspensions were subjected to reduced pressure and heat, vigorous spluttering occurred and the suspensions dried as foamy flakes on the walls of the ampoules. After 30 min, the bath was removed, the ampoules were constructed, replaced on the manifold and exposed for a further 30 min to 100° C at high vacuum, after which they were sealed *in vacuo*. The heat resistance of the sealed desiccates was tested by total immersion of the ampoules in a boiling water bath. Using "slow" rehydration procedures[3], viable counts on the desiccates were made immediately after the ampoules were sealed and at intervals during the secondary heating.

The only treatment of the three studied (Table 1) which resulted in heavy loss was treatment *C*, in which the ampoules were heated for 5 sec before evaporative cooling *in vacuo* began. With the other two treatments, where evaporative cooling began at the same time or 5 sec before the suspensions were exposed to high temperature, appreciable recoveries (5–16 per cent) of the organism were obtained. The recoveries from treatments *A* and *B* after heating the sealed ampoules at 100° C also indicate that they would yield desiccates with long storage life at ambient temperatures[3].

Table 1. RECOVERIES (LOG$_{10}$) OF *Salmonella ndelo* AFTER DRYING *in vacuo* AT A BATH TEMPERATURE OF 100° C AND AFTER FURTHER EXPOSURE OF THE SEALED DESICCATES TO 100° C

Drying treatment (see text)	Recoveries ($\log_{10}$) Immediately after ampoules were sealed	After exposure to 100° C for: 24 h	48 h
A	8·9 (16 per cent)	5·6	2·9
B	8·4 (5 per cent)	4·8	1·0
C	5·8	< 1	< 1

Number of organisms dried ($\log_{10}$) = 9·7.

No attempt has been made to measure temperature changes during the brief drying periods in these experiments. The cooling during drying *in vacuo* will be markedly modified by the heat input from the bath and the resulting temperatures could be expected to fluctuate violently.

Working at much lower temperatures, and in more slowly evaporating systems, other workers[1] found specimen temperatures within evacuated ampoules to be up to 60° C below the temperature of the bath surrounding the ampoules.

Whereas it is firmly established that glutamate protects organisms during heating in the dried state, there is no evidence from this or previous work that it does so during the early stages of drying. It is, of course, difficult to examine the two stages separately. No suggestion is made here that any of the treatments described should be seriously considered for preserving organisms, but the results do indicate that higher bath temperatures than have hitherto been contemplated could be used for drying some micro-organisms with a reasonable expectation of survival.

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Fragile Cultures of *Escherichia coli* produced by Growth in High Concentrations of Various Salts

SUCROSE-DEPENDENT mutants have been reported which permit total isolation under mild conditions of cellular ribonucleic acid and proteins from actively growing cultures[1]. The considerable time and effort required to isolate equivalent mutants in strains of other genetic provenance had led us to search for a general method to

produce fragile growing cultures. This communication reports such a technique: growth in 0·5 M sodium sulphate or comparable concentrations of other salts.

Escherichia coli strain 3000 is cultivated at 37° C in a minimal medium composed (per l.) of potassium monohydrogen phosphate, 1·12 g; potassium dihydrogen phosphate, 0·48 g; ammonium sulphate, 0·5 g; $MgSO_4.7H_2O$, 0·49 g; $FeCl_3.5H_2O$, 0·5 mg; glucose, 2 g. The addition of increments of sodium sulphate to a series of flasks produces the altered growth curves shown in Fig. 1. At 0·5 M sodium sulphate (or 1 M potassium chloride or 0·5 M potassium sulphate), growth continues for several hours, then stops. During this period, cells become increasingly fragile up to a maximum of about 70 per cent of total fragility. Fragility has been measured by treating cells with 0·5 per cent sodium deoxycholate and measuring the decrease in the percentage of scattering at 420 mμ, the release of induced β-galactosidase, or the release of RNA, detected as [^{14}C]-uracil incorporated in acid-insoluble form (Fig. 2). The most sensitive index of the degree of total lysis is RNA release, as is also found with a sucrose-dependent mutant grown in fragile form[1].

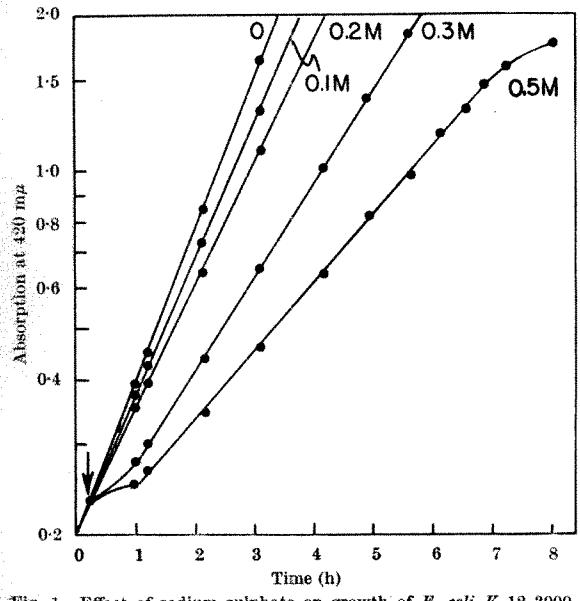


Fig. 1. Effect of sodium sulphate on growth of *E. coli K* 12 3000 in minimal medium at 37° C. At the point indicated by the arrow, the indicated levels of sodium sulphate were added to a series of cultures

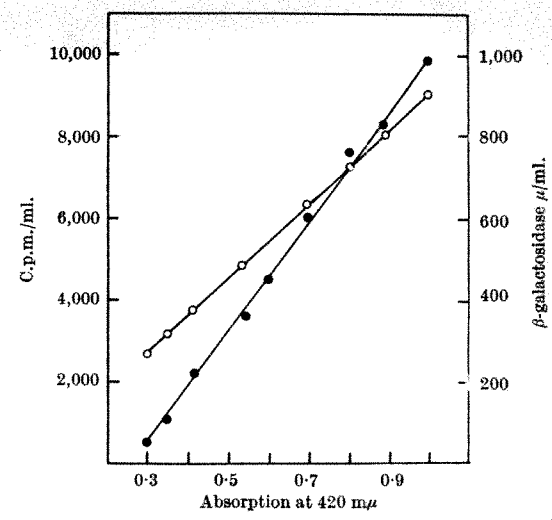


Fig. 3. β-galactosidase and RNA formation in fragile cultures. *E. coli K* 12 3000 was grown in minimal medium containing 0·5 M sodium sulphate and penicillin 15 U/ml., and 4 × 10^{-4} M IPTG. [^{14}C]-uracil (40 μg/ml., 20 μc./mg) was added at an optical density of 0·25. At intervals, samples were tested for β-galactosidase activity (— ○ —) and [^{14}C]-uracil incorporated into acid-insoluble RNA (— ● —). Each is formed in proportion to growth (measured as absorption of the culture at 420 mμ)

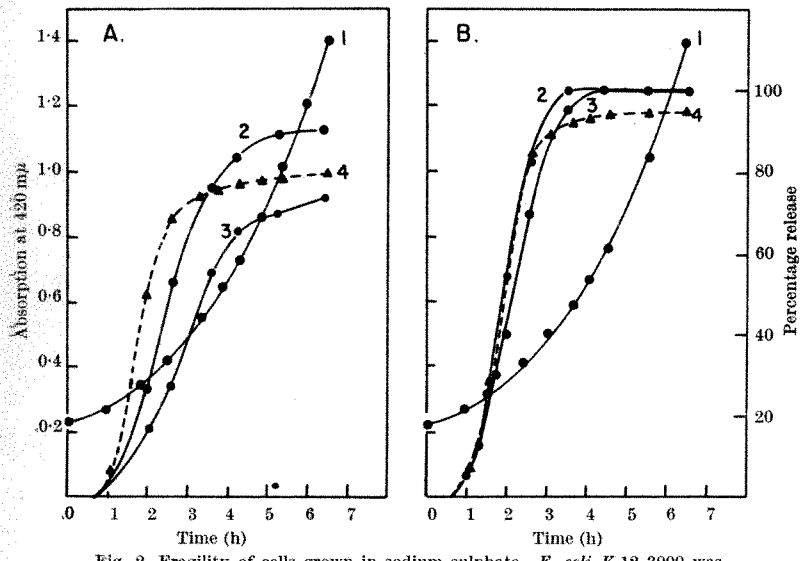


Fig. 2. Fragility of cells grown in sodium sulphate. *E. coli K* 12 3000 was grown in minimal medium containing 0·5 M sodium sulphate, 4 × 10^{-4} M isopropyl-β,D-thiogalactoside (IPTG) to induce the formation of β-galactosidase, [^{14}C]-uracil (0·02 μc./ml.), to label the RNA, and in presence of 15 U/ml. penicillin (*B*) or in its absence (*A*)
At intervals samples were centrifuged and cells were suspended in 0·5 per cent sodium deoxycholate in order to lyse them. The suspension was then tested for absorption at 420 mμ to see any reduction in scattering. Total β-galactosidase activity was also measured on toluenized cells with *o*-nitrophenol-β,D-galactoside as substrate (ref. 5). Total RNA was determined as [^{14}C]-uracil incorporated in acid-insoluble form. A portion of the same suspension was then centrifuged to remove unlysed cells and debris and the supernatant tested for β-galactosidase activity and RNA content to determine the percentage which had been released from the cells. Curve 1, absorption of the culture at 420 mμ; then, after lysis; curve 2, percentage of release of β-galactosidase activity; curve 3, percentage of release of [^{14}C]-labelled acid insoluble RNA; curve 4, percentage of decrease of optical density of cells at 420 mμ

Very similar results have been obtained with eleven *E. coli* strains and various media, although the optimum range of sodium sulphate concentration varies slightly in different cases. By contrast, *Bacillus megaterium KM* in comparable conditions goes through a fragile, spherical, non-growing stage (protoplast ?) and then lyses; the extent of the applicability of the technique to other types of bacteria is therefore not clear.

The degree of fragility can be increased to 100 per cent by the addition of 15 units per ml. of recrystallized penicillin *G* (1,500 U/mg)—a concentration which does not affect the growth of the cultures (Fig. 2) and has no detectable effect on cultures of the same strains growing in the absence of sodium sulphate.

While the synthesis of RNA or induced β-galactosidase in treated strains proceeds in proportion to growth (Fig. 3)—as does the formation of constitutive alkaline phosphatase, total protein and DNA (Fig. 4 and unpublished results)—the number of those forming colonies increases more and more slowly. The cause of this apparent discrepancy becomes obvious on observation with a microscope: high concentration of salt permits growth and the initiation of cell division, but the daughter cells sometimes remain attached in a pseudo-streptobacillar form.

Many chains break apart during handling, and all can be shaken apart (Table 1) without lysing more than 10–20 per cent

Table 1. FORMATION OF CHAINS OF VIABLE CELLS IN CULTURES GROWING IN 0·5 M SODIUM SULPHATE

Sample	Absorption at 420 mμ	Number of chains of $n = 1$ cells $n=1$	2	3	4	5	6	7	Total cells/ml. × 10^{-6}	Chains/ml. × 10^{-6}	Average No. cells/chain	Colony formers/ml. × 10^{-6}	Colony formers/cell
1	0·225	35	6						47	41	1·15	49	1·04
2	0·340	30	21						72	51	1·41	53	0·72
3	0·640	25	40	5		5			145	75	1·86	77	0·55
4	1·800	40	44	22	21	12			338	139	2·44	139	0·41
5	2·260	43	50	42	22	20	3	3	496	183	2·78	190	0·37
4*	–	217	49						315	266	1·18	310	0·98
5†	–	258	72						402	330	1·22	305	0·92

To a culture of *E. coli K* 12 3000 growing at 37° C in minimal medium, 0·5 M sodium sulphate was added at an absorption of 420 mμ of 0·210; cells continued to grow exponentially (see Fig. 1). At intervals, samples were diluted and fixed with 1 per cent formaldehyde, and total cells and number of chains containing n cells counted in a Petroff-Hausser bacterial counter. Duplicate samples were plated on rich medium to measure colony formers per ml.

* Sample 4 after 1 min agitation on a vibrator.

† Sample 5 after 1 min agitation on a vibrator.

of the cells. Nevertheless, progressively larger chains can be observed with time in carefully diluted samples; Table 1 indicates the appearance of chains noted in a typical experiment (that of Fig. 2). Because growth is rapidly arrested after two or three generations of fragile growth, it can be questioned whether the growth of treated cultures is ever truly "exponential". For many investigations, the distinction between slow exponential growth and linear growth is unimportant. Nevertheless, we have verified an exponential increase in total mass (Fig. 1.) and in the rate of uptake of [^{14}C]-leucine and [^{14}C]-uracil during growth of the cultures in sodium sulphate (unpublished results); in some experiments (Fig. 4) we have shown that, at any time during growth, induction of the internal enzyme β-galactosidase proceeds normally, producing an initial rate of enzyme synthesis which increases with growth as expected for a culture in the exponential phase. Likewise, the enzyme alkaline phosphatase, which is normally found at the cell surface[2], continues to be made, but is not released from cells; that is, very little leakage from or spontaneous lysis of these fragile cells occurs. We have confirmed the continued exponential synthesis of this "surface" enzyme, using the strain *S* 26 *C* 200 lacking alkaline phosphatase grown in sodium sulphate. This strain makes active enzyme constitutively in the presence of 5-fluorouracil[3]. Addition of 5-fluorouracil to fragile cultures at any time during growth therefore leads to the appearance of a burst of active enzyme, which is comparable in cultures growing in presence or absence of sodium sulphate (Fig. 4 compared with results of Rosen)[3].

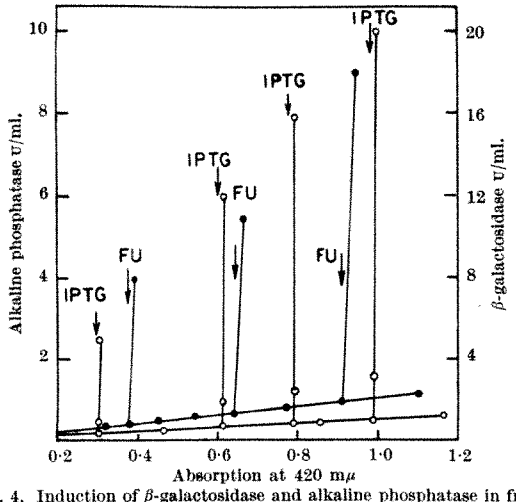


Fig. 4. Induction of β-galactosidase and alkaline phosphatase in fragile cultures. For β-galactosidase: *E. coli K* 12 3000 was grown at 37° C in minimal medium containing 0·5 M sodium sulphate and 15 U/ml. penicillin. At intervals, as indicated by the arrows, 4 ml. samples were transferred in 50 ml. flasks and 4 × 10^{-4} M IPTG was added. Immediately before the addition of IPTG and 2 min and 5 min afterwards, 1 ml. samples were chilled with frozen 20 per cent sucrose solution to stop further enzyme induction. Cells were centrifuged and resuspended in 0·5 ml. β-galactosidase activity was then determined on the toluenized cells (—○—). For alkaline phosphatase: *E. coli S* 26 *C* 200 was grown at 37° C in salts-glucose medium in presence of 0·5 M sodium sulphate and 15 U/ml. penicillin. At intervals, 3 ml. samples were transferred to 50 ml. flasks and incubated for 15 min in presence of 5-fluorouracil (2·5 μg/ml.) and thymidine (25 μg/ml.). Cultures were chilled in ice, and cells centrifuged and resuspended in 10 mM *tris*, *p*H 8·0. Alkaline phosphatase was then measured on toluenized cells using *p*-nitrophenylphosphate as substrate (ref. 6) (—●—)

In other studies we have noted that despite the sharp inhibition of *T*2 phage adsorption by high concentrations of salt, fragile cultures remain sensitive to infection by the phage, and are not sensitive to the action of actinomycin *D*. We conclude that a variety of processes (phage adsorption, resistance to actinomycin *D*, nearly complete cell division, etc.) which require a fairly intact cell envelope continue normally in cultures growing in fragile form in sodium sulphate. (In contrast, these processes are not carried out by penicillin or lysozymeversene "spheroplasts" with damaged walls[4].) Nevertheless, the fragile cultures grown in sodium sulphate can be totally lysed reproducibly, easily, and essentially instantaneously.

This investigation was supported in part by the U.S. Public Health Service. One of us (G. M.) is on leave from Istituto di Chimica Biologica, University of Genoa.

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Inhibitory Effects of Other Antibiotics on Bacterial Morphologic Changes induced by Penicillin G

ALTHOUGH it has been suggested that tetracycline and chloramphenicol antagonize the antimicrobial activity of penicillin *in vitro* and *in vivo*[1–6], several investigators do not agree with these conclusions[7–9] and have suggested that variation in methods of assay and experimental conditions makes the interpretation of results very difficult. We carried out this work in order to investigate the problem using a different method from those used in previous experiments. Tests were, therefore, carried out to determine whether the addition of tetracyclines, chloramphenicol, and other antibiotics would prevent the formation of spheroplasts, a step which precedes the lysis of bacteria exposed to penicillin.

Three sets of paper disks containing antibiotics were prepared in the following manner: the disks, 7 mm in diameter, were cut from filter paper. One set was saturated with 0·3 ml. of a solution of penicillin *G* containing ten times the quantity of antibiotic that inhibited the growth of *Vibrio comma*. A second set was treated with a hundred times the minimal inhibitory concentration of the agents listed in Table 1. A third set of disks was prepared by first treating them with each of the drugs in Table 1. After drying, they were saturated with the same quantity of penicillin as was used in the first set. Heart infusion agar containing 5 per cent horse blood was inoculated by spreading 0·5 ml. of an 18-h broth culture of *V. comma* over the surface. After standing at room temperature for 1 h, the excess fluid was removed from the surface of the

Table 1. EFFECT OF VARIOUS ANTIBIOTICS ON MORPHOLOGICAL CHANGES IN *Vibrio comma* INDUCED BY PENICILLIN

Drugs inhibiting effect of penicillin	M.I.C.* for *V. comma*	Drugs producing no inhibition of penicillin	M.I.C.* for *V. comma*
Tetracycline	2	Bacitracin	50
Chlortetracycline	—	Vancomycin	25
Oxytetracycline	—	Cephalothin	2
Demethylchlortetracycline	—	Polymyxin	16
Chloramphenicol	2	Colistine	80
Erythromycin	12	Cycloserine	250
Oleandomycin	3·2		
Kanamycin	25		
Neomycin	3·2		
Paromomycin	3·2		
Streptomycin	500		

* M.I.C., minimal inhibitory concentration (μg/ml.). M.I.C. of penicillin for *V. comma*, 35 units/ml.

agar and antibiotic disks applied. In the first experiment, the drugs were studied individually; in the second, combinations of them were used. The cultures were incubated at 37° C and cover slip impression preparations made at 2, 6 and 18 h. These were dried, fixed in absolute methyl alcohol, stained by the Giemsa method, and examined microscopically.

Long filaments and a few spheroplasts were seen in impression smears of agar cultures of *V. comma* exposed to penicillin alone for 2 h (Fig. 1). These changes rapidly progressed so that after 3 h of incubation two morphologically distinct layers, an inner one made up almost entirely of spheroplasts and an outer one consisting primarily of filamentous forms, developed at the border of the zone of growth inhibition. Increase in size of the spheroplasts was observed after 18 h (Fig. 2). When *V. comma* was exposed to 100 times the minimal inhibitory concentration of either chloramphenicol or tetracycline alone, the bacteria enlarged to several times their initial size but were not converted to filaments or spheroplasts (Figs. 3 and 4). The effects of other antimicrobial compounds (Table 1) on the morphology of *V. comma* grown in the presence of each compound individually were not studied; only the minimal quantity required to suppress growth of the organism was determined.

Spheroplasts and filamentous forms of *V. comma* did not appear when the organism was exposed to combinations of penicillin with tetracycline or penicillin with chloramphenicol; in the presence of the drug mixtures bacterial cell size increased to the same extent as was

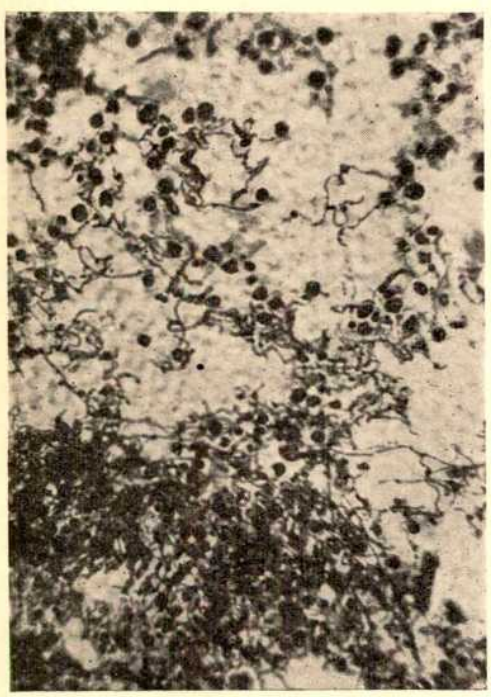


Fig. 1. *V. comma* after 2 h exposure to penicillin

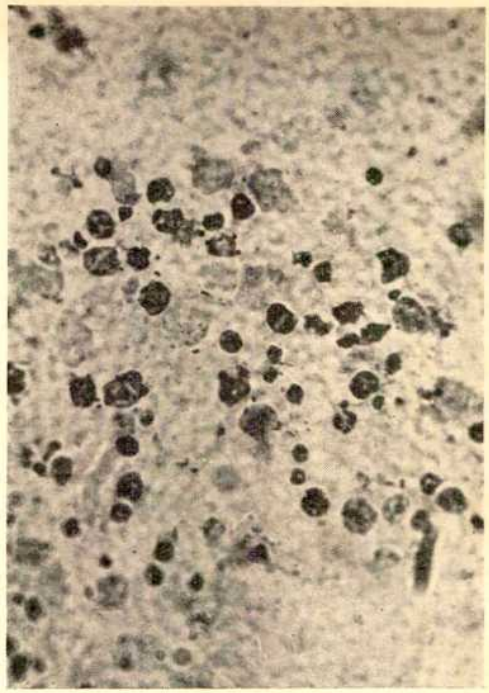


Fig. 2. *V. comma* after 18 h exposure to penicillin

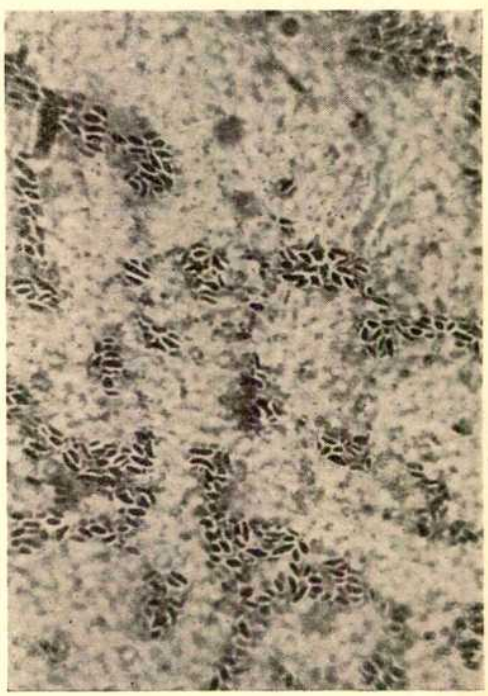


Fig. 3. *V. comma* after exposure to chloramphenicol or tetracycline alone and in combination with penicillin

observed when each of the "broad-spectrum" antibiotics was used alone (Fig. 3). Erythromycin, oleandomycin, kanamycin, neomycin, paromomycin, and streptomycin in combination with penicillin also completely inhibited the capacity of the latter to produce morphological changes. On the other hand, mixtures of bacitracin, *cyclo*serine, vancomycin, cephalothin, polymyxin, or colistin with penicillin induced morphological alterations in *V. comma* identical with those observed when penicillin alone was used.

The results of these investigations suggest that the bactericidal activity of penicillin *G* is suppressed by the

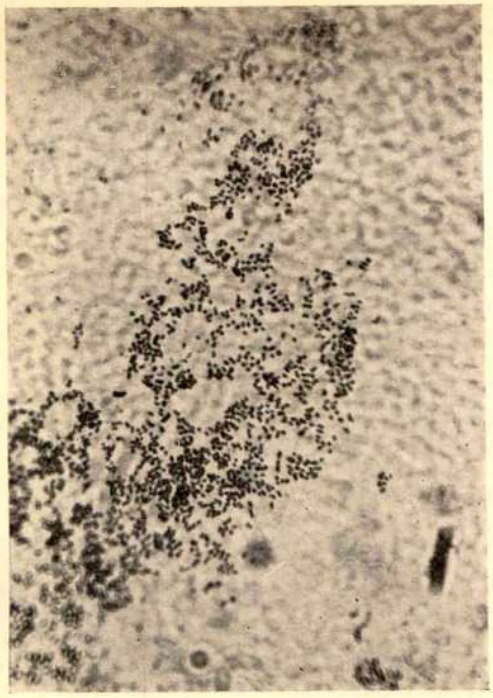


Fig. 4. *V. comma*, untreated

addition of certain classes of antibiotics but not by others. It appears that compounds which exert their antibacterial effects by interfering with protein synthesis suppress the induction of spheroplast and filament formation. Agents which act against organisms by interrupting the synthesis of the cell wall or by altering the cell membrane do not appear to interfere with the action of penicillin. These observations may cast some doubt on the efficacy of treatment of infected patients with combinations of tetracycline or chloramphenicol and penicillin—which are common forms of therapy. Furthermore, these findings may offer an explanation for the clinical failures that may result in cases where this type of antibiotic combination has been used to treat disease caused by penicillin-sensitive organisms. Removal of the "broad spectrum" agent has, in some cases, been found to result in prompt improvement and cure of the infection.

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Level of Vitamin B_{12} in Propionate-grown *Prototheca zopfii*

THE colourless alga *Prototheca zopfii* does not require vitamin B_{12} as a growth factor, whereas at least half the marine algal flagellates and diatoms which have been investigated in pure culture[1], the protozoan flagellate *Ochromonas malhamensis*[2], and *Euglena gracilis*[3], have all been shown to have a growth requirement for this compound.

It has previously been shown that *Prototheca* assimilates propionate by a route involving β-hydroxypropionate and malonic semialdehyde[4,5]. This pathway is presumably independent of vitamin B_{12}, whereas the succinyl-Co*A* pathway of propionate metabolism, present for example in *Ochromonas*[6], requires a coenzyme form of the vitamin which functions in the isomerization of methyl malonyl-Co*A* to succinyl-Co*A*. Data on the level of vitamin B_{12} in extracts of *Prototheca* grown in medium rich in propionate were obtained as further evidence that the proposed pathway of propionate assimilation is the only mechanism involved in growth on this carbon source.

The organism was grown on propionate–mineral salts–thiamine medium as previously described[4], and the cell concentration at the time of collecting was determined by counting in a haemocytometer cell. The cells were collected quantitatively and resuspended at a final population of 10^8 cells/ml. in acetate buffer (*p*H 4·6) containing one drop of 1 per cent (v/v) potassium cyanide solution per 5 ml. of buffer. After autoclaving at 10 lb./in.^{2} for 10 min, the suspension was passed twice through a French press; it was then frozen and thawed, and autoclaved once more. Microscopic examination revealed that almost all the cells had been disrupted. The presence of cyanide stabilizes any vitamin B_{12} present; the extracts were protected from strong light.

Microbiological assays were carried out by Dr. M. E. Gregory of the National Institute for Research in Dairying, Shinfield, Reading. The assay using *Ochromonas* as a test organism failed to detect any vitamin B_{12} activity. The more sensitive *Lactobacillus leichmannii* assay revealed that the extract contained 0·08 mμg of vitamin B_{12}/ml., but the possibility arises with this organism that it is deoxyriboside activity which is measured rather than true vitamin B_{12}. *Euglena gracilis* was finally used as the test organism. The extract was centrifuged and the clear supernatant was used undiluted and diluted ten-fold. The undiluted extract gave no growth, and from this it can be concluded that cyanide or some other substance was present at an inhibitory level. In the case of the diluted extract it was found that growth increased with increasing addition to the assay cultures and the results calculated at two different levels were similar. The value obtained, 0·40 mμg/ml., can be regarded as a measure of true vitamin B_{12} activity.

Droop[1] has calculated that in an alga which requires vitamin B_{12} for metabolic processes the level will correspond to about three molecules of vitamin/μ^3; Hutner *et al.*[3] have reached a similar conclusion.

Assuming a molecular weight of 1,357 for the vitamin it can be shown that the level of the compound in *Prototheca* corresponds to only 200 molecules/cell. However, if Droop's calculations are applicable to any algal species, *Prototheca* (mean volume 1,440 μ^3) would contain about 4,300 molecules/cell and it is evident that the level of vitamin B_{12} in propionate-grown *Prototheca* is some twenty times lower than would be expected if the cells required the vitamin for their metabolism.

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IMMUNOLOGY

Lack of Natural Tolerance to Allotypic γ-Globulins in Rabbits

THE induction of immunological tolerance to soluble proteins can be achieved in rabbits by introducing a suitable dose into the foetus shortly before, or the infant shortly after, birth[1]. Yet in the case of the genetically determined IgG allotypes[2,3] in rabbits, neither tolerance nor sensitization has been recorded, although the maternal IgG, which comprises virtually all the plasma IgG of the offspring at birth, being received by way of imbibition of amniotic fluid[4] can contain an allotypic determinant not represented in the offspring's genotype[5-7] and against which the offspring can afterwards be immunized. The purpose of this communication is to document this lack of tolerance, although we cannot at present provide any explanation for it.

It might be mentioned that ostensibly the allotype situation should be well suited to the demonstration of tolerance, as the dose received by the infant is prenatal and relatively large, while the antigen (as judged by "normal" rather than adjuvant assisted immunization) is not strongly immunogenic (see later).

These results arose during the course of nearly a hundred routine immunizations of rabbits for the production of anti-allotypic sera; the fifty-two recorded were in animals of accurately known parentage.

The great majority of the rabbits were immunized by injection of *Proteus vulgaris* X19 coated with rabbit anti-*Proteus* antibody of the appropriate allotype[8]. In a few cases, antigen-antibody precipitates in complete Freund's adjuvant were used[2]. Both methods were satisfactory; the first was preferred partly as being very economical of antiserum IgG, partly to avoid the complications of antibody formation to antigens present in the complex (bovine seralbumin, ovalbumin, etc.), although, of course, antibacterial antibodies against *P. vulgaris* antigens regularly appeared. A single course of immunization consisted of five bi-weekly intravenous injections (in total 3×10^{10} organisms) coated with a maximum of 2 mg of anti-*Proteus* antibody. Two or three such courses with monthly rests between were sometimes necessary. The animals were bled one week after the last injection.

In all cases (about a hundred) such immunizations resulted in anti-allotypic antibody production, though sometimes three courses were necessary for satisfactory antisera.

In four cases, intravenous iso-immunizations of up to fifteen injections with normal rabbit IgG or whole serum were used without any evidence of antibody production; all these animals later produced antibody to the allotypic determinant originally used when immunized by the *P. vulgaris* method. The success of the *P. vulgaris* method may depend on an adjuvant effect excited by the endotoxin of the bacteria, combined with an advantageous cellular localization.

Of the six well-defined allotypic determinants those of the **b** locus were strongly immunogenic by this method, As4 being rather better than As5 and As6: the **a** locus determinants were more weakly so in the order As1 > As2 > As3. At least three courses were needed to raise a satisfactory anti-As2 or anti-As3. This gradient in immunogenicity is relevant in considering the tolerance situation.

Table 2. ANTI-ALLOTYPIC IMMUNIZATION IN WHICH MATERNALLY INDUCED TOLERANCE MIGHT HAVE OCCURRED

Rabbit No.	Mother's allotype	Offspring's allotype	Age at the time of first injection (in weeks)	Anti-allotypic antibody produced
1	As1,3/4,5	As3/4	12	anti-As1
2	As1,3/4	As3/4	13	
3	As1,2/4	As2/4	33	
4	As1,3/4,5	As3/4	45	
5	As2,3/4	As2/4	22	anti-As3
6	As2,3/4,5	As2,3/5	22	anti-As4
7	As1/4,6	As1/6	28	
8	As3/4,5	As3/5	90	
9	As3/4,5	As1,3/4	10	anti-As5
10	As1,3/5,6	As3/6	15	
11	As1/4,6	As1/4	15	anti-As6
12	As1/4,6	As1,3/4	40	

The results of the immunization of fifty-two rabbits of known parentage are given in Table 1. The figures in bold type indicate in twelve of these the presence of a maternal allotype which might have been expected to be tolerogenic. Table 2 gives further details of these twelve rabbits, in particular the age at which immunization started.

Results of other investigations[1,9] suggest that the duration of neonatally induced tolerance is dose-dependent. Recent results[9] indicate that the half-life of human IgG in the infant rabbit is 13 days: that of homologous IgG derived from the mother *in vitro* is not likely to be less than this. This figure is also consistent with the figures given elsewhere[10] in the homologous situation if allowance is made for increase of weight, and with earlier results[9] with rabbit IgG in infant rabbits during the first few weeks of life. If one can estimate the dose of maternal IgG received at birth, therefore, the amount of "antigens" remaining at any time thereafter, and available for the maintenance of tolerance, can be calculated.

The IgG content of the plasma of a newborn rabbit is at least one-third of the adult[10] (all being maternally derived), that is about 2 mg/ml. If the plasma volume is 5 ml. a birth dose of at least 10 mg is likely, especially as the animal's tissues and gut are saturated with the proteins of the amniotic fluid. Of this, only about half will be "tolerogenic" as in the nature of the experiment the mother must be heterozygous for the allotype concerned. The data[9] on human IgG, human and bovine seralbumin show that doses of between 2·5 mg and 0·1 mg received by the infants transplacentally were sufficient to establish tolerance which persisted in over two-thirds of cases, until the theoretical number of molecules of antigen remaining had fallen to a hundred or less. Moreover, when tolerance did break, after 6 months or more, the antibodies produced were of restricted specificity and usually non-precipitating. With a dose of 3 mg (about 10^{16} molecules) of heteroallotypic IgG in the situation under investigation, there would still be about 10^{11} molecules after 20 weeks and 10^5 after 40 weeks (as calculated from Table 2 in ref. 9). One would expect, therefore, that nearly all the immunizations recorded in our Table 2 would have been ineffective owing to tolerance, if the autologous antigen were of comparable efficiency with the heterologous.

Table 1. ANTI-ALLOTYPIC IMMUNIZATION OF RABBITS OF KNOWN PARENTAGE

Anti-As1		Anti-As2		Anti-As3		Anti-As4		Anti-As5		Anti-As6	
No. of rabbits	Mother's allotype	No. of rabbits	Mother's allotype	No. of rabbits	Mother's allotype	No. of rabbits	Mother's allotype	No. of rabbits	Mother's allotype	No. of rabbits	Mother's allotype
1	**As1,2/4**	6	As1/4	**1**	**As2,3/4**	**1**	**As1/4,6**	**1**	**As1,3/5,6**	**2**	**As1/4,6**
1	**As1,3/4**	4	As3/4	7	As1/4	**1**	**As3/4,5**	**1**	**As3/4,5**	1	As1/4
2	**As1,3/4,5**	1	As3/5	1	As1,2/4	**1**	**As2,3/4,5**	1	As1/4	3	As3/4,5
3	As3/4,5					1	As1,3/6	2	As1,3/4		
						3	As1,3/5,6	2	As1,3/6		
						2	As3/5,6	1	As1/4,6		
								1	As2,3/4		
								1	As1,3/4,6		
Total	7		11		9		9		10		6

In all cases satisfactory anti-allotypic antisera were produced. Those in bold type indicate the twelve cases where the immunization was against an allotypic determinant present in the mother.

In all the anti-allotypic immunizations recorded there was nothing to choose, so far as the strength and reactivity of the sera went, between those in which some measure of tolerance might have been expected owing to maternal transfer, and those which it would not. It is possible that the endotoxin contained in *Proteus vulgaris X*19 might act to break tolerance when this method of immunization is used; but if tolerance had ever been established one would still expect some suppressive effect with immunizations started at 10–15 weeks. Because isologous IgG is so poorly antigenic by non-adjuvant-assisted immunization even in an animal where tolerance could not have been induced maternally, it is difficult to decide whether in fact it is possible for any degree of tolerance whatever to be induced "naturally" by placental transfer. Experiments to throw light on this situation are in progress.

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Cellular Localization of Carbon-14 Endotoxin in Cultured Leucocytes

IT is well known that the administration of endotoxin results in a very rapid increase in size of the reticulo-endothelial system (RES)[1]. The RES is enhanced not only quantitatively but also qualitatively, in that radioactively labelled endotoxin is more rapidly removed by the RES of animals which have previously received endotoxin[2]. Tritiated endotoxin administered subcutaneously is found initially in the intraendothelial cells of the lymphoid channel, and the sinusoidal cells of the draining lymph node. By 24 h the large mononuclear cells, infiltrating the area, contain the labelled material[3]. In a similar fashion, labelled antigens (such as non-endotoxin) which are administered subcutaneously localize in the macrophages of the medullary sinuses and the lymphoid follicles of the draining lymph nodes[4]. They are also more rapidly and avidly taken up by immune and immunologically tolerant animals[5].

It has been reported that labelled endotoxin is found mainly in the buffy coat platelets, only 4–8 per cent of the granulocytes, even fewer monocytes, and not at all in small lymphocytes or the red blood cells[6,7]. A recent report from this laboratory demonstrated that endotoxins stimulate lymphocyte proliferation *in vitro* and that these effects were related to the antigenicity of the endotoxins used[8]. In the present investigations, labelled endotoxin was found in the macrophages of leucocyte cultures which manifested concomitant lymphocyte proliferation.

The peripheral leucocytes from four healthy young adult human beings were obtained and prepared by methods previously described[8]. Their cell suspensions in tissue culture medium were divided into 12 ml. portions. To the first portion 0·5 ml. of isotonic saline was added, to the second 2 μg/ml. *Salmonella enteritidis* endotoxin, and to the third portion 2 μg/ml. of the same *S. enteritidis* endotoxin biosynthetically labelled with carbon-14 (specific activity 11·2 mc./mg, both kindly supplied by Dr. Edgar Ribi). The cell suspensions were then left undisturbed for 5 days at 37° C.

Four hours before collection, polystyrene particles (0·2 ml., 1 : 100 saline dilution, 1·3μ diameter, Dow Chemical Co., Midland, Michigan) and 0·33 μg/ml. 'Colcemid' (CIBA Pharmaceutical Co., Summit, N.J.) were added to each culture. The particles are often phagocytized by macrophages, thus aiding in their differentiation from non-phagocytic transformed lymphocytes[9]. 'Colcemid' arrests mitosis and therefore elevates the mitotic index to measurable levels[10]. The cell suspensions were collected, fixed with absolute methyl alcohol and glacial acetic acid (9 : 1), and slides prepared and analysed as previously described[8].

Tritiated thymidine (^{3}HTdr 0·360 c./mmole specific activity, Schwarz Bioresearch, Inc., Orangeburg, New York) was added, 4 h before collection, to those cultures which did not contain carbon-14 endotoxin from two of the subjects. Autoradiographs using 'Kodak *AR*10' were prepared and developed after 2, 6 and 20 weeks of exposure[11].

S. enteritidis endotoxin with and without carbon-14 label evoked a significant increase in the median proportion of transformed lymphocytes from 0·5 per cent in the unstimulated cultures to 10·5 and 6·0 per cent, respectively (Table 1). Concomitantly, the median mitotic index increased from zero in the unstimulated to 12/1,000 mononuclear cells in the carbon-14 endotoxin-treated cultures. Corroborating evidence of lymphocyte proliferation in response to *S. enteritidis* endotoxin was evident from the increased proportions of ^{3}HTdr labelled mononuclear cells present in the cultures. The proportion of macrophages in the unstimulated cultures was similar to the proportion in those exposed to labelled and unlabelled endotoxin. These findings indicate that both endotoxins stimulated lymphocyte transformation equally well and that the radioactive isotope did not cause sufficient cell injury to inhibit this response.

Table 1. EFFECTS OF CULTURING LEUCOCYTES FOR 5 DAYS WITH *S. enteritidis* AND CARBON-14 *S. enteritidis* ENDOTOXIN

Additive	Cell differential	Subjects A.B.	K.S.	S.P.	N.B.	Median response
Saline	Mitotic index	1	0	0	0	0
	Macrophages (%)	12	40	23	20	21·5
	Transformed lymphocytes (%)	2	1	0	0	0·5
	Mononuclear cells labelled with ^{3}HTdr (%)	—	—	0	0	0
S. enteritidis	Mitotic index	0	4	0	0	0
	Macrophages (%)	13	15	57	13	13
	Transformed lymphocytes (%)	8	4	10	4	6
	Mononuclear cells labelled with ^{3}HTdr (%)	—	—	3	3	3
Carbon-14 *S. enteritidis*	Mitotic index	0	10	16	14	12
	Macrophages (%)	33	26	30	3	14·5
	Transformed lymphocytes (%)	3	5	16	21	10·5
	Transformed lymphocytes labelled with carbon-14 *S. enteritidis* (%)	0	0	0	0	0

The autoradiographs of the carbon-14 endotoxin-stimulated cultures did not reveal any localization of label greater than that of the background over any of the small or transformed lymphocytes in any of the four subjects (Fig. 1). Unfortunately, both the platelets and red blood cells are destroyed by the method of fixation used, so that their degree of labelling could not be assessed. In the two experiments in which the friable macrophage cytoplasm was least disrupted by the method of fixation, the carbon-14 label was markedly concentrated over or near the macrophages (Fig. 2). The grain counts and the proportion of macrophages labelled could not be determined because the labelling was too heavy and the carbon-14 scatter too great.

Thus, the carbon-14 label of the *S. enteritidis* endotoxin was taken up *in vitro* only by the macrophages, and not noticeably by the lymphocytes which were stimulated to transform. This is similar to the observations of lymphocyte transformation with *Mycobacterium battei*

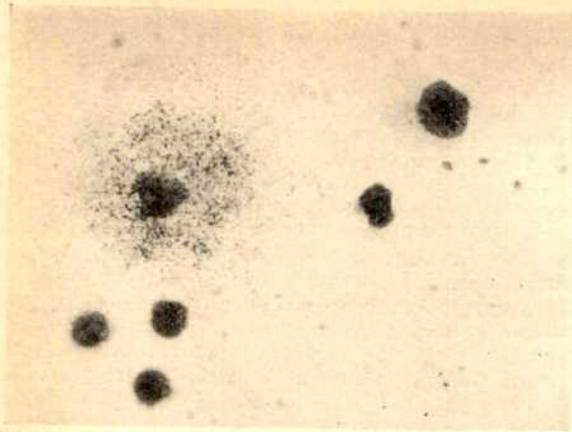


Fig. 1. Macrophage showing localization of carbon-14 endotoxin label in the region of its cytoplasm. Note the unlabelled transformed lymphocyte in the right corner of the picture, and unlabelled small lymphocytes below the macrophage (magnification × *c.* 410)

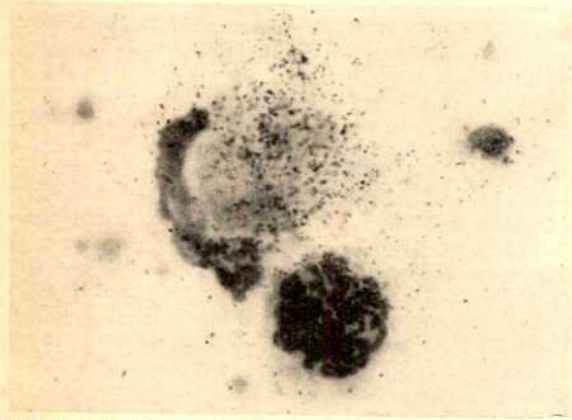


Fig. 2. A macrophage manifesting localization of carbon-14 endotoxin label flanked by an unlabelled early prophase mitotic figure (magnification × *c.* 410)

organisms. Most of these organisms are phagocytized by macrophages, and none is detectable within the transformed lymphocytes[12]. Furthermore, lymphocytes purified in nylon or glass bead columns fail to transform *in vitro* with antigens, despite the fact that these antigens are successful in transforming lymphocytes in cultures of the same leucocytes before separation[13]. These findings suggest that macrophages may play an intermediate part in the secondary *in vitro* lymphocyte transformation response to antigens.

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HAEMATOLOGY

N Blood Group System of Pigs

THE N blood group system of pigs has been identified by Hála and Hojný[1] by means of an antiserum called anti-Na. The corresponding antigen, Na, was considered to be the product of a gene, N^a. They describe a system of two alleles, N^a and $N^{(a)}$—the latter symbol denotes the absence of N^a. Two additional factors of the N system, Nb and Nc, defined by the antisera anti-Nb and anti-Nc, have been identified in this laboratory. Inheritance studies show that there are three genes: N^a, producing factor Na, N^b, producing factor Nb, and N^{bc}, producing factors Nb and Nc. N^a and N^b are a pair of contrasting alleles, forming a closed system; Nc is a sub-group of Nb, and has not been found in its absence. It was not possible to verify Hála and Hojný's claim that Na is a soluble antigenic substance.

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Large-scale Crystallization of Purified Human Foetal Oxyhaemoglobin from Cord Blood

THE crystal shape of pure human adult haemoglobin (HbA) has unanimously been described as that of well-developed, symmetrical bipyramids[1,2]. Investigations of the crystal forms of human foetal haemoglobin (HbF), however, have revealed divergent results: hexagonal rhombic plates[3], probably monoclinic forms[4] and a mixture of dodecahedra and square prisms[5] have been reported. All these investigations were carried out on cord blood. Our methods allow the investigation of pure crystalline foetal haemoglobin.

Red cells from fresh heparinized cord blood of full-term and premature infants were washed five times with 0·9 per cent sodium chloride solution and haemolysed by the addition of 1·4 parts of twice distilled water to 1 part erythrocytes. All preparations were carried out at a temperature of 4° C to exclude an increase in methaemoglobin concentration. The relative proportion of HbF was estimated as described by Betke[6]; haemoglobin and methaemoglobin were repeatedly determined as cyanmethaemoglobin. Alkali denaturation of HbA was carried out according to Chernoff[7]. 100 ml. of an approximately 15 per cent haemoglobin solution were denaturated by the addition of 800 ml. 1/12 N potassium hydroxide for 2 min to destroy HbA completely. The reaction was interrupted by the addition of a 450 ml. saturated solution of ammonium sulphate containing 0·95 ml. concentrated hydrochloric acid per 100 ml. of the salt solution. The reaction mixture was adjusted to *p*H 7·0. After centrifugation (4,000*g* for 30 min) and after decanting carefully the deep red and clear supernatant, denaturated HbA, stromata and the more insoluble proteins in the sediment were discarded. 100 ml. of 0·5 M phosphate buffer, *p*H 7·35, were added to 1 l. supernatant. The salt saturation was brought up to 75–80 per cent by gradual addition with thorough stirring of solid ammonium sulphate, while the *p*H was maintained at at least 6·5 (if necessary more phosphate buffer (*p*H 7·35) was added). The solution was then centrifuged again (4,000*g* for 30 min) and a deep red sediment containing the foetal haemoglobin appeared at the bottom. HbF was then dissolved in 0·25 M phosphate buffer (*p*H 7·4), placed in dialysis bags and dialysed

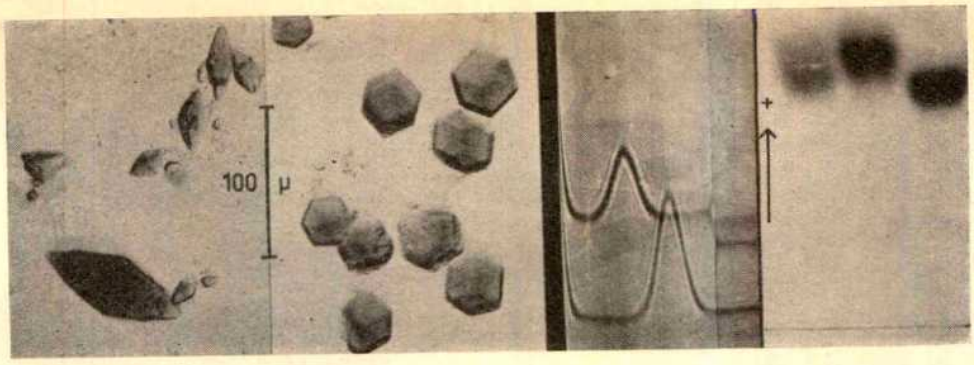


Fig. 1. *a*, HbA crystals. *b*, HbF crystals. *c*, Ultracentrifugation: 44 min, 42,040 r.p.m., 0·8 and 0·4 g per cent HbF in 0·02 M phosphate buffer, *p*H 7·4. *d*, Starch-gel electrophoresis: lithium buffer, *p*H 8·04, (120 V, approximately 12 mamp, 14 h). Left to right: cord blood, HbA, crystalline HbF (all samples at a concentration of 250 mg per cent haemoglobin)

against the same buffer to remove ammonium sulphate. The contents of the bags were centrifuged (40,000*g* for 20 min) and the supernatant HbF solution crystallized by dialysis against 2·8 M phosphate buffer of *p*H 6·8 using the method of Drabkin[2]. During these procedures, 8–12 per cent methaemoglobin developed. The addition of stoichiometric amounts of sodium thiosulphate to the dialysis fluid results in the reduction of this methaemoglobin to haemoglobin. After continued dialysis for about 12–18 h the first crystals could be identified by microscopic examination. The buffer was then renewed to allow larger growth of crystals and dialysis continued for several days to several weeks. 70–80 per cent of the original foetal haemoglobin specimen could be crystallized.

From solutions of pure foetal haemoglobin our procedure yielded exclusively isomorphous crystals, that is, dodecahedra, whereas crystals from human adult haemoglobin are typical orthorhombic bipyramids (Fig. 1*a*, 1*b*).

The completeness and uniformity of the crystallized foetal haemoglobin were examined (following separation of HbF crystals from the mother liquor by rapid filtration with suction on a Buchner funnel) as follows:

(1) *Spectroscopic examination* of dissolved HbF crystals showed the conventional plots of the haemoglobin absorption spectra, and the tryptophan fine structure band typical of HbF[8] at a wave-length between 288–289 mμ.

(2) *Ultracentrifugation* (Spinco 'Model *E*' analytical ultracentrifuge) revealed a sedimentation constant in Svedberg units (S_{20}) of 4·4 and a diffusion constant (D_{20}) of 6·6 (values extrapolated to zero concentration). The molecular weight calculated from S_{20} and D_{20} was 66,000. This figure agrees with those reported for foetal haemoglobin[9] (Fig. 1*c*).

(3) *Starch-gel electrophoresis*[10] of re-dissolved pure crystalline HbF and HbF in cord blood haemolysates showed a comparable electrophoretic mobility (Fig. 1*d*) equally with lithium buffer, *p*H 8·04, and *tris*-citrate buffer, *p*H 8·7 (ref. 11).

Measurements with the petrographic polarization microscope of crystals of pure human foetal haemoglobin showed invariably and uniformly isomorphous cubic forms (dodecahedra) so far as definite conclusions can be reached with optical investigations. Dodecahedra have been found in crystallized cord blood preparations (carbonmonoxyhaemoglobin) together with square prisms by Zinsser and Tang[5] using X-ray crystallographic measurements.

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HISTOCHEMISTRY

Staining of Paneth Cells with Thioflavine *T*

Since the thioflavine *T* (T.T.) fluorochrome method[1] was first recommended for the demonstration of amyloid its use has been extended widely. Uptake of the dye has been shown to occur with myeloma casts[2], muciphages in the intestinal mucosa[3,4], keratin[4], mast cells[5,6], myelin figures in neurones of infantile amaurotic idiocy and some of the component cells in the granular cell myoblastoma[7], acidophils in the pituitary[8], juxtaglomerular apparatus cell granules in the kidney[6,9] and zymogen granules in the pancreas of mice[6].

While investigating further possible uses of this fluorochrome it was discovered that Paneth cells in the human jejunum were also strongly positive. As a stage in the usual method, haematoxylin is ordinarily used as a non-specific quencher of tissue autofluorescence. It became apparent, however, that the uptake of T.T. by Paneth cells was largely dependent on a more specific action of haematoxylin.

In hydrated paraffin sections of formalin-fixed jejunal mucosa obtained by peroral biopsy it was possible to compare in the same section the strongly autofluorescent argentaffin cell, the muciphage and the Paneth cell. The usual staining procedure[1] with slight modification is as follows: (i) haematoxylin 5 min; (ii) two rinses in distilled water; (iii) T.T. for 5 min; (iv) rinse in 1 per cent glacial acetic acid then place in same for 3–5 min;

(v) two rinses in distilled water followed by mounting in equal parts of glycerin and distilled water. We used the haematoxylin of Krutsay[10] which is constituted as follows: 0·5 per cent haematoxylin, 5·0 per cent potassium alum, 0·02 per cent potassium iodate, 0·5 per cent hydrochloric acid and distilled water. This acid haematoxylin gave better results than those of Ehrlich, Mayer, Weigert and an alcoholic Krutsay solution. The T.T. was prepared according to Janigan[9]. Table 1 shows the results of omission and simple sequence variation of certain stages in the staining procedure. It is clear that the haematoxylin effectively potentiates the T.T. fluorescence of Paneth cells while quenching the autofluorescence of the background, nuclei and argentaffin cells.

In order to elucidate this phenomenon, two further series of sections were treated as in the standard method except that, in turn, the haematoxylin solution minus hydrochloric acid, and then each of the constituents of the solution, alone and in combination, were substituted for the full haematoxylin in the staining procedure. In one series they were mounted and examined for fluorescence after this stage—the T.T. stage was omitted. The results are shown in Table 2. Except for hydrochloric acid and haematoxylin itself, none of the other isolated constituents of the stain or combinations of them appears to play a part in the potentiation of the fluorescence of Paneth cells. A simple aqueous solution of haematoxylin gave the same results as the non-acid containing solution and thus the presence of potassium alum and potassium iodate is without effect. Haematoxylin is not acting simply as a quencher of background and non-specific fluorescence as the actual intensity of fluorescence of the Paneth cell is markedly enhanced by its use. While haematoxylin alone and hydrochloric acid alone seem to be effective, neither when used alone is as effective as the combined acid haematoxylin solution.

Table 1

	Auto-fluorescence*	Krutsay†	Krutsay-T.T.	T.T.	T.T.-Krutsay
Paneth cell	−ve	+ + bw	+ + + + lg	*M*‡	+ + + + lg
Muciphage	−ve	−ve	+ + + oy	*M*	+ oy
Argentaffin cell	+ + + oy	−ve	−ve	*M*	−ve

Ultra-violet source, Osram lamp *H.B.* 200; microscope, Leitz Wetzler; exciter filter, *U.G.*1; barrier filter, *K*.430.
bw, Blue white. lg, Lime green. oy, Orange yellow.

* Nuclei are normally autofluorescent.
† ± to + + + +, Degree of brilliance of fluorescence.
‡ *M*, Cytoplasmic and granule fluorescence almost masked by nuclear and diffuse background positivity.

Table 2

	Paneth cell	Muciphage	Argentaffin cel
Krutsay without hydrochloric acid	+ bw	−ve	+ oy
Krutsay without hydrochloric acid + T.T.	+ + lg	+ + oy	− ve
Krutsay + hydrochloric acid	+ + bw	−ve	− ve
Krutsay + hydrochloric acid + T.T.	+ + + + lg	+ + + oy	− ve
Krutsay constituents alone and in combination but without hydrochloric acid and haematoxylin	− ve	−ve	+ + + oy
,, ,, ,, + T.T.	±	±	±
Hydrochloric acid	+ bw	−ve	+ + + oy
,, ,, + T.T.	+ + lg	±	±
Aqueous haematoxylin	+ bw	−ve	+ oy
,, ,, + T.T.	+ + lg	+ + oy	− ve

It will also be noted from Table 1 that, as observed by other workers, muciphages are fluorescent after the usual staining procedure. With the filter system we used, however, a colour quite distinct from Paneth cells is produced. Moreover, if T.T. is applied before the haematoxylin the fluorescence of muciphages virtually disappears (compare Figs. 1 and 2). Why the reversal of the staining sequence has this effect is not understood, but because the Paneth cell fluorescence remains unchanged it is obvious that it has a greater affinity for T.T. That this might arise from metallic zinc, known to be present within Paneth cells, was not substantiated. Sections were stained before and after extraction of zinc by the method of Studzinski[11] without any effect on the result. As a control procedure,



Fig. 1. High-power photomicrograph of jejunum stained with the Krutsay haematoxylin-T.T. sequence. Note the strong fluorescence of both the Paneth cell granules and the cytoplasm of the muciphage. (× 1,500)



Fig. 2. Parallel section to that of Fig. 1, which demonstrates the effect of reversing the staining procedure. The Paneth cell granules fluoresce brightly while the fluorescence of the muciphage cytoplasm is scarcely apparent. (× 1,500)

sections of gastric mucosa and prostate were also treated similarly (zinc is also present in the gastric parietal cell and in prostatic epithelium). While both these cell types were not stained by T.T., the acid-producing gastric oxyntic cell showed an uptake comparable with that of the Paneth cell. This was regarded as additional con-

firmation of the importance of an acid moiety in the staining reaction.

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CYTOLOGY

Fine Structure of Experimentally Produced Subsynovial Carbon Granuloma

RECENT electron microscopic investigations of synovial joints have been concerned with the phagocytic nature of synovial membrane cells[1,2]. In order to investigate this phenomenon further, 1 c.c. of colloidal carbon in the form of Indian ink (particles 200–2000 Å in diameter) was injected into the knee joints of rabbits. 104 days later the animals were killed, and large granulomata were evident in the subsynovial tissues. This report is concerned with the structure of these carbon granulomata.

Portions of synovial membrane and subsynovial tissue were fixed in 4 per cent glutaraldehyde[3] or in Millonig's[4] fixative and embedded in 'Epon'. Thick sections (1·5μ) were prepared for investigation under the light microscope and stained with 0·5 per cent aqueous safranin. Ultrathin sections were also cut and mounted on carbon-coated grids and stained with uranyl acetate[5] and lead citrate[6]; these were investigated with an RCA *EMU*-2 electron microscope. An estimation was made of the character and relative number of subsynovial inflammatory cells present per high-power light microscopic field for each experimental stage. These cells fell into three groups: polymorphonuclear leucocytes, cells of the macrophage series (histiocytes, macrophages and primitive cells) and unclassified cells. By combining the light and electron microscopic observations, the phagocytic contributions of the synovial membrane and that of the invading inflammatory cells could be distinguished.

The cell most involved in the removal of particulate matter of this size from the joint cavity is the invading macrophage. A progressive infiltration of the macrophages could be seen: these were first observed free of carbon in the subsynovial tissues, then seen to penetrate the synovial membrane, to enter the joint cavity and finally to appear once more in the subsynovial tissues but with an abundance of colloidal carbon in their cytoplasm. Such cells appeared to migrate toward one another so that light microscopic examinations carried out 104 days after injection revealed the large carbon granulomata beneath the synovial membrane (Fig. 1). Under the light microscope, the areas where the cells congregated could be seen to be surrounded by a collagenous capsule which possessed a profuse blood supply. The cytoplasm seemed to be totally saturated with carbon particles. The nuclei contrasted sharply and often showed a prominent nucleolus.

Under the electron microscope, the configurations of the plasma membranes of adjacent macrophages varied from the more numerous long, thin finger-like processes 600–1200 Å in length to the less numerous bulb or club-shaped terminations (Fig. 2). Occasionally, when observed in cross-section, these processes exhibited a circular

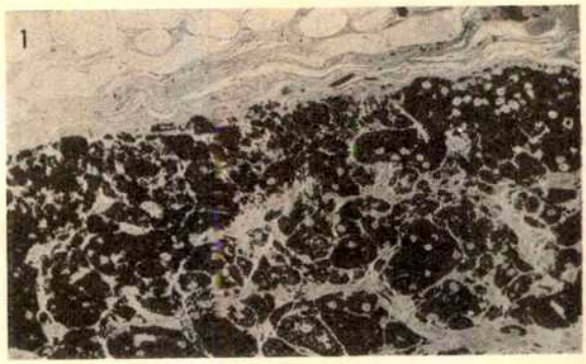


Fig. 1. Light micrograph of subsynovial tissue fixed 104 days after intra-articular carbon injection. The area of the granuloma is surrounded by fibrous tissue. The nuclei, many of which show a prominent nucleolus, contrast sharply with the carbon filled cytoplasm. Stained with aqueous safranin. (× 1260)

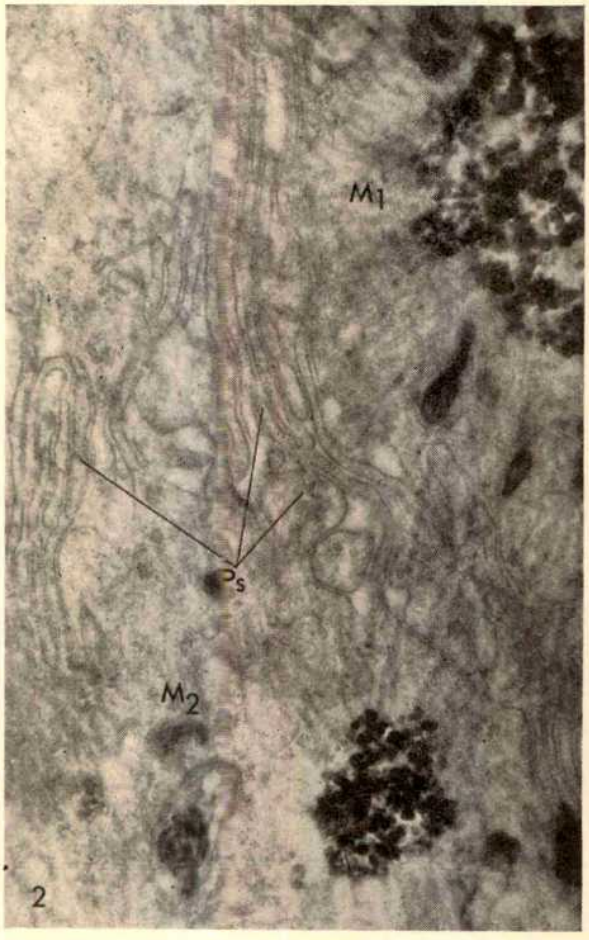


Fig. 2. Electron micrograph of adjacent macrophage-like cells (M_1 and M_2) fixed 104 days after intra-articular carbon injection. The extensive interdigitation (*Ps*) of plasma membranes by adjacent cells is well demonstrated. (× *c.* 27,700)

profile; however, an elongated profile was more usual, which indicated that most processes are probably flattened and leaf-like and vary greatly in width but are uniform in thickness.

Although extensions of plasma membrane of adjacent cells closely approached each other, direct contact between adjacent granulomatous cells was not observed. A space of 20–40 Å (Fig. 2) separated adjacent cells so that a direct cell to cell contact was not observed. A similar behaviour has been observed[7] in tissue culture and termed "contact inhibition". These plasma infoldings were carefully examined for evidence of thickenings of the plasma

membrane, condensation of submembranous cytoplasm and increased density between two cell membranes characteristic of desmosomes of adhesion plates found in epithelia[8] and in the nervous system[9]. None of these features was observed.

When the phagocytic cells involved in the formation of granulomata become closely bound together, cytoplasmic inclusions other than carbon particles for the most disappear; this is especially so in the central portion of the cytoplasm and around the nucleus. Moreover, in larger granulomata, the cells lying in the centre of a granuloma show few organelles either centrally or peripherally. A similar distribution of organelles has been noted in the giant cells of the lung[10]. Several explanations can be offered; for example, that the fixatives are not able easily to penetrate these large cytoplasmic masses. However, the two different fixation procedures used in the present work give comparable results, which indicates that the method of tissue preparation is not involved. Another possibility is that the more peripherally located cells have been added to the granuloma more recently than the centrally located cells. A fully developed granuloma as seen at 104 days post-injection is apparently neither mobile nor engaged in the production of extracellular substances (that is, proteinaceous fibres). It seems probable, therefore, that these cells would not possess as many cytoplasmic organelles as are observed in cells engaged in more active functions.

This investigation supports the previous findings[10] that giant cells are not formed, as had been suggested earlier[11,12], as a result of the presence of individual particles too large to be phagocytosed by single cells.

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Ultrastructural Transport Mechanism of Messenger Ribonucleic Acid in the Young Oocytes of Amphibians

IT is now generally accepted that the nucleus is an active centre of ribonucleic acid (RNA) synthesis and is perhaps the sole source of cellular RNA (ref. 1). Much evidence has accumulated to substantiate this view, which has raised the interesting question of the possible role of RNA as a carrier (messenger) of gene specificity to the cytoplasm, where the ribosomal RNA is thought to be involved directly in protein synthesis.

The passage of nuclear RNA into the cytoplasm has recently been observed by Balinsky and Devis[2] and Beermann[3] in the course of electron microscope studies of growing oocytes of *Xenopus laevis* and of the salivary gland cells of *Chironomus tentans*. No previous account has been given of the way in which such a passage occurs, except for the description by Beermann, in which he says that the round particles of about 300 Å in diameter possibly transport forms of the messenger, arrive at the nuclear envelope, get caught in the pore and penetrate it, and change their shape into a rod or a thread. The present communication is concerned with the ultrastructural transport mechanism of the messenger in the young oocytes of amphibians.

Small pieces of the ovaries of *Triturus pyrrhogaster* and of *Rana nigromaculata* were transferred immediately to cold 1 per cent osmium tetroxide buffered with veronal acetate. After fixation, the fragments were divided into single oocytes or small groups of oocytes. They were embedded in 'Epon 812' epoxy resin. Young oocytes ranging from 100μ to 300μ in diameter were sectioned with the Porter–Blum microtome, and thin sections were stained with uranyl acetate. They were examined with the Hitachi '*HU-11A*' electron microscope.

Electron micrographs of the young oocytes of both species reveal that the nucleoplasm is irregularly distributed, with many coarse granulated patches. These patches consist of electron-dense particles about 300 Å in diameter and fibrous structures of the lateral loop of the lampbrush chromosome conveying the particles. These particles are thought to be the result of gene activity on the lateral loop, and they may be transport forms of the messenger. Sometimes the granulated loops are observed facing the nuclear pores. In such a region, flakes of electron-dense matter can often be found in the cytoplasm, suggesting outflow of the material through the nuclear pores (Figs. 1 and 2). Indeed, high magnification of such a region clearly shows that the round particles detached from the lateral loop penetrate the pores, leaving behind their conveyor as a smooth loop (Fig. 2). On the other hand, in the cytoplasm near the nuclear pore, expelled material can be seen taking the shape of spindles or dumb-bells. It remains in this form for a time until it diffuses into the perinuclear cytoplasm. It is also found that the electron-dense flake consists of very fine filamentous structures running parallel to its long axis. It is tempting to assume that the messenger molecule, so long as it is within the nucleus, remains attached to the lateral loop, but it detaches from the loop (conveyor) when it is just ready to pass the pore. This may happen under the influence of certain preferential factors from the cytoplasm, because the granulated loops do not necessarily show this behaviour even if they lie near the nuclear pore. These particles cannot occur floating freely from the loops in the nuclear sap as is the case with the salivary gland nucleus of *Chironomus*[3]. According to Beermann, two large Balbiani rings in the salivary gland nucleus of this species produce characteristic round particles of about 300 Å in diameter. These are probably ribonucleoprotein as they are sensitive to RNase and trypsin. These particles are found floating in the nuclear sap, and, under certain conditions, they pass through the pores and change their shape into a rod or a thread. After having passed, however, they seem to disappear in the cytoplasm. He assumes that the messenger molecules are combined with transport protein, but that when they have passed through the nuclear pore they leave their vehicle protein behind in order to be picked up by ribosomes, while the vehicles themselves return to the nucleus. Balbiani rings and lampbrush chromosome loops are similar to each other in that both have a high RNA/DNA quotient and are characteristic round particles of similar dimensions. In addition, the matrix surrounding both the Balbiani rings and the lateral loops consists of RNA and protein. It is considered that two substances are actively synthesized at the activated chromosomal strands, and some quantity of RNA eventually becomes transferred to the cytoplasm. Although the mechanism of such a transfer may be different in detail, depending on the species of animals and even in different kinds of cells, it seems likely that it is fundamentally similar in all animals. At the present time it is not clear whether the differences between insects and amphibians in the time of detachment of the particles from the conveyor are due

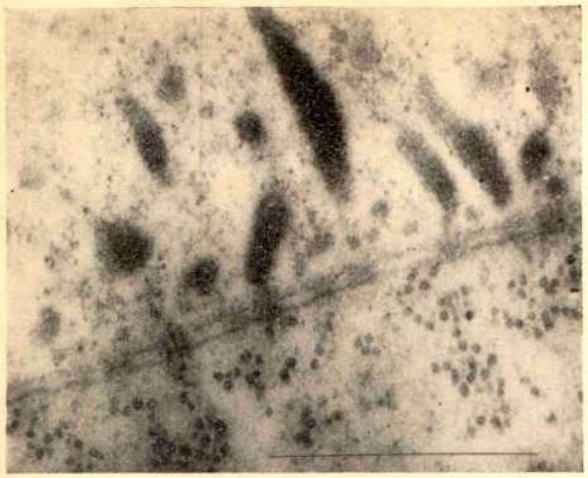


Fig. 1. Electron micrograph showing outflow of material from the nuclear pores of the oocyte of *Rana nigromaculata* (250μ in diameter). Characteristic round particles are shown attached to the lateral loops approaching the nuclear membrane and penetrating its pores, changing their shape into a rod or a thread. Particles within the nucleus remain attached to the lateral loops



Fig. 2. Electron micrograph showing the round particles attached to the lateral loops, near the nuclear pores of the oocyte of *Triturus pyrrhogaster* (150μ in diameter). Particles are shown approaching the nuclear membrane and penetrating its pores, leaving behind their conveyor as a smooth loop

to species differences or are artefactual. Recently, using whole-mount electron microscopic techniques, DuPraw[4] has shown that, in honey-bee embryonic nuclei during interphase, the individual chromosomal fibres are attached to the nuclear envelope, typically at the edge of annuli. Though DuPraw[5] showed that the functional significance of these fibre-annulus attachments is not yet known, these findings are consistent with the present observations.

Fewer flakes near the nuclear envelope are found than might be expected from the large number of granulated patches distributed within the nucleus. This may support the concept of Harris[6–8] that a large part of the nuclear RNA, particularly RNA synthesized on the chromosome, is broken down in the nucleus.

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GENETICS

Method for the Display of Avian Chromosomes

WHILE chickens and other avian species have been widely used as subjects in many areas of biology, especially embryology, karyological investigations in birds have been restricted because of inherent and procedural difficulties[1]. The relatively large number of small chromosomes seen in birds, the number and morphology of which cannot easily be ascertained, has been one discouraging factor. Another is the fact that the leucocyte culture technique developed by Moorhead *et al.*[2], while generally applicable to mammals, is unsuitable for feral birds. We have found that parasites, which are normally found in the blood, proliferate in culture and interfere with the procedure. This communication describes a simple method for the display of avian chromosomes which has been used with a variety of tissues taken from both praecoces and altrices.

Three to 3·5 h before extirpating the chosen organ or tissue, fertile eggs or young birds were injected with 0·01 mg of colcemide or colchicine (0·1 per cent solution) for each 5 g of mass. When embryos were to be used, the injection was made into the yolk sac; neonates were injected intraperitoneally. The material to be used was finely minced and teased in a drop of Hanks's balanced salt solution in a small Petri dish giving a cell suspension. The suspended cells, but not the larger pieces, were picked up with a pipette and placed in 3 ml. centrifuge tubes and Hanks's solution was added to a final volume of about 2 ml. Sediment was removed from the tubes after standing for 2–5 min.

The tubes were then placed in a centrifuge and spun at 84*g* for 6 min. Without disturbing the cells, the supernatant liquid was removed with a pipette. One to 2 ml. of 0·95 per cent sodium citrate were added and the cells were suspended by aspirating and discharging the suspension with a pipette. After standing for 20 min, the tubes were again spun and the supernatant fluid removed. The cells were suspended in a small amount of fixative (glacial acetic acid–methanol, 1 : 3) after which more was added to a volume of 1·5 ml. Following a 30 min period of fixation, the tubes were spun and the supernatant fluid removed. A small volume of either 45 per cent acetic acid or fixative solution was put in the tubes and the cells were resuspended.

One or two drops of the cell suspension were placed on a clean warmed slide and dried. While drying, the drop was moved around by rotating the slide in order to obtain uniform distribution of the cells. Slides were then stained in acetic–orcein, dehydrated in 95 per cent and 100 per cent ethanol, cleared in xylene and mounted by placing a cover slip over a drop of 'Piccolyte' or 'Permount'.

If the choice of tissue is not dictated by the experiment, it is well to take one undergoing rapid proliferation, at the particular age of the organism to be used. Very early in the period of incubation, the whole embryo is suitable. Good preparations have been obtained from 2 day chicken and 4 day turkey embryos. Following organogenesis, the liver grows rapidly and may be used. From about 11–15 days the spleen is undergoing rapid growth and yields many mitotic cells. During the later stages of incubation of praecoces the bursa of Fabricius and the thymus have been successfully used, while in young chicks, turkeys or pheasants the bursa and bone marrow have been found to be excellent sources of material. Bone marrow is obtained by splitting either tibiae or femora longitudinally and removing the marrow with a blunt probe; it is afterwards handled like other tissues.

We have not attempted to use the embryonic tissues of any altricial birds. However, young sparrows and robins taken from the nest 2–10 days after hatching have been found to be suitable subjects. The best tissues appear to be the bursa of Fabricius and bone marrow.

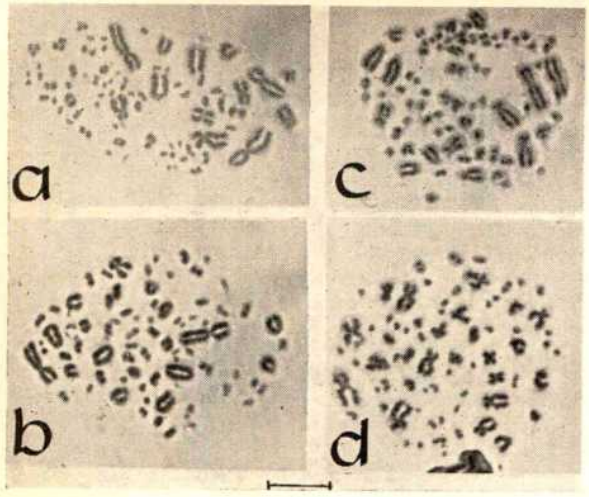


Fig. 1. Photographs of avian metaphase chromosomes. *a*, Chicken, male (*Gallus domesticus*); *b*, ring-necked pheasant, female (*Phasianus colchicus torquatus*); *c*, robin, female (*Turdus migratorius*); *d*, sparrow, male (*Passer domesticus*)

This relatively simple method, which requires no elaborate equipment, may enable a number of investigators to undertake cytogenetic investigations of avian species. The quality of the preparations that has been obtained can be seen in Fig. 1.

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Frequency of Colour Blindness among East Kentish Children

AN investigation of the frequency of colour blindness among East Kentish children was made in connexion with research sponsored by the Department of Child Development, Institute of Education, University of London. The *Ishihara Test* (second edition: 24 plates) was used under north daylight conditions, and all the children in five schools were tested. Two of the schools were in the city of Canterbury, two in agricultural communities and one in a Kentish coalfield village. The children ranged in age from 4·5 to 11·0+ years. "Colour blind" responses to 12/16 plates of figures and/or all the "path tracing" plates were required for a diagnosis of colour blindness. The frequencies found were as shown in Table 1.

Table 1

	Boys	Girls
Number tested	505	450
Colour blind	17	0
Percentage colour blind	3·37	0

The children had been tested with the *Kent (Standardised) Tests*. The range of general ability among the colour blind boys ran from 78 I.Q. to 134 I.Q., and no relationship was found between either educational ratio or intelligence quotient and colour blindness.

The frequency of 3·37 per cent of red–green blind boys in East Kent is very low, but accords with the findings of Vernon and Straker[1], who reported 3·55 per cent for Edinburgh, 3·26 per cent for Chesterfield and 4·37 per cent for Romford. Kherumian and Pickford reported 4·12 per cent in the Vosges and Moselle. These are the lowest frequencies reported for Great Britain and France, and they occur in the north and east of Britain and in those parts of France which have the poorest soils and are most removed from the Mediterranean and English Channel coasts, as was pointed out by Post[3] and Pickford[4], in their observations on the possible relaxation of natural selection against colour blindness under the conditions of civilization.

In the present investigation the expected frequencies of colour blind girls would be less than 3·37 per cent[2], namely, about one in 1,000 or fewer, and it is not surprising that none was found in a sample of 450 girls.

I thank Prof. R. W. Pickford and Miss Mary Waddington for their assistance.

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RADIOBIOLOGY

Assessment of Body Content of Radioactive Strontium following Contamination in Adult Men

ASSESSMENT of the body content of humans following an accident with radioactive strontium, particularly with strontium-89 or strontium-90, is one of vital concern. Even if equipment for body radioactivity measurement is available, its relatively low sensitivity for these isotopes makes it important to have other rapid and reliable means of estimating the body content. One of us[1] presented a simple relationship between the cumulative urinary excretion of radioactive strontium over 21 days, $\Sigma_0^{21}U$, and the injected dose R_0:

$$R_0 = \frac{\Sigma_0^{21} U}{0{\cdot}6} \qquad (1)$$

It was also shown that following a chronic ingestion of radioactive strontium there was a simple relationship between $\Sigma_0^{21}U$ and the systemic retention at the time of the last ingestion.

It has been pointed out by Macdonald *et al.*[2] that complete collection of urine over 3 weeks has practical difficulties and the inevitable delay in assessment of the body content of radioactive strontium is undesirable. Additional data from experiments in which the gamma-ray emitter, strontium-85, was administered to volunteers from the Radiobiological Research Unit have prompted us to give the problem further consideration. In Tables 1 and 2 we have compared the urinary excretion of radioactive strontium in 5, 10 and 21 days as a percentage of systemic content, R_0. For the intravenous administrations (Table 1) the original dose was taken as the value of R_0, while for the chronic ingestion (Table 2) R_0 was derived from body radioactivity measurements for each subject[3].

It will be seen that the more complete data support the original empirical equation (1). However, the gain in accuracy by extending the urinary collection to 21 days (as judged by the standard errors of the mean values) is small. To make the assessment of body content of radioactive strontium more rapid, it is suggested that the modified formula $R_0 = \frac{\Sigma_0^5 U}{0{\cdot}5}$ following intravenous injection is sufficiently exact for the present requirements.

Table 1. CUMULATIVE URINARY EXCRETION OF STRONTIUM, ΣU, AFTER INTRAVENOUS ADMINISTRATION

Subject	Expt.	ΣU (percentage of dose) 0–5 days	0–10 days	0–21 days
G.E.H.	1	40·9	49·6	56·2
	2	43·3	52·0	57·1
J.F.L.	1	51·9	59·7	64·2
	2	52·5	62·0	64·8
R.H.M.		54·5	61·3	63·9
Mean $\Sigma U/R_0$		0·49	0·57	0·61
Standard error / Mean (per cent)		5·6	4·5	1·8

Table 2. CUMULATIVE URINARY EXCRETION OF STRONTIUM, ΣU, AFTER CHRONIC ORAL ADMINISTRATION FOR 21–32 DAYS

Subject	R_0 (nc.)	ΣU (nc.) 0–5 days	0–10 days	0–21 days	$\Sigma U/R_0$ (per cent) 0–5 days	0–10 days	0–21 days
T.E.F.C.	1,050	165	226	282	16·4	23·9	26·8
G.E.H.	520	85	124	163	15·7	21·6	31·3
G.R.H.	390	92	124	158	24·6	31·8	40·5
J.F.L.	690	162	170	208	18·2	24·6	30·1
F.S.W.	610	101	142	179	16·5	20·4	29·4
Mean $\Sigma U/R_0$	–	–	–	–	0·18	0·25	0·32
Standard error / mean (per cent)	–	–	–	–	8·9	8·1	7·4

Samachson[4] and Macdonald *et al.*[2] have attempted to make this assessment from a single observation of the plasma concentration or one day's urine collection respectively, but we feel that such conclusions should not be based on a single observation. However, there will be occasions when incomplete urine collections will be unavoidable, especially when there are difficulties in enlisting the necessary co-operation of the subjects. We have, therefore, examined an alternative approach to the problem. The individual retention equations (each using three exponential functions of time)[5] for five subjects were differentiated with respect to time to give expressions for the excretion rates. From the equation for each subject, values were calculated of the retention, the total excretion rate and the ratio, F = retention/excretion rate, at various times after intake. The ratios are plotted as a function of time in Fig. 1, but to avoid confusion only those parts of the curves which define the envelope of the area enclosed are shown. For times up to 18 days after intake, the empirical equation: $F = 5{\cdot}4\ e^{0{\cdot}13t}$, where t is the time in days after exposure, predicts to within ± 30 per cent the calculated value for each subject.

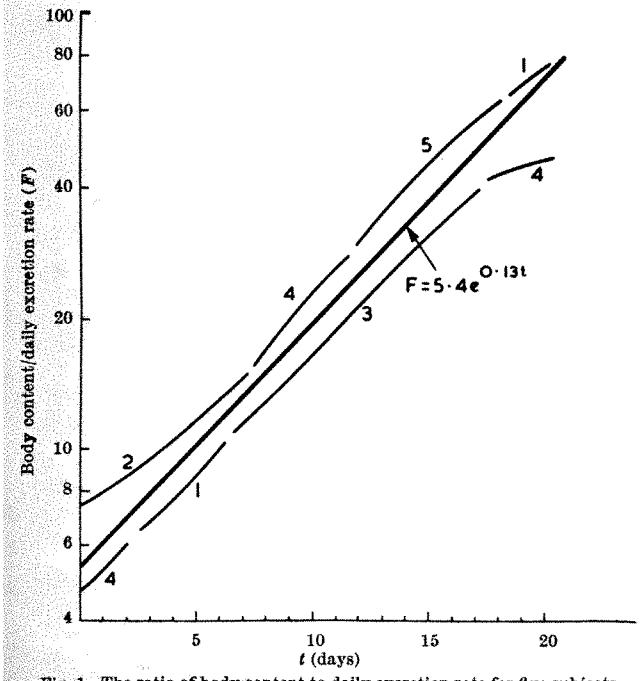


Fig. 1. The ratio of body content to daily excretion rate for five subjects, calculated from individual retention equations. Subjects: 1, T.E.F.C.; 2, G.E.H.; 3, G.R.H.; 4, J.F.L.; 5, F.S.W.

Endogenous faecal excretion is approximately 23 per cent of the total excretion[6], so the expression $7\ e^{0{\cdot}13t}$ may be used to calculate the factor by which the 24 h urinary strontium excretion is to be multiplied to give the systemic content at any time up to 18 days after an intake to the body, irrespective of the route of intake. The uncertainty of not more than 30 per cent is acceptable for purposes of radiological protection and is of the same order as the day-to-day variations in strontium excretion.

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Prevention of Nuclear Damage in Irradiated Rat Thymocytes by Post-irradiation Oxygen Deprivation

POST-IRRADIATION exposure of rats to the respiratory inhibitor carbon monoxide has been shown to reduce mortality[1,2]. On the cellular level, respiratory inhibitors (cyanide, high concentrations of nicotinamide) reduce or entirely prevent the usual post-irradiation loss of nuclear structure (pycnosis) when they are added to cultures of highly radiosensitive thymocytes after irradiation[3]. The purpose of this communication is to show that radiation-induced loss of nuclear structure in thymocytes can also be prevented by maintenance of aerobically irradiated cells in an anaerobic environment.

In the initial experiments, ten 5-week-old Sprague–Dawley rats were irradiated with 1,000 r. (dose rate: 100 r. min^{-1}) of 250 kV X-rays produced by a Seifort 'Isovolt' X-ray machine. Ten minutes after irradiation, five rats were killed and their bodies stored in an incubator at 37° C. At various times after irradiation the thymus was removed from one living and one dead rat. Thymocyte suspensions were then prepared (in medium 199). Immediately thereafter the cells were fixed in neutral formalin and stained with Delafield's haematoxylin following the method previously devised by Whitfield *et al.*[4].

Cells with structurally homogeneous nuclei began to appear in the thymuses of the living animals 1 h after irradiation (Fig. 1). By 6 h after irradiation about 85 per cent of the cells had homogeneous nuclei (Fig. 1). In the dead rats, on the other hand, there was very little development of nuclear homogeneity; the majority of the cells contained structurally normal nuclei at 6 h after irradiation (Fig. 1). Even 8 h after irradiation only 4·0 per cent of the cells in the dead animals had homogeneous nuclei while in the living rats 90 per cent of the thymocytes had such nuclei.

Because thymocytes became very fragile after 4 h in the dead rats, the observed difference could have resulted from a differential destruction of damaged cells by the mechanical stresses of the cytological procedure. However, the same results were obtained when unfixed and unstained populations were examined (cells with normal or struc-

Table 1. EFFECT OF ANAEROBIC CULTIVATION ON THE ACCUMULATION OF CELLS WITH HOMOGENEOUS NUCLEI IN IRRADIATED THYMOCYTE SUSPENSIONS

Time post-irradiation when cells were transferred to anaerobic medium (h)	Percentage of cell population with structurally homogeneous nuclei 6 h after irradiation (1,000 r.)
0	1·5
0·5	1·4
1·0	16·7
2·0	32·5
3·0	41·7
Aerobic control	74·2

turally homogeneous nuclei could be readily recognized in such untreated preparations).

The striking difference between the rates of radiation-induced nuclear changes in living and dead animals probably arose from the absence of respiration in the anaerobic environment in the dead animal. However, to ascribe the effect to anaerobiosis and not to some other post-mortem phenomenon, we were obliged to show that oxygen deprivation after irradiation could reduce nuclear damage in cultures of thymocytes.

Thymocytes were suspended in a glucose–phosphate medium containing 15 mM sodium monohydrogen phosphate and buffered at *p*H 7·2 with 5·0 mM *tris* (hydroxymethyl) aminomethane. This medium and the method of isolation of thymocytes have been described in detail by Whitfield *et al.*[4]. The kinetics of nuclear homogenization in cells maintained in this medium are identical to those in the living animal. All suspensions were irradiated under aerobic conditions at 37° C with 1,000 r. (dose rate: 100 r. min^{-1}). The oxygen tension in the medium was reduced from the normal level of 3·70 mg/l. to 0·14 mg/l. by initially bubbling nitrogen through the (cell-free) medium and then continuously passing nitrogen through the gas phase of the cell culture during the subsequent period of incubation. Bubbling nitrogen through the culture was avoided as the mechanical stress and anaerobiosis combined to cause a rapid and complete lysis of the cell population.

Reduction of the oxygen tension during the first 3 h after irradiation prevented, or very much reduced, the loss of nuclear structure (Table 1). Transfer of cells to the anaerobic medium as late as 3 h after irradiation could not undo the damage which had already been done but it greatly reduced any further loss of nuclear structure in the population. As an illustration of this, the percentage of cells with homogeneous nuclei in aerobically maintained populations 2 h after irradiation was 26·4 per cent and when the cells were transferred to the anaerobic medium the percentage rose to only 32·5 during the next 4 h (Table 1); under aerobic conditions, the percentage would have risen to 74·2 (Table 1). Three hours after irradiation, the percentage in the aerobically maintained population had risen to 39·3 and when the cells were transferred to the anaerobic medium the percentage rose to only 41·7 during the next 3 h (Table 1).

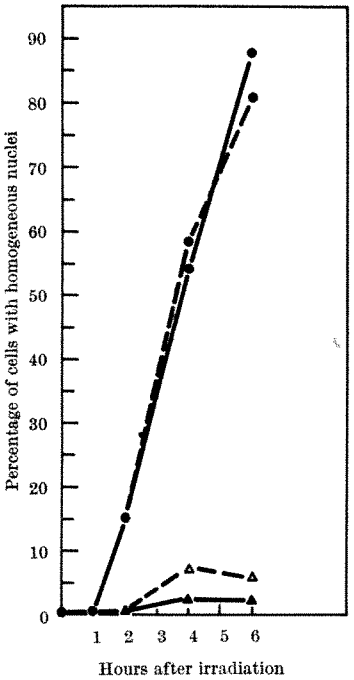


Fig. 1. The accumulation of cells with structurally homogeneous nuclei in the thymuses of living and dead irradiated (1,000 r.) rats. ●———●, In living rat, stained preparations; ● - - - - - ●, in living rat, unfixed and unstained cells. ▲———▲, In dead rat (killed 10 min after irradiation), stained cells; △ - - - - - △, in dead rat, unfixed and unstained cells

We conclude now that oxygen deprivation after irradiation can reduce the amount of nuclear damage in very radiosensitive cells such as thymic lymphocytes which when irradiated suffer an early death in interphase. An identical effect has been observed in preliminary experiments with the equally radiosensitive bone marrow. The effectiveness of oxygen deprivation for a relatively long time after irradiation under aerobic conditions rules out any involvement of the classical oxygen effect (reduction of the number of oxidizing radicals formed during radiation in the absence of oxygen).

On the bases of cytological[4,5] and biochemical[6–8] observations the post-irradiation loss of nuclear structure in thymocytes can be attributed to a phosphate-induced separation of histones from the deoxyribonucleic acid of the interphase chromatin. Such a separation would cause the highly condensed nucleoproteins of the chromatin granules and threads of the interphase nucleus to disaggregate into a structureless, basophilic mass[4,8].

During the first hour after irradiation, the ability of the thymocyte nucleus to synthesize oxidatively adenosine triphosphate is inhibited[9,10] while the uptake of inorganic phosphate and respiration are unaffected at this time[3,10]. The free phosphate which enters the nucleus can dissociate the histones from the irradiated (and certainly unstable[11]) deoxyribonucleoproteins[8]. Because phosphate uptake by such cells is propelled by respiration[12], any agent or condition which stops or slows respiration would be expected to prevent, or at least reduce, the loss of nuclear structure. This has already been shown to be true using respiratory inhibitors[3], and the present observations fully support this expectation.

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Radiation-induced Calcium Release and its Relation to Post-irradiation Textural Changes in Fruits and Vegetables

Loss in texture is the most characteristic change in fruits and vegetables on exposure to ionizing radiation[1–9]. Relatively low doses induce extensive softening in tissues. Consideration of the radiation-induced chemical changes in pectins—important structural elements of the middle

lamella[10]—led to the suggestion that their *in situ* degradation may contribute to softening[11]. A decrease in protopectin and an increase in the pectin and pectate fractions of pears and peaches irradiated with 300, 600, or 900 krads of γ-radiation have been correlated with textural changes[12]. Glegg and Kertesz[13] considered that degradation of cellulose was probably a major factor in post-irradiation softening.

Although, in biological adhesion, the role of certain divalent cations, especially calcium, has long been known, it has received little attention in investigations of radiation-induced textural changes. Adhesion of cells has been reviewed in detail by Weiss[14]. Even though the data presented are, for the most part, based on animal tissue, many of them are relevant to plant tissue. Gelation is known to occur on addition of calcium ions to alginate sol; this results in cross-linking of molecular chains through the coulombic forces which exist between the free carboxylic group and the divalent calcium ion. The dependence of the strength of a gel on the concentration of calcium present is strikingly illustrated by data provided by Blood[15]. The middle lamella is considered to be a gel composed largely of insoluble calcium salts of pectic and low methoxyl pectinic acids. Other ions, forming sparingly soluble pectates, could be involved which stabilize the intercellular gel. Also, other substances of macromolecular origin certainly contribute to intercellular bonding. The evidence for the part played by divalent cations in intercellular bonding was provided by Letham[16,17]. The separation of cells from plant tissues on treatment with ethylenediamine tetraacetate (EDTA) or hexametaphosphate (HMP) was shown to be largely dependent on the chelation of calcium and possibly magnesium ions. After treatment with HMP, shaking results in considerable separation of cells from apple tissue. Addition of either divalent or trivalent ions before shaking results in the cells becoming recemented.

This communication describes the release of calcium from *Avena* coleoptiles and from peaches irradiated over a dose range of 50–1,000 krads. Irradiations were carried out at ambient temperatures. After irradiation, the tissues were transferred to a dialysing bag and suspended in conductivity water (190 ml.) and shaken (100 strokes per min). Changes in specific conductivity were measured with a conductivity bridge. Compared with the controls, the irradiated tissue very rapidly lost electrolyte, as was shown by increases in conductivity. In exploratory experiments, calcium was detected by reaction with glyoxal-*bis*-2 hydroxy-anil reagent and extraction with chloroform; the appearance of a red colour in the organic phase indicating the presence of calcium[18]. In quantitative experiments, the combined dialysate and washings from the dialysing bag were evaporated to dryness. The residue was digested with a mixture of perchloric and nitric acids (3 : 1), 5 ml. On evaporation to dryness, the residue was dissolved in water and diluted to 25 ml. The amount of calcium released was measured by the standard flame photometric method[19]. The amount of calcium retained in the tissue was proportional to the dose up to 600 krad, after which about 80 per cent of it was released. If the dose was increased beyond 600 krads, there was no significant increase in calcium release. This observation could be significant in investigations concerning the nature of intercellular bonding. The calcium release induced by irradiation is probably independent of enzymatic mediation. Irradiation at 0° C did not decrease the calcium release over the dose range investigated.

Experiments at the University of Michigan[20] with tomato leaves which had absorbed calcium-45, and with detached strawberries which had been stem-fed with the same isotope, independently substantiate these results.

Release of calcium also occurred in model experiments in which calcium pectate was suspended in water and irradiated. Furthermore, a certain degree of decarboxylation was observed on irradiation of a pectic acid–water suspension, as was evinced by the reduction in neutralization number. Such results indicate that the electrovalent links between calcium or other divalent cations and the carboxyl groups of pectic acid are broken on irradiation. This could result in loss of adhesion between cells of a tissue. Even though covalent bonds exist in the middle lamella which possibly hold together other macromolecules, it can be concluded that the release of calcium plays an important part in radiation-induced softening of fruit and other plant tissue. A complete release of calcium is probably not obtained because of its involvement in other modes of combinations.

Although no explanation was offered by Somogyi and Romani[12] for the relatively reduced softening of tissue in a nitrogen atmosphere, it is likely that in aerobic irradiation ozone and free radicals associated with the presence of oxygen are more critical factors. A period of anaerobiosis will rapidly increase the covalent bonding, which in turn will affect the strength of the tissue concerned[21,22].

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Strontium-90 in Beagle Hair

IN 1963, Hopkins *et al.*[1] reported the accumulation of significant amounts of strontium-90 in the hair of rats which had acquired known body burdens of the radionuclide either by injection or *in utero*. They further suggested that the analysis of hair could provide a non-destructive method of measuring bone seeking radioelements. The Davis Beagle Project offered a unique opportunity to test this hypothesis as the diet and the gut-kidney discrimination factors are well controlled and known. Accordingly, we assayed the concentration of strontium-90 in the hair of beagles while the animals were on a chronic ingestion regimen of the radionuclide, at constant ratios of strontium-90 : calcium, as well as afterwards. The concentration of strontium-90 in the hair was followed up to 1 year after we stopped feeding the diet containing strontium-90, and was correlated with the ratios of strontium-90 : calcium for plasma during the same periods.

Table 1. STRONTIUM-90 IN BEAGLE HAIR

Level*	No. dogs	g Ca/g ash	μc.^{90}Sr/g Ca			Observed ratio†	
			Hair	Plasma	Diet	Hair/plasma	Hair/diet
D3	3	0·12	0·16	0·17	0·35	0·9	0·46
D4	1	0·13	0·31	0·30	1·11	1·0	0·28
D5	12	0·12	1·38	1·33	3·20	1·0	0·43
Mean		0·12				1·0	0·42
Range		(0·11–0·15)				(0·6–1·5)	(0·23–0·65)

* The dietary levels *D3*, *D4*, *D5* are maintained at a 1 per cent calcium content (by weight) with a calcium/phosphorus ratio of 1·5 and a three-fold increment in the ratios of strontium-90 : calcium.

† The value of the observed ratio is given by the $\frac{\text{Sr-90/Ca sample}}{\text{Sr-90/Ca precursor}}$

Samples of beagle hair (5–20 g) were obtained by clipping the hair of the lower neck and upper chest, and in a few cases hair was taken from the flanks. Repeat samples of hair were obtained from the same areas at about 100-day intervals, or when hair regrowth had completely blended with the surrounding hair. The hair was weighed dry, washed with water containing a detergent, and rinsed with tap water, then deionized water, and finally 1 N hydrochloric acid. After drying, the hair was ashed at 600° C for about 12 h, weighed and dissolved in 1 N hydrochloric acid to a standard volume. The strontium-90 and calcium content of hair and plasma were determined on the ashed samples using methods described earlier[2].

The deposition of dietary strontium-90 in the hair of beagles during exposure to three ingestion levels of the radionuclide is shown in Table 1. The calcium content of hair is about 12 per cent of the ash weight, or 0·04 per cent of the fresh weight. During the course of the chronic ingestion of the radionuclide, the ratios of strontium 90 : calcium for the plasma and hair are essentially equal and constant. The strontium-90 : calcium ratios of plasma relative to those of the diet represent an overall reduction by a factor of more than two resulting from the combined gastro-intestinal and renal discriminatory processes[3]. The value of the observed ratio of bone : diet in the beagle has been found to be about 0·45. Thus, it seems that the ratio of strontium-90 : calcium for plasma governs the ratio of strontium-90 : calcium for other compartments, that is, hair and bone, unless there are other intermediate and intervening membrane barriers which result in further discrimination, as in the placenta or mammary gland.

The relative specific activities, in μc. strontium-90/g calcium, of plasma and hair during and after the chronic ingestion of strontium-90 are shown in Fig. 1. The data for all dogs are presented as mean values and ranges which have been normalized to the highest ingestion level (*D5*) by multiplication of the values by the appropriate factors which separate the strontium-90 concentrations of the different dietary levels. Thus, the ratios of strontium-90 : calcium for hair and plasma were nearly constant at 1·5 μc. strontium-90/g calcium with the observed ratio of 0·45 for all dietary levels.

After we stopped feeding the diet containing strontium-90, there was a rapid decrease in the strontium-90 : calcium ratio of plasma to a normalized value of 0·1 μc. strontium-90/g calcium at 200 days after feeding the animals the radionuclide (740 days of age). In contrast, the ratio of strontium-90 : calcium for hair obtained at consecutive 100-day intervals was consistently greater than that of plasma taken at the same time, so that at 1 year after feeding with strontium-90 the ratio of strontium-90 : calcium for the hair was about 1·5–2 times higher than that for the plasma.

Because of the integral nature of hair growth, the ratios of strontium-90 : calcium for hair and bone will demonstrate similar relative specific activities during the chronic feeding of the radionuclide and, following the end of feeding, the ratios of strontium-90 : calcium for hair will reflect the continually decreasing relative specific activities of plasma during the period of growth. Furthermore, the hair : plasma ratio will increase as a function of the interval between clippings if the plasma is sampled at the time of clipping. However, if the plasma were sampled at the midpoint of the growth period these differences would be considerably smaller.

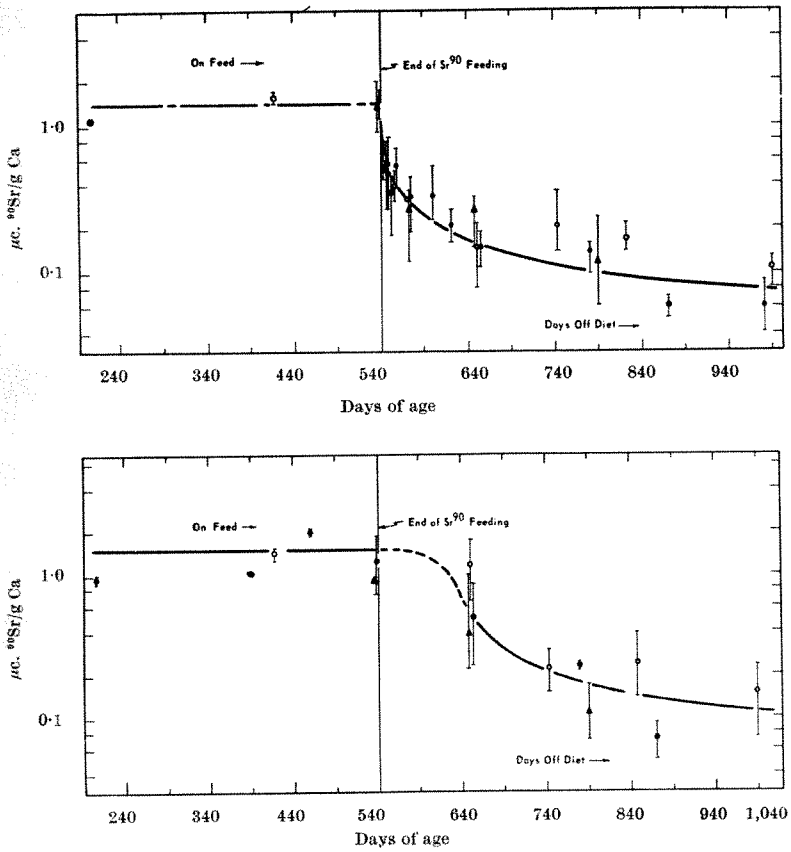


Fig. 1. Mean normalized "*D5* level" plasma (top) and hair (bottom) concentrations, μc. strontium-90/g calcium, while on strontium-90 feed and following the end of the period during which strontium-90 was fed to the animals. Mean values and ranges for each ingestion level obtained from six dogs *D3* level (○); eleven dogs *D4* level (▲); and nine dogs *D5* level (●)

The accumulation of strontium-90 in hair during periods of exposure to the radionuclide affords the opportunity of estimating the strontium-90 concentration of the diet. If all the factors in the metabolism of strontium relative to its transfer from diet to blood, and then to bone and hair, are fully understood, this form of non-traumatic bio-assay offers a less variable approach to strontium-90 contamination investigations than either excreta or diet investigations. The usefulness of hair in a bio-assay method could be extended to other radionuclides, for example caesium-137, as a possible index of body burden and dietary concentration.

In the Davis experimental beagles, the constant relationships between the ratios of strontium-90 : calcium in the diet, plasma, bone, and hair during the period of continual strontium-90 feeding affords an estimate of the strontium-90 concentration in the body. When the dog is taken off the dietary strontium-90, the uniformly labelled skeleton provides an endogenous source of the radionuclide, by way of the plasma to the hair, and thus supplies an indirect assessment of that portion of the skeleton which undergoes remodelling during the period of hair growth.

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SOIL SCIENCE

Fluoride Interaction with Clays in Relation to Third Buffer Range

Considerable acceptance of a ready exchange of structural OH of clay minerals by fluoride ions has been reported[1]; however, so far the evidence rests mainly on the appearance of OH in an extraction solution of potassium fluoride. Recently it was found[2] that the hydrochloric acid titratable OH formed on the reaction of clay minerals and soils in neutral 1 N potassium fluoride solution results from the complexing reaction by fluoride ions and dissolution of structural aluminium or iron from the minerals, by a reaction that can be represented as:

$$Al(OH)_3 + 6KF \rightarrow K_3AlF_6 + 3KOH \qquad (1)$$

No fluoride that could be analytically detected remained in the clay or soil sample. A series of carbon replicas of micas shows (Fig. 1) that biotite and muscovite surfaces are disrupted by the reaction in neutral 1 N potassium fluoride solution for 12–24 h. Furthermore, potentiographic evidence reveals that, on disruption of the surfaces of the sesquioxic and the alumino-silicate minerals, acidic functional groups such as $^{4}X\text{Al–OH}_2$ and $X\text{Fe–OH}_2$ develop in the minerals and this results in the appearance of a third buffer range[5]. This indicates that the third buffer range not only arises from the neutralization of excess protons of Al–OH_2 groups in the positively charged hydroxy aluminium polymers formed in the interlayer surfaces of expansible clays, but also from sesquioxic surfaces in general. The reaction of the titrating base with the substituted hydronium surface or edge groups ($X\text{M}^{3+}\text{–OH}_2$) of the oxides of iron, hydroxide of aluminium and the alumino-silicates (including allophane) is as large as 50–100 m.equiv./100 g. This third buffer range is of considerable importance in mid-range soil acidity and agricultural liming practice as well as in the retention of sodium in alkaline soils.

The present investigation showed that third buffer range developed in the titration with sodium hydroxide of $Al(OH)_3$ (Merck) and natural gibbsite ($< 20\mu$) treated with potassium fluoride could be reversibly titrated with hydrochloric acid (Fig. 2, *b* versus *c*). After the reaction of sodium hydroxide with the proton–donor groups on the natural gibbsite treated with potassium fluoride, the mineral suspension was sedimented by centrifugation and washed five times with an ethanol–water mixture as is usual in the measurement of cation exchange capacity. The third buffer range again appeared in the sodium hydroxide titration of the washed residue (Fig. 2*d*), which shows that the sodium is only weakly held and readily hydrolysed in water. These results further strengthen the argument that the third buffer range which appeared in the sodium hydroxide titration curves of minerals made to interact with potassium fluoride resulted from the neutralization of the proton–donor groups on the mineral surfaces[2,4], and proceeded by way of equation (2):

$$\text{Mineral Al (or Fe)} \begin{matrix} \text{OH}_2^{0.5+} \\ \text{OH}^{0.5-} \end{matrix} + \text{NaOH} \longrightarrow$$

$$\text{Mineral Al (or Fe)} \begin{matrix} \text{OH} \\ \text{OH} \end{matrix} \text{Na} + \text{H}_2\text{O} \qquad (2)$$

In addition, fluoride-saturated 'Dowex 21*K*' (*RF*) was used to interact with the hydrogen–aluminium vermiculite (2–0·2μ in diameter). The mixture of clay and 'H–Amberite, *IR* 120' (*HR*) was shaken for 2 h, while that of clay and *RF* was shaken overnight. The clay suspension was separated by suction on a glass filter. Potassium

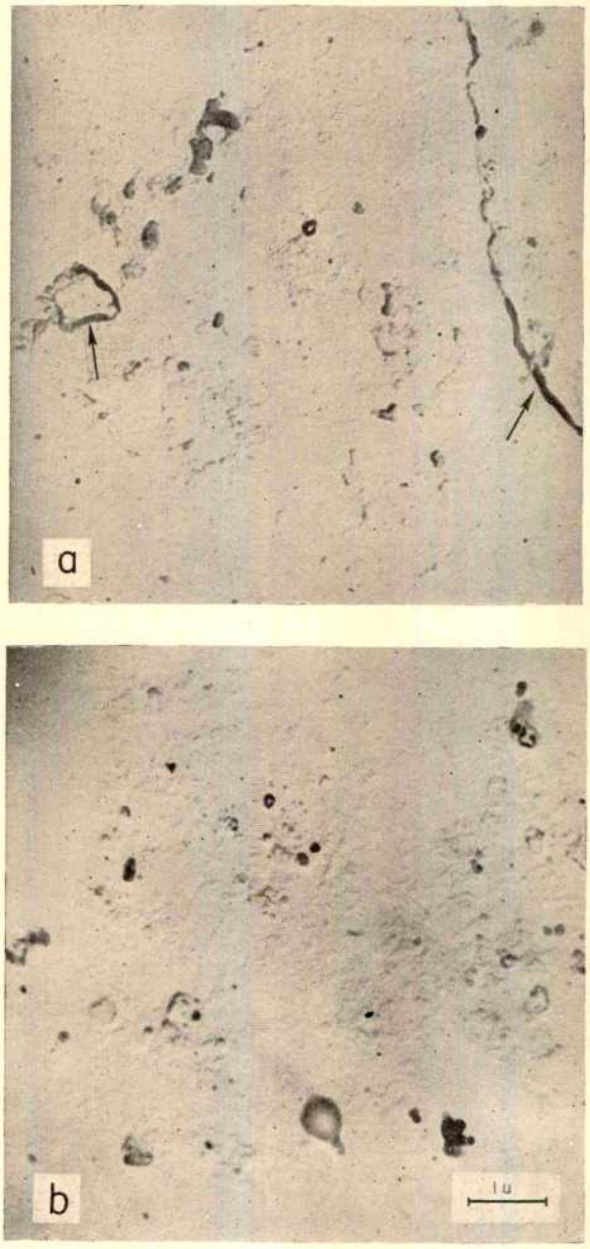


Fig. 1. Electron micrograph of carbon replica of the cleavage surfaces of (*a*) biotite and (*b*) muscovite, showing the corrosion and fractures caused by interaction with neutral 1 N potassium fluoride for 12 and 24 h, respectively. Scrolling (arrows) results from fractures as shown by Raman and Jackson (ref. 3), together with smooth untreated biotite and muscovite cleavages. (We thank Charles Roth for his assistance in the electron microscopy)

nitrate was then added to make 1 N potassium nitrate and 0·5 per cent clay in the suspension. The suspension was shaken for 1 h before it was titrated with sodium hydroxide. (The titration apparatus used was a Metrohm potentiograph; the speed of titration was 0·0065 m.equiv./min.) The titration curves (Fig. 3) show that the total base consumption of the clay, created by *HR* interaction, was one-third reduced on the interaction of *RF*. This can be attributed to the removal from the clay of aluminium or iron that can be complexed to form fluoraluminate or fluorferrate[6] in solution, and to release OH which then reacts with part of the acid clay. The decrease in acidity of the hydrogen–aluminium clays by *RF* interaction is compensated on treating them again with *HR*—

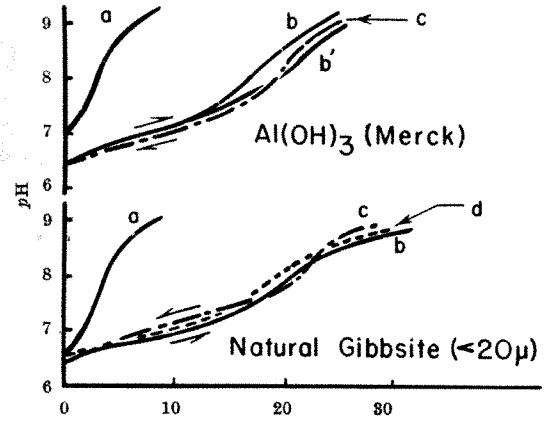


Fig. 2. Potentiograms showing the effect of fluoride treatment on the titration curves of gibbsite suspended in 1 N potassium chloride. *a*, Control, with no potassium fluoride treatment, sample washed with potassium chloride and then with water–ethanol, and then titrated with sodium hydroxide. *b*, Sample washed with potassium chloride, reacted with neutral 1 N potassium fluoride for 24 h, and then washed with water–ethanol and titrated with sodium hydroxide. *b'*, Duplicate of *b*. *c*, Sample treated as in *b* and then back titrated with hydrochloric acid (curve plotted backward, arrows). *d*, Sample treated as in *b* then washed with water–ethanol and titrated again with sodium hydroxide, showing that the full acidity was restored by hydrolysis during washing

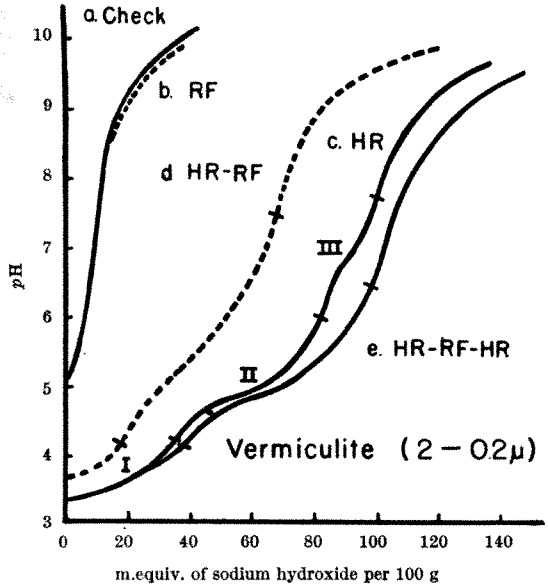


Fig. 3. Potentiograms showing the influence of hydrogen resin (*HR*) and fluoride resin (*RF*) treatment on the sodium hydroxide titration curves of vermiculite suspended in 1 N potassium nitrate. *a* and *b*, Controls, with no treatment with *HR* and *RF* for (*a*) and sample (*b*) showing no reaction with *RF* during 24 h. *c*, Sample reacted with *HR* for 2 h. *d*, Sample treated as in *c* and then reacted with *RF* for 24 h. *e*, Same as *d* followed by another treatment with *HR*; note that the third range has been removed by the *RF* treatment (curve *e* compared with curve *c*)
Proton donor ranges: I, OH_2^+; II, $Al(OH_2)_6^{3+}$; III, $[Al_x(OH)_{3x-n}(OH_2)_m]^{n+}$

through saturation of all cation exchange sites of the clay with hydrogen (and aluminium released the spontaneous alteration[7] of hydrogen clays to aluminium clays).

The results obtained in the present investigation indicate that the interaction of fluoride resin with hydrogen–aluminium clays brings about the removal of acidic functional groups from the minerals, particularly the third range (Fig. 3, curve *e*). In contrast, the reaction of neutral 1 N potassium fluoride resulted[2] in the formation of the proton–donor groups on the mineral surfaces of the third range. The *RF* action is considered to be predominantly that of removal of polyalumina which readily forms[5,8] in acid vermiculite, while the potassium fluoride rapidly removes trivalent ions from the structure, which creates weakly acidic XM^{3+}–OH_2 groups on the surfaces and at the same time forms an excess of potassium hydroxide (*p*H 8–11) by equation (1). We attribute the third range buffering[5] (Fig. 3), which resulted from treatment of aluminosilicate clays with *HR*, to alumina surface groups rather than to aluminosilicate surface characteristics. This conclusion is based on the large third range buffering which arose on the surfaces of pure iron oxides and alumina[2] (Fig. 2) in the absence of a silica component.

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Toxicity of Boron to Rice

IN the course of a study on the chemical retardation of soil reduction as a remedy for physiological disorders of the rice plant, we observed that despite this retardation by calcium nitrate and manganese dioxide the foliar symptoms persisted in one of the four soils studied.

The symptoms were intervenal chlorosis of the upper portions of the older leaves, especially along the margins, and later large dark brown elliptical spots in the affected parts which ultimately turned light brown, dried and rolled up. They were almost identical with the symptoms of boron poisoning in rice grown in culture solution as described by Lockard[1]. The boron content of the straw at harvest was 80 p.p.m. compared with 26–30 p.p.m. for normal plants.

The soil was a saline silt loam from the Silo region on the coast of Taiwan and had a *p*H of 8·1. Soil solutions of the flooded soil contained 9·1 p.p.m. boron (this figure contrasts with 1 p.p.m. boron for two normal Philippine lowland rice soils).

To ascertain whether the symptoms observed in the Silo silt loam were indeed those of boron toxicity, the same variety of rice was grown on two lowland rice soils (Maahas clay—*p*H, 6·6; organic matter, 2·0 per cent and Luisiana clay—*p*H, 4·6: organic matter, 4·1 per cent treated with 0, 20, 40 and 60 p.p.m. of boron given in the form of borax and on untreated Silo silt loam.

Table 1. INFLUENCE OF ADDED BORON (AS BORAX) ON THE CONCENTRATION OF WATER-SOLUBLE BORON (P.P.M.) AND THE SEVERITY OF SYMPTOMS OF BORON TOXICITY TO RICE ON THREE SOILS

Soil	Added boron as p.p.m. of the dry soil							
	0		20		40		60	
	Boron	Symptoms	Boron	Symptoms	Boron	Symptoms	Boron	Symptoms
Silo silt loam	9·1	Moderate	—	—	—	—	—	—
Maahas clay	0·9	None	9·3	Slight	17·6	Moderate	25·6	Marked
Luisiana clay	0·6	None	6·7	Moderate	12·9	Marked	20·1	Very marked

Within a week, the symptoms characteristic of boron poisoning in solution culture were observed in the plants treated with 60 p.p.m. boron. The same symptoms, but in a milder form, appeared a week later in plants grown in the untreated Silo silt loam and in those grown in the Maahas and Luisiana soils treated with 20 and 40 p.p.m. boron. The foliar symptoms observed on plants grown on Silo silt loam were comparable with those on Luisiana soil, to which 20 p.p.m. boron or 6·7 p.p.m. water-soluble boron had been added. Table 1 shows that, although the concentration of water-soluble boron was lower in Luisiana clay than in Maahas clay at all levels of added boron, the toxic effects were more severe in the former. This means that the critical toxic concentration of water-soluble boron for rice may vary from soil to soil.

As coastal soils derived from marine sediments may contain from ten to fifty times as much boron as inland soils[2], boron poisoning may be a hitherto unsuspected factor limiting rice production on such soils.

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AGRICULTURE

Identification of Gibberellins A_4 and A_7 in Immature Apple Seeds

FOR many years, plant physiologists have attempted to isolate and identify the growth substances which control plant growth. Many early attempts failed, largely because of the minute quantities of these compounds present in plants. Recently, microtechniques such as thin-layer chromatography have greatly stimulated interest in their identification.

Luckwill[1] investigated the auxins present in apple seeds, but did not identify them chemically except to report the absence of indole-acetic acid[2]. Nitsch[3] demonstrated the presence of gibberellin-like substances in apple seeds, and Kato and Ito[4] extracted such substances from apple shoots. On the basis of relative mobility values for the gibberellins in paper chromatography, MacMillan *et al.*[5] postulated that the apple seed gibberellins demonstrated by Nitsch might be A_5 and A_9.

In an attempt to identify some of the growth substances present in apple seeds, approximately 2 tons of fruitlets of the 'Golden Delicious' variety were collected from a commercial orchard south of Paris between June 14 and 26, 1961. At this time, the fruitlets were approximately 3·5 cm in diameter. The fruitlets were kept in storage at a temperature of 3° C until the seeds could be removed, a process requiring 5 weeks from the first date of collection.

The bioassays used to detect gibberellin activity in the extract were the *Avena* mesocotyl test[6,7], the dwarf pea test[8], the lettuce hypocotyl test[9,10], and the cucumber hypocotyl test[11–13]. The *Avena* mesocotyl test was performed as described by Nitsch and Nitsch[6].

Pea seeds, variety 'Little Marvel', were planted in soil in the greenhouse, 50 μl. of the solutions to be bioassayed were applied to the first true leaf 6–8 days after planting, and the first three internodes above this leaf were measured 5 days after treatment.

Lettuce seeds, variety 'Parris Island', were placed on filter paper disks in vials 19 mm in diameter containing 0·3 c.c. of the solution to be bioassayed. The hypocotyls were measured after 5 days of continuous illumination (450 ft.-candles) at 25° C and 100 per cent relative humidity. Ten hypocotyls were measured in each of two vials for each treatment.

Cucumber seeds, variety 'Spartan 27', were germinated on moist filter paper under continuous light (450 ft.-candles) at 25° C and 100 per cent relative humidity. After 3 days, the seed coats were removed and the seedlings planted in vermiculite under the same environmental conditions. Twenty-four or 48 h after transplanting, 20 μl. of the solutions to be bioassayed were applied to the epicotyl and adjacent cotyledons, and the length of the hypocotyl measured 2 days after treatment.

'Tween 80' (0·1 per cent) was included as a surfactant in all solutions, and all control plants were treated with this concentration of 'Tween'.

On removal from the fruits, the seeds were dropped into cold methanol and transferred to a freezer at −20° C. The seeds were ground and extracted repeatedly with fresh methanol, the methanol filtered through paper and evaporated *in vacuo*, and the tissue discarded. The aqueous residue remaining after removal of the methanol was frozen and lyophilized. The residue was dissolved in water, the *p*H adjusted to 3·0 with hydrochloric acid, the solution exhaustively extracted with diethyl ether, and the water phase discarded. The ether was evaporated *in vacuo* and the residue chromatographed (ascending) on 'Whatman No. 17 filter paper using isopropanol/water/ammonia (80:19·9:0·1) as the developer. The area of the chromatograms active in the *Avena* mesocotyl test (R_F 0·5–1·0) was eluted with 80 per cent methanol. The solvent was removed *in vacuo*, and the residue chromatographed in batches on a column of silica gel 22 mm in diameter and 140 mm long, using increasing concentrations of secondary butanol in hexane, both solvents having been previously equilibrated with 0·5 formic acid[14]. Two hundred and fifty c.c. of each of the following solvents were used in elution: 0·5, 10, 25, and 100 per cent secondary butanol in hexane. The first 75 c.c. of the 10 per cent solvent contained approximately one-half of the gibberellin activity (dwarf pea test). This fraction (fraction *A*) was reduced to dryness *in vacuo*, the residue dissolved in water, and the suspension, containing a green precipitate, filtered. The filtrate was evaporated to dryness, and the residue extracted with ethyl acetate. The ethyl acetate fraction, containing the major part of the gibberellin activity, was reduced to dryness (fraction *B*.

Table 1. ELUTION PATTERN OF GIBBERELLINS IN COLUMN CHROMATOGRAPHY ON SILICA GEL

Gibberellin	Solvent (percentage of *sec*-butanol in hexane)				
	0·5	5	10	25	50
A_1				X	
A_2				X	
A_3				X	
A_4		X			
A_5		X	X		
A_6		X	X		
A_7	x	X	x		
A_8					X
A_9	X				
Fraction *A*		X	x		

Activity detected by bioassay of eluates in dwarf pea test. *X*, major activity; *x*, minor activity.

Table 1 compares the elution patterns of the nine gibberellins with that of fraction *A* in column chromatography on silica gel as described above, with the following modifications: column 14 × 140 mm; 100 c.c. per solvent. The residue from each solvent was bioassayed, using the dwarf pea test. The gibberellin activity in the extract coincided with that of gibberellins 4, 5, 6, and 7, which could not be separated with this series of solvents. Activity was clearly separated from that of the remaining five known gibberellins.

Table 2. COMPARISON OF ACTIVITIES OF NINE GIBBERELLINS AND FRACTION *B* IN THREE BIOASSAYS
Height in millimetres

Gibberellin*	Bioassay: Dwarf pea	Lettuce hypocotyl	Cucumber hypocotyl
A_1	57 *cd*	6 3 *c*	24*a*
A_2	42*ab*	4·1*a*	27*a*
A_3	90 *e*	9 9 *e*	27*a*
A_4	42*ab*	8·1 *d*	48 *c*
A_5	48 *bc*	7·5 *d*	26*a*
A_6	44*ab*	5·2 *b*	23*a*
A_7	67 *d*	11·9 *f*	56 *d*
A_8	38*ab*	3 6*a*	24*a*
A_9	36*ab*	5·9 *bc*	37 *b*
Fraction *B*†	44	9·5 *e*	37 *b*
Control	30*a*	3 5*a*	25*a*

* Each gibberellin used at 3×10^{-10} moles per plant (pea and cucumber) or per tube (lettuce).
† Used at 1 g equiv. per plant or tube (lettuce and cucumber) and at 0·56 and 5·6 g equiv. per plant (pea). Value for pea test adjusted to 1 g equiv. per plant by interpolation on semi-log scale.
abcdef, Means not followed by the same letter are significantly different from one another at the 5 per cent level.

The activity of fraction *B* is compared with that of the nine gibberellins in three bioassays in Table 2. The extract was active in all three assays, but activity in the cucumber hypocotyl assay is particularly noteworthy, since both A_4 and A_7 are active, while A_5 and A_6 are not. Hence it appeared that the unknown was either A_4 or A_7.

In order to characterize further the unknown, thin-layer chromatography was used according to the method of MacMillan and Suter[15]. Silica gel *G* was used as the adsorbent, and benzene–acetic acid–water (8 : 3 : 5) as the solvent. Plates 150μ thick were equilibrated over the lower phase overnight, then developed with the upper phase at 25° C. Gibberellins were detected by spraying the plates with 5 per cent sulphuric acid in 95 per cent ethanol, heating for 15 min at 110° C, and noting the pattern of fluorescence under ultra-violet light. Considerable variability was encountered from one set of plates to another using this system. Each set of plates was first checked with a mixture of gibberellins A_4 and A_7 to assure that separation would occur. When separation was obtained, a sister plate was used to compare the R_F's of the known gibberellins with that of the apple gibberellin(s).

Fig. 1 shows the appearance of such a plate after spraying with the reagent and heating. The extract contained two distinct fluorescent areas corresponding closely with the R_F's of gibberellins A_4 and A_7 and separated clearly from gibberellins A_5 and A_6. Additional weak fluorescent

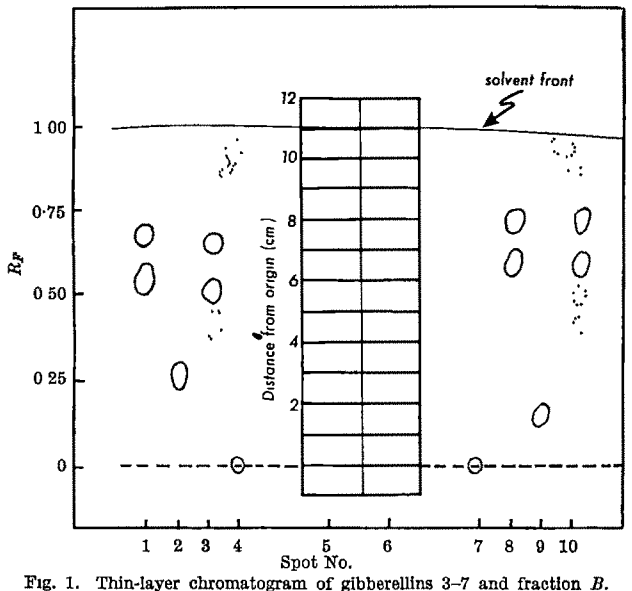


Fig. 1. Thin-layer chromatogram of gibberellins 3–7 and fraction *B*. Spots 1, 5 and 8, mixture of GA_4 and GA_7, 0·4 μg total per spot; 2, GA_5, about 1·0 μg; 3, 6, and 10, fraction *B*, 10 g equiv. per spot; 4 and 7, GA_3, 0·4 μg per spot; 9, GA_6, about 1 0 μg. Centre area covered by glass plate before spraying with 5 per cent sulphuric acid in ethanol and heating. Areas which fluoresced under ultra-violet light after heating are indicated by circles

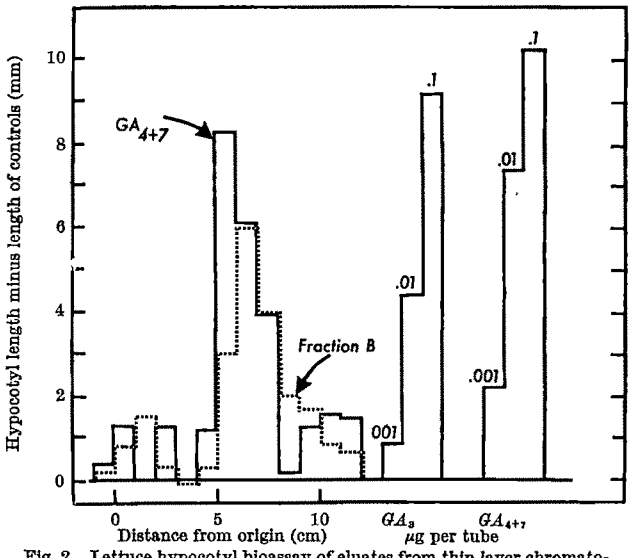


Fig. 2. Lettuce hypocotyl bioassay of eluates from thin layer chromatogram of GA_{4+7} (solid line) and fraction *B* (broken line)

areas appeared just below the position of GA_7 and near the solvent front. The central portion of the chromatogram, to which (*a*) the unknown, and (*b*) a mixture of gibberellins A_4 and A_7 were separately applied, was covered with a glass plate before spraying, and the edges sealed with tape. After spraying, sections of silica gel 1 cm in height by 2 cm in width were scraped from the plate (Fig. 1) and individually extracted with 1 ml. of 0·1 per cent 'Tween 80'. The eluates were filtered, the filtrates frozen and lyophilized, and the residues dissolved in 1 c.c. of water. These solutions were bioassayed in the lettuce hypocotyl test, using 0·3 c.c. of each solution, and the mean length of ten hypocotyls is shown graphically for each section in Fig. 2. Gibberellin activity occurred at the R_F's of A_4 and A_7 in both the extract and in the GA_{4+7} mixture, although the width of the sections used did not allow for separation of the two in the bioassay. The other fluorescent zones in the extract were inactive. The unknown is therefore a mixture of A_4 and A_7.

Since gibberellin activity is not confined to the fractions described above, work is continuing in an attempt to identify some of the more polar gibberellin-like substances present in the extract.

We thank the Pépinières Thuilleaux for the apple fruitlets, Dr. P. W. Brian and Dr. J. MacMillan for samples of gibberellins A_1 to A_9, and Abbott Laboratories, North Chicago, Illinois, for a larger quantity of gibberellin A_3. This investigation was supported in part by a grant from the National Science Foundation.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (preferably with a degree or equivalent qualification in biochemistry) to work on nucleic acid metabolism in cancer tumours—The Assistant Secretary General (S5), Brunel University, Woodlands Avenue, Acton, London, W.3 (August 19).

SENIOR LECTURER IN THE DEPARTMENT OF BACTERIOLOGY—The Registrar, The University, Liverpool, 3, quoting Ref. CV/169 (August 19).

ASSISTANT LECTURER or LECTURER IN VETERINARY MEDICINE (Small Animals)—The Registrar, The University, Liverpool, quoting Ref. CV/177/N (August 20).

JUNIOR RESEARCH FELLOWS (2) IN BOTANY—The Registrar, The University, Sheffield (August 20).

LECTURER IN PHARMACEUTICS to teach to honours degree standard and to undertake research—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, quoting Ref. 490/6 (August 20).

SENIOR RESEARCH ASSISTANT (with a science or engineering degree) IN THE DEPARTMENT OF ENGINEERING SCIENCE, to carry out research in the field of thermodynamics in collaboration with Prof. Russell Hoyle—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (August 20).

RESEARCH OFFICERS or SENIOR RESEARCH OFFICERS (2), and a RESEARCH ASSISTANT IN THE COMPUTING CENTRE, to assist in computer programming arising from research in all departments of the University—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 21).

SENIOR TECHNICIAN (with experience in electronics) IN THE DEPARTMENT OF CHEMISTRY for assistance with research in electron spin resonance spectroscopy—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 22).

TEMPORARY ASSISTANT LECTURER (preferably with a special interest in political and moral philosophy) IN PHILOSOPHY—The Registrar, The University, Manchester, 13, quoting Ref. 180/66 (August 24).

ASSISTANT LECTURER or LECTURER IN PSYCHOLOGY—The Registrar, The University, Keele, Staffordshire (August 27).

EXPERIMENTAL OFFICER (with a degree or equivalent qualification in physics, chemistry, or in an appropriate technological subject, and experience in physical methods of analysis) IN THE ELECTRON PROBE AND X-RAY SECTION OF THE DEPARTMENT OF EARTH SCIENCES—The Secretary, Department of Earth Sciences, The University, Leeds, 2 (August 27)

LECTURER IN PHYSICAL OR INORGANIC CHEMISTRY—The Assistant Registrar (Science and Engineering), The University of Birmingham, Birmingham 14 (August 27).

RESEARCH ASSISTANT IN THE SOLID-STATE ELECTRONICS GROUP OF THE DEPARTMENT OF ELECTRICAL ENGINEERING AND ELECTRONICS for work on metal-semiconductor junctions, under the direction of Prof. E. H. Rhoderick—Prof. E. H. Rhoderick, Department of Engineering and Electronics, The University of Manchester Institute of Science and Technology, Manchester, 1 (August 30).

ASSISTANT LECTURER IN AERONAUTICAL ENGINEERING—Prof. A. V. Stephens, The Queen's University, Belfast, Northern Ireland (August 31).

LECTURER IN VETERINARY PARASITOLOGY IN THE SCHOOL OF TROPICAL MEDICINE—The Registrar, The University, Liverpool, quoting Ref. CV/187/N (August 31).

LECTURER or ASSISTANT LECTURER IN MICROBIOLOGY IN THE DEPARTMENT OF BOTANY—The Registrar, Queen Mary College (University of London), Mile End Road, London, E 1 (August 31).

RESEARCH ASSISTANT (with an honours degree in zoology and preferably a special interest in electron microscopy or nerve physiology) IN THE MARINE SCIENCE LABORATORIES, to work with Dr. D. A. Dorsett on the fine structure of invertebrate nervous systems—The Registrar, University College of North Wales, Bangor, North Wales (August 31).

RESEARCH OFFICER IN THE DEPARTMENT OF PHYSICS to investigate damage occurring in high-power laser systems—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (August 31).

ASSISTANT LECTURER (with special qualifications and research interests in petrology, geochemistry or economic geology) IN THE DEPARTMENT OF GEOLOGY—The Registrar, The University, Nottingham (September 1).

JUNIOR LECTURER IN BACTERIOLOGY—The Secretary, Trinity College (University of Dublin), Dublin, Republic of Ireland (September 1)

LECTURER or ASSISTANT LECTURER (with an honours degree in engineering or mathematics and some experience in structural and/or machine vibration problems) IN THE INSTITUTE OF SOUND AND VIBRATION RESEARCH—The Deputy Secretary, The University, Southampton (September 1).

LECTURER or DEMONSTRATOR (with interests in the genetics and biochemistry of micro-organisms) IN THE DEPARTMENT OF GENETICS—Dr. Sydney Smith, Department of Zoology, The University, Downing Street, Cambridge (September 1).

LECTURER (with good academic qualifications and postgraduate experience, a special interest in the analysis and design of power generation and distribution systems, and familiarity with modern methods of analysis and simulation of systems, using computer techniques) IN ELECTRICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (September 1)

RESEARCH SCIENTIST or SENIOR RESEARCH SCIENTIST (Physicist) (with a Ph.D. degree in physics or postgraduate experience of equivalent standard and duration, supported by satisfactory evidence of research ability) IN THE DIVISION OF APPLIED PHYSICS, Commonwealth Scientific and Industrial Research Organization, located at the National Standards Laboratory, University Grounds, Chippendale, Sydney, Australia, to undertake magnetic research—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 750/364 (September 3).

UNITED STEEL COMPANIES FELLOW (engineering graduate) IN MECHANICAL ENGINEERING for research in the field of fluids or fluid power control—The Registrar, The University, Sheffield (September 3).

SCIENTIST (with a Ph.D. or honours degree, preferably with some additional experience in ruminant nutrition) AT RUAKURA AGRICULTURAL RESEARCH CENTRE, New Zealand, to assist in a research programme in dairy cattle nutrition aimed at evaluating pasture as a feedstuff for growing, lactating and fattening stock and at measuring the voluntary intakes of grazing stock, the factors which affect these and the feed requirements of grazing animals—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B13/2/73/2823 (September 5).

POSTDOCTORAL FELLOW IN THE DEPARTMENT OF CHEMISTRY to work on new methods of peptide synthesis in collaboration with Dr. C. J. M. Stirling—Dr C. J M. Stirling, Department of Chemistry, King's College (University of London), Strand, London, W.C.2 (September 9).

LECTURER or SENIOR LECTURER (preferably specializing in bacterial or virus diseases of plants) IN PLANT PATHOLOGY IN THE DEPARTMENT OF AGRICULTURAL BIOLOGY, University of Ibadan, Nigeria—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 10).

SENIOR LECTURER (graduate in animal production, agriculture or science, with postgraduate specialization in any one of the major branches of animal production) IN ANIMAL PRODUCTION at the University of the West Indies, Trinidad—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 12).

LECTURER or ASSISTANT LECTURER (interested in any of the fields of animal physiology) IN PHYSIOLOGY—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (September 15).

LECTURER or SENIOR LECTURER (with special interests in physical chemistry) IN CHEMISTRY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 16).

LECTURER (with a special interest in and preferably some practical experience in electrical power engineering) IN THE DEPARTMENT OF ELECTRONIC AND ELECTRICAL ENGINEERING—The Registrar, The University, Sheffield (September 16).

SENIOR LECTURER IN PHYSICS, and a SENIOR LECTURER IN THEORETICAL PHYSICS at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S W 1 (London and Brisbane, September 16).

UNIVERSITY LECTURER (preferably with an interest in experimental taxonomy) IN BOTANY and Curator of the Claridge Druce Herbarium—The Administrator, Botany School, The University, South Parks Road, Oxford (September 20).

RESEARCH FELLOW IN THE SEDIMENTOLOGY RESEARCH LABORATORY OF THE GEOLOGY DEPARTMENT, to conduct an investigation into the sedimentology (including geochemistry, physical properties, and clay mineralogy) of facies variation in the Great Oolite Series of the Malmesbury area—The Registrar (Room 39, O.R.B.), The University, Reading (September 30).

SENIOR LECTURER IN PSYCHOLOGY at the University of Western Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 1).

ASSISTANT PLANT CYTOGENETICIST or ASSOCIATE PLANT CYTOGENETICIST (with an M S. or Ph.D. degree or equivalent, and training in plant cytogenetics) IN THE DEPARTMENT OF GENETICS—The Director, Agricultural Research Station, University of Puerto Rico, Rio Piedras, Puerto Rico (October 31).

CHAIR OF MICROBIOLOGY (Medical) at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

AGRICULTURAL OFFICER/STATISTICIAN, Scientific Officer Grade (with a degree in agriculture, botany or allied subject with a sound knowledge of statistics or a degree in statistics with an interest in agriculture) for duties which will be concerned with the planning and analysis of scientific investigations undertaken by the Board—The Establishment Officer, Potato Marketing Board, 50 Hans Crescent, Knightsbridge, London, S.W.1.

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (with an interest in biophysics) to be responsible for the operation and development of techniques using the analytical ultracentrifuge—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 66 12.

BASIC GRADE BIOCHEMIST (university graduate in biochemistry or chemistry or A R.I.C.) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY for duties which will include routine steroid assays—The Clerk to the Governors, St. Bartholomew's Hospital, London, E.C 1, quoting Ref. No. ASC/112.

BIOCHEMICAL RESEARCH ASSISTANT to work on effects of stress in rats—Dr. G Curzon, Institute of Neurology, The National Hospital, Queen Square, London, W C 1.

BIOCHEMIST (Basic Grade, probationary or post-probationary) IN THE ASSISTED VENTILATION UNIT, for duties concerned primarily with blood gas analysis and biochemical aspects of investigation into metabolic disturbances, associated with respiratory failure—The Personnel Officer, Royal Infirmary, Edinburgh, 3

EDITORIAL ASSISTANT (with a degree in science and preferably some editorial experience) for the *Biochemical Journal*—The Editorial Board, The *Biochemical Journal*, 20 Park Crescent, London, W.1.

EXPERIMENTAL ECOLOGIST or LIMNOLOGIST IN THE DEPARTMENT OF BOTANY—Prof. N. P. Badenhuizen, Department of Botany, University of Toronto, Toronto 6, Ontario, Canada.

EXPERIMENTAL OFFICER (with a sound working knowledge of chemistry and experience of several instrumental methods) IN THE DEPARTMENT OF CHEMISTRY for work concerned with the development and application of instrumental analytical techniques and methods of relevance to chemical industry and new processes—The Registrar, The University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1, marking envelope "Experimental Officer—Analytical".

HISTOLOGIST (with an honours degree in physiology or zoology or an equivalent qualification and preferably previous experience of histological techniques) IN THE PHYSIOLOGY DEPARTMENT to be in charge of the histological work of the Department and to collaborate in research, mainly in the field of endocrinology—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 21/N/66.

HISTOLOGY TECHNICIAN (preferably with at least one year's experience in histology and with I.M L.T. or appropriate G C E. "A" level qualifications) IN THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY, Chester Beatty Research Institute, Fulham Road, London, S W.3—The Secretary, Institute for Cancer Research, Royal Cancer Hospital, 34 Sumner Place, London, S W.7, quoting Ref. 301/B/240.

PHYSICIST required for one year to develop some diagnostic uses of radioactive isotopes including scanning—The Secretary, St. Mary's Hospital Medical School, London, W 2.

RESEARCH ASSISTANT (with a degree or higher degree in aeronautical, civil or mechanical engineering, or physics, with some research experience) IN THE DEPARTMENT OF AERONAUTICS, to engage in fundamental research connected with the forces on buildings and structures due to atmospheric wind—Prof. P. R. Owen, Department of Aeronautics, Imperial College of Science and Technology, London, S.W.7.

RESEARCH FELLOW (with postdoctoral research experience and interested in the possibility of a permanent University appointment) IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY to assist Prof. K. A. Munday in an investigation into the effect of osmotic changes on biochemical properties of subcellular particles of invertebrates—The Deputy Secretary, The University, Southampton.

SENIOR TECHNICIAN or TECHNICIAN IN THE DEPARTMENT OF MEDICINE to be responsible for technical work for a research programme investigating the chemical control of respiration—Prof. W. I. Cranston, St. Thomas's Hospital Medical School, London, S.E.1.

TECHNICAL OFFICER (with a knowledge of electronics) IN THE DEPARTMENT OF GEODESY AND GEOPHYSICS to help with the design, construction and

maintenance of new instruments for research and to take part in short expeditions abroad on land and sea—Secretary of the Faculty Board, c/o Department of Geophysics, University of Cambridge, Madingley Rise, Madingley Road, Cambridge.

TECHNICIAN (experienced in histological techniques) as personal assistant on a research project in the DEPARTMENT OF ZOOLOGY (the work will involve tissue culture and histology)—The Registrar, The University, Liverpool 3, quoting Ref. 154/N.

REPORTS and other PUBLICATIONS

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THE NUMBERS GAME

It takes courage to go among physicists and suggest that the United Kingdom may be spending too much on nuclear physics. That is the first thing to be said about the Presidential Address by Sir Gordon Sutherland (see p. 792) to the Institute of Physics and the Physical Society (which should long ago have found a less cumbersome description for itself). It is also courageous to stand up in public and consider how and when to call a halt to the steady growth of spending, in the past two decades, on research and development. Lord Bowden did a lot to set this ball rolling during his stay at the Department of Education and Science. The Council for Scientific Policy, in its last report, took the process an important stage further by publicizing its preoccupation with the problems of balancing the limitless opportunities which exist for spending money against the understandable wish of the British Treasury that the pace of growth should be reduced. Obviously the council will find itself exposed to even greater pressures in the months ahead, and if it is to know when to yield gracefully and when to dig in its heels, it will have to be provided with a rational philosophy for deciding what should be spent on research and development. If what Sir Gordon has been saying should stimulate a search for the objective yardsticks which circumstances demand, much will have been accomplished.

But what yardsticks should there be? This is where the fun begins. One difficulty is that there is bound to be confusion between the questions of how to allocate resources between different branches of science and how to determine what proportion of a nation's entire wealth should be spent on science as a whole. Another is that there is no simple univalent rule for calculating how much to spend on research and development but, rather, an untidy jumble of incommensurable arguments, some of them in conflict with each other. Often it is only possible to establish lower limits to the amounts of money that should be spent on science and technology. Certainly the familiar yardstick which consists of the fraction of the Gross National Product spent on research and development is only the crudest rule of thumb. By making it the central pillar of his argument to the physicists, Sir Gordon Sutherland may have oversimplified the issues and—in the process—may have given comfort to the British Treasury.

There is no natural law to decree what proportion of a nation's wealth should be spent on science and technology. The fact that the United States and the United Kingdom spend roughly the same is not in itself an assurance that these are the proportions that sensible nations ought to spend. Everything depends on the goals they set themselves, and on the speed with which they seek to attain them. In any event, in the comparison between Britain and the United States, the absolute amounts of effort being spent may be more significant than the corresponding proportions of the G.N.P. Except in space research, where Britain has been mercifully modest, the smaller nation has not openly limited its ambition in science and technology. Should it not therefore spend on research and development an amount comparable to what is spent in the United States? In other words, should not the United Kingdom be spending 10 per cent of its G.N.P. on research and development? Certainly if it chooses to spend a mere 2·7 per cent, it should more openly acknowledge that this is only a poor man's pittance, and that a great many of the things which are done well in the United States cannot be done at all in Britain.

The comfortable arithmetic based on fractions of the G.N.P. conceals the sad truth that in British research and development, resources are spread too thinly, and that the most immediate need is to find some way of concentrating on a manageable portfolio of ambitions. On the long view, it may be necessary for smaller nations to acknowledge that the only way of keeping up with the Joneses is to spend a disproportionately large proportion of their wealth on scientific research and development. The time may come when really well-off nations spend their wealth on leisure—golf and foreign travel—while their less successful imitators have to keep on slaving away in the laboratories. In other words, there is no particular reason why the spending on science in Britain should now level off a respectful interval below the plateau (as a fraction of the G.N.P.) which seems to have been attained in the United States. Another way of putting this is to suggest that if spending on research and development is linked to anything, it is to the expected growth of the G.N.P. and not to its absolute amount.

In Britain, there are other reasons for believing that the growth in expenditure on research and development should continue for some time yet. For one thing, those engaged on research and development are not always fully provided with the resources which make for effective work. Not all laboratories are as well equipped as the National Physical Laboratory. Possibly there are similar problems in the United States, and statistics recently compiled by the OECD suggest that in 1962 the average spending for each qualified scientist engaged on research and development amounted to $40,000, compared with $30,000 in the United Kingdom. The difference is scarcely enough to account for the differences in rates of pay. Yet in both countries it is essential that the fullest use should be made of the limited stock of skilled manpower. In both places there is probably room for more growth in spending simply so that people now at work shall be effectively employed.

In Britain there is a specific reason why growth should continue for some years to come. The universities and similar institutions are at present turning out more than 20,000 qualified scientists each year, and it must be hoped that at least a quarter of these will find their way into research and development. Indeed, for some years to come, the annual increase of the labour force in research and development must be expected to grow by 7 or 8 per cent a year if the fullest value is to be derived from the expansion which the universities have been asked to undertake. In other words, the annual cost of research and development should be expected to increase by some £60 million even without the inflationary pressure of the steadily increasing cost of research and development of all kinds. Even if, as now seems likely, the G.N.P. does not change very much for some years to come, it would be folly to fail to make full use of the skilled manpower which has been expensively trained in recent years. There are ways of finding lower limits to what should be spent on research and development—of suggesting how governments should make proper use of resources which exist already, or which will exist in the years immediately ahead. But what of the more distant future? How many qualified scientists and engineers will Britain need in the eighties? How much will have to be spent each year if each is to work effectively? There are no ready answers to questions like these, yet there is no sign that the trend towards increasing dependence on science and technology which has been the history of the past three decades will now quickly come to a halt. Indeed, things point mostly in the other direction. It will be a great surprise if the proportion of the G.N.P. being spent on research and development comes suddenly to a halt, in Britain or in the United States, in 1966. In the months between now and Christmas, when British government departments will be trying to decide how money should be spent in 1967–68, the Council for Scientific Policy will be saddled with the task of persuading the Treasury that growth is probably inescapable. The job would be far easier if the available statistics were more reliable. It will be impossible if there is too much dependence on fractions of the G.N.P.

MALE AND FEMALE

IT is naturally disappointing to read, on page 863 of this issue, that an interesting attempt to pre-determine the sex of cattle has not so far been successful. For some years now, work has been under way at the Institute of Animal Physiology of the Agricultural Research Council, and British farmers (as well as British newspapers) have been awaiting its outcome with the greatest interest. They should not now be too downcast, for success at this stage may have been almost too much to hope for.

Dr. Keynes and his colleagues at Babraham have been following a lead provided by Dr. B. C. Bhattacharya several years ago, in some experiments with rabbits. Then it seemed that if rabbit semen were cooled to 1° C to bring to a halt the movement of individual sperms, two distinct fractions would separate under the influence of gravity, that tending to sink more quickly tending to contain a predominance of sperms carrying the *X* chromosomes which determine female offspring. The less dense fraction of the semen, on the other hand, would tend to contain a majority of *Y* chromosomes and would, to that extent, produce buck rabbits when inseminated into fertile females. By all accounts it is extremely difficult to ensure that separation under gravity is not disturbed, which may be one explanation for the negative result of the experiment with cows at Babraham. But it may also be that such differences as exist between the sperms which carry *X* and *Y* chromosomes may not be significant in semen from bulls but just enough to make a perceptible difference in rabbits. More may be known about this when there has been a chance to improve on the statistical validity of the work with rabbits. In any event, however, progress is bound to be unsure until much more is known about the mechanism by which the supposed separation of sperms under gravity can be accomplished. The fact that *X* chromosomes are larger and therefore heavier than *Y* chromosomes is not a sufficient explanation, for the difference of density that would result is insufficient to explain the segregation which is observed. But may there not be physiological differences between sperms of the two kinds? This is what needs most urgently to be determined. Until much more is known about the ways, if any, in which the sex chromosomes can influence the macroscopic behaviour of the sperms which carry them, those who attempt to segregate fractions of semen will necessarily be working in the dark.

It does not follow, of course, that there can be no hope of determining the sex of cattle until all the more fundamental research has been successfully carried out. On the contrary, there is such a great economic benefit to be won from a way of making sure that dairy herds do not waste nearly half their breeding potential on unwanted bull calves that experiments are bound to continue, and deserve to be supported generously. But there are also other lines of enquiry to be followed. Talk of implanting fertilized ova of determined sex in fertile females is not entirely fanciful, even if there can be no assurance that methods which work with difficulty in the laboratory, and with laboratory animals, can be adapted to the trying circumstances of agriculture where, for example, surgical techniques are out of the question. Even if further developments should show that it is unreasonable to look for success, the comparatively modest cost of the work which needs doing would be an entirely prudent gamble. And fortunately there is no immediate prospect that it will be possible to apply any of these techniques to human beings, though it may not be too soon to ask how society should adapt itself to such a possibility.

NEWS AND VIEWS

Tribology for Some

THE Minister of Technology, Mr Anthony Wedgwood Benn, has now announced the formation of what is to be known as the Committee on Tribology. This is the first sign, so far, of the attitude of the British Government to the report of a working party under Mr. Peter Jost which appeared under the imprint of the Department of Education and Science earlier this year. The committee, soon to be established, is said by the Minister of Technology to be a means of deciding "how the objectives of the report can be most effectively pursued". It is not, therefore, to be confused with the committee whose formation was recommended in the report of the working party, and whose function was supposed to be the co-ordination of research and the provision of a forum for the exchange of information. According to Mr. Benn, one of the first questions to be asked of the new committee is whether the encouragement of better lubrication practices in British industry is really dependent on the development of what the report called "Institutes of Tribology".

Since the publication of the report, the Institutes of Tribology have become the acid test which separates the tribologists from the non-believers. The original working party wanted to see the creation of "several national centres in lubrication engineering to become Institutes of Tribology". The institutes were to undertake research in tribology, provide opportunities for post-graduate education and "form and maintain a two-way bridge with industry", partly by attracting industrial men and women to research and teaching in tribology, and partly by providing "a specialist tribological service to industry on an *ad hoc* as well as a regular basis" Institutes were to be placed near academic institutions for the sake of the education, and near industrial centres so as to function effectively as consultants.

This proposal has fallen foul of critics for several different reasons. Some, this journal among them (*Nature*, **210**, 338; 1966), have argued that the proposals are needlessly grandiose. Others have opposed them because they seem to cut across the legitimate interests of established institutions, and in particular because they would be certain to duplicate work that is being done, or which should be done, by existing organizations for industrial research. The Department of Education and Science has remained somewhat unenthusiastic about the proposals in the report, though there is said to have been a welcome for them by some members of the University Grants Committee. The Ministry of Technology has been hesitant, although there is reason to believe that an announcement on tribology was at one stage to have been made by the previous Minister of Technology, Mr. Frank Cousins, on the eve of his resignation from that post. From this point of view, the decision to create a new committee should be a welcome opportunity to resolve the differences between the tribologists and their critics. If the outcome should be a demonstration of how engineering departments in universities can more effectively help industry to some of the benefits, often exaggerated, of better tribology, nobody will complain. If the result is merely a better understanding of what the working party on tribology was really getting at, even that will be something gained.

ELDO under Scrutiny

THE view that the European Launcher Development Organization (ELDO) has not, so far, been "value for money" for the British Government has been made public by a sub-committee of the Estimates Committee of the House of Commons (*Second Report from the Estimates Committee*, 1966–67, *The European Space Launcher Development Organization*, H.M.S.O., 19*s*.). The report stands out among such documents for its topicality; it carries the history of the negotiations between the British Government and its European partners up to the meeting in Paris on July 7 at which a re-allocation of the costs of ELDO among the participants was finally agreed. Some of the verbatim evidence in the report is no older than a month.

For all the reputation of the Estimates Committee as a counter of candle ends, the enquiry on ELDO seems to have been concerned as much with the future as the past. It is made clear, however—possibly for the first time—that the French proposal at the beginning of 1965 for the virtual replacement of the original ELDO programme by a much more ambitious venture was, at the time, as much of a shock as the circulation by the British Government of a memorandum expressing serious doubts about ELDO in February 1966 and its public declaration to that effect at the beginning of June. The sub-committee, by its questioning of officials from the Foreign Office, did very little to define for public purposes how the Government's doubts could have been resolved by the decision that the scale of its contribution to future costs should be 27 per cent and not 38 per cent, although it is also now written into the agreements on ELDO that future increases of cost will not automatically be passed on to unconsenting partners.

On the value of the ELDO programme, the committee was concerned to establish that the present ELDO PAS programme will yield by 1970 a launching vehicle capable of putting into a geostationary orbit satellites of 150 kg or so, which will carry some 2,500 voice channels of communication. It was common ground among the witnesses that such satellites will not be capable of direct television broadcasting, but there was disagreement about the extent to which they would be able to form part of a world-wide satellite network by the early seventies. ELDO was supported with scant enthusiasm by the representatives of the British Post Office, who held firmly to the view that the United Kingdom could secure her interests in the future of telecommunications by negotiations within the international organizations for the time being responsible for the development of the satellite network. The same exchanges disclosed that British companies had been invited to tender for the six satellites now being developed for INTELSTAT, and had not felt able to put in a bid.

The sub-committee's general conclusion is that the decisions just reached on ELDO "are not a solution to the real problem", and that there is a need immediately to take decisions about the long-term future of the organization, with adequate guarantees of long-term finance. It promises that it will soon turn its attention to the general question of space research in Britain, and its present report is heavy with hints

of how France has arranged that there should be a national space programme in parallel with ELDO. This prospect of continuity has prompted some suggestions that the sub-committee may emerge as a British equivalent of the congressional committees in the United States, but it is only proper to point out that the minutes of evidence now published show that it has a long way to go to earn this title. Vital pieces of evidence remain unpublished. The pursuit of truth is often impeded by gentility. Holding these enquiries in public would help to give them point. The lack of full-time secretariat plainly shows.

More Power for *Dragon*

AT least one dragon is trying to avoid obsolescence. The *Dragon* Project of the European Nuclear Energy Agency is moving rapidly ahead with designs for what is hoped will be a competitive 1,250 MW (thermal) power reactor plant generating 540 MW of electricity. The seventh annual report of the Project for the period from April 1, 1965, to March 31, 1966, reviews the advances that have been made, especially since the high temperature gas cooled reactor at Winfrith Heath, Dorset, was completed and its full design output was achieved. The results have been so encouraging that, despite the competition of other operative reactor designs such as the United Kingdom's fast breeder reactors, a further £1·5 million has been allocated to extend the agreement from April, 1967, to the end of that year. There remains the possibility of another extension to March, 1970.

The *Dragon* project was set up in 1959 under an agreement concluded between 12 member countries of the European Nuclear Energy Agency, six of which belong to the Euratom Commission; the original agreement was for five years, but in 1962 this was extended to eight years with an increase in the budget from £13·6 million to £25 million. An agreement in 1964 on collaboration with the United States Atomic Energy Commission provided for the exchange of information and documents and reciprocal visits by scientists. A similar agreement was concluded in July, 1965, with the Thorium High Temperature Reactor Association at Julich, Germany, and permits joint use of the *Dragon*'s major research facilities.

What to Buy?

THE perennial question of how a library should spend its money on scientific journals may be a little easier to answer after the Center for Research Libraries at Chicago has finished the programme of research which it has persuaded the National Science Foundation to finance. The intention is to help libraries to decide whether it is better to subscribe to a journal or, alternatively, to borrow from another library or to buy photo-copies of those items in journals when—and only when—they are needed. If speed of access is the prime consideration, there may be no substitute for acquisition and storage on the spot, although the centre points out that libraries may often be able to provide a better service for the same cost if they acquire several copies of frequently used journals and rely on borrowing the others. With journals the contents of which fade rapidly into limbo, it may be more economical to buy but not to bind. Sometimes it will be best for libraries to borrow all but a few serial publications.

Librarians and the academic committees which advise them are familiar enough with these alternatives. The Center for Research Libraries hopes to be able to help by making a detailed analysis of the use of serial journals, and of the costs to libraries of the various stages in handling them. The first product of the research programme will be a set of mathematical formulae by means of which librarians can hope to decide objectively how best to use their budgets. But there should also be a wealth of information to augment the present scanty knowledge of what really happens to the individual copies of the journals after they reach the shelves of libraries. The Center for Research Libraries has obviously done something to win the heart of the National Science Foundation by pointing out that some scientific papers which appear in journals are read by no more than twenty-five people. Much, of course, depends on the meaning of the verb *to read*.

Healthy Seeds

DR. JOHN GRAINGER writes: The International Seed Testing Association has begun to publish Series 3 of its *Handbook on Seed Health Testing* (Secretariat of the Association, Bennenhaven 1, Wageningen, The Netherlands; working sheets 1–33, except No. 15 and No. 23, $1.00 plus $1.00 for special cover). This is a loose-leaf folder of thirty-one sheets, each of which gives directions for methods of revealing the presence of particular seed-borne pathogens. It is introduced and largely compiled by Dr. Mary Noble of the Department of Agriculture and Fisheries for Scotland, East Graigs, Edinburgh, but individual sheets have been prepared by Dr. Rohloff of Germany, Dr. Meiri of Israel and Dr. Wallen of Canada. The present series deals with oat (three pathogens), *Brassica* seeds (four pathogens), carrot (two), soya bean (one), barley (five), lettuce (one), flax (one), rice (seven), opium poppy (one), pea (one), sesame (one), wheat (three) and *Zinnia elegans* (one pathogen). This list alone shows the international scope of the project. Methods used for examination are mainly incubation on or between blotters or in a rolled paper towel, sowing in brickstone or sand, or the placing of seeds on an agar plate. These simple, successful methods are, however, made precise by the specification of standard conditions such as depth of sowing, length of incubation at a particular temperature, and sometimes the character and diurnal variation of illumination.

Particular interest attaches to a method of revealing seed-borne lettuce mosaic virus by culture in controlled environment—a standard peat medium at a temperature of 10° C for 3–5 days in the dark, then 13–19 days at 25°–27° C in continuous illumination by warm-white fluorescent tubes. Mosaic symptoms appear on the first three true leaves of the lettuce if the disease is indeed carried by the seed. Washing the seed sample may reveal the presence of some pathogens, while the herbicide, 2 : 4-dichlorophenoxyacetic acid (2 : 4-D), is occasionally necessary to prevent germination when, for example, cabbage seeds are examined for *Sclerotinia* rot. Loose smut of barley can only be revealed by the microscopic examination of embryos. Each sheet is clearly illustrated by excellent half-tone blocks and line drawings of the pathogen as revealed by the methods considered. In this the present volume is

complementary to another part of the Handbook (Series 4 (1)) by Malone and Muskett, which described seventy-seven species of seed-borne fungi for purposes of recognition. Since the publication of Series 3, I have received 'working' copies of eleven further working sheets, an earnest that this most important international section of agricultural science is being expanded energetically.

Liquid Inorganic Laser

A LIQUID laser radiating at 1·06 microns and based on a solution of neodymium oxide in selenium oxychloride has been developed by Dr. Adam Heller and Dr. Alexander Lempicki, of General Electric and Telephone Laboratories Inc., according to the current issue of *Applied Physics Letters*. The device is a successor to an earlier laser based on a solution of a rare-earth in an organic chelating compound, the efficiency of which was limited by energy absorption by light atoms in the solvent. The working characteristics of the selenium oxychloride laser have not yet been determined, but pulses of 1 joule have so far been obtained with an input of energy measured as some hundreds of joules.

Long Range Weather

THE Meteorological Office is cheerful if restrained about the success of its long range weather prediction service in its annual report for 1965—the year that saw Professor B. J. Mason succeed Sir Graham Sutton as director-general (*Annual Report on the Meteorological Office*, H.M.S.O., 9*s*.). Long range forecasts for a period of thirty days ahead have been published twice a month for the past three years. On the basis of the first two years of the service, the Meteorological Office says that results have been slightly better than originally expected. According to the annual report for 1965, forecasts are assessed after the event by a panel of meteorologists, and "marks are given for the accuracy of forecasts of temperature, rainfall and additional information". Predicting temperature seems to be the easiest, with twenty-eight out of fifty forecasts ranking for the mark "good agreement". On rainfall, however, agreement between forecasts and reality was good on fourteen occasions, moderate on seventeen and deserving the description "little agreement" on nineteen occasions.

The long range forecasts are based on searches of records going back to the middle of the nineteenth century for analogous patterns of mean temperature and mean pressure in the northern hemisphere, and of successions of daily weather patterns in the British Isles. To predict for September 1966, for example, a search is made for weather patterns in August in earlier years which resemble that of August 1966. The long range weather forecasters are cheerful if they can find several years in which very similar August patterns are followed by September patterns which also resemble each other, and they then predict with confidence (though not necessarily with success) that September 1966 will be much the same as the earlier Septembers. Evidently this happy set of circumstances is not often repeated, however, and the report also points out that much will have to be done before the parameters which are really significant in this technique of analogous forecasting can be identified.

What might be called objective long range forecasting is reckoned to be an extremely distant prospect. Routine forecasts produced by computers have been available for nearly a year, and are said to be already as good as those produced by experienced forecasters. According to the report, the possibility of producing reliable computed forecasts for five days ahead is being seriously considered in the United States, and is also the motive for the proposed programme of World Weather Watch now being considered by the appropriate international organizations, and in which the Meteorological Office thinks it may be asked to play an important part.

Academics make Maps

DESPITE the fact that many European geological surveys rely largely on university personnel for their routine geological mapping, the sentinels of British geological surveying have long argued against such an arrangement. They have claimed that the system does not lead to the building up within the organization of the stock of experience and knowledge which the staff of a good survey needs. Moreover, they are at pains to point out that the survey man is a very different specimen from the academic geologist. Although the latter tends to follow his fancies and become a specialist, the professional surveyor must give—and be seen to give—his wholehearted and impartial attention to all facets of the geology which he encounters. In the past the Geological Survey of Great Britain has suffered somewhat from an inborn fear that a taste of academic bliss could entice away members of its staff.

Recently, with the advent of new geophysical and geochemical methods of surveying—most of which incur organization and finance beyond the reaches of many university departments—the boot has moved gradually over to the other foot. The Survey has become less churlish about entering into co-operative schemes with universities. The most recent contract to go to a university—and the first to deal with the production of a one inch geological map—speaks much for Sir James Stubblefield's efforts during his directorship of the Survey to foster relationships with universities. Through Prof. Scott Simpson's persuasiveness, the Department of Geology in the University of Exeter has received a contract from the Natural Environment Research Council on behalf of the Survey (now incorporated in the Institute of Geological Sciences). Over a period of 5 years, six postgraduate students will work under the joint supervision of the University of Exeter and the Geological Survey to re-survey the region covered by *G.S.E.W. One Inch Sheet* 339 (*Teignmouth*). This particular sheet was chosen because of the need to revise the Devonian and Carboniferous stratigraphy and structure of South Devon in the light of recent advances in the stratigraphy of South-West England, and because it is believed that the re-investigation of the Bovey Beds and the Haldon gravels will yield useful results. Although some of the ground has been re-surveyed on a scale of six inches to the mile, and although there were airborne surveys in the mid-fifties, the map itself has not been revised since 1909.

Sir James Stubblefield has made it clear that the Geological Survey would be prepared to consider approaches from other university departments with the view of further joint projects.

Parliament in Britain

THE Minister of Technology, Mr. Wedgwood Benn, announced in the House of Commons on August 9 that the first Institute for Advanced Studies of Design will soon be established on the site of the National Engineering Laboratory at East Kilbride, Scotland. Mr. Benn was replying to a question on plans for implementing the recommendation of the Fielden Committee to establish such institutes. He said that the first institute would be devoted to advanced machine and control technology and that a director will soon be appointed who will also be a full member of the University of Strathclyde. The Establishment will be under the Ministry of Technology, but the Steering Committee will represent industries, trade unions and the university.

ROUGHLY 55 per cent of the domestic computer business is conducted on rental, according to Mr. Anthony Wedgwood Benn in a written reply to a question about the renting of industrial machinery on August 12. He said that the ministry has no information about the extent of renting for other types of machinery, but that he is hoping that the new arrangements for investment grants will lead to "more flexible leasing terms" for new types of machinery.

RECRUITMENT of staff for the National Reference Library for Science and Invention was said by Mr. Goronwy Roberts, Minister of State at the Department of Education and Science, to be well under way. His written reply to a question on August 11 was that thirty-eight librarians and others had been recruited since the beginning of the year, although only sixteen of these would fill new posts. As yet it appears that the part of the National Lending Library housed at the British Museum is not open to the public, but that what remains in the old Patent Office Library is still accessible.

THE National Foundry College is no longer receiving government assistance because it is "a small monotechnic". The subject was raised in the House of Commons on August 12, and in a written reply Mr. Goronwy Roberts stated that the decision was based on the extensive development of the provision by local education authorities for higher technological education since the college was set up in 1948. In the future a more broadly based institution within the local authority sector will be better able to meet the needs of the foundry and other industries.

FOLLOWING the recommendations of the Geddes Report on the shipbuilding industry, a Shipbuilding Industry Board will be set up under the chairmanship of Mr. William Swallow, until recently chairman and managing director of Vauxhall Motors, Ltd. The board's task is to promote the reorganization of the industry, principally by holding discussions with firms and unions in advance of legislation. This announcement was made by Mr. Douglas Jay, President of the Board of Trade, in a statement to the House of Commons on August 9. He added that implementation of the productivity bargains will be deferred for six months, in accordance with the White Paper, but that both the Government and the Industrial Re-organization Corporation were prepared to give financial assistance if required.

University News: London

PROF. J. BROWN, at present professor of electrical engineering at University College, has been appointed to the chair of light electrical engineering tenable at the Imperial College of Science and Technology. Mr. L. Castillejo, at present lecturer in theoretical physics and fellow of Wadham College, has been appointed to the second chair of physics tenable at University College. The title of professor has been conferred on the following: Dr. R. A. Kekwick (biophysics, in respect of his post at the Lister Institute of Preventive Medicine); Dr. S. J. Pirt (microbiology, in respect of his post at Queen Elizabeth College); Dr. J. C. Sloper (experimental pathology, in respect of his post at Charing Cross Hospital Medical School); Dr. R. E. B. Hudson (pathology, in respect of his post at the Institute of Cardiology).

Southampton

MR. H. CHARNOCK, at present principal scientific officer in the National Institute of Oceanography, has been appointed to the chair of physical oceanography.

Appointments

DR. R. S. F. HENNESSEY, at present head of the Therapeutic Research Division of the Wellcome Foundation Ltd., has been appointed assistant research director with responsibility for scientific administration. Prof. M. Weatherall, at present professor of pharmacology at the London Hospital Medical College, has been appointed head of the Therapeutic Research Division of the Wellcome Research Laboratories.

DR. B. R. NIJHAWAN, director of the National Metallurgical Laboratory, Jamshedpur, has been appointed to the United Nations Organization as senior adviser (Inter-Regional), Centre for Industrial Development (Technological Division) in New York.

Announcements

THE American Society of Plant Physiologists has chosen Prof. Daniel I. Arnon, of the University of California (Berkeley), as the recipient of its Stephen Hales Award; Prof. Kenneth Thimann, of the University of California (Santa Cruz), for the Barnes Life Membership Award; and Prof. C. B. van Niel, of California, for the Kettering Award. At the annual meeting of the Society held at the University of Maryland recently, Prof. R. S. Bandurski, of Michigan State University, was elected president.

A NATIONAL Medal of Science has been established in the United States to recognize individuals who have made outstanding contributions in the physical, biological, mathematical and engineering sciences, and the first awards have been made to: PROF. R. H. BING, professor of mathematics in the University of Wisconsin; PROF. M. CALVIN, professor of chemistry in the University of California; PROF. H. E. CARTER, professor and head of the Department of Chemistry and Chemical Engineering in the University of Illinois; DR. P. CHENEA, vice president for academic affairs in Purdue University; PROF. B. L. CRAWFORD, JUN., professor and chairman of the Department of Chemistry in the University of Minnesota; DR. J. C. FLETCHER, president, University of Utah; DR. H. J. HOLLOMON, assistant secretary of commerce for science and technology; PROF. P. J. KRAMER, professor of botany in Duke University; PROF. G. LINDZEY, professor and chairman of the Department of Psychology in the University of Texas; PROF. W. MCDERMOTT, professor of medicine in Cornell University; DR. R. REVELLE, director of the Harvard Center for Population Studies in Harvard University; DR. A. M. WEINBERG, director of Oak Ridge National Laboratory.

A COURSE of instruction in powder technology leading to a master of science degree or to the college postgraduate

diploma will commence at King's College London in October. The course will last for one year, and is designed for students who have a first degree in engineering, physics, chemistry, or certain other approved branches of applied science. Further information can be obtained from Mr. A. Davies, King's College London, Strand, London, W.C.2.

A DISCUSSION group for users of gel permeation chromatography will be held at Shell Centre, London, on September 13. Further information can be obtained from A. Lambert, Shell Research Limited, Carrington Plastics Laboratory, Urmston, Manchester.

AN international conference on "Forensic Activation Analysis" will be held in San Diego during September 19–21. Further information can be obtained from Dr. V. P. Guinn, General Atomic, P.O. Box 608, San Diego, California.

A CONFERENCE on "The Physical Basis of Yield and Fracture", arranged by the Stress Analysis Group of the Institute of Physics and the Physical Society, will be held in the University of Oxford during September 28–30. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A CONFERENCE on "Nuclear and Particle Physics", arranged by the Nuclear Physics Sub-Committee of the Institute of Physics and the Physical Society, will be held in the University of Glasgow during September 21–23. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A CONFERENCE on "The Physics of Semiconducting Compounds", arranged by the Institute of Physics and the Physical Society, will be held at the University College of Swansea during September 21–23. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Plastics and Polymer Technology" will be held at the Borough Polytechnic, London, during October 27–28. Further information can be obtained from the Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

A SYMPOSIUM on "Solid State and Chemical Radiation Dosimetry in Medicine and Biology" will be held, under the auspices of the International Atomic Energy Agency, in Vienna during October 3–7. Further information can be obtained from the appropriate national authorities responsible for atomic energy matters.

A SYMPOSIUM on "Combustion in Advanced Gas Turbine Systems" will be held at the College of Aeronautics during April 4–6, 1967. Papers are being sought for the symposium and any correspondence should be sent to Dr. I. E. Smith, The College of Aeronautics, Cranfield, Bedford.

A SYMPOSIUM on "Microwave Measurement", organized by the Scientific Society for Measurement and Automation, member organization of IMEKO in Hungary, will be held in Budapest during October 12–14. Further information can be obtained from the Organizing Committee of the IMEKO—Symposium on Microwave Measurement, P.O.E. 457, Budapest 5.

THE third symposium on "Flames and Industry", arranged jointly by the Institute of Fuel and the British Flame Research Committee, will be held at the Imperial College of Science and Technology, and the Royal Institute of British Architects, during October 18–19. Further information can be obtained from the Secretary of the Institute of Fuel, 13 Devonshire Street, Portland Place, London, W.1.

A SYMPOSIUM on "Physico-chemical and Biophysical Factors affecting the Activity of Pesticides", under the auspices of the Pesticides Group of the Society of Chemical Industry, will be held at the London School of Pharmacy during April 11–12, 1967. Further information can be obtained from Dr. M. B. Green, Imperial Chemical Industries, Ltd., Mond Division, P.O. Box 8, The Heath, Runcorn, Cheshire.

A SYMPOSIUM on "Spectroscopy of Macromolecules" is part of the programme of the thirteenth Colloquium Spectroscopicum Internationale, sponsored by the Canadian Association for Applied Spectroscopy and the Society for Applied Spectroscopy, to be held at Carleton University during June 19–23, 1967. Further information can be obtained from Mr. A. W. Pross, Central Research Laboratory, McMasterville, Quebec.

A SYMPOSIUM on "Ceramic Materials and their Properties", sponsored by the American Ceramics Society, the National Bureau of Standards, and the American Society for Testing and Materials, will be held at the National Bureau of Standards in Gaithersburg, Maryland, on October 14. Further information can be obtained from Dr. Wachtman, National Bureau of Standards, Washington, D.C.

THE NIGHT SKY IN SEPTEMBER

All times are in Universal Time

MOON

New Moon	14d 19h
Full Moon	29d 17h

CONJUNCTIONS WITH THE MOON

Venus	13d 16h, 4° S.
Mars	12d 01h, 4° S.
Jupiter	11d 03h, 5° S.
Saturn	1d 16h, 2° N. 28d 18h, 2° N.

PLANETS

Name	R/S	Times of rising (R) and setting (S) during the month Beginning	Middle	End	Mag.	D_θ (10^6 miles)	Zodiacal position
Mercury	—	Unfavourable for observation			—	130	Virgo
Venus	R	3h 20m	4h 20m	4h 50m	−3·4	153	Leo
Mars	R	1h 50m	1h 45m	1h 40m	+1·9	211	Cancer
Jupiter	R	1h 15m	0h 20m	23h 40m	−1·6	534	Cancer
Saturn	R	19h 20m	18h 30m	—	—	—	—
	S	—	—	4h 50m	+0·9	796	Pisces→Aquarius

D_θ is the distance of planet from the Earth on the 15th of the month.

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	R/D	Time	Mag.
139 Tau	R	9d 03h 00·3m	+4·9
ω Sgr	D	23d 18h 32·7m	+4·8
60 Sgr	D	23d 20h 16·1m	+5·0
ψ^3 Aqr	D	28d 01h 57·5m	+5·2

(D, disappearance; R, reappearance)

OTHER PHENOMENA

28d 12h, Autumnal Equinox.

HOW TO SPEND MONEY?

The following are extracts from the Presidential Address to the Institute of Physics and the Physical Society, entitled "Rate of Growth of Expenditure on Science", delivered on May 3, 1966, by Sir Gordon Sutherland, Master of Emmanuel College, Cambridge.

THE expenditure of the research councils has grown over the past 25 years as shown by the figures in Table 1 (taken largely from the report of the Advisory Council for Scientific Policy for 1963/64). In 1938/39 the total expenditure of the research councils was £1.43 million. By 1964/65 this had grown to nearly £50 million. The overall compound annual per cent increase is 14·6 over the 26 years. It is on the basis of these figures that the statement is often made that Britain has been doubling her expenditure on science every 5 years.

The expenditure of the universities on research in science and technology shows a similar trend, although the period for which reasonably accurate figures are available is much shorter. The figures are given in Table 2. Here we find that expenditure has risen from £20 million in 1958/59 to more than £50 million in 1964/65. If we divide this interval into two periods of 3 years, then the compound annual per cent increase increased from 13·3 for the first three years to 19·1 for the last three. Thus in the research councils and the universities the annual expenditure is not only rising but the rate at which it is rising is also increasing. It is this increase in the rate of increase which is rightly beginning to cause concern. If these trends were to continue for even 10 years, the expenditure of the research councils would by 1974/75 have reached a total of about £240 million, which is considerably more than all government departments were spending on civil research (£204 million) in 1964/65. A similar extrapolation of the university figures leads to a total for the universities from the University Grants Committee alone of about £235 million. Thus the combined total for research carried out at universities and by the research councils would be about £475 million in 1974/75—a total which is nearly equal to the estimated combined expenditure on road construction (£192 million) and the whole of the Ministry of Aviation (£299 million) for the year 1965/66.

Table 1. EXPENDITURE BY THE U.K. RESEARCH COUNCILS (£ MILLION)

	1938/9	1947/8	1955/6	1962/3	1964/5
D.S.I.R.	0 89	3 08	6·48	21·87	
A.R.C.	0 32	0·97	4·0	7 71	
M.R.C.	0·22	0·77	2·2	5 86	
N.C.	—	—	0·21	0·66	
	1·43	4·82	12·89	36·10	49·4
Compound annual per cent increase	14 4	13·0	15·8	16·6	

It is conceded by all scientists that the exponential growth of expenditure on science cannot continue indefinitely at a figure (15–20 per cent per annum) which is so out of line with the exponential rate of growth of the G.N.P. of the country as a whole (about 6 per cent per annum at current prices between 1956 and 1965). How can we decide (*a*) when to start cutting back the rate of increase, (*b*) how much and at what rate to cut back, and (*c*) the correct annual rate of increase at which we should finally stabilize?

One obvious line of attack is to examine the figures available for expenditure on all research in various countries and to relate them to the gross national product. For Britain, the relevant figures available are given in Table 3. It will be seen that in 1955/56 the total expenditure by government and industry, private and nationalized, was only 1·64 per cent of the G.N.P. By 1961/62, this had risen to 2·5 per cent, and the best current estimate for 1964/65 leads to much the same figure (2·44 per cent). Over the past three years of this period, total expenditure on research by the four councils and the universities rose from 0·21 per cent of the G.N.P. to 0·28 per cent. Over the same period, expenditure by all industry showed a much smaller rise, that is, from 0·98 per cent of the G.N.P. to 1·04 per cent. Thus the compound annual rate of increase of expenditure by the research councils and the universities (10·1 per cent) was more than five times that of all industry (2·0 per cent) when expressed as a percentage of the G.N.P. Extrapolating these figures, we find that in 1974/75 the research councils and universities would be spending 0·75 per cent of the G.N.P., while the whole of industry would be spending 1·3 per cent. Are such figures reasonable?

We have seen that Britain's total expenditure on research and development seems to have stabilized at around 2·5 per cent of the G.N.P. However, if the rates we have been discussing were maintained, then by 1974/75 all industry together with the research councils and universities would be spending 2·05 per cent of the G.N.P. If we merely maintain all the other expenditure on research as a constant factor of the G.N.P. (1·04 per cent), then the grand total of expenditure on research and development by 1974/75 would be 3·1 per cent of the G.N.P. According to a recent O.E.C.D. investigation, the percentages of the G.N.P. spent on research in 1962 by a number of highly industrialized countries were as follows:

United States	3·1
United Kingdom	2·2
Netherlands	1·8
France	1·5
Germany	1·3
Belgium	1·0

In view of the fact that France and Germany each have achieved an economic situation which is much stronger than the British, it would seem difficult to justify Britain going up to the United States figure of 3 per cent of the G.N.P. in the next ten years, and surely it would be wrong to spend one quarter of that money on basic research. Some decrease in the rate of growth of expenditure by the research councils and the universities on research seems inevitable in the near future.

The techniques used in pure scientific research are becoming more and more expensive as new discoveries in one field become applicable to an entirely different branch of science; for example, nuclear magnetic resonance and the Mössbauer effect, discovered by physicists, are now widely used in chemical research; electron microscopes and computers are gradually being used in nearly every branch of science. This is often referred to nowadays as "the sophistication factor", that is to say, the additional cost required in order to keep a research laboratory up to date technologically.

We might take the figures for a recent three-year period at the National Physical Laboratory to illustrate these trends in salaries and equipment costs. The N.P.L. is sufficiently large and varied to be representative of a cross-section of research laboratories in non-nuclear physics, metallurgy, aerodynamics and ship hydro-

Table 2. EXPENDITURE ON RESEARCH BY UNIVERSITIES (£ MILLION)

	1958/9	1959/60	1960/1	1961/2	1962/3	1963/4	1964/5
U.G.C.	16 4	18 2	19·0	21·2	26 4	31·0	37·1
R.C.	3·6	4 6	6·1	7·9	10 1	12·1*	14·8†
	20·0	22·8	25·1	29 1	36·5	43·1	51·8
Percentage of compound growth rate		13·3 over 3 years				19·1 over 2 years	

* Provisional.
† Estimated.

Table 3. EXPENDITURE ON CIVIL RESEARCH RELATED TO U.K. GROSS NATIONAL PRODUCT

	1955/6	1959/60	1960/1	1961/2	1962/3	1963/4	1964/5
G.N.P. at current prices in £000 million	18·32	22·7	24·3	25·3	27·0	28·9	30·8*
Total R and D in £ million	300			634			750
(% of G.N.P.)	(1·64)			(2·5)			(2·44)
Government expenditure on civil research in £ million	?			137	154	174	204
(% of G.N.P.)				(0 54	(0·57)	(0·6)	(0·66)
R.C. and university expenditure in £ million	?			52·8	62 5	72·5	86·5
(% of G.N.P.)				(0 21	(0·23)	(0·25)	(0 28)
Private industry and public corporations in £ million	77·3			247·7			320
(% of G.N.P.)	(0 42)			(0·98·			(1·04)
Universities alone in £ million		22·8	25·1	29 1	36·5	43·1	51·8
(% of G.N.P.)		(0·1)	(0·1)	(0·12	(0·135)	(0·15)	(0·17)

* Preliminary figure.

dynamics, doing a 50/50 mixture of pure and applied research. During the period under review some rather expensive equipment was being acquired (for example, payments for a *KDF* 9 computer of £74,000 were made in 1962/63 and of £148,000 in 1963/64 and the total staff increased from 1,350 to 1,425, nearly 6 per cent in the two years). The cost of new equipment for each scientist went up from £2,670 to £3,350 in two years. In 1956/57, the corresponding figure was less than £1,100. Whereas new equipment formed only 16 per cent of the annual expenditure in 1956/57 that figure had risen to nearly 32 per cent by 1963/64.

The annual increase of 15 per cent in expenditure on basic research by the universities and the research councils must start to drop eventually, but it could be maintained for a few more years if the rate of increase of defence expenditure on research and development were still further diminished. Moreover, for the past four years the expenditure of the Atomic Energy Authority on research and development has been almost constant at about £54 million. If the expenditure is constant (at current prices), this means that the effort is actually being reduced. It could perhaps be further reduced, because several of the tasks for which Harwell and Aldermaston were created have been accomplished.

Table 4. ANALYSIS OF N.P.L. EXPENDITURE (IN £)

	Salaries and wages	Percentage of increase	Equipment	Percentage of increase	Cost of new equipment per scientific officer	Percentage of increase
1961/2	1,378,000		594,000		2,670	
		5		10		25 5
1962/3	1,446,000		651,000			in two
		8·5		22·5		years
1963/4	1,594,000		797,000		3,350	

Undoubtedly the most satisfactory way of producing more money for research and development would be to increase the G.N.P. at a faster rate. The annual growth rate in the G.N.P. of 6 per cent is misleading, because this figure is based on current prices for each year. At constant prices, the real increase in the G.N.P. over the past five years is only about 3·4 per cent per annum. This rate has been surpassed by a considerable factor by countries like Germany, Japan and France, countries which have been devoting a much smaller fraction of their G.N.P. to research and development. Although the United States is the country which devotes the largest fraction of its G.N.P. to research, it must be remembered that this is only so because of a huge programme in space research. If this item is eliminated, then the United States would be spending a slightly smaller fraction of its G.N.P. than Britain. Until about twenty years ago, the United States grossly neglected basic science, yet its applied science and technology were superb and surely played a great part in making its productivity far greater than the British in nearly every manufacturing industry. During the nineteenth century and the early twentieth, Britain was in the forefront technologically as well as scientifically, but we have lost our technological supremacy in many fields, not only to the United States, but to countries like Japan and Sweden. The reasons for this change are many and too complex to be analysed here, but it is generally conceded that we are not getting a sufficiently high fraction of our ablest young men to go into applied science and technology.

Because our battle to reassert ourselves technologically will take some time to win, it is worth considering whether we are making the best use of the government money at present being devoted to civil research, and whether some changes in the distribution of this money might not be made with advantage to our technological and economic future.

Table 5. SOME FIGURES FROM THE CIVIL ESTIMATES FOR 1966/7 (£ MILLION)

Department of Education and Science		Ministry of Aviation	
S.R.C.	33 9	Contribution to ELDO	11·3
M.R.C.	11 9	Other space research	3 3
A.R.C.	10·3		
N.E.R.C.*	5·4	Total	14 6
S.S.R.C.†	0·7		
		Ministry of Technology	
Total	62 2	Total budget excluding U.K.A.E.A.	24·4

* Natural Environment Research Council.
† Social Science Research Council.

An examination of the Civil Estimates for 1966/67 reveals some figures which provide food for a good deal of thought. These are set out in Table 5. It will be seen that expenditure by the Ministry of Aviation on space research is greater than half the total budget of the Ministry of Technology. However, that is not the whole story. Through the Science Research Council an additional £5·5 million is being spent on space research, giving a total of more than £20 million, a sum almost as large as the combined expenditure of the Medical Research Council and the Agricultural Research Council. Is the expenditure of this amount of money justifiable in terms of its returns to Britain scientifically and technologically? It is often argued that "technological fall-out" justifies a large expenditure on space research, and it is certainly a field from which we should not wish to opt out completely and especially because of the potential return in the field of satellite communication. But it is possible to be active in space research with a much smaller expenditure of money.

Nuclear physics is another very expensive field of research. The estimated direct expenditure on nuclear physics by the Science Research Council during 1966/67 amounts to £14·4 million and of this £1·9 million will go as grants to university departments. The total is about 30 per cent more than the total expenditure of the Medical Research Council or the Agricultural Research Council, and more than three times the corresponding sum allocated to chemistry, metallurgy, biology, technology and the rest of physics. Is such an imbalance justifiable? Is it in the best interests of British science and technology? Is it in the best interests of British physics? Would it not be wiser to divert a larger slice of the Science Research Council's cake to solid state physics, to metallurgy, to the environmental sciences, to technology and to chemistry? The technological fall-out from nuclear physics (at least for the next few years) is surely far less than we can anticipate from all these other branches of science on each of which we spend a small fraction of what we spend on nuclear physics.

If therefore we are able to decrease our current expenditure on space research and nuclear physics, it would be quite possible to maintain an annual compound growth rate of 10–15 per cent on university research and the remainder of the research council research work for at least another three years. By this time, the new universities (the creation of which has been partly responsible for the recent steep increase in the bill for university research) might be reasonably well equipped.

BUILDING ON SPRINGS

By R. A. WALLER
W. S. Atkins and Partners, Epsom, Surrey

A CONSTANT source of annoyance that has to be endured by all who live near railways, especially underground railways, is vibration. This is not necessarily so, however, where new lines are being built, or where urban areas are being redeveloped over or alongside railways. Such redevelopment, however, costing each year about £50 million in London alone, represents a considerable problem. Recently in London a building was mounted on springs in order to eliminate vibrations generated by a railway and transmitted to it through the ground (see Fig. 1). The design was to be such as to provide an acceptable level of amenity in what were to be high-quality flats. Similar specifications in further buildings could well require that they also be mounted in this way: a method of establishing whether this is worthwhile has been developed[1].

The problem of mounting a complete building on springs presents the engineer with many interesting and challenging problems. Perhaps the most difficult problems to overcome are the "psychological" ones met in obtaining acceptance for such a scheme. Springs are associated with flexibility and not with the ability to carry load. Now that a precedent has been created it could be that these particular problems will disappear.

When a radical change is made in a well-established form of construction there are likely to be many side effects apart from the obvious problems associated with the design of the springs. The following description will illustrate how many (undoubtedly not all) of these effects and problems were allowed for in the design.

The first step was to define the design requirements, in particular the flexibility required in the springs. This followed from a knowledge of the ambient vibrations in the ground and of the levels of vibration which would be acceptable in the building. The level of vibration in the ground was established by direct measurement using velocity sensitive probes. The ground vibrated predominantly in the frequency band of 20–25 c/s although there were some smaller components at lower frequencies (the overall velocity amplitude was about 0·04 in./sec). These readings were taken while the building that was to be demolished to make way for the new one was still intact. The new building is about the same weight as the old and it was considered that the site conditions would not alter significantly or, alternatively, if there was a change, that the predominant frequencies might be somewhat higher because of the reduction in the mass

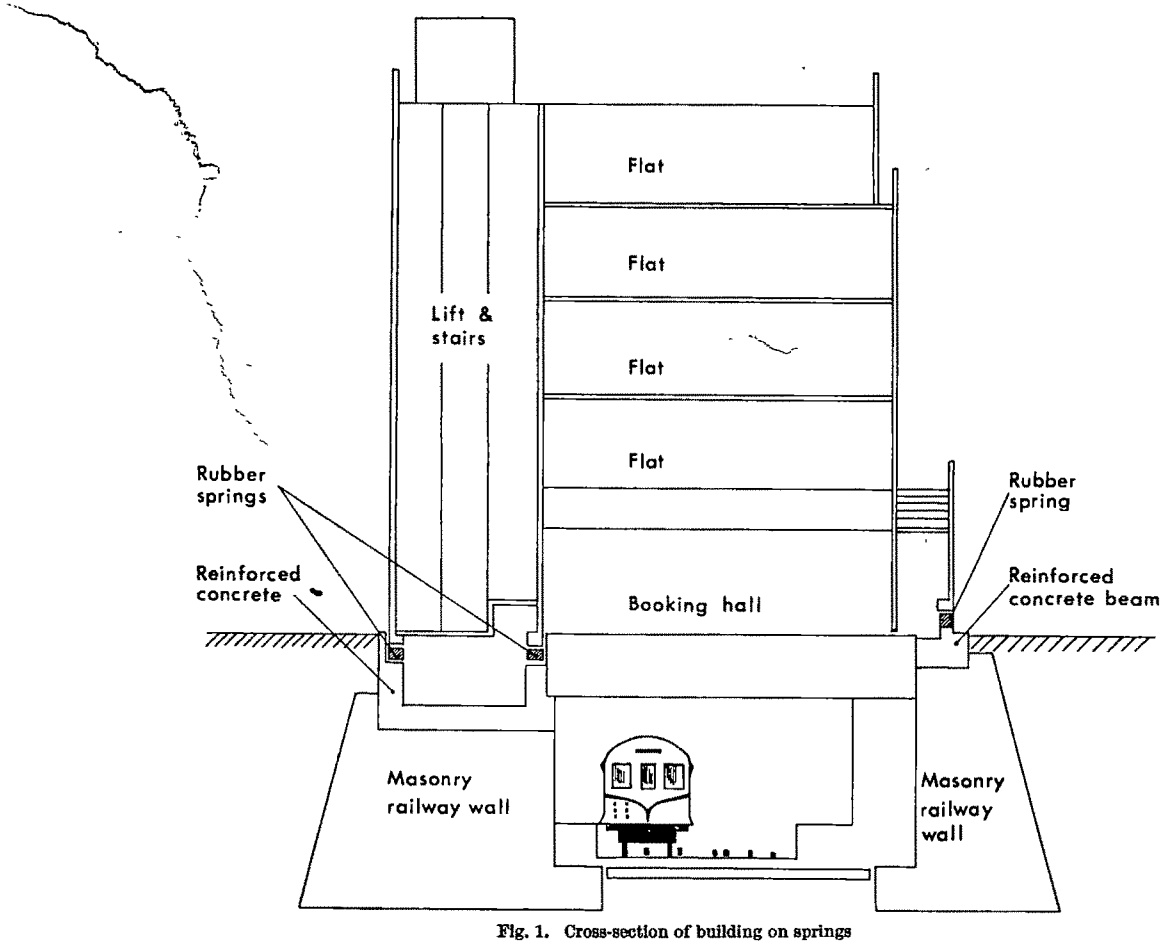


Fig. 1. Cross-section of building on springs

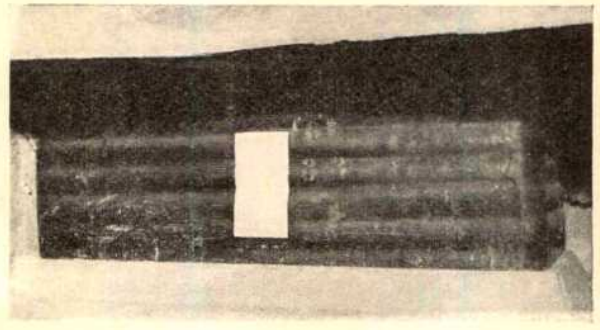
rigidly connected to the ground. (The only change measured on the completion of the new building was that the lower frequency components were slightly more significant.)

The thresholds of perception of vibration have been widely studied[2]. Experiments have been carried out with people standing, sitting and lying down both parallel and perpendicular to the direction of motion. These have almost all been for sinusoidal single frequency vibration which unfortunately in no way represents the pattern of vibrations produced by the trains. At these low frequencies, however, peak velocity seems to be a reasonable measure of sensitivity and this has been used as the criterion. Owing to the considerable variations in sensitivities from person to person it is not possible to fix a level that would be imperceptible to everybody. As with all engineering problems, it was necessary to compromise and the level was fixed (0·01 in./sec) such that only about a sixth of the population could notice the vibrations. The pattern of vibrations generated by the trains consists of a number of peaks superimposed on a background vibration of significantly lower level and the level fixed above was applied to the second highest peak recorded. This was an arbitrary decision made to allow for the transient nature of the vibrations. The difference between the level of the ambient vibration and the level which would be acceptable in the building determined the flexibility required from the springs and the vertical natural frequency of the mounting was fixed at 7 c/s. This allowed for the fact that the low frequency components would be magnified.

Apart from the flexibility the other major requirement for the springs was the ability to carry the load of the building. The flexibility and load carrying capacity determined the type of spring which it would be most economic to use. In this case the appropriate type is a laminated rubber spring with steel reinforcing plates[3]. Rubber is elastic but virtually incompressible—its bulk modulus is much the same as that of water. Between two reinforcing plates the rubber tends to flow outwards restrained only by its shear stiffness (Fig. 2). The shear strain is a maximum at the edge of the plates and limits the load that can be applied and the vertical deflexion. The thinner the rubber layer the more able it is to carry vertical load but the stiffer it becomes. In fact it can be shown* that for given load and stiffness requirements the volume of the springs is approximately constant. This is important because it is the volume which, to a first approximation, determines the cost of an individual spring. The engineer has, therefore, a reasonably free hand to determine the proportions he requires for the springs. At one extreme the rubber layers are very thin and numerous but because of the high load carrying capacity the area of the spring is small; at the other extreme the spring has a large area and consists of one single thick layer of rubber.

Generally it will be found best to equate the compressive stress in the springs to that carried in the [illegible]. This provides continuity of load carrying [illegible] the load does not have to be spread unnecessarily from a column in the building to accommodate a spring. In a reinforced concrete building the compressive stress in the columns will generally be 1,000 lb./in.² and, as in the present building, there is no difficulty in designing the rubber springs to an identical figure. In steel structures the compressive stresses are higher. While some designs of rubber springs have gone up to 2 tons/in.², and while it is theoretically possible to design springs to match the compressive stress carried by steel (10 tons/in.²), it is not at the moment economic to manufacture such springs.

Rubber springs are non-linear their stiffness increases with the speed of loading and their stiffness at small amplitudes is greater than at large amplitudes. Allowance was made for all these factors in the design of the individual springs which entailed a vertical deflexion of 0·4 in. Without these effects an ideal linear spring would have required a deflexion of 0·2 in. Rubber has the advantage, however, of providing damping which is useful in causing a rapid decay in any transient disturbance.



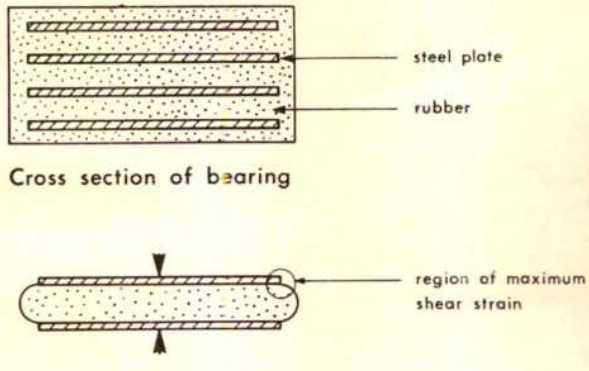


Fig. 2. Rubber spring.

It is worth noting in passing that temperature can have a significant effect on the behaviour of rubber although, in the present instance where natural rubber was used, it was of no significance. The flexibility is affected in two ways. There is an instantaneous increase [illegible]ess as the temperature is lowered; for example, natural rubber doubles its stiffness. Secondly, [illegible] increases gradually with time owing to [illegible]t low temperatures; for example, natural [illegible]rystallize significantly at −20° C. The

* *Note.* Design rules (ref. 4) available for [illegible] expansion movements in bridges gi[illegible] rubber slab:

fc (the allowable [illegible]

Table 1. PROPERTIES OF THE NATURAL RUBBER SPRINGS

Ratio actual stiffness to stiffness of a linear spring with same deflexion	1·5–1·7 (measured)
Ratio dynamic to static stiffness	1·14 (measured)
Ratio stiffness at service amplitudes to stiffness at amplitude used in dynamic test	1·00–1·01 (estimated)
Damping (phase angle)	6° (measured)

Perhaps the paramount problem in designing springs for incorporation in the foundation of a building is the prediction of their useful life. It is quite possible to jack up a building in order to replace the springs, but this is expensive—probably too expensive. There are three major ways in which the life of a laminated rubber spring could be limited and these were tackled in turn as follows:

(1) *Corrosion.* The corrosion of the steel reinforcing plates was prevented by embedding them 0·25 in. into the rubber.

(2) *Atmospheric attack.* Natural rubber is attacked by ultra-violet radiation, oxygen and ozone. Fortunately these are no longer serious problems. The ultra-violet radiation, which might have initiated oxygen attack, is absorbed by the carbon black which is used in the compounding of the rubber. The oxygen attack which results in deterioration of the physical properties of the rubber and the ozone attack which results in the cracking of the rubber where it is strained are both inhibited by the additives used in the manufacture of the rubber. While oxygen and ozone are still, none the less, something of a problem with small sections of rubber (for example, car trims), with the large volumes involved in the springs used here, the main body of rubber is very effectively protected by the outer layers. In particular the critical point at the edge of the bond between the rubber and the steel reinforcing plates is well protected.

(3) *Creep.* It is difficult to estimate the extent to which rubber springs will creep over long periods of the order of 50 years. There are two mechanisms of creep; one results from the oxygen attack which would occur continuously over the years, but in our present springs this can be discounted because of their size. The more important mechanism is due to the effect of loading which produces a creep of approximately 10 per cent of the initial deflexion every decade, that is, every increase in time by a factor of ten. This can be reduced by overloading the springs before putting them into service. In this case with an initial deflexion of 0·4 in. it is estimated that in 50 years the springs might creep a further 0·1 in. from when the building was completed.

It was stated earlier that the introduction of springs into a conventional type of building could have a number of side effects. The effect of rolling a grand piano from one side of the building to the other is fortunately insignificant, but other, perhaps less obvious, effects can cause significant problems. The biggest of these is the effect of wind. Wind loads are not steady and are generated by gusts occurring more or less at random. The problem of wind gusting is one of random vibration and is amenable to some extent to analysis and prediction[5]. Wind gusting can produce significant vibrations in buildings [illegible] it is necessary to examine whether the introduction of flexibility into the foundations might aggravate [illegible] introduce the problem where it might not [illegible] existed.

sary to provide additional lateral springs. While, from the point of view of wind gusting, a higher frequency still would have further reduced the risk of vibrations generated by wind, a higher frequency would have interfered significantly with the isolation of the vibrations borne through the ground in all directions.

Wind can also generate steady vibrations in a building owing to the shedding of a regular pattern of vortices; this generally occurs with fairly rounded shapes or with sections with extreme shapes such as those approaching the flat plate[6]. In this case, with an approximately square shape and with sharp edges, a steady pattern of vortices is unlikely to be set up. The natural frequency chosen to avoid problems from gusting would, as it happens, also prevent trouble on this score because the vortices cannot be generated at a sufficiently high frequency for the amplitude of vibration to build up.

A small margin of safety was allowed in the springs because the vibrations which were borne through the ground occurred at frequencies which correspond with the range of natural frequencies to be found in building components. So far as possible the floors in particular were designed to have natural frequencies away from the frequencies of vibration occurring in the ground. It is virtually impossible to avoid some magnification. However, if severe magnification had occurred, allowance had been made for the construction to be modified and in an extreme case additional damping layers could have been added[7].

Most buildings have to be designed so that in the event of fire they will not collapse before the occupants have had a reasonable chance to get out. It is probable that the rubber springs would burn slowly because of the protection that would be afforded them by a charred outer layer. It is almost certain that they would continue to support a load longer than the structure itself (in any event, stops were provided to limit the settlement of the building to 0·25 in.).

The construction of the building was straightforward; it was built on the springs; the rate of building was kept uniform and materials were stacked where they were to be used in order to ensure that the load was divided between the springs in accordance with the design. Having introduced springs into the foundation it was essential that nothing "bridged" them; all services, for example, sewers and gas, were provided with flexible connexions. In the same way that, for psychological reasons, the major problem in design was obtaining approval for the scheme in principle, during construction, the major problem was ensuring that the gap under the building was not filled with unwanted material. Workmen are accustomed to plugging such gaps and no matter how often they are told they will probably unconsciously continue to fill any gap that can be filled. As much attention needs to be paid to designing unbridgable gaps as to designing cheap and effective springs.

In spite of all these problems the building was completed, [illegible] nobody sensitive enough to feel any vib[illegible] in the building. The economics [illegible] well for a difficult site. The cost [illegible] t 5 per cent (springs alone 2

Amenity Tech. Commun.,

SATELLITE WAKE

Interactions of Space Vehicles with an Ionized Atmosphere

Edited by S. F. Singer. (International Series of Monographs in Aeronautics and Astronautics. Division 9: Symposia, Vol. 18.) Pp. viii + 510. (London and New York: Pergamon Press, Ltd., 1965.) 150*s*. net.

THE classical problem of the motion of a particle in a central force field takes on some complications when interactions between more than one such particle have to be considered and when the radial variation of the force is, in consequence, no longer as the inverse square of the distance. This problem has a number of applications—the accretion of electric charge by dust particles in the interstellar gas, the theory of the operation of the Langmuir probe and the development of a satellite wake in the ionosphere. This last example forms the central topic of this book.

The book is based on material originally presented at a symposium of the American Astronomical Society in Washington, D.C., but an attempt has also been made to cover the subject more completely by means of additional papers. In all there are fourteen contributions, divided into two parts. As is often the case with such books, this one presents a difficult problem for the reviewer on account of the varying standard of the contributions, and also of their completeness of coverage, which combine to make it difficult to indicate how thoroughly different portions of the subject have been covered.

Part I deals principally with the interaction with a plasma of a charged body which may be moving. This is discussed in considerable generality in the first contribution by E. J. Opik in a chapter which represents a most valuable contribution to the subject, especially in the discussion of central force fields where the index of the force law is greater than 2. The following chapter, by E. H. Walker, develops the ideas of the first for two applications—the potential distribution about a stationary sphere in a plasma and about a hypersonic moving object. The first of these is the ordinary Langmuir probe; for some reason the greater part of the discussion is for the physically unrealized case in which both ions and electrons are all reflected at the surface of the sphere, though the case which actually occurs is also treated. Results for the moving object—the case of a satellite wake—are also given. It is unfortunate that no comparison with Russian work, that of Al'pert and his collaborators, is attempted as this is in many respects complementary. In fact, it is a noticeable feature of the book generally that the references to other work, particularly that done outside the United States, are often inadequate. This is possibly partly due to its having been a long time in preparation, but it is a weakness.

The next chapter, by R. T. Bettinger, deals with probe methods of measurement in the ionosphere. This is the longest single contribution; from the acknowledgments it appears to have been written as a [illegible] reproduced in its entirety, an im[illegible] review (actually a very p[illegible] any event, it was a cl[illegible]

Soon after the launching of *Sputnik I* a number of different radio-wave propagation effects aroused some interest, such as long-distance reception of the satellite transmitters. Another effect observed by J. D. Kraus at the Ohio State University was thought to be scattering of WWV transmissions from the satellite or its wake, the remarkable feature being the strength of the received signal which corresponded to a scattering cross-section of 10^4–10^6 m^2, several orders of magnitude greater than the geometrical area. To explain this observation provided the original incentive for much of the work on satellite wakes. However, radio signals are also reflected from ionized meteor trails, and the association of some signals with satellites has always been controversial. Part II is devoted to this topic, and the first three contributions (by J. D. Kraus, by T. G. Hame and W. D. Stuart, and by T. A. Croft and O. G. Villard) describe different experimental investigations of this correlation. In the work by Kraus, an effort is made to correlate specific strong bursts with satellites; it is concluded that positive results are obtained. In the next two papers a more statistical approach is used but negative conclusions are reached—neither investigation yielded effects which could be ascribed with certainty to a satellite. The general impression left on the reader is that this conclusion is more likely to be correct. The next three papers (by E. H. Walker, by L. Peters and W. G. Swarner, and by K. M. Chen) discuss the calculation of the scattering of electromagnetic waves by a satellite wake. Rather oddly, in view of the above conclusion, it is found that because of the rod-like form of the trail sufficient energy might be focused in an approximately normal direction to give effects comparable with those observed.

The uneven quality of its subject-matter makes it difficult to recommend this book without reservations, though, at best, as in the chapter by Opik, it is very good. The subject-matter for the most part covers a rather narrow field and will, therefore, interest specialists. On the other hand, three contributions have some general application in astronomy and plasma physics and will attract a wider audience. The index is comprehensive and, for the most part, well selected. A. P. WILLMORE

QUANTUM APPROXIMATIONS

JWKB Approximation

Contributions to the Theory. By Nanny Froman and Per Olof Froman. Pp. viii + 138. (Amsterdam: North-Holland Publishing Company, 1965.) 32*s*.

THE subject of this book is the mathematical approximation method, which to most authors in theoretical physics is known as the Wenzel–Kramers–Brillouin (WKB) method. Apart from an orthodox derivation of the WKB formulae in Chapter 2, the book is based entirely on the original work of the authors.

Their aim has been to provide a mathematical apparatus [illegible]gorous treatment and systematic applica[illegible]. In this, they have added considerably [illegible] of the method. The important [illegible]imits of error in applications [illegible]d is clearly discussed in

The general solution of this system of equations is obtained in the form of four convergent series expansions. For the derivation of the exact solution and subsequent discussion of its properties (Chapters 4–7) a particularly elegant matrix notation is introduced which greatly condenses and simplifies the presentation.

In the final three chapters (9–11) the important points emerging from the general theory are illustrated by detailed applications to some well-known physical problems; these are barrier transmission problems, the bound states of a particle in a potential well, and the radial wave equation for a spherically symmetric potential.

The material in this book, which is clearly presented and well illustrated with contour diagrams, should be of interest to many theoretical physicists. D. W. Wood

HYDROGENATION

Catalytic Hydrogenation

Techniques and Applications in Organic Synthesis. By Robert L. Augustine. Pp. xii+188. (London: Edward Arnold (Publishers), Ltd.; New York: Marcel Dekker, Inc., 1965.) 72*s*. 6*d*. net.

For more than half a century the techniques and mystiques of catalytic hydrogenation have been fruitfully used in synthetic organic chemistry, and a great deal of information of what catalyses which reaction and under what conditions lies scattered throughout the chemical literature. Dr. Augustine has performed a useful function in summarizing what is known in this field and presenting it in a form which will be readily assimilated by experimental organic chemists.

After a brief introduction, Chapter 2 treats apparatus and techniques with quite detailed descriptions of equipment used both at atmospheric pressure and at elevated pressure, with proper emphasis in the latter case on safety precautions. Chapter 3 deals with catalysts and conditions; the preparation, properties and catalytic usefulness of hydrogenation catalysts in common use (Raney nickel, copper chromite, Adams platinum oxide, palladium on charcoal, etc.) are described. The reader could, however, be pardoned for forming the erroneous impression that there is only one manufacturer of supported noble metal catalysts.

It is in the second part of this chapter that Augustine's presentation is weakest. The proper choice of reaction conditions is of paramount importance in determining the yields of products, but the effect of agitation is dismissed in one sentence without mentioning diffusion or transport control, and although efficient use of the catalyst is clearly not a prime consideration in preparative organic chemistry, it is well established that agitation conditions can effect selectivity in multi-stage reactions. The effect of temperature is likewise dismissed somewhat lightly. There is no mention of the fact that the increase in vapour pressure of the solvent with temperature serves to reduce the partial pressure of hydrogen and that this can under certain circumstances give an apparently negative activation energy. The effect of quantity of catalyst is also discussed without reference to diffusion limitation in a monumentally cumbersome sentence. The author's style in this section is often obtuse, and sometimes ambiguous. The sentence (page 46): "All hydrogenations, except that of nitro groups, in which a nickel catalyst is used, increase in rate with the addition of base", besides being ungrammatical, is capable of two distinct interpretations: such is the power of the mighty comma!

There follow two chapters on the hydrogenation of functional groups in which the author considers in turn the various reducible groups encountered in organic chemistry: he starts each section by stating a general recipe for the hydrogenation, and subsequently enlarges and qualifies this by means of illustrative examples. Perhaps wisely, the author eschews any mechanistic discussion, although some further attempt at rationalization of the observations would not have come amiss. It is, however, these two sections, and the following chapter on hydrogenolysis, which will be most widely appreciated and used.

It is commonplace, to-day, to complain of the price of scientific books: many, I fear, will wonder whether 72*s*. 6*d*. is not too high a price to pay for less than 200 pages, whatever their value. G. C. Bond

LIVING STRESS

The Physiology of Human Survival

Edited by O. G. Edholm and A. L. Bacharach. Pp. xxii+581. (London Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 140*s*.

In their introductory remarks the editors state that this book arose from discussions between themselves about the factors affecting the survival of the individual and his ability to adapt to changing conditions. They have gathered together articles by sixteen writers, physiologists and psychologists, relating primarily to the responses of human beings to a wide variety of external and internal stresses ranging from those of extremes of temperature and pressure on the one hand to sleep deprivation and pregnancy on the other. The editors aimed at bringing together all the important information at present available on the effects of environmental stress on man including, where it is necessary for a more complete understanding, accounts of the effects on other mammals. It is relatively easy to pick out areas which have not been dealt with in this book, but the editors point out that they did not intend it to be all-embracing and in certain respects it may be regarded as being complementary to the volume in the Handbook of Physiology series published by the American Physiological Society on *Adaptation to the Environment* which dealt with the whole animal kingdom. The contributors were asked not to produce a complete review of their subject but rather to concentrate on their own fields of research within the subject allocated to them. This request has been met by a variety of responses so that the scope of the articles ranges from a comprehensive review of the effects on man of deficiencies and excesses of water and salt intake by W. S. S. Ladell to a relatively detailed account by J. D. Hatcher of his own work on the humoral mechanisms responsible for the increase of cardiac output induced by acute anoxic anoxia.

The first six chapters of the book deal with the responses of man to stresses associated with changes in his physical environment. The first two chapters, one on the effects of cold by L. D. Carlson and A. C. L. Hsieh, and the other by R. H. Fox on the effects of heat, reflect the different interests of these authors. Thus, while the chapter on cold is based on a mathematical treatment of heat exchange, Fox deals at length with the problems of finding a reliable index of heat stress and the techniques for studying and determining the physiological changes responsible for heat acclimatization. Hatcher's account of the cardiovascular and respiratory responses to acute anoxia is followed by a comprehensive description by L. G. C. E. Pugh of the adaptive responses to hypoxia which occur on prolonged exposure to high altitudes. A brief review of the physiological effects of exposure to high pressures by H. J. Taylor shows the many phenomena in this field which invite further study. Progress in one particular field is illustrated by P. B. Bennett's article on the narcotic action of inert gases. An authoritative account by P. Howard of the effects of high and low gravitational forces brings out the contrast between extensive present-day knowledge of the mechanisms which underlie man's reactions to the stress of increased gravitational field and the paucity of

information concerning the physiological effects of the weightless state.

The second section of the book consists of articles which deal with nutritional stress and exercise. In a discursive review of nutritional changes, R. A. McCance and E. M. Widdowson point out that man has himself created the nutritional stress of a surfeit of calories. There follows W. S. S. Ladell's extensive account of the effects of the disturbance of water and salt balance. J. V. G. A. Durnin presents in a provocative chapter an objective evaluation of the value of exercise, and emphasizes the need for adequate physical exercise in modern society. A chapter by F. E. Hytten and A. M. Thomson on the stress of pregnancy presents a summary of the physiological changes which occur in this condition, and discusses in detail the associated changes of food stores and energy requirements.

In the next section of the book, Mary C. Lobban calls on the wealth of experimental data which she has collected in her studies at Spitsbergen to present some of the challenging problems associated with the 'biological clocks' of the human organism. R. T. Wilkinson describes the difficulties which have been encountered in attempts to demonstrate physiological and biochemical changes as a result of deprivation of sleep. Indeed, he points out that it has only recently been possible to demonstrate objectively the decrement of performance of which one is aware after a sleepless night.

In a stimulating article A. T. Welford describes the present state of knowledge with respect to the psychological and physiological mechanisms involved in fatigue. This chapter, taken in conjunction with the succeeding one by S. M. Hilton, in which he describes his studies on the neurophysiological mechanisms underlying emotional expression, demonstrates how the artificial barriers which have for so long separated psychology and physiology are being broken down.

The final chapter of the book by R. W. Brauer, which is entitled "Irreversible Changes", emphasizes the areas in which further experimental work is required in order that the physiological processes which underlie human reactions to stress may be better understood.

I enjoyed reading this book and it will no doubt be of value to those concerned with human biology. The specialist working in one of the more applied fields of human physiology will find much of interest, particularly in regions other than that with which he is directly concerned. The editors are to be congratulated on the result of their efforts to obtain uniformity of expression between the sixteen contributors to this book. J. ERNSTING

BREATH OF LIFE

Oxygen

(Proceedings of a Symposium sponsored by the New York Heart Association.) Pp. 283. (London: J. and A. Churchill, Ltd., 1965.) 42*s*. 6*d*.

FEW substances are as familiar as oxygen. With these words the chairman of the symposium committee begins his general introduction to the proceedings of a symposium sponsored by the New York Heart Association. Let us hope the spate of books on the biological importance of oxygen does not make us contemptuous. However, this volume makes a valuable contribution to a most important field, despite its appearance so soon after the larger work, *Oxygen in the Animal Organism*, published in the International Union of Biochemistry Symposium Series.

The work is divided into four parts, each introduced by an eminent authority and followed by a discussion.

In the first part, introduced by D. Rittenberg, M. Dole describes the "natural history" of oxygen, beginning with its cosmic generation and going on to the formation of molecular oxygen in the Earth's atmosphere. The main part of the first paper is concerned with an attempt to quantitate the mechanisms for the production of molecular oxygen. The first of these before the appearance of life on Earth is the photolysis of water vapour and the second photosynthesis. The production of oxygen permitted the appearance of other living systems for two reasons: it provided an energy-producing mechanism, the reduction of molecular oxygen, and in addition the appearance of oxygen, at about 1 per cent of the present level, was followed by the formation of ozone, which acted as a barrier to ultra-violet light. This event occurred in the Cambrian era and was followed by an "evolutionary explosion".

The second paper, by H. Taube, is concerned with mechanisms of oxidation with oxygen. It begins with a discussion of the relevant aspects of the electronic structure of oxygen and goes on to consider oxidation mechanisms generally as biological models.

The second part, on oxygen-transporting pigments, is introduced by R. Forster. Only two pigments are considered: haemoglobin and myoglobin. They illustrate two different types of transport mechanism. Haemoglobin transports oxygen in the blood from the lungs to the tissues by increasing the capacity of blood for oxygen, whereas myoglobin in the tissue facilitates the transport of oxygen by a rather different mechanism. That myoglobin may facilitate the diffusion of oxygen in tissue is a recent hypothesis, and J. Wittenberg considers some of the possible mechanisms. In the second paper in this section, P. Urnes discusses the important question as to how far the magnificent work on the structure of crystalline myoglobin is relevant to the structure of the molecule in solution. From observation of the ultraviolet optical rotatory dispersion of the solution, he concludes that no appreciable change in conformation occurs on solution. F. J. R. Roughton's masterly account of recent work on haemoglobin closes this section. His main theme is the possible role of aggregation of the haemoglobin molecule on oxygenation. There seems to be a range, from the lamprey, in which aggregation is of fundamental importance to the reaction, to the sheep, whose haemoglobin shows minimal change of aggregation on oxygenation.

V. Dole introduces the third section, on oxygen consumption in the cell. In the first paper, entitled "Oxidation and Peroxidation", P. Nicols considers the reaction of the haem enzymes with molecular oxygen and discusses a different kind of theory of the mechanism. R. Butow and E. Racker, in their paper on the mechanism of respiratory control, discuss the properties of their new model in which the "loosely coupled' sub-mitochondrial respiratory particle is made "tightly coupled" by the addition of *o*-phenanthroline.

The section is closed by B. Chance, who considers the way in which the oxygen and control reactions described in the previous two papers could co-operate to establish a purposeful metabolic control *in vivo*.

The final section, introduced by S. Granick, is entitled "Non-Haemoproteins and Oxygen". In the first paper, Q. Gibson describes the mechanisms of reaction of some flavoprotein enzymes with oxygen. These differ from the haem enzymes in that oxidation and reduction involve two equivalents and the evidence for the existence of intermediately oxidized forms is considered. In the second paper, E. Frieden, S. Osagi and H. Kobayashi survey the interaction of the copper proteins and oxygen and consider the correlation between structure and function. Finally, H. Goldfine in his paper "The Evolution of Oxygen as a Biosynthetic Agent" presents a completely different aspect of the biological role of oxygen. All the other papers were essentially concerned with the role of oxygen in bioenergetics, but this final paper considers it as a synthetic agent, as an integral part of cell constituents.

Each of the four sections is followed by a general discussion, which has clearly been carefully edited but without losing the spontaneity of some of the comments. The book is well printed and reasonably well bound; the index, however, contains some errors but it is of a higher standard than is sometimes found in published proceedings. I. S. LONGMUIR

STAFF OF RUSSIAN LIFE

Biochemistry of Grain and Breadmaking

Edited by V. L. Kretovich. Translated from the Russian by N. Kaner. Edited by E. Seijffers. Pp. xi+259. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 81*s*.

FOOD technologists, plant breeders and cereal chemists in particular will read this book with interest and profit. It is a collection of thirty papers read at the All Union Conference on Biochemistry and Breadmaking in Moscow in 1960. The Conference was held by a decision of the twenty-first Congress of the Communist Party of the Soviet Union as part of the National Seven Year Plan. Twenty-six of the papers are concerned with wheat and the remaining four with maize, rice, barley and sorghum. The Conference was held in 1960 and it is easy to say that the papers are now dated. This would be most misleading. Some of the problems may have lost a little of their novelty, but they still remain, and this book brings them together, defines them very clearly and, wherever possible, relates them.

The Conference was opened by N. M. Sisakyan, biological secretary of the Academy of Sciences, who stated that the Soviet Union is "a grain producing world power with unlimited possibilities", and in which "the crop yields and grain quality should be the highest in the world". He read out two resolutions approved by the Academy of Sciences, one of which is certainly worth pondering over: "The importance of a more concentrated effort in tackling the major research problems must be impressed on scientists and scientific organizations. They must be encouraged to display the maximum possible initiative and creative ability, and must keep informed on the progress of science and participate in the discussions taken on the scientific research policy".

A masterly paper by Prof. Kretovich opened the Conference and outlined the scope of the field of research involved in the decision of the twenty-first Congress. Here and there he highlighted certain aspects with important practical applications, such as the possibilities of breeding maize and wheat with a high lysine content, the protein complex of flour and the changes it undergoes during breadmaking, and the proper drying of damp grain. He also emphasized the part that electronics can play in the development of automatic analytical methods for use in the handling of cereals and in the operations of milling and baking.

It is invidious to single out individual papers, but I read with particular interest that on the effects of irradiation on the baking properties of wheat by Sosedov and Vakar, on the biochemical effects of wheat conditioning by Zotova and Kretovich, on the structure of cereal proteins by Reznichenko, and on the action of oxidizing and reducing agents on gluten by Shklovskii. Another paper, by Auerman *et al.*, in making a strong case for the widespread use of ascorbic acid as a bread improver, is of particular interest in Britain in the light of recent developments in rapid breadmaking in which ascorbic acid is an essential ingredient.

My most striking impression is perhaps the close similarity both of the problems and of the manner in which they are being tackled in the U.S.S.R. and in Britain. This perhaps is not surprising, yet apart from some references to the work of Chibnall and Sanger on the chemistry of proteins, there are practically no references to papers published outside the Soviet Union. But then how many references to Russian work would be found even in a review paper on any aspect of cereal chemistry published in Britain? The book certainly suggests that an International Scientific Conference focused on the staff of life would present no difficulties over a programme, would be well worth while, and any recommendations regarding future research would be unanimous. As Academician Sisakyan pointed out, the biochemistry of grain and that of breadmaking are of the utmost scientific and practical importance and play a major part in his country's economy. In this respect the U.S.S.R. is not unique. T. MORAN

Weak Interaction of Elementary Particles

By L. B. Okun'. Translated from the Russian by S. and M. Nikolić. Translation edited by J. Bernstein. (International Series of Monographs in Natural Philosophy, Vol. 5.) Pp. x+292. (London and New York: Pergamon Press, Ltd., 1965.) 60*s*. net.

THIS book by Prof. L. B. Okun' of the Institute of Theoretical and Experimental Physics, Moscow, is intended for graduate students who are specializing in high energy physics. It really starts from the beginnings of the subject, so that it can be put into the hands of a research student as soon as he starts work, provided only that he has had a reasonable general grounding in quantum mechanics.

In just under three hundred pages it goes systematically through the subject of weak interactions. It starts with a general discussion of the fundamental interactions and defines the operations of space, time, and particle (charge) reversal. Then follows a series of chapters on pure leptonic, semi-leptonic and non-leptonic decays. Calculations are presented in considerable detail, and there is no resort to "it can be shown" or "it is easy to see".

The book is based on lectures originally given in 1960 and still carries the flavour of that period with its major emphasis on parity non-conservation. Some revision has been made during the translation in 1964, which consists mainly of a long additional chapter on unitary symmetry. There is, of course, no mention of the recent discovery that charge-parity is also not universally conserved. This matters less than it might, because it has not yet been incorporated in any satisfactory way into the general theory, but remains for the moment an isolated freak effect of the neutral K-complex.

Okun' is one of the best-known high-energy theoretical physicists of the U.S.S.R. with a world-wide reputation for his clear thinking and incisive critical approach. The translation has been edited by J. Bernstein, also a high-energy physicist of international repute, who has contributed to the *New Yorker*, and takes a pride in finding the felicitous phrase. The result is something rare—particularly when the transition is from Russian into English—a first-rate translation of an excellent book.

P. T. MATTHEWS

Selected Problems in Quantum Mechanics

Collected and edited by D. ter Haar. Revised and augmented second edition of Gol'dman, Krivchenkov, Kogan and Galitskii, "Problems in Quantum Mechanics". Pp. vii+402. (London: Infosearch, Ltd., 1964. Distributed outside the U.S.A. by Macmillan and Co., Ltd., London.) 50*s*.

THE second edition of this excellent collection of problems is certainly to be welcomed. The problems, with detailed solutions, cover most aspects of non-relativistic quantum mechanics, with a range of difficulties covering the whole spectrum of undergraduate and postgraduate teaching. The changes from the first edition are an increase in the number of problems from 333 to 404 and a greatly improved typography.

A collection of problems such as this is usually consulted both to test one's knowledge and to further it. In many ways these are separate functions, and perhaps the only real criticism of the book is that the two functions are not separated well enough. Undergraduate students in particular would often want to use the book as a practice for examinations, and it is a pity that the simpler problems are not differentiated from the others in some way: often the only way for such a student to discover that an apparently simple problem requires mathematical techniques beyond his ability, apart from wasting hours of effort, is to look firstly at the solution in the back of the book! On the other hand, more senior students who are using the book as advanced reading might well prefer the difficult problems to be clearly grouped together in each chapter.

This is a small point, however, which should not prevent this second edition being as widely read as the first, as it well deserves. F. H. READ

Ultrasonic Engineering

By Julian R. Frederick. Pp. xii+379. (New York and London: John Wiley and Sons, Inc., 1965.) 113*s*.

THE author of this book is an associate professor of mechanical engineering in the University of Michigan and as therefore might be expected he deals very competently with the engineering aspects of ultrasonics. He has also had the earlier benefit of being associated with Firestone when the latter was developing his ultrasonic flaw detection systems, and so Chapter 7 dealing with this subject is a very authoritative contribution to the literature. Inevitably, through compression into a single chapter, the basic theory has suffered somewhat in places through inadequacy of explanation, but these are small blemishes in a book which is written in a lucid style and where the text is supported by excellent line diagrams. It contains a wealth of practical and theoretical information which is by no means restricted to American sources. There is an excellent set of references and a most useful guide to ultrasonic literature which the growing field of users of ultrasonics will find invaluable.

R. W. B. STEPHENS

Parliament and the Executive

An Analysis with Readings. By H. V. Wiseman. Pp. xvi+271. (London: Routledge and Kegan Paul, Ltd., 1966.) 18*s*.

APART from an introduction, largely historical, of 46 pages, this book consists of 120 short extracts from writings about the relations between Parliament and the executive. They range from standard authorities such as Dicey and Bentham, Bagehot and Mill to the current writings of Sir Ivor Jennings, D. N. Chester, K. C. Wheare and Lord Campion, and are classified in eleven sections dealing with such subjects as collective and individual ministerial responsibility, the power of the Cabinet, the role of the House of Commons, questions and the use of committees. Scientific aspects of the formation of policy are not specifically discussed in these extracts, although the introduction shows some awareness of these aspects in explaining the choice and arrangement of the extracts. There is in fact little of direct interest to the scientist in this book and, while the variety of the extracts indicates the extent of the field to be considered, the work is unlikely to prove more satisfactory for the general reader. The extracts generally are too short and the introduction fails to gain the coherence which a well-chosen anthology should possess. To those embarking on a first study of British history or politics it may well provide a useful introduction to the literature, but Prof. Wiseman contributes disappointingly by way of criticism himself, though his selection of extracts is representative and well designed to assist the formation of a sound opinion on the role of Parliament in relation to the increased power of the executive. R. BRIGHTMAN

MEETINGS

DRUGS FROM ANIMALS

THE first international symposium on "Drugs of Animal Origin" was held at the Istituto di Ricerche Farmacologiche Mario Negri in Milan, during May 12–13, organized by Prof. Garattini (chairman) and Prof. Leonardi (secretary). As expected from the all-embracing nature of the title, the topics discussed varied enormously. They ranged from recent developments in the chemistry and pharmacology of hormones in well-established endocrine fields, to the introduction of new concepts about the part played by the body as its own pharmacologist and dispensing chemist.

The prostaglandins provided the largest single topic, and Prof. Bergstrom (Karolinska Institutet, Stockholm) opened the discussion with an excellent review of the present knowledge of their structure, biosynthesis, and metabolism. With this knowledge available, Dr. Vogt (Max Planck Institut, Germany) extended the scope to the release of prostaglandins from guinea-pig lung by cobra venom and by such milder stimuli as hypertonic saline. It would appear that prostaglandins provide a normal constituent of the slow-reacting substance released in this manner. The wider distribution of prostaglandins outside the site from which they take their name (the prostate) was clearly shown both by Prof. Horton (St. Bartholomew's Hospital Medical School, London) and by Dr. Paoletti (University of Milan). Prof. Horton's work demonstrated the presence of various prostaglandins in the central nervous system of the cat and the fowl, and showed the unusual pharmacological responses which injection of these drugs can induce. Dr. Paoletti's work was concerned with the systemic pharmacological and biochemical actions (for example, on blood pressure, heart rate, mobilization of glucose).

Another subject dealt with by a number of authors was the control of blood calcium levels. Until quite recently it was believed that the level of blood calcium was essentially under the control of parathormone—the hormone of the parathyroid gland. Dr. Aurbach (National Institutes of Health, Bethesda) presented a summary of the chemical, biological and immunological properties of parathormone, and suggested a probable structure. However, another hormone, thyrocalcitonin, has recently been isolated from the thyroid gland, and has been shown to possess an important plasma calcium-lowering property. This topic was briefly mentioned by Dr. Andreoli (Istituto di Patologia Medica, Rome) when considering the broader aspects of synthesis, metabolism and actions of thyroid hormones. A more detailed study was given by Dr. MacIntyre (Postgraduate Medical School, London), who described classical endocrine experiments in which perfusion of the vascularly inseparable parathyroid/thyroid gland of the dog was performed. Perfusion with blood containing high and low calcium levels resulted in low and high systemic blood levels respectively. Use of the goat (in which the thyroid and parathyroid glands are anatomically separate) strongly suggested that the hormone is secreted by the thyroid. The hormone was shown to act independently of the parathyroid hormone, and to produce its effect by increasing bone uptake of calcium, an effect probably arising from a reduction in the breakdown of bone. The possible clinical applications of thyrocalcitonin in hypercalcaemia and other diseases in which calcium metabolism is upset were discussed, both by Dr. MacIntyre and by Dr. Milhaud (Institut Pasteur, Paris), but so far only limited clinical results are available.

Other subjects discussed show the varied nature of the meeting. Dr. Schofield (Royal Veterinary College,

London) presented evidence obtained from experiments in guinea-pigs and sheep which demonstrated the action of the placenta on the myometrium, and indicated its possible function in labour. Dr. Jung (Universität Frauenklinik, Germany) demonstrated a substance of myometrial origin which activated the rat uterus, while inhibiting the human uterus.

Prof. Robson and Dr. Robinson (Guy's Hospital Medical School, London) presented evidence for the presence of an anti-inflammatory substance at sites of inflammation, with some preliminary data on its possible chemical nature. Dr. Masek (Institute of Pharmacology, Prague) described the pharmacological effects of palmitoyl ethanolamide, a compound which occurs naturally in some mammalian tissues, and which has been shown to possess anti-inflammatory, anti-anaphylactic, and analgesic actions.

Several papers dealt with various aspects of assay techniques used to estimate trophic factors associated with central control of endocrine function. Dr. De Wied (University of Utrecht, The Netherlands) reviewed the *in vivo* and *in vitro* methods available for the assay of corticotrophin releasing factor. Prof. Martini (University of Milan, Italy) then discussed a new technique known as the "pituitary depletion method", which has recently been developed in his department. This enables simultaneous evaluation of the activity of hypothalamic extracts on all pituitary hormones, and has facilitated investigation of the physiological significance of the hypothalamic control factors. Finally, in this sphere, advances in the techniques of extraction, separation and purification of human follicle stimulating hormone were reviewed by Dr. Reichert (Emory University, U.S.A.), who then went on to discuss the value of available biological assay methods. The preparation of purer samples of follicle stimulating hormone has, over recent years, made possible much greater accuracy in therapeutic dosage in cases of amenorrhoea, thus reducing the risk of multiple ovulation.

B. V. ROBINSON
J. M. ROBSON

NON-LINEAR ACOUSTICS

SIXTY-TWO people attended the one-day symposium on non-linear underwater acoustics, sponsored by the newly formed British Acoustical Society and organized on its behalf by Professor D. G. Tucker, which was held in the Department of Electronic and Electrical Engineering at the University of Birmingham on July 19. Professor Tucker took the chair for the morning session and Dr. R. W. B. Stephens (Imperial College of Science and Technology) in the afternoon.

Three of the five papers presented discussed the sound field arising from the interaction of two coincident acoustic waves. Professor S. Tjøtta (University of Bergen) presented a rigorous mathematical analysis based on the hydrodynamical equations while Dr. H. O. Berktay and Mr. B. V. Smith (University of Birmingham) gave alternative (and simplified) engineering approaches to the same problem. These papers showed that, as a result of the non-linear relationships of acoustic pressure with density on one hand and compressibility on the other, two coincident plane (or spherical) primary waves of differing frequencies produce virtual sources in the water at the harmonic and intermodulation frequencies. These virtual sources form a virtual acoustic array in the medium itself, and Dr. Berktay discussed in detail some possible transmitting applications of the virtual array formed at the difference frequency and illustrated these by experiments. He showed that a number of desirable results could be obtained economically, including high directivity at low frequencies with small transducers and a substantially constant beamwidth over a wide range of transmitted frequencies. These possibilities could completely revolutionize the design of echo-sounders, wide band sonars and transmit-beam scanning sonars. A small scale model of a non-linear sonar system was also demonstrated; a narrow beam of acoustic waves at a frequency of 100 Kc/s was formed from two primary waves in the region of 3 Mc/s. Professor Tjøtta made the further point that if one of the primary waves was used as a "pump" and the other consisted of an echo-signal from a distant target, parametric amplification of the weaker signal could be effected. During the discussion, the similarity between the parametric amplification of acoustic and electronic travelling waves was noted, particularly in regard to the need to suppress the sum frequency wave.

Mr. R. T. Smith (Imperial College of Science and Technology) showed that an initially sinusoidal wave of high amplitude becomes progressively more triangular owing to changes in the velocity of propagation of various parts of the wave. He also discussed the formation of shock waves in liquids and related this to the physical properties of the medium.

In the final paper Dr. V. G. Welsby and Mr. M. H. Safar of the University of Birmingham described investigations of the generation of harmonic and subharmonic frequencies when the amplitude of a single primary wave is increased to the region of the cavitation threshold of the medium. As the acoustic pressure is increased, the subharmonic level increases; but sometimes on approaching the cavitation threshold the subharmonic decreases slightly and then, after cavitation, rapidly increases. (On other occasions the dip near cavitation is less pronounced or even entirely absent; the factors governing its appearance have not yet been fully investigated.) On the basis of their experiments the authors posed a number of questions about the physical mechanisms involved, and discussion made it clear that there is considerable uncertainty on this topic.

The British Acoustical Society seems to have got off to a good start with a successful first meeting.

B. K. GAZEY

OBITUARIES

S E. Hollingworth

THE sudden death in London on June 23, 1966, of Professor Sydney Ewart Hollingworth, on the eve of retirement after 20 years in the Yates-Goldsmid chair of Geology at University College, London, was a shock to his many friends. He was the twelfth well-known British geologist to die since March 1965. Before becoming professor he had served 25 years with the Geological Survey of Great Britain where he had established a high reputation as a field geologist with an independent and critical outlook.

He was born on November 7, 1899, and educated at Northampton School. After a short period of Army service, he went to Clare College, Cambridge, in 1918, where he graduated with first-class honours in both Part I and Part II of the Natural Sciences Tripos, and in 1921 he was awarded the Harkness Scholarship. In the same year he took the external B.Sc. honours degree of the University of London and joined the Geological Survey. In his first winter he revised some of the geological boundaries for publication of the Shaftesbury one-inch map. Hollingworth then took his place in the Survey's Cumberland unit first under B. Smith and later T. Eastwood to become part-author of the Carlisle, Brampton, Whitehaven, Gosforth and Cockermouth maps. Besides studying the Ordovician, Carboniferous and other bedded rocks, he showed that the Ennerdale Granophyre was a

stock and not, as previously supposed, a laccolith. He identified the components of the Carrock Fell igneous complex and inferred that they were intruded as steeply inclined sheets or elongated lenses after the main Caledonian folding and during a trough-like subsidence of the region. He also threw light on the glacial retreat phenomena of western Edenside and the Solway Basin, drainage problems of the River Eden and solifluxion phenomena. Inspired by data from Cumberland he made a statistical analysis of high-level erosion surfaces in Britain.

After two years spent mapping in the Droitwich neighbourhood he was transferred to work on the one-inch maps of the Huntingdon and Cambridge areas. In the war years he worked mainly on the structure, stratigraphy and reserves of the sedimentary iron ore field developed in the Northampton, Wellingborough and Kettering areas. As a substantial legacy to geology he contributed a demonstration, with other colleagues, that some structures revealed in the Jurassic rocks in the ironstone field were superficial Pleistocene features caused by adjustment forces producing valley bulging and valley side cambering. In all, Hollingworth was part author of twelve of the Geological Survey's one-inch maps as well as a memoir on gypsum and anhydrite. The latter subject he amplified later when dealing with facies problems in the Permo-Triassic related to evaporite deposition and stratal correlation.

Hollingworth was selected for the Geology Chair at University College, London, in 1946, in succession to W. B. R. King, who moved to Cambridge. He proved to be an invigorating teacher. His special attributes were to develop competence in field geology and an appreciation of the industrial applications of the subject. He initiated research by his students in south-west Ireland and, subsequently, he established research groups in the Caledonian orogenic belt of northern Norway and later in Chile.

Hollingworth had exceptional gifts in debating the results of almost any geological research and thus was a stimulating but always friendly critic. These gifts were canalized into the affairs of the Geological Society of London, which he served as Secretary from 1949 to 1956 and as Vice-President (1956–58 and 1962–64). He was President from 1960 to 1962 and received the Society's Murchison Medal in 1959. He leaves a widow and two sons.

C. J. STUBBLEFIELD

Prof. P. Maheshwari

THE sudden and untimely death of Professor Panchanan Maheshwari on May 18, 1966, at Delhi, where he was head of the Department of Botany at the University, is a sad loss to botany not only in India but throughout the world. His was a dynamic and stimulating personality and nobody who met him could fail to be impressed by his energy, sincerity and simplicity, even if these good qualities were occasionally masked by an arrogance that could be misunderstood by those who failed to see beneath it. Maheshwari was a man of boundless energy and high ideals who set himself and his colleagues a very high standard in all the scientific work which they undertook. He could tolerate nothing that was second rate and he did not mince his words when he encountered inefficiency or obstruction in any shape or form. He was a good teacher with a broad outlook who not only inspired but also really cared for his students, who held him in high regard. And yet this broadness of vision was accompanied by ability to conduct research of a very high order in a specialized field, and his life's work on the embryology of plants is known throughout the botanical world.

Maheshwari was born at Jaipur on November 9, 1904, where he later went to school before proceeding to the Ewing Christian College, Allahabad, in 1921, graduating in 1925. Here, ably influenced and guided by the late Dr. Winfield Dudgeon, an American missionary teacher, he took his M.Sc. and D.Sc. degrees at the University of Allahabad in 1927 and 1931, respectively. Joining the staff of Agra College, Agra, in 1930, he became associate professor in 1936. Subsequent appointments were at the University of Allahabad (1937–39), Lucknow (briefly in 1939), and he became reader and head of the department of biology at the University of Dacca later the same year. The best-known period of his career began in 1949 when he was appointed professor and head of the Department of Botany at the University of Delhi, a post which he continued to hold until he died.

Maheshwari travelled widely. During his first visit to Europe in 1936–37 he was greatly inspired by meeting the eminent embryologist Professor Karl Schnarf of Vienna, who gave him the idea of writing *An Introduction to the Embryology of Angiosperms*, which was completed at Harvard University in 1945. He was a member of and held office in numerous scientific societies, but, of these, perhaps particular attention should be directed to the fact that he was president of the Indian Botanical Society in 1951 and became founder and first president of the International Society of Plant Morphologists in the same year. This last society is responsible for publishing the journal *Phytomorphology*, first issued in 1951 and of which Maheshwari has been the sole editor.

Maheshwari's *An Introduction to the Embryology of Angiosperms* has for many years been widely recognized as the leading textbook on the subject written in English, and a Russian translation has also been published. The more recent *Advances in the Embryology of Angiosperms*, edited by Maheshwari and published in 1963, is also widely used. It is impossible here to survey his numerous publications in scientific journals and it must suffice to say that until quite recently most of them fell into the broad field of descriptive morphology with special emphasis on the embryology of flowering plants, using the term embryology in the broadest possible sense. With his marked capacity for sifting and arranging factual information, Maheshwari, having mastered a wealth of detail, classified and clarified the main lines of variation in plant embryology that are known to occur. He also discussed the taxonomic and phylogenetic conclusions that can be drawn from embryological knowledge. In recent years he turned his attention to the experimental side of embryology, and some investigations on this important aspect were still in progress when he died.

Because of Maheshwari's influence, the study of plant embryology and floral morphology has assumed an important role in the development of botany in India in recent decades, especially among Ph.D. students. There may be some who feel that these research exercises have been too repetitive. It must be remembered, however, that theoretical conclusions based on descriptive aspects of botany are likely to be invalid unless they are supported by factual information drawn from investigations that are extensive and thorough. It was because Maheshwari realized that this is so, and because of his heavy teaching and administrative duties, that he adopted the only course open to him in seeking the aid of many collaborators and students to achieve his aim. The result must be judged from the synthesis of these exercises which Maheshwari achieved rather than on the individual researches themselves, however valuable these may be. Viewed in this light, Maheshwari's contribution to botany is a notable one, especially when we remember that he himself regarded his own work as only a part of the even broader synthesis of descriptive botany that will become possible when his contribution has been added to that of related morphological disciplines.

Maheshwari, who was married at the age of 13, is survived by his widow and by three sons and three daughters.

I thank Professor B. M. Johri and other colleagues of the late Professor Maheshwari for providing some of the particulars included in this notice.

C. R. METCALFE

INVERSE COMPTON EFFECT IN QUASARS

By M. J. REES and DR. D. W. SCIAMA
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THE importance of the inverse Compton effect in intense radio sources has been emphasized on several occasions[1-4]. In particular, Hoyle, Burbidge and Sargent have suggested[4] that Compton losses are so serious as to rule out some models for variable quasi-stellar sources (quasars) unless these objects are very much closer, and less luminous, than they would be if their red-shifts indicated a Hubble recession. The nature of the red-shifts of quasars has not yet been conclusively settled by observations, and therefore interest attaches to theoretical arguments which give any clue to this problem. In this article we present a further discussion of the effect of inverse Compton scattering, and hope to show that there are plausible models which assume that quasars are superluminous objects at cosmological distances.

Hoyle, Burbidge and Sargent considered a model for the variable part of 3*C* 273 with the following properties: (i) the radius of the region emitting the optical continuum is $\sim 10^{17}$ cm; (ii) the optical continuum is synchrotron radiation; (iii) all the electrons are accelerated within a volume with dimensions $\ll 10^{17}$ cm, and the radius of the source is determined by the distance which the radiating electrons can move before losing their energy.

Assumption (i) was adopted in view of the fact that the optical brightness of 3*C* 273*B* varies with a time-scale of a few months[5], and the light was assumed to be synchrotron radiation because of the observed optical polarization[6]. The variations are assumed to be caused by changes in the rate of production of relativistic electrons, and (iii) was adopted because of the difficulty of synchronizing the different parts of a larger region. We shall discuss this assumption later. We note, with reference to (ii), that polarized light could also be produced by inverse Compton scattering of polarized radio frequency radiation. We have previously[2] suggested a model in which the visible light is indeed produced in this way, and we shall discuss this possibility further in this article. (There is, incidentally, no evidence that the variable component of the light from 3*C* 273 is polarized.)

Hoyle, Burbidge and Sargent claimed that their assumptions could not be simultaneously satisfied if the power output has the value appropriate to a quasar at a cosmological distance. We shall show that their assumptions, strictly interpreted, do not in fact permit models of quasars even if they are "local" objects. However, we shall argue that assumption (iii) is not physically reasonable, and that, if it is dropped and we take a plausible value for the size of the region where the electrons are accelerated, it is possible to interpret the optical continuum radiation from quasars such as 3*C* 273*B* as either synchrotron or inverse Compton radiation, even if these objects are at cosmological distances.

Estimates of the Importance of Inverse Compton Scattering

In Fig. 1 the quantity τ_{sync} (defined as c times the synchrotron lifetime) of electrons emitting synchrotron radiation at the optical frequency of $\sim 3 \times 10^{14}$ c/s is plotted as a function of magnetic field H. We also plot the radius $r_{\text{s.a.}}$ of a self-absorbed source emitting synchrotron radiation at a rate $\sim 10^{31}$ ergs/sec/(c/s) at this frequency (the intensity appropriate to 3*C* 273*B* if it is at a cosmological distance). If the electrons are all accelerated in a region which can be treated as a point, the larger of these two numbers will determine the radius of the source (if we neglect for the moment the effect of Compton losses). If $r_{\text{s.a.}} > r_{\text{sync}}$ the lifetime of the electrons will be prolonged by the reabsorption of synchrotron radiation, so they will be able to reach the boundary of the emitting region despite their very short synchrotron lifetimes.

The "Compton distance" r_{comp} is also plotted on the same diagram. We define this to be $c\tau$, where τ is the time in which an electron would lose half its energy as a result of inverse Compton collisions, if it moved in a radiation field with energy density equal to the mean synchrotron energy density within the source. If r_{comp} is less than the radius of the source, Compton losses will be important, and the model will need to be modified to take them into account.

The point emphasized by Hoyle, Burbidge and Sargent —that Compton losses are more important than synchrotron losses, and that source models of this kind calculated ignoring the Compton effect are consequently not self-consistent—is shown clearly by the diagram, because r_{comp} is much less than the radius of the source for any reasonable value of the magnetic field strength. If the magnetic field were tangled, the source radius might be $\ll r_{\text{sync}}$ when there is no self-absorption. The discrepancy between r_{comp} and the source radius would then be even larger. The effect of the inclusion of self-absorption, which was not taken into account by Hoyle, Burbidge and Sargent, is to reduce the disparity as the field becomes very strong, but H would have to become impossibly large before the discrepancy disappeared altogether.

At first sight it may seem surprising that the occurrence of self-absorption can reduce the importance of the Compton losses, as one might expect these to be even more

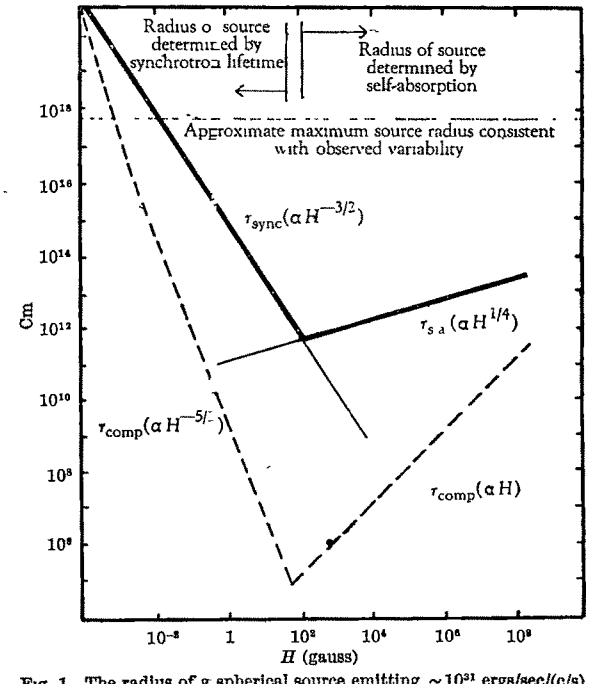


FIG. 1. The radius of a spherical source emitting $\sim 10^{31}$ ergs/sec/(c/s) at $\sim 3 \times 10^{14}$ c/s, in which the electrons are all assumed to be accelerated at its centre. The radius is approximately equal to the larger of r_{sync} and $r_{\text{s.a.}}$, where r_{sync} is c times the synchrotron lifetime of the appropriate electrons, and $r_{\text{s.a.}}$ is the radius of a self-absorbing source emitting the required flux. The graph also plots r_{comp}, which is defined to be the distance which the electrons would travel in a radiation field with the mean energy density of the synchrotron radiation within the source, before losing half their energy as a result of inverse Compton collisions. These quantities are only plotted for values of H for which the gyro-frequency of non-relativistic electrons is less than 3×10^{14} c/s. The small values which this graph gives for the source radius are not physically reasonable, for reasons which are given in the section headed "Dimensions of the Region where Electrons are Accelerated"

dominant if the net synchrotron radiation loss of the electrons is reduced. However, this is not so for two reasons: (i) the effect of self-absorption is to increase the dimensions of the source, and so reduce the radiation energy density; (ii) in a strong field the energy of the electrons which emit synchrotron radiation at a given frequency is less, and low energy electrons survive longer in a radiation field of given energy density.

If the electrons are all accelerated at the centre of the source, it is clearly not consistent to treat the Compton losses as a mere perturbation when r_{comp} is less than the source radius. Furthermore, we can easily see that no attempt to take the Compton effect properly into account can then lead to a consistent model, for the radius of such a model would obviously be smaller than the radius we calculated when we ignored Compton losses. The mean radiation density within the model would be correspondingly greater, and there would be an even greater discrepancy between the "Compton distance" and the source radius. If electrons were ejected from a negligibly small region, they would in fact radiate their energy predominantly in the form of γ-rays (produced as a result of repeated Compton scatterings) with energy comparable with that of the individual relativistic electrons. The radiation would come from a very small sphere opaque even to γ-rays, and a negligible fraction of the energy would be emitted at radio frequencies. Obviously such a source could not emit a detectable radio flux unless there were an absurdly high γ-ray flux.

It was stated by Hoyle, Burbidge and Sargent that this effectively rules out a model in which the optical frequency flux from a quasar such as 3*C* 273*B* results from Compton scattering of radio frequency photons by the same electrons which are responsible for the radio (synchrotron) emission. It is true that such models could not produce vastly more energy by the Compton process than by synchrotron emission without emitting unreasonably large amounts at X-ray and γ-ray frequencies by repeated scatterings. However, it is certainly possible for the two losses to be of comparable importance. One is encouraged to attempt to devise such a model for 3*C* 273*B* by the fact that the energy radiated in the optical continuum is almost certainly less than that emitted at radio frequencies $\lesssim 3 \times 10^{11}$ c/s (wave-lengths $\gtrsim 1$ mm). A model of this kind has been previously suggested by us[2], and also by Shklovski[3]. We shall later on describe such a model in greater detail, but Fig. 2 immediately suggests that such a possibility is likely to be quantitatively reasonable. Fig. 2 shows the same quantities as Fig. 1, but this time plotted for a source emitting synchrotron radiation with an observed intensity of ~300 flux units at 3×10^{11} c/s (which is consistent with Epstein's observations[7] of 3*C* 273 at millimetre wave-lengths). We see that the "Compton distance" exceeds the radius of the source when the field strength $\gtrsim 30$ G (and also when $H \lesssim 10^{-3}$ G, though we may ignore this case because the source would then be too large to exhibit rapid variations). We emphasize that this result is only obtained when allowance is made for synchrotron self-absorption. If the radius of the source were determined by the very short synchrotron lifetime of electrons in a field $\gtrsim 30$ G, the radiation density would be very high, and the Compton losses would be too great.

Dimensions of the Region where Electrons are Accelerated

In the preceding section it was assumed (following Hoyle, Burbidge and Sargent[4]) that Compton losses would not be serious if the Compton lifetime of electrons moving in a radiation field with the mean energy density of synchrotron radiation within the source exceeds the time they would take to cross the source. However, we shall show that, if the electrons all originate in a small region at the centre of the source, Compton losses are more important than this criterion would suggest. This is because the energy density of the scattered radiation is bound to exceed the synchrotron radiation density (implying that the model requires drastic modification) at points close enough to the centre, even if the original criterion indicates that Compton losses are not important.

If synchrotron self-absorption occurs, the energy density of synchrotron radiation will be almost uniform throughout the source. We can assume, as a rough approximation, that the electrons have a mean outward velocity ($\sim c$) which is independent of r. The electron number density will then be proportional to r^{-2}, and the rate of production of Compton scattered photons, per unit volume, will also be proportional to r^{-2}. Provided that the scattered photons escape from the source without undergoing further scattering, the energy density of the Compton scattered radiation within the source $\propto r^{-1}$. If, on the other hand, synchrotron self-absorption is negligible throughout most of the source, the synchrotron radiation energy density $\propto r^{-1}$ (implying incidentally that there must be a small self-absorbing region near the centre), and the Compton scattered radiation density will be $\propto r^{-2}$. In both these cases, therefore, the energy density of inverse Compton radiation relative to that of synchrotron radiation $\propto r^{-1}$. So however small this ratio at the boundary of the source, it will become large at points sufficiently near the centre. The model then becomes inconsistent, for the reasons discussed in the preceding section. It is therefore impossible to devise a model producing a significant amount of synchrotron radiation in which the relativistic electrons are assumed to originate at a point, even if r_{comp}, as defined earlier, is much greater than the source radius. This remark applies equally strongly if quasars are 'local' objects, and the required luminosity is comparatively low.

Other physical considerations obviously set lower limits to the size of the volume within which the relativistic electrons can be generated. The total energy which a quasar at a cosmological distance would radiate during its lifetime may well be in excess of 10^{60} ergs, and an object capable of liberating this amount of energy must have a very great mass. Because the Schwarzschild radius of a mass of, say, 10^{43} gm is $\sim 10^{15}$ cm, it is clear that the relativistic particles in a realistic model cannot be assumed to originate in a region many orders of magnitude smaller

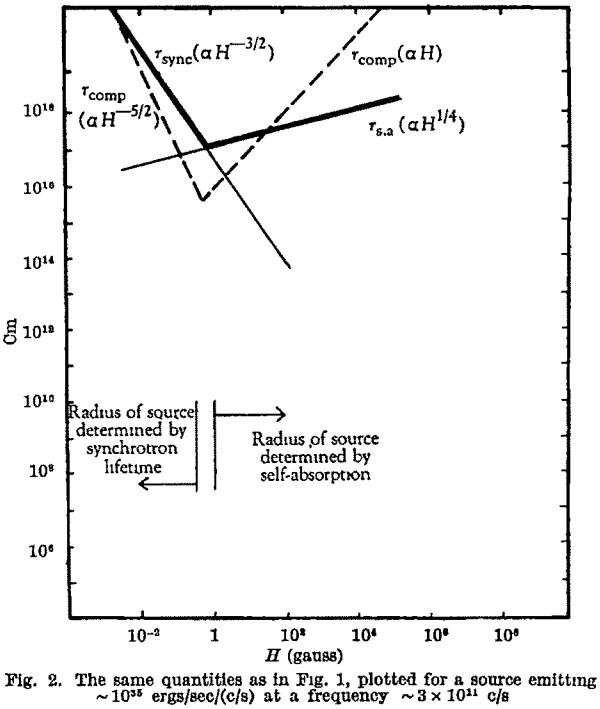


Fig. 2. The same quantities as in Fig. 1, plotted for a source emitting $\sim 10^{35}$ ergs/sec/(c/s) at a frequency $\sim 3 \times 10^{11}$ c/s

than the whole variable source. The larger this region has to be, the more difficult it becomes to synchronize variations in the rate of electron production over the whole volume. (It was for this reason that Hoyle, Burbidge and Sargent preferred not to assume that the electrons were being accelerated throughout the source.) We shall suggest in the next section some possible models.

Models for 3C 273 and Similar Variable Quasars

The first point to be made is that there *are* reasonable models capable of accounting for the optical variability of quasars on the assumption that they are at cosmological distances, and that the optical continuum is synchrotron radiation. For example, if the particles are being accelerated throughout a sphere of radius $\sim 10^{16}$ cm containing a magnetic field $\gtrsim 100$ G, the synchrotron lifetime of electrons radiating at visible frequencies will be shorter than the Compton lifetime (though these will both be much shorter than the time it would take for an electron to cross the source). The Compton losses will therefore be comparatively unimportant.

But there are also possible models in which the optical flux results from Compton scattering. If the observed flux at 3×10^{11} c/s (~ 300 flux units) is synchrotron emission, Fig. 2 shows that the dimensions of the emitting region must be $\sim 5 \times 10^{17}$ cm if $H \sim 100$ G. The electrons responsible for this synchrotron radiation will have $\gamma \sim 50$, and the radiation produced when such electrons scatter millimetre wave-length photons will be in the optical range. The observed flux density of the optical continuum is $\sim 0{\cdot}03$ flux units[8,9], so the energy radiated in this range is ~ 10 per cent of the total emission at frequencies $\lesssim 3 \times 10^{11}$ c/s (allowing for the factor $\sim 10^3$ difference in the frequency ranges). Using this fact we can easily determine the minimum size of the region within which the electrons are accelerated. We require the ratio of the Compton to synchrotron energy densities to be $\sim \frac{1}{10}$ at the boundary of the source ($\sim 5 \times 10^{17}$ cm radius). This ratio rises like r^{-1} within the source, and within an energetically reasonable model it must not become $\gg 1$. Therefore, we must take the dimensions of the region where the electrons are accelerated to be $\gtrsim 5 \times 10^{16}$ cm (see Fig. 3). Is this region too large to vary synchronously in time-scales of a few months? Fortunately not, because if the magnetic field strength ~ 100 G, the Alfven speed $\gtrsim c/10$ provided that the gas density $\lesssim 10^9$ atoms/c.c. This is an entirely reasonable requirement, and indeed unless the gas density were less than this the ionization losses would be so great as to prevent the acceleration of the electrons. The total energy in the magnetic field $\sim 10^{57}$ ergs, which is plausible on energetic grounds.

It follows from the usual self-absorption arguments that the radio flux at frequencies $\ll 3 \times 10^{11}$ c/s cannot come from the same small volume as the higher frequencies. The variable component of the flux observed[10–12] at frequencies $\gtrsim 1{\cdot}4 \times 10^9$ c/s would be emitted in a region a few parsecs in radius, as we have discussed in previous papers[2,13], and the non-varying low frequency flux could of course come from an even larger region (in which $H \ll 100$ G). The existence of lines in the optical spectrum of *3C 273B* implies the presence of excited gas, which is likely to be in the form of a shell surrounding the intense source of the continuum radiation[2,8,14,15], in which case it would absorb energy at optical and radio frequencies from the interior.

The inverse Compton effect plays an important part in quasars, and restricts the properties of permissible models. If the relativistic electrons are accelerated throughout a sphere of radius 10^{16}–10^{17} cm, there are models in which the optical continuum can result from either synchrotron or inverse Compton emission. The magnetic field in such models ~ 100 G, and the Alfven velocity in such a strong field will be so high ($\gtrsim c/10$) that

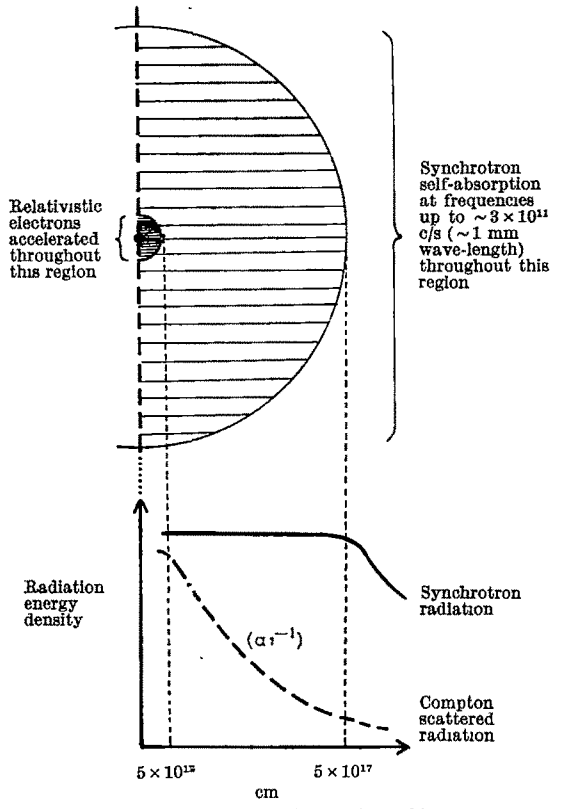


Fig. 3. A model for the central part of *3C 273B* in which the optical continuum arises from inverse Compton scattering of radio frequency synchrotron radiation, $H \sim 100$ G, and the particles are accelerated in a sphere of radius $\sim 5 \times 10^{16}$ cm which contains a massive object. The observed flux at $\sim 3 \times 10^{11}$ c/s (~ 1 mm wave-length) comes from a sphere of radius $\sim 5 \times 10^{17}$ cm. The ratio of the energy densities of synchrotron radiation and visible light is 10 : 1 at the outside, but increases to about 1 : 1 at the surface of the inner sphere. The radio flux observed at frequencies of less than 3×10^{11} c/s must come from a larger volume

the variations in the rate of acceleration of particles could be synchronized over a region of the required size in such a way as to give rise to the observed variations. It would be difficult to distinguish observationally between these two alternatives. If the "local" theory is adopted, the radiation density within the variable part of the quasar can be reduced by a large factor, and this obviously simplifies the construction of a model. However, we believe that there are no compelling arguments which force us to abandon the cosmological hypothesis.

Note added in proof. Optical observations of *3C 273* by J. B. Whiteoak (*Z. Astrophys.*, **64**, 181; 1966) cast doubt on the reality of the linear polarization reported in ref. 6. Although polarization of the optical continuum would strongly suggest that some of the light was synchrotron or inverse Compton radiation, this hypothesis would remain very probable even if the light from quasars were found to be unpolarized.

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MILLISECOND RADIO PULSES FROM JUPITER

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AN experiment has been conducted to study the characteristics of the very short duration pulses which are sometimes observed in the decametre wave radiation from Jupiter. These pulses have been observed on a number of occasions during the 1965–66 apparition. Preliminary results indicate that the occurrence probability of this type of radiation is related to the System III Central Meridian Longitude (1957·0) somewhat differently from the more normal type of radiation, being relatively larger for the subsidiary sources *B* and *C* than for the main source *A*. Furthermore the correlation with satellite Io is more pronounced for events containing the short pulses than for other events.

The bursts of radiation from Jupiter usually have durations of between 0·2 and 10 sec with a gradual rise and fall in intensity and a fairly smooth profile. Following the *Recommendations for Standardization of Reporting Procedures*[1] recently proposed, *S*, *N* and *L* type bursts can be distinguished as defined in Table 1. Several observers[2–6] have reported receiving bursts with durations of the order of milliseconds, and for the purpose of discussing these another class of bursts, *I*, is arbitrarily defined having durations less than 50 msec. These latter pulses have sometimes been referred to as "spitting" pulses in the literature.

Table 1. BURST CLASSIFICATION

Character of emission		Suggested	
Burst type	Time scale in seconds	NASA classification	Name
Very short	Less than 0 05	1	*I**
Short	0 05–0·2		*S*
Normal	0 2–2 0	2	*N*
Long	Greater than 2 0	3	*L*

* Arbitrarily assigned for this article.

Observations have been made of the left- and right-handed components of the radiation at 16, 18 and 22 Mc/s, and of the total power at 14 Mc/s. The polarization measurements have been made using the method of Barrow[7] utilizing crossed five-element Yagi antennae, hybrid rings and separate receivers for the two senses of polarization to avoid the uncertainties that might arise from switching techniques. Identification was by normal aural monitoring techniques[8], but in addition the total power was recorded at 18 Mc/s using a broadside array having a null in the direction of Jupiter. This antenna served as a monitor to help discriminate static pulses which might have the same appearance as Jovian *I*-pulses. A phase-switched interferometer was operated at 18 Mc/s throughout observing periods as a further means of identifying that Jupiter was active during the period. The outputs of the polarimeters, the 14 Mc/s receiver and the 18 Mc/s null receiver were recorded by an eight channel electric writing high speed recorder using chart speeds of up to 50 mm/sec and post-detection time constants down to 5 msec. Most of the recordings of storms containing significant numbers of *I*-pulses were made using a time constant such that the minimum measurable pulse width at half-height was 7 msec. The reception of faint stations was eliminated by manually tuning the receivers over about 100 kc/s whenever signals were received. Static pulses were easily identified by their wide bandwidth and lack of polarization. The null monitor antenna and the interferometer were primarily for increasing confidence in the identification.

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Jupiter radio storms were observed on ninety-two occasions on 62 days during the period November 21, 1965–March 17, 1966. The burst structure showed a continuous range extending from the slowly varying *L*-bursts lasting for tens of seconds to isolated *I*-pulses with durations of 10 msec or less and separations of the order of a second. About half the storms included a few *N*- and *L*-bursts present with *I*-pulses superimposed. These *N*- and *L*-bursts were usually among the most intense in the storm and the *I*-pulses were seldom present for more than 1 or 2 min.

There were eighteen storms which contained appreciable *I*-pulse activity. In sixteen of these storms *I*-pulses occurred for at least 15 min and the *I*-pulses were as intense as, or more intense than, any *N*- or *L*-bursts which might have been present at the same frequency. On occasions, the *I*-pulses were isolated as in Fig. 1 but as the storm progressed they tended to cluster together so that the envelopes of their peaks looked like *N*-bursts. Frequently they seemed to have such short separations (10 msec or less) that the recorder pen would not return to its zero level between pulses. On many occasions *I*-pulses were present on one frequency while *N*-bursts were present on others. On these occasions, however, the profiles of the *N*-bursts were usually very irregular. Two sections from one of these *I*-pulse storms are shown in Figs. 2 and 3. In Fig. 2 there is strong *I*-pulse activity at 22 Mc/s which exhibits both senses of polarization with mostly *N*-bursts at 14, 16 and 18 Mc/s. There is no activity on the left-hand 16 Mc/s channel. In Fig. 3, 17 min later, *I*-pulses continue on the 22 Mc/s right-hand channel but there is little or no activity on the left-hand channel. At 16 and 18 Mc/s the activity is confined to *S*- and *N*-bursts with very weak left-hand channel activity at 18 Mc/s but appreciable left-hand channel activity at 16 Mc/s. *N*-bursts are also present at 14 Mc/s. It is clear that both the burst structure and the polarization can vary considerably from one frequency to another during a single event. The *I*-pulses were usually not present for the entire period of the storm. In most cases they were preceded or succeeded by *N*- or *L*-bursts on the same frequency. This behaviour is summarized in Table 2 which shows the System III (1957·0) longitudes for which the *I*-pulse storms were observed.

A histogram of a number of events against System III (1957·0) longitude is shown in Fig. 4 for all the storms observed. Observations at all frequencies have been combined for this purpose. Also shown in Fig. 4 is a similar histogram for periods of *I*-pulse activity only. The histogram for all the storms has the typical shape

Table 2. SYSTEM III (1957·0) LONGITUDES FOR MAJOR *I*-PULSE EVENTS

Date	Event duration		*I*-pulses	
	Begin	End	Begin	End
November 21, 1965	164	187	164	187
November 28, 1965	150	190	166	186
November 30, 1965	85	144	103	143
December 14, 1965	96	136	117	126
December 20, 1965	300	344	303	340
December 28, 1965	224	296	239	250
January 18, 1966	211	248	213	222
January 21, 1966	277	341	277	329
January 22, 1966	96	146	108	146
January 28, 1966	241	336	245	336
January 29, 1966	124	185	124	178
January 30, 1966	157	232	157	223
February 2, 1966	279	4	302	354
February 23, 1966	129	187	129	180
March 1, 1966	1	18	1	18
March 2, 1966	117	202	117	184
March 8, 1966	348	354	348	354
March 16, 1966	160	190	160	190

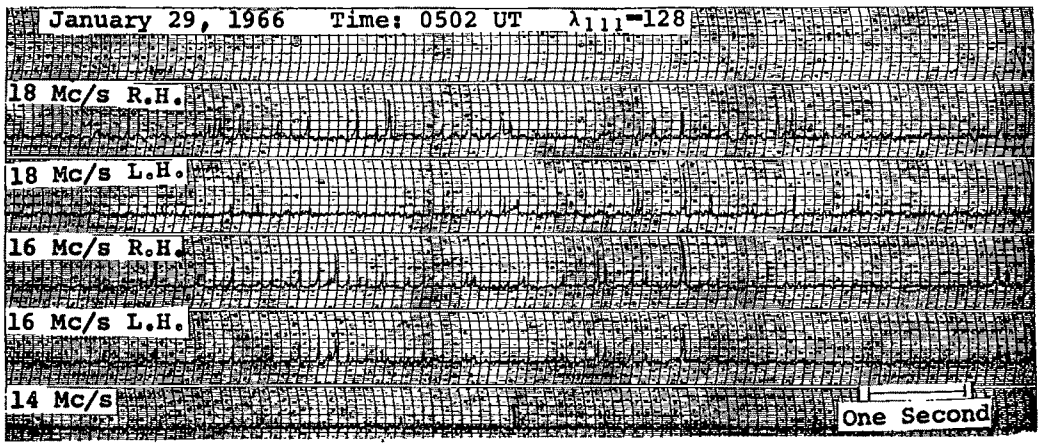


Fig. 1. Specimen record showing isolated *I*-pulses at 16 and 18 Mc/s

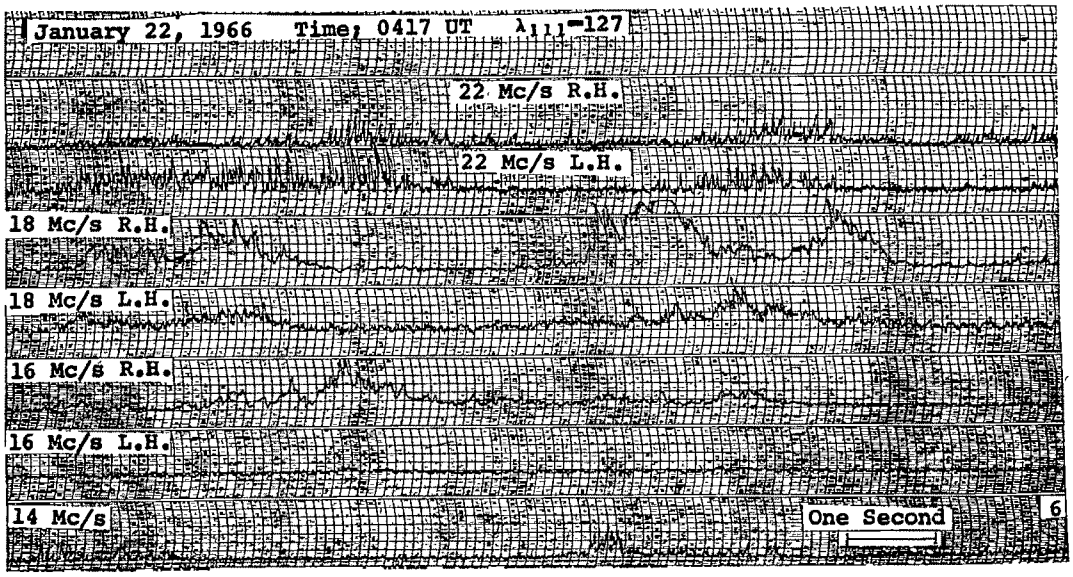


Fig. 2. Specimen record showing strong *I*-pulse activity at 22 Mc/s with *N*-bursts at 16 and 18 Mc/s

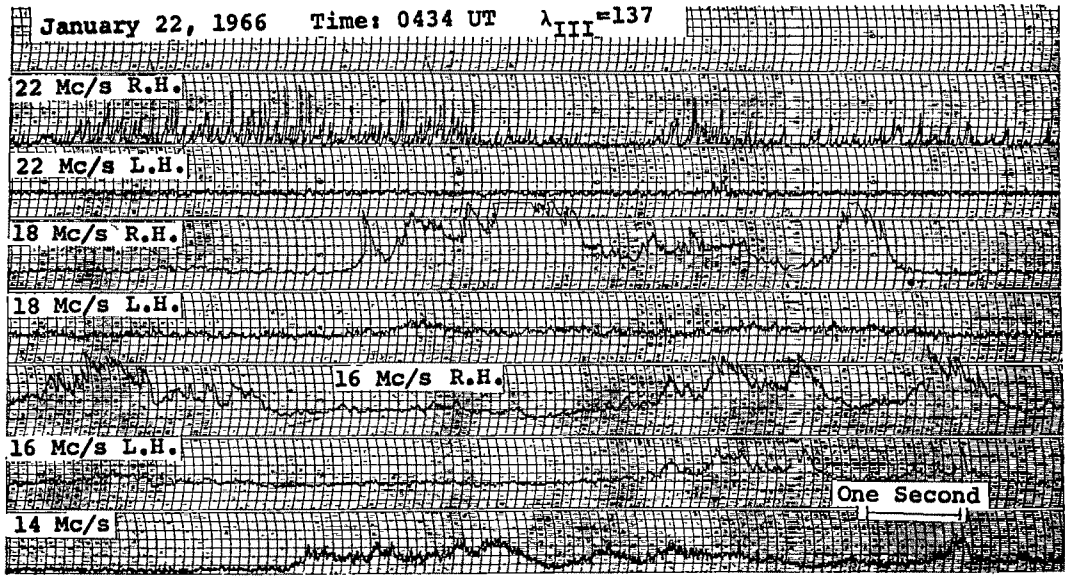


Fig. 3. Specimen record showing *I*-pulses at 22 Mc/s simultaneously with *L*- and *N*-bursts at 14, 16, and 18 Mc/s. Note the almost complete absence of activity on the left-hand channels at 22 and 18 Mc/s compared with 16 Mc/s and compared with the section of record taken 17 min earlier in Fig. 2

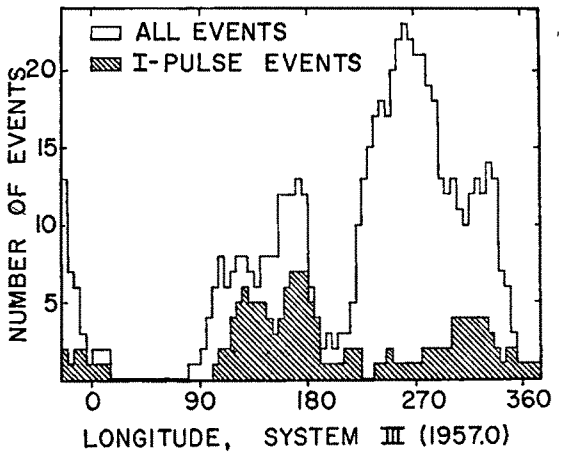


Fig. 4. Histogram of a number of events against System III (1957·0) longitude for all events and for *I*-pulse events only

observed in previous years with "sources" *A*, *B* and *C* defined by longitudes 190°–280°, 70°–190° and 280°–360° (ref. 1), respectively (sources *A* and *C* are unresolved). The interesting feature, however, is that most of the *I*-pulse storms are associated with sources *B* and *C* rather than source *A*.

Smith and Douglas[9] have suggested that *L* and *N* type burst structure may result from diffraction in the interplanetary medium. The present work suggests two possibilities. First, that the radiation associated with the *B* and *C* sources is produced within a smaller region than that from the source *A*, giving rise to a finer diffraction pattern which is produced by the interplanetary medium. (We thank Dr. W. C. Erickson for this suggestion.) The presence of *I*-pulses at one frequency and not at others may be an indication of the different depths and thus sizes of the source regions in the Jovian magnetosphere at which the different frequencies are produced. Alternatively, the *I*-pulse structure may be present in the radiation when it leaves Jupiter and not be produced during propagation through the interplanetary medium. Further study of *I*-pulses may thus prove valuable in clarifying the production mechanism of all Jovian decametric emission.

It is worth noting that, for the ninety-two events considered in this preliminary investigation, the correlation with satellite Io (ref. 10) is more clearly indicated for events containing *I*-pulses than for other events. More observations are required, however, to establish a conclusive result in this respect.

In conclusion, it might be appropriate to summarize the evidence that the *I*-pulse radiation originated from Jupiter. (*a*) The *I*-pulses formed an integral part of a Jupiter storm as identified by the usual techniques[8] and also by the 18 Mc/s phase-switched interferometer. The *I*-pulses were received on aerials pointing towards Jupiter but not on an aerial with a null in that direction. (*b*) The *I*-pulses were only observed as part of a normal Jupiter storm and were usually preceded and/or succeeded by *N*-bursts at the same frequency. The *I*-pulses usually clustered together so that the envelope of a group showed the form of an *N*-burst as in Figs. 2 and 3. They were usually accompanied by *N*- or *L*-bursts at other frequencies and less often by *I*-pulses at other frequencies. (*c*) The bandwidth of the *I*-pulses was always less than 4 Mc/s and individual pulses never occurred simultaneously on the different frequencies. A large proportion of the pulses were 100 per cent circularly polarized as shown in Fig. 3. Terrestrial static pulses have bandwidths considerably greater than 4 Mc/s and produce simultaneous pulses over a wide range of separated frequencies. Also, static is not polarized and does not occur in long series of closely spaced pulses of the type shown in Figs. 2 and 3. (*d*) The *I*-pulse radiation seems to be correlated with two central meridian longitude regions of Jupiter. Also, no *I*-pulse storms occurred when the central meridian longitude was between 20° and 100°.

In view of the foregoing it would seem to be most unlikely that these *I*-pulses were not connected with Jupiter in some manner.

We thank Mr. J. D. Merritt, Mr. D. P. Morrow and Mr. G. M. Resch for assistance with various phases of the work. The research is supported by the National Aeronautics and Space Administration.

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RELATIVISTIC STATES OF POSITRONIUM AND STRUCTURE OF FUNDAMENTAL PARTICLES

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THERE is much to suggest that the interrelationship of the fundamental particles, which is the basic problem of particle physics, may turn out to be analogous to the interrelationship between the various atoms in the Periodic Table of the Elements. It has been too readily forgotten that the simple pictorial models of Rutherford and Bohr provided the key to a successful theory of atomic structure, and are probably indispensable as a conceptual guide for the application of wave mechanics. In this article my aim is to find an equivalent model for particle structure. The particle states will be distinguished from the atomic states in that they are relativistic.

Consider the relativistic positronium system, in which an electron and a positron rotate about their common centre of mass, *C*, with a velocity, *v*, which is close to the velocity of light, *c*. We shall adopt the rest frame of the centre of mass, *C*, the inertial frame. The radial distance to either particle will be denoted by *r*, so that the particles are separated by $2r$. The particles will be assumed to rotate about *C* in a common circular orbit at opposite ends of a diameter. The force exerted on either particle will be due to the time-symmetric (half-advanced and half-retarded) field of the other, since the particles do not radiate in stationary orbits.

We wish to calculate the fields of the positron at the position of the electron. If the positron were at rest in the inertial frame then the required field would be simply the electric field, $\mathbf{E}' = e2\mathbf{r}'/(2r')^3$. In a frame of reference in which the positron moves with velocity $\mathbf{v}$ in a direction normal to $\mathbf{r}'$, let the electric and magnetic fields be $\mathbf{E}$ and

H respectively. Then from the transformation of fields[1] we know that:

$$\mathbf{E} = \gamma\mathbf{E}'; \quad \mathbf{H} = \gamma\mathbf{v} \times \mathbf{E}'/c \tag{1}$$

where $\gamma = (1 - v^2/c^2)^{-\frac{1}{2}}$. These fields will exert a Lorentz force on the electron, the instantaneous velocity of which is equal and opposite to that of the positron. Noting that $\mathbf{H} = \mathbf{v} \times \mathbf{E}/c$ according to equation (1), we obtain:

$$\mathbf{F} = -e(\mathbf{E} - \mathbf{v} \times \mathbf{H}/c) = -e[\mathbf{E} - \mathbf{v} \times (\mathbf{v} \times \mathbf{E})/c^2] = \frac{e^2\gamma(1 + v^2/c^2)\mathbf{r}}{4r^3} \tag{2}$$

where we have used $\mathbf{r}' = \mathbf{r}$. We assume that this force law is valid instantaneously in the case of circular motion ($v = \omega r$).

The question of the magnetic interactions between the particles such as spin$_1$–orbit$_1$, spin$_1$–orbit$_2$, spin$_1$–spin$_2$, or orbit$_1$–orbit$_2$, requires a brief digression. Let us adopt the attitude that the Bohr–Sommerfeld (B–S) theory has the status of an alternative language to quantum theory in the sense that every phenomenon will have a description in either language. We contend that with suitable generalization, B–S theory is capable of yielding predictions to any required accuracy. We view the B–S quantization rules as boundary conditions which are imposed on a continuum of classical field momentum-energy by the act of measurement. The field will satisfy Maxwell's equations, generalized by introduction of the non-linear term $T^{\alpha\beta}v_\beta$ in the source current density, where $T^{\alpha\beta}$ is the Maxwell tensor for the field and v_β is the 4-velocity of the charge the motion of which is being considered. It is in respect of the non-linearity of the generalized classical field that the deviation with quantum electrodynamics occurs, because in the latter theory linear superposition of states is an axiomatic postulate. The postulate leads to great simplifications (particularly in many electron systems), but it is achieved at the expense of having to introduce operator algebra. Thus in the Jordan–Wigner quantum field version of electrodynamics, the wave-function is not a classical field variable only because it is subject to anticommutation rules. These rules are a direct expression of the Pauli Principle, so that this Principle may be regarded as a consequence of the artificial linearization of the "field", and it will not be required in classical field cum B–S theory.

To return to the question of magnetic couplings, we know that spin effects were first introduced in quantum theory by means of the Pauli spin matrices as an empirical "extra" to Schrodinger's wave mechanics. Later the spin effects emerged automatically from Dirac's relativistic wave mechanics. We expect the same to happen in the classical field cum B–S language; that is, spin effects may be introduced either as an extra to a non-relativistic theory or they may emerge automatically from relativistic equations.

The former type of approach is well recognized. By imagining a spinning sphere with charge distributed uniformly over the surface the combination of magnetic and electric fields gives rise to electromagnetic momentum which bears a ratio to the magnetic moment of one-half the ratio for orbital revolution. If the angular momentum of the spinning electron is **S**, then the magnetic moment is $\mu = (e/mc)\mathbf{S}$. The spin–orbit coupling can now be derived. In the rest frame of the electron the electric field of the nucleus, **E**, is transformed into the magnetic field, $\mathbf{H} = \mathbf{v} \times \mathbf{E}/c$, which interacts with the moment to give the coupling energy $\boldsymbol{\mu}.(\mathbf{v} \times \mathbf{E}/c)$. To see how this energy is transformed when we return to the rest frame of the nucleus we express it in the form $\boldsymbol{\omega}_L.\mathbf{S}$, where ω_L is the Larmor precessional frequency of the moment about the field **H**. On transforming back to the rest frame of the nucleus there arises in addition to the orbital motion of the electron the Thomas precession, $\boldsymbol{\omega}_T = -(\mathbf{v} \times \mathbf{a})/2c^2$, which has to be added to the Larmor precession (**a** is the acceleration of the electron). Inserting $\mathbf{a} = -\mathbf{E}e/m$, we see that $\boldsymbol{\omega}_T = -\frac{1}{2}\boldsymbol{\omega}_L$. Hence the spin–orbit coupling becomes $\frac{1}{2}\boldsymbol{\mu}.(\mathbf{v} \times \mathbf{E}_r c)$ (ref. 2). For the case of an observer who chooses to remain in the rest frame of the nucleus, the coupling arises from the interaction of **E** with an electric moment of the electron. The electric moment arises because the relativistic addition of the orbital and spin velocities of the charged sphere gives the sphere a distorted appearance causing the centre of charge to be displaced from the centre of mass. Since the charge is piled up on the side with the highest velocity and reduced on the other side, the spin axis of the electron tends to align itself normally to **E** and **v** (in order to maximize coupling energy). It can be shown that the orbital and spin moments precess about the direction of the resultant moment[3].

The second type of approach is well exemplified by the famous Bohr–Sommerfeld treatment of the relativistic hydrogen atom. This treatment, it will be recalled, was actually superior to the early Schrodinger wave mechanics, and the agreement of the result with that of Dirac's wave mechanics is described by Eisberg (ref. 2, page 356) as "one of the most amazing coincidences to be found in the study of physics". We contend that the result is not a coincidence, but that because the theory is relativistic, spin–orbit coupling is implicitly taken care of (without introduction of any spin parameter). In the same way spin effects will emerge from our relativistic equations. It would be superfluous and wrong to add terms to describe magnetic interaction.

With this justification for the force law (2), we proceed to equate it to relativistic centrifugal force, obtaining:

$$\frac{-e^2}{(2r)^2}\left(1 + \frac{v^2}{c^2}\right) = \frac{\gamma mv^2}{r} \tag{3}$$

For the case of a circular orbit without precessional periods, the B–S quantization rules yield:

$$2\gamma mvr = n\hbar \tag{4}$$

Eliminating mr between equations (3) and (4), we obtain:

$$4\eta^2\gamma^4 - (4\eta^2 + 1)\gamma^2 + (\eta^2 + 1) = 0 \tag{5}$$

where:

$$\eta = \frac{1}{2\pi}\left(\frac{e^2}{\hbar c}\right) = \frac{\alpha}{2n} = \frac{1}{274n} \tag{6}$$

The quadratic equation (5) has the two solutions:

$$\gamma_1^2 = \frac{1}{8\eta^2}[(4\eta^2 + 1) - (1 - 8\eta^2)^{1/2}] \simeq 1 + \eta^2 \tag{7a}$$

$$\gamma_2^2 = \frac{1}{8\eta^2}[(4\eta^2 + 1) + (1 - 8\eta^2)^{1/2}] \simeq \frac{1}{4\eta^2} - \eta^2 \tag{7b}$$

where we expand the square root term up to terms of order η^4 in the small quantity, η.

Since η is small the first solution gives non-relativistic motion, while the second gives ultrarelativistic motion. In the former case we can put $\gamma_1^2 \simeq 1 + v_1^2/c^2$. Thus to good approximation:

$$\frac{v_1}{c} = \frac{\alpha}{2n} = \frac{1}{274n} \tag{8a}$$

$$\gamma_2 = \frac{n}{\alpha} = 137n \tag{8b}$$

Using these results, the radii of the stationary orbits as obtained from equation (4) are:

$$r_{n_1} = \frac{n^2\hbar^2}{me^2} \tag{9a}$$

$$r_{n_2} = \frac{1}{2}\left(\frac{e^2}{mc^2}\right)\frac{n/\alpha}{(n^2/\alpha^2 - 1)^{1/2}} \simeq \frac{e^2}{2mc^2} \tag{9b}$$

Formula (9a), with $n = 1$, gives the radius of the first Bohr orbit of the hydrogen atom, a_0. The electron and positron are therefore separated by $2a_0$. If we had adopted the rest frame of the positron rather than the centre of mass frame we should have obtained the same result by

introducing a reduced mass, $m^* = \frac{1}{2}m$. Thus, the first solution yields the ordinary non-relativistic states of positronium with which we are already familiar.

The second solution, however, gives something quite new. The stationary orbits all have radii very close to $\frac{1}{2}r_0$, where $r_0 = e^2/mc^2 = 2{\cdot}818 \times 10^{-13}$ cm. The particles are therefore separated by r_0. The constant r_0 is sometimes known as the classical electron radius, but this significance must not be taken literally. In fact, there has recently been a return to the old view that the mass of the electron is purely electromagnetic, in both classical[5] and quantum theories[6,7]. Rohrlich[8] has shown that the energy and momentum in the field of the classical electron form the 4-vector, $-\int T^{\alpha\beta}v_\beta d\tau_0$, where $d\tau_0$ is an invariant 3-volume element; this removes an old difficulty.

The energy states for the second (relativistic) solution are:

$$E_n = 2\gamma_n mc^2 - \frac{\gamma_n e^2}{4r_n}\left(1 + \frac{v_n{}^2}{c^2}\right) \qquad (10)$$

$$\simeq \gamma_n mc^2$$

$$\simeq 137nmc^2$$

where equation (3), with $v \simeq c$, has been used, and γ_n has been obtained from equation (8*b*).

Sternglass[9] was the first to obtain relativistic positronium states of this type. However, his force law appears to be in error because he does not work out the centrifugal force in an inertial frame before transforming to the rest frame of the positron, and also because he introduces the Thomas precession as an "extra" when quantizing angular momentum, not realizing that this precession is already implied by relativistic equations. His stationary energy states have twice the value (10). Schild[10] has taken advantage of the time-symmetry of the fields in deriving a force law from a Fokker or Wheeler–Feynman action principle, in which the field variables do not appear. He fails to obtain the relativistic energy states for reasons that are not immediately apparent. We may note, however, that the relativistic energy states would disappear from our analysis also, if the $+v^2/c^2$ term of the force law (2) were replaced by a $-v^2/c^2$ term (this would be wrong because the instantaneous velocities of the electron and positron are opposite).

With the value of γ_2 given by equation (8*b*) for $n = 1$, the interaction potential energy of the electron and positron becomes:

$$V = \frac{137e^2}{4r}\left(1 + \frac{v^2}{c^2}\right) = \frac{\hbar c}{2r_0}\left(1 + \frac{v^2}{c^2}\right) \simeq \frac{\hbar c}{r_0} \qquad (11)$$

The rotation of the electron constitutes a current, $e/c\tau$, where $\tau = \pi r_0/v$. The orbital magnetic moment ($\pi r_0^2/4$ times this current) is therefore $er_0/4$ since $v \simeq c$. The spin magnetic moment of the electron is $(e/mc)\frac{1}{2}\hbar$. If μ_0 and μ_s denote the orbital and spin magnetic moments, respectively, we have for the interaction energy of these dipole moments the formula:

$$V^1 = \frac{\mu_0\mu_s}{r^3} = \frac{8\mu_0\mu_s}{r_0{}^3} = \frac{c\hbar}{r_0} \qquad (12)$$

This has the same magnitude as our relativistic interaction potential energy (11). Thus the coupling given by the force law (2) in the relativistic limit is of the form $(\text{spin})_1$–$(\text{orbit})_2$, where the suffixes 1 and 2 denote the two particles. It would appear that forces of the same order as strong nuclear forces can be obtained from relativistic electrodynamics. All nuclear forces may be electrodynamic in nature.

To return to the significance of our new energy states (10), it will be noted that the energies are positive. This is in contrast with the non-relativistic case where the positive energy states are continuous. Evidently each of the two leptons (we restrict this term to refer to an electron or positron, and later we shall include a neutrino or antineutrino) contributes $\frac{1}{2}(137)mc^2$ to the ground state energy of the relativistic positronium system. This system probably decays too rapidly to be observed as a fundamental particle. However, in view of the magnetic character of the coupling, it is not unreasonable to postulate that a relativistic system containing N leptons would have rest energy close to $\frac{1}{2}N(137)mc^2$. It is a curious and hitherto unexplained fact that the rest energies of the mesons are indeed close to integral multiples of $\frac{1}{2}(137)mc^2$. This empirical law has been known for some time[11–13]. In Table 1 the rest masses predicted by the empirical law and the observed rest masses are compared. The muon would contain 3 leptons, the pion 4, the kaon 14 and the proton 27 (for the more massive particles accumulated corrections will become significant). The π^0 has a rest energy which is $9mc^2$ less than that of π^+ or π^- and about $10mc^2$ less than predicted. The π^0 consists of two relativistic positronium units which may be coupled by this energy. The η meson, a resonance particle with a rest mass of 1072·0 (expressed in units of mc^2), could be regarded as built up from $\pi^+ + \pi^- + \pi^0 + \pi^0$, which yields a total rest mass of 1074·8.

Table 1. PREDICTED AND OBSERVED REST MASSES

Particle	Spin	N	$\frac{1}{2}(137)N$	Observed mass (mc^2)
μ^+, μ^-	$\frac{1}{2}$	3	205 5	206·8
π^0	0	4	274·0	264 2
π^+, π^-	0	4	274 0	273·2
κ^0	0	14	959·0	974·5
κ^+, κ^-	0	14	959·0	966 8
ϱ	1	27	1,849·0	1,836·0

If the spin vectors of the leptons within a composite particle are aligned parallel, the resultant spin will be half-integral or integral depending on whether the number of leptons is odd or even. The spins of the composite particles do indeed appear to obey this rule (Table 1).

In the case of the muons charge is conserved. We can say that the μ^+ consists of two positrons and an electron, the μ^- two electrons and a positron. No neutral muon has been discovered. The neutral pion also presents no problem since it consists of two electrons and two positrons, and not unexpectedly decays into two photons. The charged pions, however, present a difficulty if charge is conserved. On the grounds that each constituent lepton contributes $\frac{1}{2}(137)mc^2$ to the rest energy we have assigned four leptons to the charged pion, and this is consistent with its spin of zero. One of the leptons, while making the standard contribution to the rest energy, does not apparently contribute to the charge. Could this particle be a neutrino, for which we have as yet found no role? But how is a neutral particle able to interact magnetically with electrons and positrons?

Perhaps the answer is that the charged pions are excited states of the muons, and therefore contain only three charged leptons. The distinction between the first excited state of a three-lepton system and the ground state of a four-lepton system may be without significance. For example, rather than describe the π^0 as two positronium units we might, in accordance with equation (10), regard it as the first excited state of a single positronium unit. The neutrino would then acquire a significance like that of the photon—it would be a means of carrying away a half-integral quantum of angular momentum. In the decay of the muon, $\mu^\pm \rightarrow e^\pm + \nu + \emptyset$, we see that an electron pair may annihilate into a neutrino pair.

If helium ions are bombarded with electrons, it is found that the capture cross-section is greatest at certain kinetic energies. These resonances occur when the kinetic energy plus the ionization potential for the level into which the electron is captured are just sufficient to excite the electron in the ground state of the helium ion into a discrete level, resulting in a doubly excited state which we shall denote by He'' (ref. 14). The reverse process is the autoionization of He'', namely, $He'' \rightarrow He^+ + e^-$, and is known to occur in times of order 10^{-12} to 10^{-13} sec. It is now possible to create doubly excited states directly from helium by absorption of radiation in the vacuum ultra-violet[15]. We wish to point out the strong analogy

between the autoionization of He″ and a fundamental particle decay. In the case of the autoionization, the energy available to liberate one electron comes from the fall of the other electron from an excited level to the ground level and is discrete. Hence the electron is liberated with a preferred kinetic energy. By analysing the kinetic energies of the decay products of certain fundamental particles analogues of He″, termed resonance particles, have been discovered.

The short lifetime of the resonance particles (10^{-23} sec) indicates that they decay by allowed transitions. The longer-lived mesons, with lifetimes of the order of 10^{-10} sec, are really metastable states, the decay of which must proceed by a forbidden transition. The spontaneous decay of fundamental particles is possible because their energies are positive.

We would argue, therefore, that all matter is built up from electrons and positrons, in accordance with a previous suggestion[16]. Only electromagnetic and gravitational fields will exist in Nature. If the mass of the electron is purely electromagnetic, then the energy in the electromagnetic field will be the source of the gravitational field, and the neutrino (as described here) will become the elementary particle of the gravitational field.

If all matter is built up from electrons and positrons, one has to explain the absence of antimatter. Gray[17] has suggested that the conservation of parity in chemical syntheses may have an analogue in the evolution of a universe of particular parity from an initial chance asymmetry.

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RADIAL SPREAD OF A LIQUID STREAM ON A HORIZONTAL PLATE

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IT is a common observation, seen, for example, in a kitchen sink, that a stream of liquid falling vertically on to a horizontal plate spreads out radially in a particular manner. Around the point of impingement there is a circular zone in which a thin layer of liquid moves radially with a high velocity. At the edge of this region the liquid layer abruptly becomes much thicker and consequently flows radially at a much lower velocity. This transformation is called the "hydraulic jump". This article deals primarily with the region inside the jump.

Recently, in one of the very few papers on this subject, Watson[1] applied the boundary layer theory to the analysis of flow inside the jump. The flow is characterized by a boundary layer that begins at the stagnation point and increases in thickness with radial spread. The expenditure of energy due to the creation of new surface was not considered. The fluid velocity outside the boundary layer was considered to remain almost constant and essentially equal to the falling stream velocity, U_0. By assuming a Blasius flat-plate profile for the velocity distribution, Watson obtained an approximate solution of the boundary layer equation in the usual manner.

Beyond a certain radial distance $r > r_0$, the viscous stresses are taken to affect the free surface such that the whole flow is considered to be of boundary layer type. Therefore, for distance $r > r_0$, the surface velocity decreases with increasing radial distance.

According to Watson's analysis, the merging of the boundary layer and the surface takes place at $(r_0/a)R^{-1/3} = 0{\cdot}3155$, where Reynolds number $R = Q/\nu a$ (Q being the volume flow rate of the falling stream, ν its kinematic viscosity, and a its radius). Although some experiments were carried out by Watson on the spread of a falling stream on a horizontal plate, his results do not prove or disprove the applicability of the theoretical analysis given.

In the present investigation several experiments were conducted to provide insight into the nature of the region within the jump and speculations are made concerning the applicability of simple theoretical analysis to the hydrodynamics of this system.

The falling streams were generally formed at an orifice in the base of a constant head calming tank. The head of the liquid in the tank was 9·9 cm or 18·3 cm, and the free-fall height of the jet to impact was 10·2 cm. The volumetric flow-rate of the liquid was varied from 9 to 111 cm³/sec by varying the orifice radius from 0·16 to 0·40 cm. The experiments were carried out using different liquids, for example, methylene bromide, water or water–glycerine solution, such that the kinematic viscosity could be varied from 0·004 to 0·052 cm²/sec. The stream diameters at the deflexion plate were calculated.

The impingement of water from a conventional tap on a plate was also observed. By careful adjustment of the tap, a relatively smooth stream of water could be obtained. The diameter of the stream was measured with callipers close to the surface of the deflexion plate. The volumetric flow-rate in these experiments was varied from 41 to 182 cm³/sec.

In the first series of experiments, an attempt was made to determine the kinetic energy of the liquid inside the jump as a function of radial distance. The jet was directed to the centre of horizontal disks having diameters such that the liquid flies from the edge, forming an "umbrella". If surface tension effects were negligible, the profile of the umbrella would clearly be parabolic with apex at the edge of the disk (like a ball or projectile launched horizontally). However, it was observed that this profile was decidedly not parabolic. This marked departure is shown in Fig. 1. In all cases the initial slope of the profile is definitely not zero, and furthermore, actual contraction of the umbrella occurs for sufficiently great fall. Clearly surface tension plays a marked part.

In the second series of experiments, the thickness of the liquid layer inside and outside the hydraulic jump was measured as a function of radial position for a number of jets, using different liquids. In these experiments the stream was directed normally on to the surface of a large

glass plate and depth measurements were made with a vernier height gauge having a needle pointer.

Some of the results obtained on thickness profiles using different liquid streams are given in Fig. 2. The curve drawn by dotted lines for a particular case is calculated film thickness, h, for inviscid flow, that is, $h = a^2/2r$. The observed difference between the measured film thickness and those computed for inviscid flow manifests the viscous flow effect within this region. Depending on the Reynolds number of the falling stream and the kind of liquid used, the average film thickness inside the jump was within the range 0·01–0·09 cm, and after hydraulic jump, the thickness increased to 0·1–0·3 cm. Outside the jump little or no variation in film thickness was noted with increasing radial distance.

Several high-speed motion pictures (2,000 frames/sec) were taken of various falling liquid streams spreading radially on a horizontal plate. The high-speed photographs in Figs. 3 and 4 are examples showing the appearance of the falling stream and of the radial flow for water and a water–glycerine solution. Because of the low kinematic viscosity of water, that is, $\nu \simeq 0{\cdot}01$ cm²/sec, the stream (Fig. 3) is rough and consequently is responsible for the formation of ripples on the surface of the region inside the jump. The height of these ripples could not be measured with any degree of accuracy; furthermore, as seen from the photograph in Fig. 3, the ripples interfere

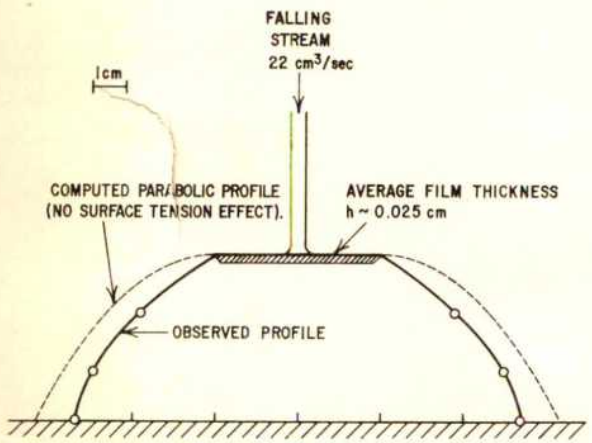


Fig. 1. Experimental umbrella profile for a methylene bromide stream impinging on a small disk

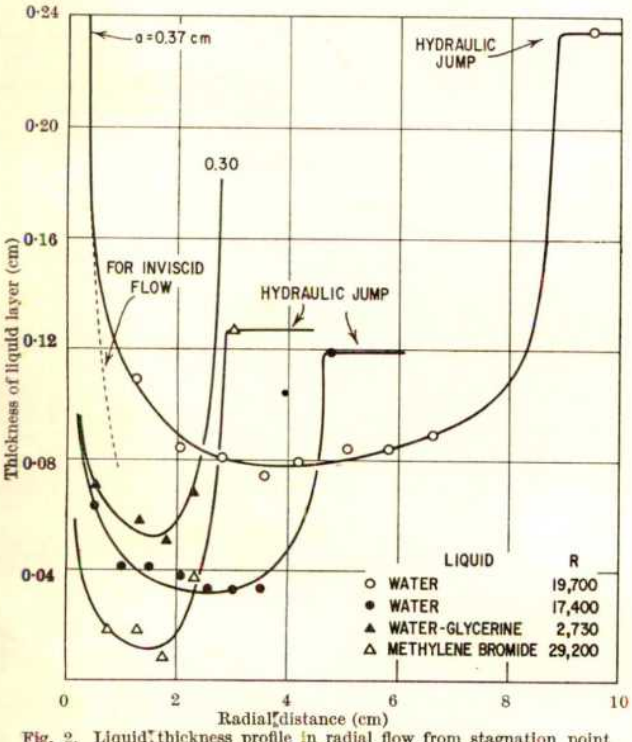


Fig. 2. Liquid thickness profile in radial flow from stagnation point using different liquids



Fig. 3. High-speed photograph (1/2,000 sec) of radial flow of water stream impinging on a horizontal plate. R = 14,800, a = 0·25 cm, rj = 5·3 cm

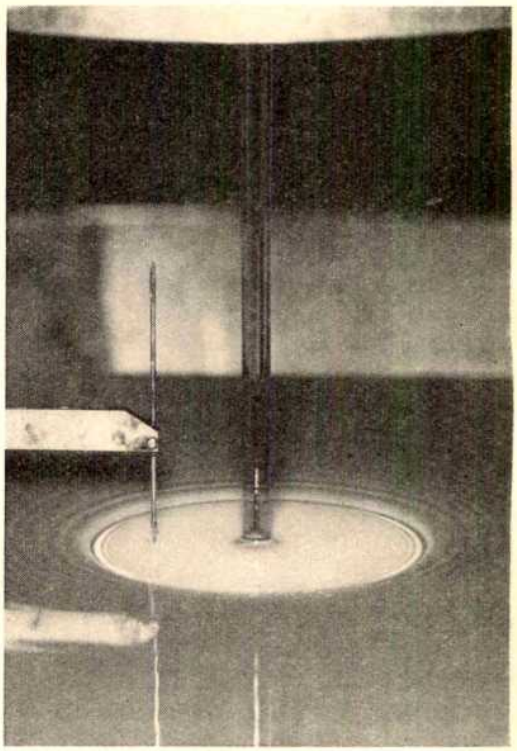


Fig. 4. High-speed photograph (1/2,000 sec) of radial flow of water-glycerine solution stream impinging on a horizontal plate. R = 2,730, a = 0·24 cm, rj = 2·8 cm

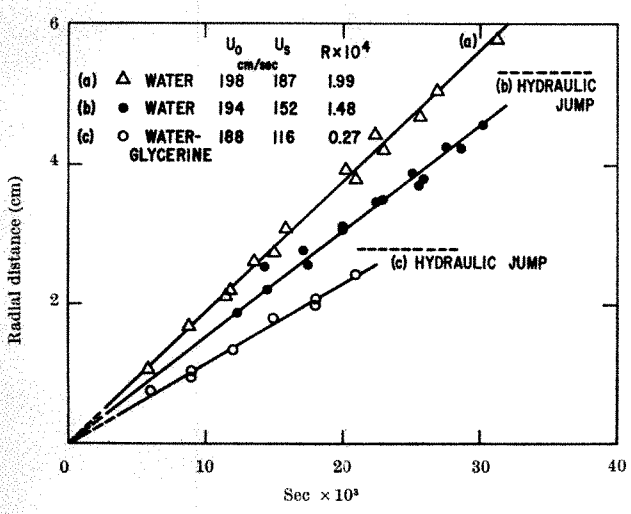


Fig. 5. Surface velocity, U_s, of the liquid inside the jump using water and water–glycerine solution

to some extent with measurements of film thickness. In the case of water–glycerine (Fig. 4), having $\nu = 0{\cdot}05$ cm²/sec, both the stream and the radial flow are very smooth.

When high-speed motion pictures were taken, very small pieces of cork were dropped on to the region within the jump and were used to measure the surface velocity of radial flow. As can be seen from the examples given in Fig. 5, the surface velocity remains essentially constant almost up to the position of the hydraulic jump, for which $(r_j/a)\ R^{-1/3}$ is between 0·84–0·89 for the examples considered. That is, according to the present experimental work, the boundary layer does not extend to the surface at $(r/a)\ R^{-1/3} = 0{\cdot}3155$ as derived by Watson from the application of the boundary layer theory but at a much greater radial distance, if at all.

It should also be mentioned that the surface velocity estimated from the rate of propagation of the ripples (Fig. 3) agrees reasonably well with those determined from the motion of cork particles on the surface of the liquid. The attempts to measure the surface velocity outside the jump have not been successful.

Another point of interest is that although the surface velocity, U_s, inside the jump remains constant, it is decidedly less than the velocity of the falling stream, U_0. However, the indications are that the ratio U_s/U_0 approaches unity with increasing Reynolds number of the falling stream. The significance of this observation is not clear at present.

The experimental data have given some indication of the complexity of the system under investigation. It is clear that even an approximate solution of the Navier–Stokes equation for the radial flow considered becomes a very complex mathematical problem.

Under these circumstances, all that can be done is to look for some empirical correlation which may summarize the data in a more condensed form. As an example, Fig. 6 shows the variation of the jump radius r_j as a function of Reynolds number for various liquids. In order to obtain a meaningful correlation for several liquids together, surface tension effects would also have to be considered. Since the present experiments were conducted over a narrow range of surface tensions, no such correlation was attempted.

The decrease in the magnitude of the liquid stream momentum brought about by the radial spread can be estimated from the height, h, and radius of the hydraulic jump. The momentum flux of the falling stream is $\rho Q U_0 (= \rho Q^2/\pi a^2)$. If it is assumed that the velocity profile of the thick, relatively slow-moving region beyond the hydraulic jump is fairly flat, the momentum flux just beyond the jump is $\rho Q U_j (= \rho Q^2/2\pi r_j d_j)$. Consequently, the ratio of their magnitudes is given by:

$$\frac{\text{momentum flux after jump}}{\text{momentum flux of falling stream}} = \frac{a^2}{2r_j d}$$

which is found in the present experiments to be within the range 0·02–0·04

The momentum flux just before the jump is obtained by adding the momentum flux required to maintain the standing wave to the momentum flux just beyond the jump. This gives:

$$\rho Q^2/2\pi r_j d_j + \pi r_j \rho g\ (d^2 - h^2)$$

where h is the film thickness just before the jump and g is the gravitational constant. Since h is small compared with d, the ratio of magnitudes is given by:

$$\frac{\text{momentum flux before jump}}{\text{momentum flux of falling stream}} = \frac{Q^2/2\pi r_j d_j + \pi r_j g d^2}{Q^2/\pi a^2}$$

In all the experiments carried out, this ratio was found to be within the range 0·07–0·16.

Observed variations in these ratios could not be correlated as a function of independent variables of the falling stream.

The corresponding ratios of kinetic energy are obtained by squaring the momentum ratios. The kinetic energy ratio is quite small before the jump and effectively zero after the jump.

It is possible to estimate the portion of kinetic energy loss brought about by expansion of the surface. For the expansion to the jump radius the rate of loss of kinetic energy due to the surface tension effect at the free surface is given by:

$$\frac{dE}{dt} = 2\pi r_j U_s \gamma$$

where γ is the surface tension of the liquid. The kinetic energy of the falling stream is equal to $1/2\ \rho Q U_0^2$. Since the kinetic energy of the stream is almost entirely lost at the jump, the fraction of kinetic energy loss due to surface expansion is given by:

$$\frac{4\pi r_j \gamma U_s}{\rho Q U_0^2}$$

In the several experiments in which surface velocity was measured, this ratio for water was about 50 per cent and for the water–glycerine solution about 20 per cent. The remaining kinetic energy losses are primarily due to viscous effects.

It is to be expected that an increase in viscosity (with all other things remaining the same) will result in a greater loss of energy due to viscous effects per unit area. Since the kinetic energy available is limited, this will result in

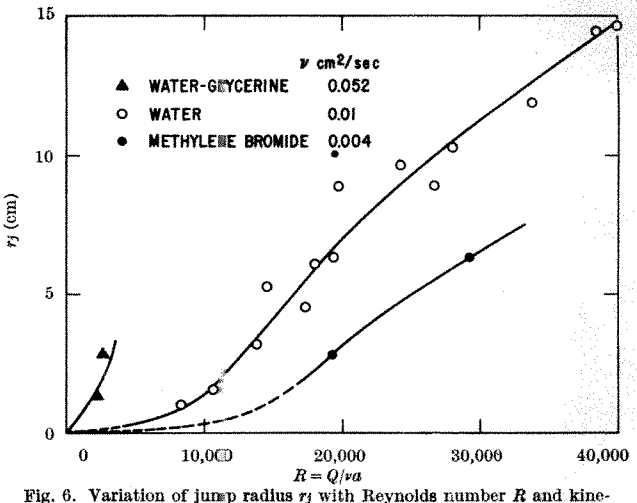


Fig. 6. Variation of jump radius r_j with Reynolds number R and kinematic viscosity of liquid

a smaller hydraulic radius and consequently a smaller share of the kinetic energy loss caused by surface expansion.

Since most of the kinetic energy of the stream is lost at the jump, further expansion of the surface beyond the jump will be slight. As a result, the product rU_s in this region will tend to be constant. If it is again assumed that the velocity profile remains fairly flat throughout this region (for example, $U = \frac{Q}{2\pi rd}$), it can be seen that the liquid thickness, d, also tends to remain constant for a given case.

The thickness d of the liquid layer can be increased, as desired, by placing a ledge around the splash plate. An increase in d will, of course, reduce the jump diameter. Beyond a critical value of d, the hydraulic jump will disappear completely.

Summary

As a falling liquid stream spreads radially on a horizontal plate, the surface velocity of the region within the jump remains essentially constant almost up to the position of the hydraulic jump. It is found experimentally that the ratio of the surface velocity to the initial velocity increases from 0·6 to 0·94 as the Reynolds number for the stream increases from about 3,000 to 20,000. The kinetic energy of the falling stream is essentially completely dissipated at the hydraulic jump. The fraction of kinetic energy expended in the creation of new surface is shown to be quite large.

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TERNARY AND QUATERNARY AQUEOUS SYSTEMS CONTAINING BILE SALT, LECITHIN, AND CHOLESTEROL

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THE solubilization in aqueous phases of insoluble amphiphilic substances by association with more soluble similar substances is of interest, not only from a purely physicochemical point of view but also because of its industrial (detergents) and biological applications.

The particular problem to be discussed here concerns the solubilization of phospholipids and cholesterol in biological fluids—particularly bile. About six hundred mixtures representing all possible proportions of bile salt, lecithin, cholesterol, and water were prepared and examined with a polarizing microscope and 250 of these analysed by X-ray diffraction. Pure sodium cholate was used as the bile salt (solubility in water at 20° C = 56 per cent). Cholesterol recrystallized four times from hot methanol (final melting-point 148° C was used), and the pure egg lecithin was prepared in this laboratory[1]. In order to maintain the *p*H of the mixtures greater than 8 (at which there is almost no free bile acid), the distilled water used was brought to *p*H 10 with sodium hydroxide and stored under nitrogen. All experiments were carried out in the region of 22°–24° C.

Bile salt is very soluble in water, but cholesterol is almost totally insoluble. Lecithin is not soluble in water but swells to form a paracrystalline phase. Even in very large volumes of water lecithin simply forms a dispersion of the swollen phase[2].

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Ternary Systems. Before examining the quaternary system, the three ternary systems: (*a*) bile salt–cholesterol–water, (*b*) lecithin–cholesterol–water, and (*c*) bile salt–lecithin–water, were investigated. Ternary phase diagrams representing these systems are presented in Fig. 1. It can be seen from Fig. 1*a* that the ability of sodium cholate to solubilize cholesterol is very weak: the quantity of cholesterol solubilized in water represents at the most 2 per cent of the weight of sodium cholate present, regardless of the quantity of water. All mixtures with more than 2 per cent cholesterol, or more than 56 per cent sodium cholate (as a percentage of weight of all three components), form either two or three phases. The phases are: (1) the isotropic solution containing 2 per cent or less cholesterol solubilized by the bile salt; (2) the crystals of sodium cholate; (3) the crystals of cholesterol. However, it can be seen in Fig. 1*b* that up to 33 per cent cholesterol (which corresponds to a molecular ratio with lecithin of 1 : 1) can be incorporated in a paracrystalline phase given by lecithin and water. This system, however, never forms an isotropic micellar solution.

The system bile salt–lecithin–water is of some interest as it gives three paracrystalline phases labelled I, II and III in Fig. 1*c* and an isotropic solution (phase IV). Phase I is analogous to the "neat soap" of the aqueous soap systems. All the mixtures with compositions falling into this area show a lamellar structure with the long spacings (D) ranging from 38 Å to 75 Å according to

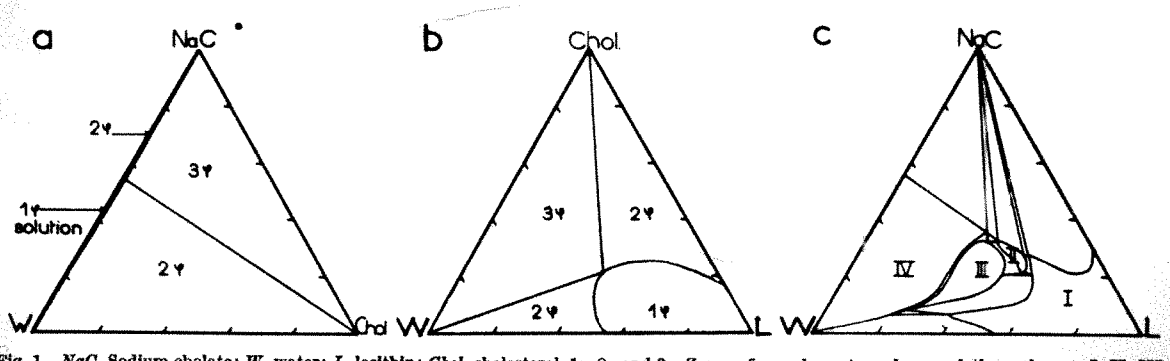


Fig. 1. *NaC*, Sodium cholate; *W*, water; *L*, lecithin; *Chol*, cholesterol. 1φ, 2φ and 3φ, Zones of one phase, two phase and three phases. I, II, III : paracrystalline phases (see text); IV : isotropic solution (see text)

the composition. From the measured long spacings (D) and the quantity of water present the thickness (d_w) of the interstitial water layer and the thickness (d_L) of the lipid layer can be calculated. The thickness (d_w) of the water varies between the extreme values of 0 (anhydrous) and 34 Å. As for the lipid layer, its calculated thickness (d_L) depends not only on the amount of water present but also the amount of bile salt in the lipid layers. Thus, for example, the thickness (d_L) may fall from 42·5 Å to 33·5 Å with increasing hydration and, at a certain constant water concentration, d_L falls from 42 Å to 31 Å with increasing bile salt concentration. The calculated area per molecule in this layered structure varies from 70 Å² to 59 Å². These mean molecular areas correspond to those obtained with similar proportions of lecithin and cholic acid spread as monolayers and compressed to a pressure of 25–30 dynes/cm (ref. 3).

Phase II, although isotropic, is very thick, almost rigid and gives long spacings corresponding to a face-centred cubic lattice. This phase is probably made up of dodecahedrally deformed spherical micelles of lecithin and bile salt packed in a face-centred cubic lattice. It is analogous to the cubic phase described by Luzzati *et al.*[4] for certain soap and detergent systems. In this phase D ranges from 39 Å to 43·5 Å.

Phase III is analogous to the "middle soap" in aqueous soap systems and gives long spacings corresponding to hexagonally packed indefinitely long cylinders as described by Luzzati *et al.*[4] for the "middle soap". Depending on the proportions of the components, the axis to axis distance (D) between the cylinders ranges from 39·5 Å to 70 Å. The calculated diameter (d_L) of the lipid cylinders ranges from 32·5 Å to 49·5 Å. Either an increase in hydration or an increase in bile salt content of the cylinders produces a decrease in the diameter of the cylinders. The calculated water thickness between the adjacent cylinders ranges from 5 Å to 25 Å. The calculated area per molecule in this phase varies from 96 Å to 116 Å². These areas are the same as those found with similar proportions of lecithin–cholic acid mixtures spread as monolayers compressed to a pressure of 6 dynes/cm (ref. 3).

Phase IV is an isotropic micellar solution. This phase extends up to and includes a mixture containing nearly equal weights of the three components (36 per cent bile salt, 34 per cent lecithin and 30 per cent water). In order to gain some information about the micellar dispersion in this phase preliminary light-scattering measurements were performed on aqueous solutions containing 2 per cent sodium cholate and increasing amounts of lecithin. The micellar weight rises gradually from 1,650 for a 2 per cent sodium cholate solution to 16,500 for a solution containing about 3 per cent lecithin and 2 per cent sodium cholate. Further addition of lecithin up to 5 per cent effects a slight fall in micellar weight to 15,000.

The zones between these four different areas represent compositions which produce two coexisting phases or three phases of fixed composition. These phases can be crystalline (sodium cholate crystals), paracrystalline (I, II, III) or the isotropic solution (IV).

The effect of changing the nature of the bile salt should be emphasized here. The same series of systems was prepared, but this time a mixture of naturally occurring conjugated bile salts extracted from beef bile was substituted for the pure sodium cholate[1]. The diagram obtained was very similar, so far as the paracrystalline phases I, II, and III were concerned, when compared with the diagram obtained with pure sodium cholate. Even the lower limits of the isotropic phase (IV) were similar; the only difference was the disappearance of the upper linear frontier, which meant that an increasing amount of bile salt could be added without there being any separation into crystals. This is understandable when it is remembered that the mixture of naturally occurring bile salt is soluble in water in all proportions—contrary to pure sodium cholate the maximum solubility of which is 56 per cent.

The effect of substituting this conjugated bile salt mixture for sodium cholate in the bile salt–cholesterol–water system (Fig. 1*a*) was simply to extend the upper border of the solution from a point at 56 per cent bile

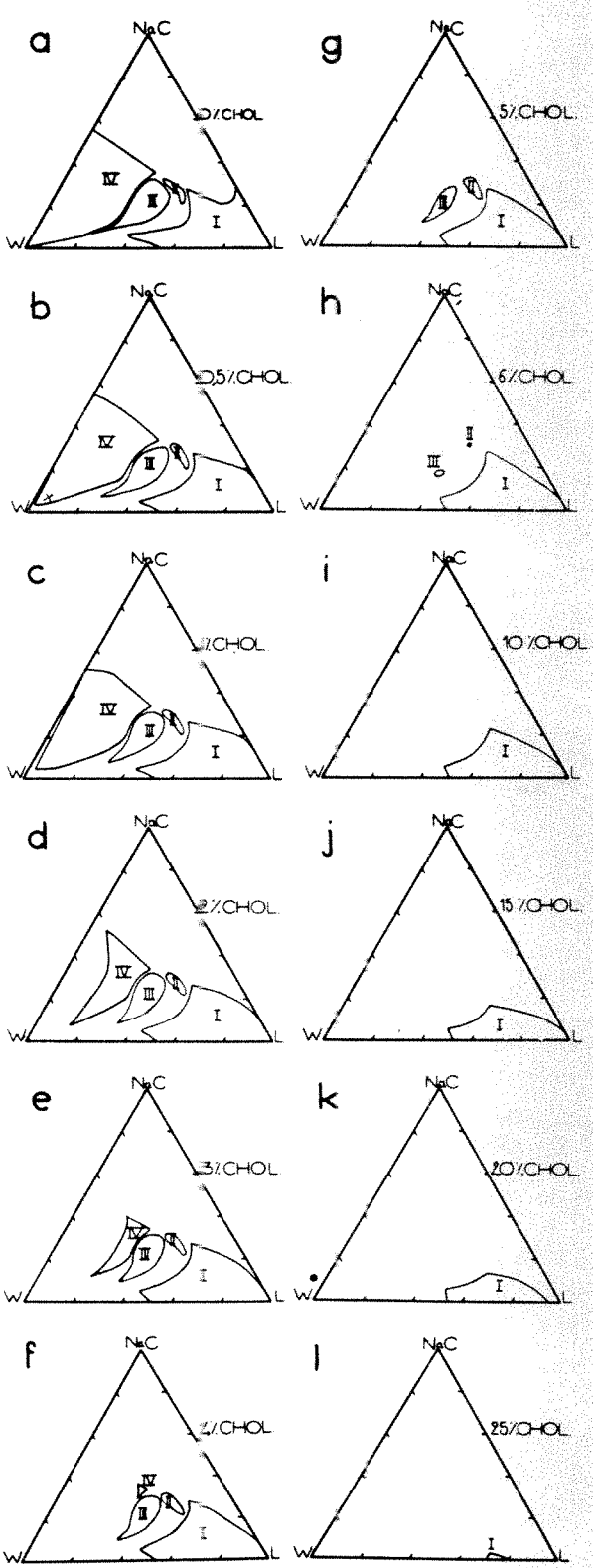


Fig. 2. *NaC*, Sodium cholate; *W*, water; *L*, lecithin; *Chol.*, cholesterol.
I, II, III paracrystalline phases } see text
IV: isotropic solution

salt for sodium cholate to 100 per cent bile salt for the mixture. The maximum cholesterol which could be solubilized by this mixture of conjugated bile salt was 1·7 per cent at 24° C as opposed to 2 per cent for the sodium cholate. While the solubility of the conjugated bile salt mixture is much greater in water than sodium cholate, its capacity to solubilize cholesterol is almost the same.

Quaternary systems. It seemed that the best way to present the quaternary system was to examine the modification of the ternary diagram (bile salt–lecithin–water) when cholesterol was added as a fourth component. Indeed, the addition of cholesterol to the various phases of the ternary system did not change their general structure but simply modified the frontier of their corresponding areas on the diagram. This is clearly shown in the series of diagrams in Fig. 2. Each of these triangular diagrams corresponds to a constant overall percentage of cholesterol—only the percentages of the other three components vary. Thus Fig. 2*a*, which corresponds to 0 per cent cholesterol, is simply a reproduction of the ternary diagram Fig. 1*c* simplified to show only the contours of the four separate phases. The others, Figs. 2*b*–*j*, correspond to overall percentages of cholesterol 0·5 per cent, 1 per cent, 2 per cent . . . 25 per cent.

It can be seen that, in phase I, up to 25 per cent cholesterol can be associated with the lecithin and bile salt. X-ray analysis indicated that in these quaternary systems, phase I remained lamellar, phase II cubic, and phase III hexagonal. The presence of cholesterol in these phases caused a slight increase in the long spacing (D) compared with the corresponding points in the ternary system, just as if the lipid layer was in a more condensed state. This finding is in accordance with what is observed with mixed films of lecithin and cholesterol spread on water[5]. Here the presence of cholesterol reduces the partial molecular area of lecithin, showing that the chains of the lecithin are in a more condensed state.

With these diagrams at our disposal, and given that bile is an aqueous solution containing as its main soluble component bile salt and as its primary insoluble components lecithin and cholesterol, the question naturally arises as to which region of the diagram the gall bladder bile falls in and what might result from variation in its composition.

An average normal human gall bladder bile contains about 92 per cent water, 3·5 per cent bile salt, 3 per cent lecithin, 0·5 per cent cholesterol and 1 per cent other components—for example, bile pigments (mostly in the form of water soluble bilirubin diglucuronide), protein, and inorganic anions and cations. If we neglect the minor changes in Fig. 2 which these other components may produce, we can compare bile with a system having a composition of about 93 per cent water, 3·5 per cent bile salt, 3 per cent lecithin and 0·5 per cent cholesterol, the corresponding point of which falls in the region of the isotropic phase IV at the point marked X in Fig. 2*b*.

The composition of gall bladder bile, especially in pathological cases, has been shown to vary widely[6–8]. If bile is to remain a micellar solution, however, its composition must be within the confines of phase IV. Indeed, from an examination of these diagrams it is possible to predict that a change in the percentage of any of the components (cholesterol, lecithin, bile salt and water) may lead to the formation of crystals of cholesterol or one of the paracrystalline phases (I or III).

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A CHEMICAL REACTION WITH A MEMORY EFFECT

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A MECHANISM frequently encountered in reaction kinetics consists of an equilibrium:

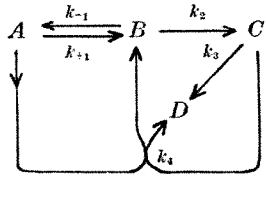
$$A \underset{k_{+1}}{\overset{k_{-1}}{\rightleftarrows}} B, \text{ for which } k_{-1} \gg k_{+1}$$

followed by an irreversible reaction of B:

$$A \underset{k_{+1}}{\overset{k_{-1}}{\rightleftarrows}} B \xrightarrow[k_2]{} C \xrightarrow[k_3]{} D \rightarrow \text{etc.} \qquad \text{(I)}$$

During such a reaction the concentration of B will be smaller than that corresponding to the unperturbed equilibrium $A \rightleftarrows B$, in particular if k_2 is not small relative to k_{-1}.

The present article reports a type of reaction in which the set of equations (I) is contained, but in which during the reaction the concentration of B becomes greater than that corresponding to an unperturbed equilibrium $A \rightleftarrows B$. This unusual behaviour is due to a reaction (4) of A and C, regenerating the intermediate B and yielding the final product D:



(II)

The assumption that $k_{-1} \gg k_{+1}$ is retained throughout this article. Moreover, it is assumed that

reaction (4) is fast* relative to (−1), (2) and (3), and reaction (−1) is not much faster than (2) } conditions α

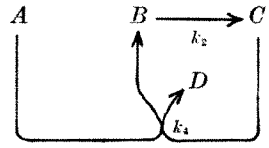
Then each quantity of B reacting to give C will immediately be replenished by reaction (4). As a result, the concentration of B and thus the reaction rate, $-\mathrm{d}[A]/\mathrm{d}t$, which under these conditions tends to $k_2[B]$, will decrease only slowly, while A may be consumed rapidly. Under these conditions the consumption of A proceeds mainly by way of reaction (4) and only to a small extent by way of (1).

Reactions proceeding by way of mechanism (II) under the conditions α possess some properties which are at first sight paradoxical. For example, the reaction rate is not a single valued function of the concentration of A, because the 'history' of the system also has its effect. This is seen most clearly when the conditions α are fulfilled in the extreme, namely when:

k_{+1}, k_{-1} and k_3 are zero, while $k_4 \rightarrow \infty$ (at least 'very large') and $[B]_0 \neq 0$ } conditions β

* The quantities compared here are the products of rate constants and concentrations.

The condition that B should be given an initial value obviously has to be made in order to start the reaction. Under conditions β the system reduced to:



(III)

In such a system B remains constant and equal to any initially given value $[B]_0$, since any B disappearing by way of (2) is instantaneously regenerated by (4).

Since $[B]$ is constant, the reaction rate $-\mathrm{d}[A]/\mathrm{d}t$ will also be constant, although $[A]$ continuously decreases:

$$\frac{\mathrm{d}[B]}{\mathrm{d}t} = 0 = -k_2\,[B] + k_4\,[A]\,[C]$$

$$-\frac{\mathrm{d}[A]}{\mathrm{d}t} = k_4\,[A]\,[C] = k_2\,[B] = k_2\,[B]_0 = \text{constant}$$

The concentration of C thus increases, being inversely proportional to $[A]$ during the reaction.

(An apparent objection to this reasoning seems to be that, since $\mathrm{d}[B]/\mathrm{d}t + \mathrm{d}[C]/\mathrm{d}t = 0$ irrespective of the values of k_2 and k_4, while $\mathrm{d}[B]/\mathrm{d}t \to 0$ when $k_4 \to \infty$, $[C]$ would have to be constant. In the extreme case β, $[C]$ is indeed constant, namely zero, and the product $k_4\,[A]\,[C]$ can remain constant by virtue of the indefinite value of the product $0 \times \infty$. When k_4 is finite, although $\gg k_2$, $[C]$ will be very small relative to $[B]$. Then $\mathrm{d}[B]/\mathrm{d}t$ will no longer be zero, but have a small value, approximately zero so long as $[A]$ does not tend to zero. The relative changes in $[B]$ will be small, but, since $\mathrm{d}[B]/\mathrm{d}t + \mathrm{d}[C]/\mathrm{d}t = 0$, the relative changes in $[C]$ will be appreciable.)

Now if k_{+1} and k_{-1} are not truly zero, but reactions $+1$ and -1 are negligibly slow relative to the other reactions in mechanism (II), then $[B]_0$ will be a function of $[A]_0$, for example:

$$[B]_0 = K_1\,[A]_0^n \qquad \text{(condition } \gamma\text{)}$$

The initial rates of such a system will then be found to be n^{th} order in $[A]$, if a series of experiments with different initial concentrations of A is carried out. During each experiment, however, the rate will be zero order in $[A]$, since each experiment conserves the initial rate until $[A]$ has dropped to zero. For $n = 1$ this situation is shown in Fig. 1.

It can also be seen from Fig. 1 that the rate $-\mathrm{d}[A]/\mathrm{d}t$ of such systems is not a single-valued function of $[A]$. At a given value of $[A]$, say x, the rate can have any value $\geqslant y$, depending on the "history"—that is the initial concentration of A—of the system.

The phenomena displayed in the most extreme form by mechanism (III) under the conditions β and γ will be referred to as the "memory effect", which has the following characteristics:

(*a*) The rate $-\mathrm{d}[A]/\mathrm{d}t$ is of zero (or very low) order in $[A]$ so long as $[A]$ does not drop to very low values, while the initial rate is of a higher order in $[A]_0$.

(*b*) Consequently, the reaction rate is not a single-valued function of $[A]$—except at the start of the reaction—but is co-determined by the "history" of the system. (The term "memory effect" will be used in connexion with homogeneous systems. In heterogeneous systems the "ageing" of, for example, solid phases provides a simple manner of making the behaviour of the system dependent on its history. A long-known example of a reaction possessing different orders with respect to concentration and to time is the thermolysis of acetaldehyde at 500° (ref. 1).)

If the conditions for a mechanism (II), α, are fulfilled but not in the extreme (like β), then the non-single-valuedness of $-\mathrm{d}[A]/\mathrm{d}t$ as a function of $[A]$ remains, but the rate of separate runs is not exactly constant. An experimental example of this situation is given in Fig. 2. In the course of the reaction the reaction rate decreases far more slowly than the measured concentration.

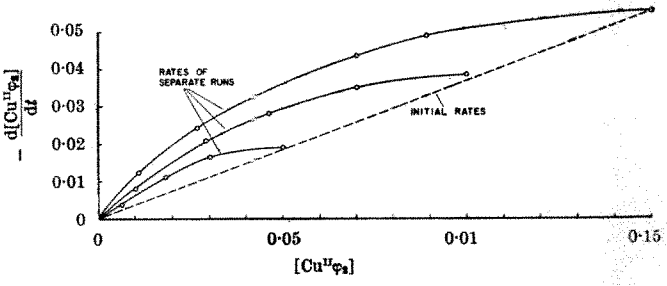


Fig. 2. Plot of reaction rate against concentration in systems $Cu\varphi_2(NO_3)_2$ (0·05 – 0·1 – 0·15 mmole), $NaOCH_3$ (0·2 mmole) and $(t\text{-}C_4H_9)_2NO$ (0·002 mmole) in 30 ml. methanol, 30° C, anaerobic conditions

The differential equations describing the kinetics of mechanism (II) cannot be solved analytically, even for the simplified problem (III), with $k_4 \neq \infty$. The systems (II) and (III) are, however, easily simulated on an electronic analogue computer.

Kinetics of the aforementioned type were encountered in the reduction of cupric–phenanthroline complexes by methanol, catalysed by the stable free radical di-*t*-butyl nitroxide $(t\text{-}C_4H_9)_2NO$.

Solutions of cupric nitrate and *o*-phenenthroline (1 : 2 ratio) in methanol containing some $NaOCH_3$ are stable at 30° C in the absence of oxygen, unless a catalyst is added. In such solutions the copper ion and phenanthroline (φ) largely combine to a complex ion $Cu^{II}\varphi_2$. (The reaction is described in detail elsewhere[2]. In the present article those complications which do not contribute to the "memory effect", such as the further co-ordination of $Cu^{II}\varphi_2$ with φ, CH_3O^- or CH_3OH or the existence of two parallel oxidation reactions of methanol, have been ignored for simplicity.) The latter is comparable with compound A in diagram (II). To a small extent this complex dissociates according to:

$$Cu^{II}\varphi_2 \rightleftarrows Cu^{II}\varphi + \varphi$$

The kinetics indicate that only the complex $Cu^{II}\varphi$ (B in diagram (II)) is reduced by methanol and a catalyst, to form $Cu^{I}\varphi$ (C). The fate of methanol and catalyst in this reduction is irrelevant to the present discussion. Suffice it to say that their concentrations remain constant throughout the reaction. The co-ordinatively unsaturated cuprous complex $Cu^{I}\varphi$ can be converted into the much more stable $Cu^{I}\varphi_2$ (D) in two ways: (*a*) The reaction with free phenanthroline:

$$Cu^{I}\varphi + \varphi \to Cu^{I}\varphi_2$$

which will be relatively slow because $[\varphi]$ is small. (*b*) An electron transfer reaction with $Cu^{II}\varphi_2$:

$$Cu^{I}\varphi + Cu^{II}\varphi_2 \to Cu^{II}\varphi + Cu^{I}\varphi_2$$

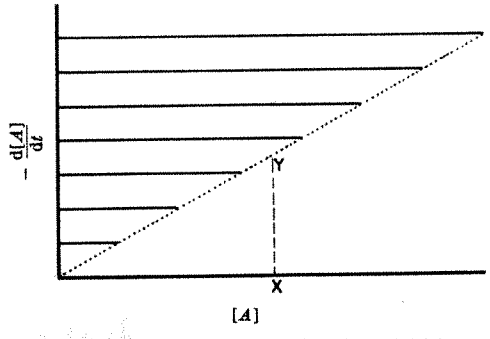
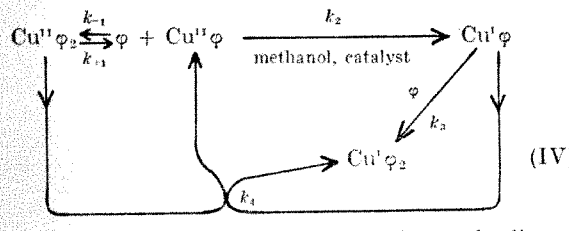


Fig. 1. Full lines, rates of separate runs; dotted line, initial rates. As a function of time the systems run from right to left

(reaction 4 in (II)), which may be expected to be very fast. It provides an efficient way of stabilizing the concentration of the reactive intermediate $Cu^{II}\varphi$.

The individual reactions participating in the catalysed reduction of the cupric phenanthroline complex by methanol can be assembled in a diagram analogous to (II):



(IV)

Experimentally, the reaction is started by adding the catalyst to the methanolic solution of the cupric phenanthroline complex. The total concentration of cupric complexes as a function of time is followed on a recording spectrophotometer. The reaction rates as a function of the concentration are plotted in Fig. 2 for three systems. The initial rates are approximately first order in $[Cu^{II}\varphi_2]$; during the reaction this order is considerably lower. (On the basis of an equilibrium $Cu\varphi_2 \rightleftarrows Cu\varphi + \varphi$, the initial concentration of $Cu\varphi$ and the initial rate would be expected to be proportional to $[Cu\varphi_2]_0^{\frac{1}{2}}$, since $[Cu\varphi]_0 = [\varphi]_0$. The complications giving rise to an approximate first order in $Cu^{II}\varphi_2$ are discussed in ref. 2.) The rate is not a single-valued function of $[Cu^{II}\varphi_2]$, because the system 'remembers' its initial conditions.

The reactions of diagram (IV) were simulated on an electronic analogue computer. k_{-1} and k_3 (which were assumed equal for simplicity) were varied, while k_{+1}/k_{-1}, k_2 and k_4 were given constant values such that the time needed for completion of the reaction was of the order of 5–10 min. It was seen that k_4 needs to be only about $10 \times k_{-1}$ in order to obtain a memory effect comparable to that illustrated in Fig. 2.

I thank Dr. H. A. Vreedenberg for helpful discussions.

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SENSE RECEPTORS OF *Multicotyle purvisi* DAWES, 1941 (TREMATODA, ASPIDOBOTHRIA)

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IT is generally assumed that one of the adaptations to a parasitic way of life in Platyhelminthes is a reduction of their sense receptors. It is, therefore, of great interest that in a species of trematode sense receptors were found which are not only more diversified than known from any trematode, but even more complex than in free-living turbellarians.

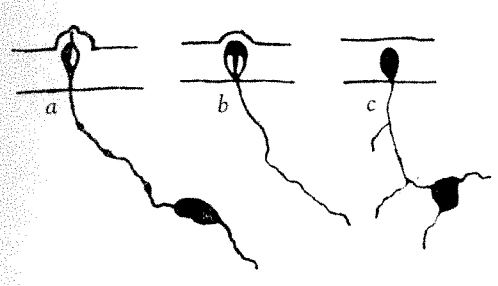


Fig. 1. Three types of sensory bulbs in the oral sucker of *Cercariaeum*. (After ref. 4)

While free-living turbellarians may have, besides eye-spots and statocysts, a variety of sensory bristles, some serving as chemoreceptors, others as tangoreceptors or rheoreceptors (see, for example, ref. 1), the only sense receptors known from digenetic trematodes, are free nerve endings below the cuticle (see especially refs. 2 and 3) and bulb like terminal swellings of neurosensory cells in the cuticle[3–5] (Fig. 1). Eye spots are often present in free larval stages, but are generally lost in the adult parasitic stage. For monogenetic trematodes, Wright and MacCallum[6] describe simple "tactile organs" with a long tactile hair. The marginal organs of Aspidobothria, which were considered to be sense organs by some early authors, are not sensory in nature, but ampulla-like structures filled with glandular secretion.

Zernecke[7], Blochmann[8] and Pintner[9] have shown that cestodes have simple types of sense receptors similar to those of trematodes.

In the course of histological investigations of *Multicotyle purvisi* Dawes, 1941 (Fig. 2), using the slightly modified urea–silver nitrate method as described by

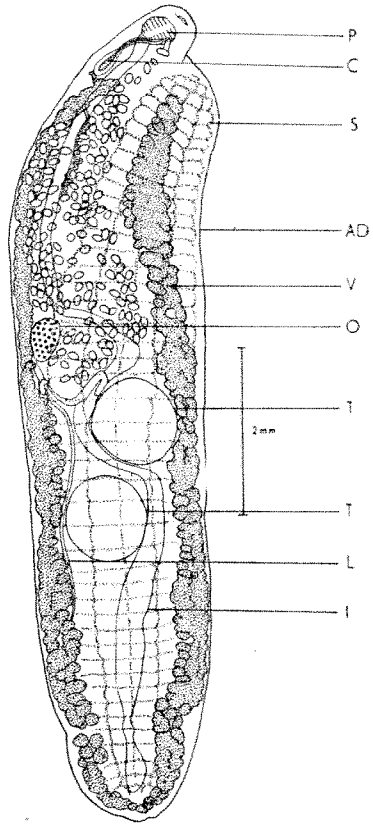
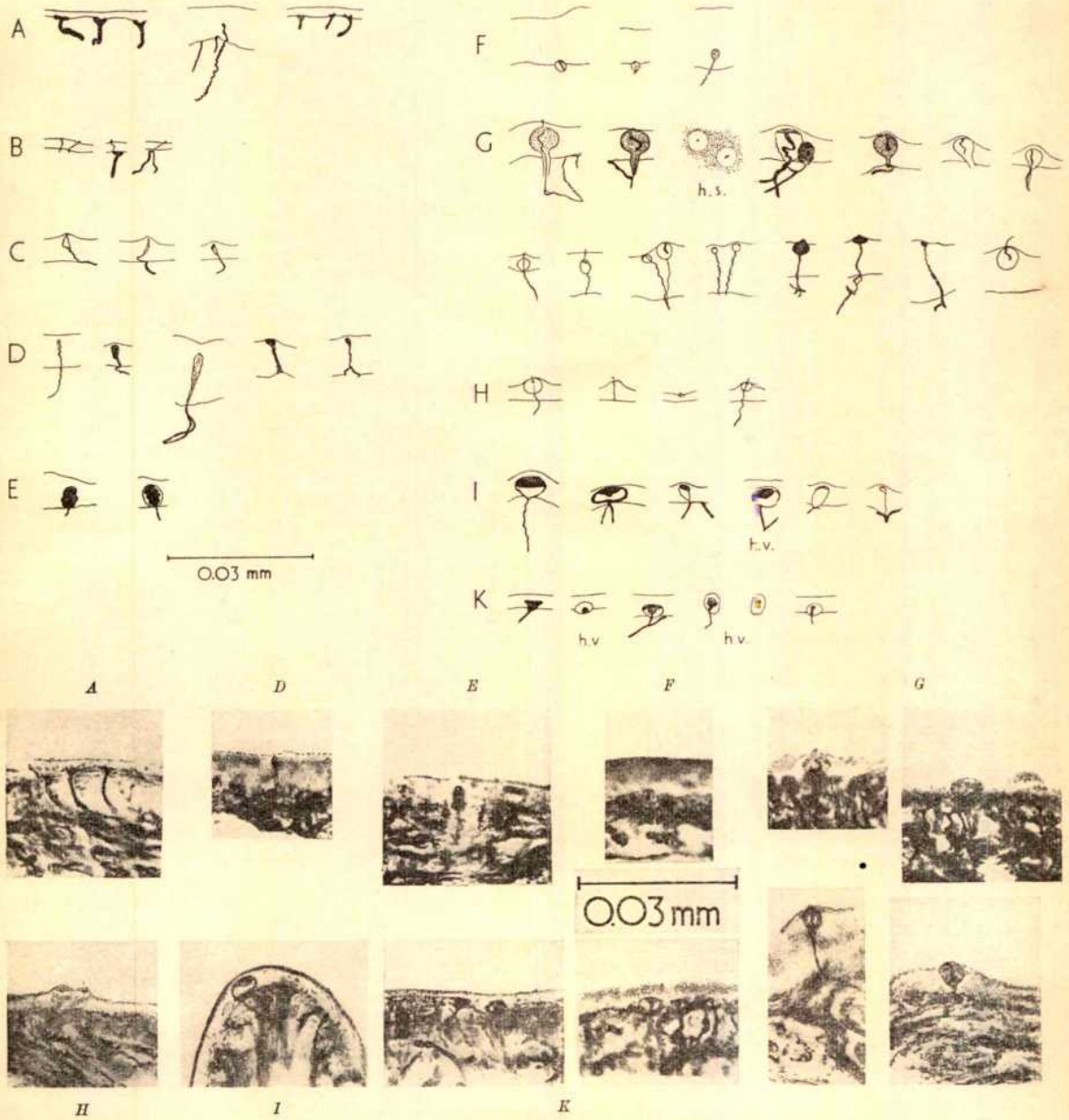


Fig. 2. *Multicotyle purvisi* Dawes, 1941. Whole mount. *P*, pharynx; *C*, cirrus pouch; *S*, suckerlet of adhesive disk; *AD*, adhesive disk; *V*, vitellaria; *O*, ovary; *T*, testis; *L*, Laurer's canal; *I*, intestine

Gurr[10], an astonishing variety of sense receptors was found. This variety is even greater than that of free-living turbellarians. The statement that parasitism in Platyhelminthes leads to a reduction of their sense receptors cannot therefore be applied to this species.

In *Multicotyle*, the least specialized sensory elements are free nerve endings which can be found below the cuticle in most parts of the body (Figs. 3 and 4*A*). They are most common in the suckerlets of the adhesive disk and on the walls separating them, and in the oral opening and the region surrounding it and behind it. They occasionally penetrate a short distance into the cuticle. Often their ending is branched in a T-like fashion. They must not be confused with the network of connective tissue the fibres of which often look very similar, especially those in the suckerlets of the adhesive disk. Because of this similarity, the connexion of a fibre to large nerves was used as the only criterion for its nervous nature. Free sense hairs are very common on the adhesive disk and probably in the oral opening; they arise from the subcuticular nerve endings and pierce the cuticle to form a small knob-like projection on its surface (Fig. 3*B*). On rare occasions, such sensory hairs were also seen penetrating the thick dorsal cuticle. It is, however, possible that these are in fact secretory ducts. (In the suckerlets, the hairs could often be traced to the free nerve endings which supports the view that they are of a sensory nature. However, it cannot be ruled out that at least some of the "hairs" are secretory ducts and the knob-like projections secretory droplets. Final clarification of this point would be possible by investigations with an electron microscope.)

Relatively few nerve fibres penetrate into the cuticle-forming loops (Fig. 3*C*) or clubs consisting of a coiled up nerve fibre (or fibres ?, Figs. 3 and 4*D*). While the former were mainly found on the adhesive disk, the latter are thinly scattered on the lateral and dorsal parts of the main body. Sometimes, the clubs are solidly stained, thus concealing any fibres.



Figs. 3 and 4. Sense receptors of *Multicotyle purvisi* Dawes (Fig. 3, camera lucida drawing; Fig. 4, photographs). *A*, Free subcuticular nerve endings; *B*, free sensory hairs; *C*, loops of nerve fibres in cuticle; *D*, coiled up nerve fibres and small clubs in cuticle; *E*, large clubs in cuticle around mouth opening; *F*, small subcuticular capsules; *G*, capsules in cuticle; *H*, small capsules in cuticle of adhesive disk; *I*, sensory bodies in cuticle of walls separating suckerlets of adhesive disk; *K*, small sensory bodies in and around mouth; *h.s.*, horizontal section; *h.v.*, horizontal view

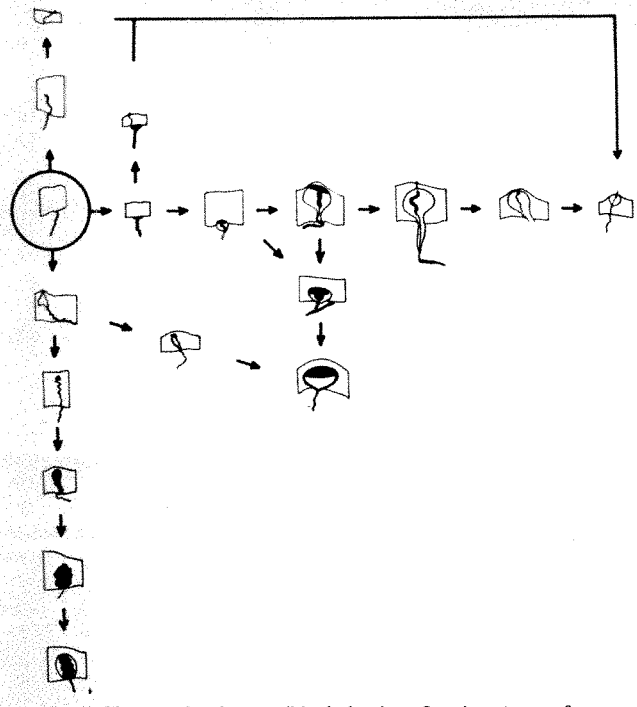


Fig. 5. Diagram showing possible derivation of various types of sense receptors

Common in the region around and behind the oral opening (but absent in the mouth cavity) are large, solidly stained bodies which are sometimes surrounded by a capsule (Figs. 3 and 4*E*, maximum size 6·0 × 3·6μ). It is possible that these are derived from the smaller clubs in other parts of the body.

Small capsules are dispersed throughout the region below the cuticle of the main body. They can be considered to be free T-shaped nerve endings surrounded by a capsule (Figs. 3 and 4*F*, maximum size 5·4 × 3·6μ, usually about 2·5 × 2·5μ). The terminal solid plate is often absent. Occasionally, the capsules penetrate for a short distance into the cuticle. An apparent further development of these small subcuticular capsules are large capsules which are distributed in most parts of the body. They are often located in an elevation of the cuticle and show a considerable variability in size (Figs. 3 and 4*G*, maximum size 7·0 × 7·4μ). They are largest and most common in the regions dorsal and lateral to the oral opening (but not in the mouth cavity) and rarely occur on the adhesive disk. Typically, the capsule is a pear-shaped body with a long projection reaching below the cuticle. A thick nerve fibre crosses the capsule, forming an S-shaped figure in its widest part. Terminally, it either forms a solid plate which sometimes bears a short hair-like process passing beyond the surface of the cuticle, or it occurs without such a plate, and becomes very thin and often passes to the surface where it forms a knob-like projection or a long sensory hair. In horizontal sections, the capsule appears homogeneous and surrounded by a strongly granular cuticle. A modification of this kind of capsule is the smaller capsule found on the ventral disk, in the lumen of the mouth cavity, on the lip surrounding the mouth opening, and in the regions ventral and lateral to the mouth opening (Figs. 3 and 4*H*, maximum size 3·4 × 3·6μ). In this type, there is no thick S-shaped fibre in the capsule. Instead, the fibre is usually straight and extremely thin and forms a very long sensory hair at the surface. Normally, there is a small plate-like swelling of the fibre at the base of the hair which probably corresponds to the body of discrete amorphous material described by Dixon and Mercer[5] in the sensory bulb of the cercaria of *Fasciola hepatica*.

Large bodies are very conspicuous and common on the walls separating the suckerlets of the adhesive disk (Figs. 3 and 4*I*, maximum size 6·0 × 7·2μ). The large terminal plate of this sense receptor seems to consist of a fused nerve fibre. Sometimes the end plate is very thin, which indicates a possible relationship with the simple nervous loop (see Fig. 3*C*). It is also possible that they are derived from the large pear-shaped capsules (see Figs. 3 and 4*G*) in which the terminal plate has become abnormally thick. This is indicated by the structure of smaller capsules which are very common on the lip around the oral opening and inside the mouth cavity (Figs. 3 and 4*K*, maximum size 3·0 × 4·1μ). These capsules resemble the receptors illustrated in Figs. 3 and 4*G*, but they have an extremely thick terminal nerve fibre and usually a very thick terminal plate. The possible relationship of the various types of sense receptors is illustrated in Fig. 5.

The number of sense receptors is greatest around and behind the mouth and on the adhesive disk. In a specimen, 6·1 mm long, the following numbers of receptors were counted: in the prepharyngeal region (310μ long), dorsal, 360; ventral, 260; in the mouth cavity, 140. Most common in the mouth cavity and on the lip surrounding it were sense receptors, as illustrated in *K*, but there were also many '*H*'s'. In the regions dorsal, lateral and ventral to the oral opening there were mainly '*G*'s', but also a considerable number of '*E*'s'. Besides the sense receptors, numerous free nerve endings were present.

In another specimen, 3·1 mm long, the posterior half of the prepharyngeal region (100μ long), excluding the mouth cavity, contained about 160 sense receptors, while a portion of the body, 300μ long and beginning 600μ behind the anterior end, contained about thirty-five '*I*'s', sixty '*H*'s', thirty-five '*C*'s' and a great number of sensory hairs and free nerve endings on the adhesive disk, and seventy '*F*'s' and '*G*'s' and five '*D*'s' on the main body, primarily its dorsal portion. A part of the body, 300μ long and beginning 2·9 mm behind the anterior end, contained about forty '*I*'s', 115 '*H*'s', ten '*C*'s' and a great number of sensory hairs and free nerve endings on the adhesive disk, and twenty-five '*F*'s' and '*G*'s', five '*H*'s' and two '*D*'s' on the main body. There were hundreds of free nerve endings and at least some dozen free sensory hairs in each suckerlet of the adhesive disk.

The numbers given are only approximate and probably minimum values, because it is often difficult to count all sense receptors, especially in areas where they are closely packed. Most difficult to find are the small bulbs located below the cuticle ('*F*').

After staining in hot 2 per cent silver nitrate solution, the openings of the sensory bulbs appear as black spots on the cuticle. It is doubtful, however, whether all bulbs are stained, especially those without a hair piercing the cuticle. Thus, this method cannot be used for counting the sense receptors.

Regarding the function of the different receptors, it seems probable that '*A*', '*F*', '*I*' and '*K*' are tango-receptors, because they do not possess hairs which might be stimulated by chemicals. No statement about the function of the other types is possible at present.

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MORPHOLOGICAL AND SEROLOGICAL ANALYSIS OF LEPTOSPIRAL STRUCTURE

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THERE is no serological or chemical information about the nature of the morphologically identifiable structures of the leptospiral cell nor have there been investigations of the cellular localization of antigenic sites. This information is of importance in microbiology because of the unique structure of the genus *Leptospira* and its relationship to the structure of other genera of spirochaetes, and of taxonomic importance because of the complex and increasingly confusing serological relationship between serotypes of leptospires. An understanding of leptospiral structure may help elucidate some unexplained aspects of the pathogenesis of leptospirosis[1]. This article reports the morphological identification and partial purification of cell-walls from pathogenic *L. icterohaemorrhagiae*, describes perimural fibrils and suggests the nature of antigens corresponding to various cellular components.

It has been generally agreed that leptospires have three main structures, a protoplasmic cylinder, an axial filament and a cell envelope[2]. As a result of thin sectioning of leptospires Babudieri[3] showed that the axial filament was covered by and located inside the envelope. Simpson and White[4] reported that the protoplasmic spiral was a hollow tube which contained material of low electron density.

Serologically-active antigenic fractions of leptospires have been extracted with ethanol[5–8], deoxycholate[9] and phenol[10], but no morphological observations were made of the cellular location of extracted material. Recently, Ginger[11] identified muramic acid in *Borrelia duttoni* and *L. biflexa*. He suggested that spirochaetes should have cell-walls structurally similar to the bacteria.

In the present experiments *L. icterohaemorrhagiae* was cultivated in Korthof's medium with 8 per cent rabbit serum for 4–7 days at 30° C, washed twice with 0·01 M phosphate buffer, *p*H 7·2, and resuspended in buffer to one-twentieth of the volume of the original culture.

Initially, serologically antigenic fractions were extracted from the washed cell suspensions by the following agents: ethanol (final 50 per cent, 4° C overnight, repeated more than twice), *n*-butanol (final 50 per cent, shaken at room temperature for 5 min, 4° C overnight, aqueous phase used), ethyl ether (excess amount, shaken for 5 min and kept overnight at room temperature, aqueous phase used), phenol (final 50 per cent, 65° C for 10 min, aqueous phase used), sodium deoxycholate (L. Light and Co., final 1 per cent in 0·01 M phosphate buffer solution, *p*H 7·2, 45° C for 4 h), trypsin (B.D.H., 1 mg/ml. in 0·06 M phosphate buffer solution, *p*H 7·8, 37° C up to 3 h), lysozyme (Armour, 20–1,000 μg/ml.), penicillin (10–100,000 U), DNase (Sigma, 0·5 mg/ml.), RNase (N.B.C., 0·5 mg/ml.). A Hughes bacterial press[12] was also used. Ethyl ether, *n*-butanol and ethanol were removed from the aqueous phase by evaporation at 37° C. The extracts or soluble parts were separated from the cell residue by centrifugation and used as antigens for precipitin reaction in gels. Cell residues, examined by light and electron microscopy, were also used as antigens in agglutination. Precipitin reaction in gels and agglutination-lysis tests followed standard techniques. Homologous strain antiserum was obtained by immunizing rabbits with whole cultures of intact leptospires, yielding an agglutination-lysis titre of 10^{-4}.

For electron microscopy, residual cells after washing and further treatment were fixed overnight at 4° C in 0·5 per cent osmium tetroxide in veronal buffer, *p*H 7·3. Fixed leptospires sedimented by centrifugation at 17,000–25,000*g* for 30 min and washed twice with distilled water were mounted on Formvar' grids, and either shadowed with gold–palladium at an angle of 30° or stained with 2 per cent phosphotungstic acid which was adjusted with 1 N potassium hydroxide to *p*H 7·2 or were hardened in 2 per cent agar, dehydrated, embedded in 'Araldite' and sectioned with an '*LKB* 4800*A*' ultratome. Sections were stained with either phosphotungstic acid or lead[13] and examined in a Siemens 'Elmiskop I' electron microscope at initial magnifications up to 40,000 times.

Estimations of muramic acid and glucosamine were made spectrophotometrically following the method of Park, described by Perkins and Rogers[14].

Washed leptospires were treated as described and the antigenicity of the supernatant fractions was examined by precipitin reactions in gels with immune serum against whole organisms. Ethanol and *n*-butanol extracts each contained an antigenic fraction which gave three similar lines. Phenol extracted a similar antigenic fraction which gave a faint line. No appreciable antigenicity was found in the extract or supernatant of the cells treated with ether, trypsin, lysozyme, or penicillin and in the cells disrupted with the Hughes press.

Treatment with deoxycholate produced a fraction containing antigens which formed five lines, including all three of those found in ethanol, *n*-butanol and phenol extracts. The common lines were spaced near the anti-serum well or between the anti-serum and antigen wells, while the characteristic lines of deoxycholate extracts were formed close to the antigen well (Fig. 1). Residues of the cells treated with ethanol or *n*-butanol could be further extracted with deoxycholate. The extract thus produced gave three or four lines, which included all the lines characteristic of deoxycholate and also one or two lines common to ethanol or *n*-butanol extracts.

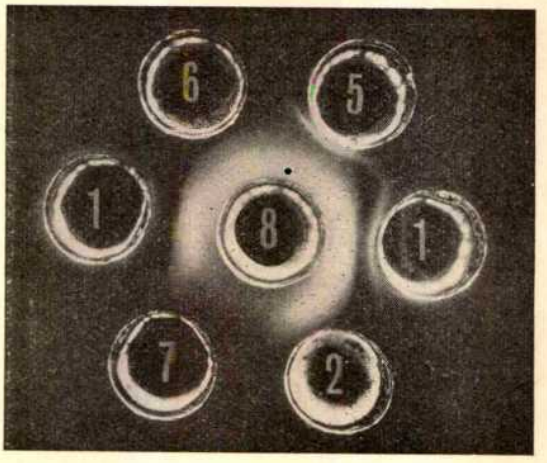


Fig. 1. Immunodiffusion of rabbit antiserum to whole *L. icterohaemorrhagiae* (8) with the following extracts of homologous leptospires: deoxycholate (1), butanol (2), deoxycholate extract of ethanol-treated cells (5), ethanol (6) and phenol (7)

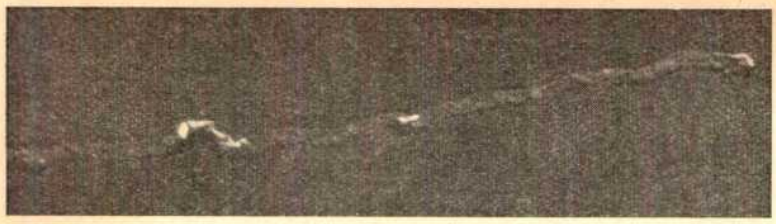


Fig. 2. Cell wall of *L. icterohaemorrhagiae*. A vestige of spiral structure remains where there is adherent perimural material. Shadowed electron micrograph. (× *c.* 11,850)

The optical density of leptospiral suspensions was reduced within 0·5–1 h when treated with deoxycholate and was further reduced after washing and trypsinization. Suspension density was also reduced after pressing but not by other treatments. Under dark-field, leptospires were seen to retain their shapes. Trypsin caused a slight reduction and deoxycholate a great reduction in refractility which was not appreciably changed by ethanol, *n*-butanol, ether or phenol. When the cells were treated with trypsin after deoxycholate, the refractility was greatly decreased. Pressing caused leptospires to agglutinate into large clumps allowing the individual apparently disrupted cells to be seen only with great difficulty.

Under the electron microscope, whole leptospires clearly showed their three basic structures—namely, a protoplasmic cylinder, an axial filament and a cell envelope. A regular fold or apparently cord-like structure twisting all over the protoplasmic body was noticed under the envelope.

Ethanol treatment caused incomplete removal of the envelope and the axial filament but the electron density was not reduced. After deoxycholate treatment with constant shaking, the outermost cell envelopes were removed and the axial filaments were completely lost. Cells thus treated appeared to have surfaces rougher than when untreated, and their electron density was lowered.

Trypsinization of cells already treated with deoxycholate made the leptospires flat, straight and less electron-dense. Occasionally a fibrous structure was revealed on the cell body, details of which are described in a later paragraph. Similar results were also obtained when trypsinization was applied to the cells treated with both ethanol and deoxycholate. Almost all nucleic acid, measured by the absorption of 260 mμ of a 1 N perchloric acid extract (70° C for 20 min), was lost from the deoxycholate and trypsin-treated cells. Agglutinability was reduced by these treatments (Table 1).

Table 1. AGGLUTINABILITY OF LEPTOSPIRES AFTER CHEMICAL TREATMENT

Leptospiral antigen suspension after treatment with	Morphology	Reciprocal agglutination-lysis titre (50 per cent agglutination end-point)
—	Whole cells	10,000
50 per cent ethanol, nine times	Cells only partially deprived of envelopes and axial filaments	1,000
Deoxycholate and DNase	Cells deprived of envelopes, axial filaments and DNA	100
Deoxycholate, trypsin, DNase and RNase	Only cell walls and some protoplasmic material	100

Table 2. MURAMIC ACID AND GLUCOSAMINE IN LEPTOSPIRES AFTER CHEMICAL TREATMENT

Leptospires treated with	Morphology	Muramic acid (per cent dry wt.)	Glucosamine (per cent dry wt.)
—	Whole cell	0·7	2·4
Deoxycholate and DNase	Cells deprived of envelopes, axial filaments and DNA	2·6	3·3
Deoxycholate, trypsin, DNase and RNase	Cell walls with some cytoplasmic materials	3·7	4·0

After shaking successively with deoxycholate, trypsin, DNase, and RNase, the preparations under the electron microscope showed filamentous cell residues with some intracytoplasmic material still remaining. Estimation of muramic acid and glucosamine (Table 2) showed that the proportionate muramic acid content of leptospires was gradually increased during progressive purification. This is evidence that the flat filamentous shape contains muramic acid and that it could be cell wall. Glucosamine content was similarly increased progressively during purification but the increase was less than in the content of muramic acid (Table 2). These findings confirm the conclusion that the long filamentous shape was the leptospiral cell wall and thus the basic morphological entity of leptospires. Cell walls were further purified by more drastic procedures including pressing which did not clump cell walls as it did whole cells. The preparation obtained after treatment with deoxycholate, DNase, trypsin, pressing, deoxycholate, DNase, trypsin and RNase is shown in Fig. 2. Many of the cell walls were straight, very flat and contained apparently internal granules. Occasionally foldings were seen which may have been an indication of their original spiral. The width of the purified cell walls was about half that of the whole cells. Thin sections of leptospires were examined to observe particularly their cell walls. A section of a whole leptospira (Fig. 3) showed the three main structures and also demonstrated a three-layered cell wall consisting of a less dense layer between two denser layers. Partially purified cell walls showed a lightly staining tubular appearance when sectioned, but bounding membranes were not clear.

In the course of electron microscopy, fine fibrils were observed in preparations which had been treated with deoxycholate without shaking and immediately fixed with osmium tetroxide. Ten min trypsinization after deoxycholate treatment also clearly showed several fibrils twisted around cell walls (Fig. 4) but longer trypsinization of 0·5–1 h destroyed them. They were never seen in whole cell preparations not treated with deoxycholate. Some fibrils remained attached to the cell body by one or both ends, while others were released at one or both ends. On each leptospira there appeared to be six or more fibrils each estimated to be 60–80 Å in individual diameter, and wound or tightly coiled around the leptospiral cell wall as seen in shadowed preparations. The fibrils were clearly different from the axial filament because they were coiled or wavy, were never seen in untreated cells, and were observed either in cells where an intact axial filament could be seen attached to one end, or after treatments where the axial filament was completely released. The most suitable treatments to demonstrate the fibrils were different from those required to show cell walls. The protein composition of the fibrils was suggested by their susceptibility to prolonged trypsin treatment and their staining with phosphotungstic acid. Loss of the spiral shape coincided with the loss of fibrils



Fig. 3. Thin section of a leptospira, (Karkovsky lead strain) (× 40,000). A triple-layered structure corresponding to cell wall is seen between the two darkly-stained masses of axial filament

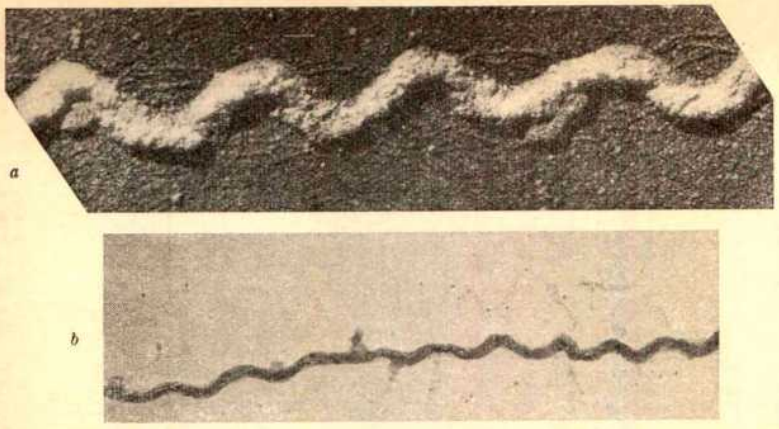


Fig. 4. (*a*) Spiral fibrils between leptospiral spirals seen after treatment of a leptospira with deoxycholate and trypsin. The axial filament of this leptospira was attached at one end, but apparently intact (× *c.* 27,000). (*b*) Fibrils positively-stained with phosphotungstic acid in a parallel preparation to that shown in *a* (× *c.* 13,500)

or their matrix after trypsinization. This body of evidence points to the fibrils as a structural entity distinct from cell wall and axial filament, possibly responsible for the spiral shape by coiling like a tight spring around the cell wall to produce a spiral structure.

The findings show that leptospires are bacteria with cell walls structurally and chemically similar to other bacteria. However, methods such as crushing or decompressive rupture[15] appear to be unsuitable for preparing cell walls without chemical treatment beforehand. Sodium deoxycholate does not cause complete lysis, as is commonly supposed, but strips the outer cellular layers, causing reduction of optical density and of refractility in dark-field, and immediate leakage of intracellular enzymes[16].

Three main layers may now be recognized in the leptospira. The outermost envelope is removed both by 50 per cent ethanol and deoxycholate, which also loosens and removes the deeper perimural fibrillar layer. Although the ethanol extract contains the erythrocyte sensitizing (HL) carbohydrate[5,17] it also removes protein antigens on repeated extraction[8], probably from layers deeper than the envelope. Since agglutinability is reduced after repeated alcohol extraction and almost completely absent in cell wall preparations (Table 1) and anti-HL antiserum does not agglutinate (unpublished observations), the agglutinating antigens are deeper than the envelope and in the perimural layer. The second layer comprised the perimural fibrils and their matrix. It is possible that the perimural fibrils correspond with those thought by Mölbert[18] to originate in the axial filament, by Simpson and White[4] to be in the thick wall of the protoplasmic cylinder, and seen in "degenerating" leptospires[19].

The fibrillar perimural structure may be of taxonomic importance among members of the Treponemataceae. Fibrils, usually thought to originate from the axial filament or bundle, may be found in *Borrelia* or *Treponema*[20]. Further experiments are clearly required to elucidate whether they may not correspond to the perimural fibrils of *Leptospira*. It would also be important to establish to what extent differences in the nutritional environment are reflected in metabolic changes manifested as qualitative or quantitative changes in antigenic constitution and in structure. The third layer beneath the fibril-containing perimural layer is the triple-layered cell wall proper, containing muramic acid and enclosing the cytoplasmic contents. It is not spiral but is a straight, hollow, collapsible tube. Given that the axial filament is flexible as well as straight, these observations are compatible with and explain the infinite flexibility of spirochaetes in semi-solid environments and their immediate return to a straight form in liquids.

The last main structural component is the axial filament which Ritchie and Ellinghausen[19] showed to be multi-stranded within a sheath and suggested was double. Neither duplicate nor multiple axial filaments were seen. However, although the evidence for more than one axial filament is inadequate, it is very likely on theoretical grounds that two axial filaments may be found. The axial filament may be regarded as a locomotor organelle attached to an end-knob inserted into or originating from an intracellular specialized apparatus[19] from which originates a presumably electrical contractile impulse. In a fluid the movement of the axial filament causes the spiral to rotate and the leptospira to move. The possible arrangements are (1) a single axial filament inserted at one end, (2) a single axial filament inserted at both ends, (3) a double axial filament inserted at one end, and (4) two axial filaments inserted one at each end.

In order to explain the movement in both directions in cases 1 and 3 it is necessary to assume that an end-knob apparatus can generate such an impulse either away from it or towards it. It is hard to envisage a mechanism to explain a centripetal impulse, so that it is unlikely that there can be only one end-knob. Furthermore, in cases 1 and 3 the possibilities on division are that (*a*) dissimilar daughter cells will be formed in one of which the axial filament is unattached and thus non-functional, (*b*) the daughter cell which did not receive a maternal end-knob will form one and unite it with the existing fragment of inherited axial filament; this idea envisages a unique and highly improbable mechanism for the newly-formed intracellular end-knob to "trap" and unite to form a functional physiological unit with an existing axial filament which lies outside a considerable layer of cell wall and perimural material. Alternatively a new end-knob may be formed, leading to the growth of a new axial filament or filaments in addition to the unattached fragment derived from the mother cell. In case 2, the axial filament may divide with cell division but in subsequent generations the problems must be the same as described for (1) and (3), unless the alternative is envisaged that there is formation of a new end-knob and "trapping" of the axial filament as in (*b*). If a new end-knob and axial filament were grown, the daughter cells would have two axial filaments as in (4). In case 4, two axial filaments are each attached to a terminal end-knob. On division, there would be two similar daughter cells, each capable of prompt formation of a new end-knob at the newly-formed end, able to give rise to an axial filament which would elongate as the leptospira lengthened, until the form of the parent cell was attained. Thus the criteria of morphological similarity of daughter cells with mother cells and with each other, of simplicity and of microbiological and biological probability are fulfilled. It would be possible to test this hypothesis of axial filament formation by investigating the distribution of forms corresponding to all stages in (4) in cultures during conventional or during synchronized growth. A similar mechanism of replication of multiple axial filaments in *Treponema* species has been published by Listgarten and Socransky[21].

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Department of Medical Illustrations, University of Sydney, for the photographs.

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ONCOGENICITY OF BRYAN STRAIN ROUS SARCOMA VIRUS FOR RODENTS

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ALTHOUGH certain strains of Rous sarcoma virus (RSV) were known to induce tumours in rodents[1–3], it was not until very recently that the Bryan strain of RSV showed evidence of similar properties. Rabotti *et al.*[4] described gliomata induced in hamsters inoculated intracerebrally with high doses of Bryan RSV and it was suggested by these authors that the virus may contain a mixture of two types of RSV, one affecting only birds and the other affecting both birds and mammals. The latter, if present in small amounts, would only become apparent if high doses of Bryan RSV were used to inoculate mammals. Eidinoff *et al.*[5] obtained subcutaneous sarcomata in hamsters injected with high doses of Bryan RSV, but so far no reports have appeared of tumour induction in mice and rats with this virus.

The RSV used in this laboratory was originally obtained from Dr. W. R. Bryan (National Institutes of Health, Bethesda) in 1959 as a frozen sample of "high titre" virus. This was inoculated into young (4-week-old) East Lansing line 15 White Leghorn chicks obtained through the courtesy of Dr. C. le Q. Darcel from a flock reared in isolation by the Canada Department of Agriculture, Animal Diseases Research Institute (Western), Lethbridge, Alberta. The resulting tumour tissue was used as a source of virus for further inoculation and ever since then either crude cell-free tumour homogenate or tumour cells themselves have been passaged weekly in the same line of chicks in order to maintain a constant supply of virus or cells. Throughout this time many assays of the RSV content of the tumours have been made. These have been performed either on the chorioallantoic membranes of fertile eggs or in tissue culture by the method of Temin and Rubin[6]. Eggs and embryos from a flock of White Leghorns maintained in isolation by the Poultry Science Department of this University were chosen for this purpose after considerable testing of available sources. Over the years the assays have consistently shown that the tumours yield 10^6–10^8 or more pock or focus forming units of virus per gram.

During the past 6 years we have frequently attempted to induce tumours in rodents with our RSV without success. The first indication that this virus was oncogenic for mice came to our attention as a result of an experiment designed to test the possible effects of inositol protection[7,8] of RSV on its infectivity for other species and the "activation" of RSV by a factor in fast-growing tissues as described by Stewart and Landon[9].

Crude RSV was prepared by homogenizing tumour tissue either in Hanks's balanced salt solution (BSS)[10] or 5 per cent myo-inositol solution. Mouse embryo extract (MEE) was prepared by homogenizing near-term Swiss embryos in an equal volume of BSS or 5 per cent inositol and centrifuging to clear the homogenate of cells and debris. Portions of MEE were mixed with part of the corresponding virus suspensions and BSS or 5 per cent inositol were added to other samples of the virus suspensions so that each animal received the same amount of virus. The original virus preparations were assayed in fertile eggs and the virus mixtures were inoculated subcutaneously into newborn (24 h old) Swiss mice. These mice were originally obtained from Connaught Medical Research Laboratories, Toronto, and have been pen-bred and maintained for several years at this University.

Table 1. INCIDENCE AND TIME OF APPEARANCE OF TUMOURS AFTER THE INOCULATION OF RSV IN VARIOUS MEDIA INTO NEWBORN SWISS MICE

Litter inoculated with 10^5 P.F.U.* RSV in the following media	No. of mice	No. of tumours	Time of appearance of tumours (weeks)	Tumour incidence (%)
BSS	21	14	9–30	66
5 per cent inositol	16	10	10–28	63
BSS + MEE	13	5	10–30	38
5 per cent inositol + MEE	18	7	9–22	39

* Pock forming units (chorioallantoic membrane assay).

It is apparent from Table 1 that tumours appeared in all groups of mice within 9–10 weeks after inoculation. No evidence was found of any enhancing effect of either inositol or MEE on the ability of RSV to infect Swiss mice.

Several of the tumours arising were transplanted to both newborn and adult Swiss mice and one is at present in its fifth passage. The latent period of appearance of this tumour is now approximately 2 weeks in adults given 0·2 ml. of a 1 : 10 tumour cell suspension subcutaneously. Attempts to transplant several of the tumours to 4-week-old chicks have failed, which indicates that no infective RSV is elaborated by the cells. Histological sections were made of many of the neoplasms and stained with haematoxylin and eosin. Most of them could be characterized as anaplastic spindle cell sarcomata and, in some cases, fibrosarcomata (Fig. 1). Rhabdomyoblast-like cells were observed when invasion of skeletal muscle occurred, as described for hamster RSV-induced tumours by Eidinoff[5] (Fig. 2). The results of this experiment were surprising in view of our failure to show any ability of Bryan strain RSV to infect mice in previous tests with similarly potent virus extracts. It was of interest, therefore, to see if the virus could induce tumours in rats as well.

Two litters of newborn Wistar rats were inoculated with approximately 10^5 pock forming units RSV subcutaneously. Within 10 days, fluid-filled cysts developed in some of the animals. At 3 weeks after inoculation, one animal was killed and on autopsy was found to have two cysts each approximately 1·0 cm in diameter and a solid tumour weighing 1·3 g. Within 3 months all but one of the

seventeen rats injected had developed multiple cysts and tumours (often weighing several grams), usually with both types of lesions appearing in the same animal. It was frequently observed that small tumours grew from the interior walls of the cysts and this was confirmed by histology. The tumours were all anaplastic spindle cell sarcomata which tended to remain encapsulated until well advanced (Fig. 3). No evidence of metastasis was found in mice or rats even in animals dying with tumours they had carried for several months. Attempts to transplant the rat tumour cells to both rats and chickens failed, the latter observation indicating that no RSV was produced by the cells as was the case with the mouse tumours.

We have never used Schmidt-Ruppin RSV in our laboratory. However, in view of the close similarity of the behaviour of our RSV with the Schmidt-Ruppin strain it was felt of importance to try to establish by neutralization tests whether our RSV had altered its antigenic properties. Through the courtesy of Dr. Robert M. Dougherty, of the Department of Microbiology, Upstate Medical Center, Syracuse, New York, we obtained samples of chicken antisera to both Bryan strain and Schmidt-Ruppin RSV. Neutralization tests were performed by mixing known amounts of our RSV with known amounts of the antisera and incubating for 1 h at room temperature before assaying on chick embryo cells. The results of two tests are given in Table 2.

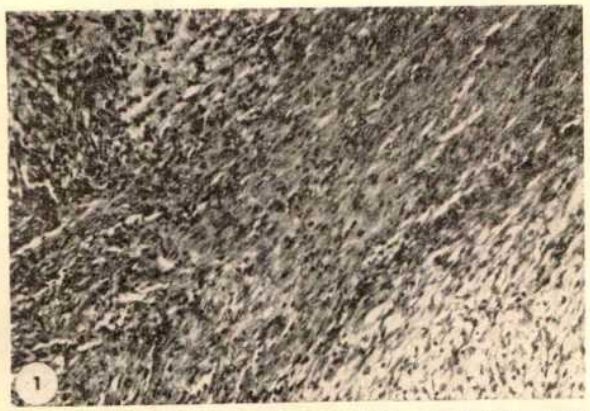
Table 2. EFFECT OF INCUBATING OUR RSV WITH CHICKEN ANTISERA TO BRYAN STRAIN AND SCHMIDT-RUPPIN RSV FOR 1 H AT ROOM TEMPERATURE BEFORE ASSAY IN TISSUE CULTURE

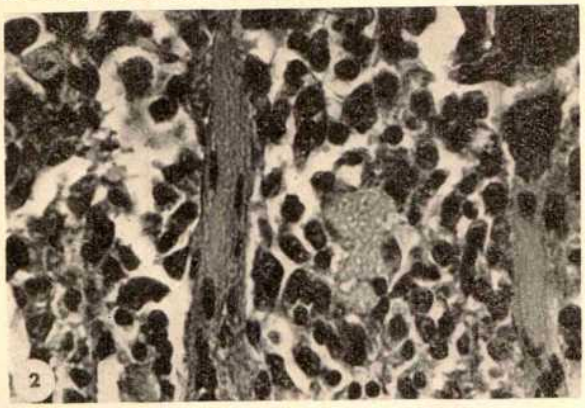
Type of antiserum	Focus-forming units per plate Exp. 1	Exp. 2
None	170	300
Anti-Bryan (> 250 units)	0	0
Anti-Schmidt-Ruppin (> 50 units)	11	65

Although only small amounts of Schmidt-Ruppin antiserum were available for the tests, it is apparent that our RSV was neutralized by both types of antiserum. It should be pointed out that no cross-neutralization occurs when these antisera are reacted with the heterologous virus. Comparison of the morphology of the foci formed in tissue culture by our virus with those formed by Bryan and Schmidt-Ruppin viruses showed that our virus converted chick fibroblasts to typical Bryan foci consisting mainly of round cells and quite unlike those produced by Schmidt-Ruppin virus. This was confirmed for us by Dr. Dougherty[11].

Three properties of our RSV confirm that we still maintain in this laboratory a virus which has retained the characteristics of Bryan strain RSV. These are: (1) consistent and high virus titres found in tumours resulting from its inoculation into young chicks (10^6–10^8 pock-forming or focus-forming units per gram of tissue); (2) complete neutralization by Bryan chicken antiserum; and (3) the round-cell morphology of foci produced in tissue culture. The last property is shared by some other strains of RSV, but not by Schmidt-Ruppin virus. That a change has occurred in the virus is borne out by its neutralization by Schmidt-Ruppin antiserum and by its ability to produce tumours in mice and rats. Further evidence for such a change is provided by the observation that the virus will produce tumours in newborn hamsters with the very short latent period of 2 weeks. Whether or not the antigenic change noted here has anything to do with the oncogenicity of our RSV for rodents is open to question, especially in view of the recent report of Eidinoff *et al.*[5]. These authors used RSV obtained directly from Dr. Bryan for their demonstration of tumour induction in hamsters.

It is true, however, that there now seems to be no difference between the European and North American strains of RSV in their oncogenicity for rodents except for their relative virulence. Further work with our strain is under way including immunological and cytological investigations. These will be reported at a later date, but preliminary cytological results indicate that the mouse tumours exhibit typical mouse chromosomes.





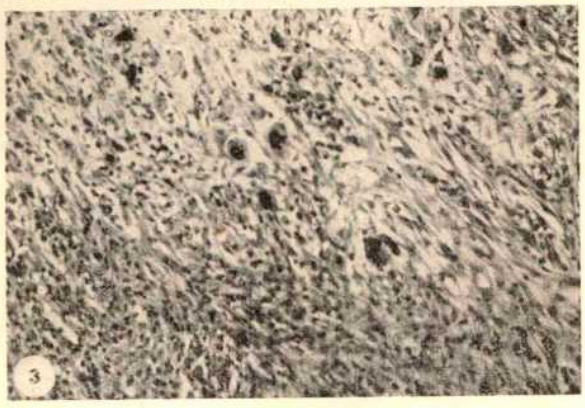


Fig. 1. Section of mouse sarcoma induced with Bryan RSV. Anaplastic spindle cell sarcoma (haematoxylin–eosin stain, × 80)

Fig. 2. Section of mouse sarcoma induced with Bryan RSV. Rhabdomyoblast cells suggesting rhabdomyosarcoma in areas where muscle tissue is affected (haematoxylin–eosin stain, × 320)

Fig. 3. Section of rat sarcoma induced with Bryan RSV. Anaplastic spindle cell sarcoma (haematoxylin–eosin stain, × 80)

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NUCLEAR VARIATION BETWEEN DIPLOID ANGIOSPERMS

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Extensive polyploid series in many plant genera, particularly in Angiosperms, testify to the prominence and importance of quantitative variation in nuclear genetic material during the evolution and divergence of numerous species. In sharp contrast, but not uncommonly, are other plant genera which show little or no trace of polyploidy, and are composed of species with consistently diploid chromosome numbers. In these diploid groups it would appear on the face of it that variation in the amount of nuclear material has played little or no part in their divergence and evolution. However, this impression of constancy in nuclear material in the diploids may well be more apparent than real. For example, McLeish[1] has reported a ten-fold difference between the nuclear DNA contents of two diploid *Liliaceous* species and a smaller, though considerable variation between two *Allium* species[2]. The indication is that large changes in the amount of nuclear material may take place quite independently of change in chromosome numbers such as polyploidy. This article is a brief account of a survey of species in *Lathyrus*, *Vicia* and *Lolium*, meant to determine the extent of quantitative variation in nuclear material, particularly in DNA, between diploid species in the three genera. Evidence is also presented which relates to the origin of such variation and, finally, the consequences of such variation in genetical terms are briefly discussed.

Lathyrus

This genus comprises, almost exclusively, diploid species with fourteen chromosomes. Although the chromosome number is constant, there is a considerable variation in chromosome size between species (Fig. 1) and therefore a suggestion of variation in the amount of genetic material. However, a difference in chromosome size cannot with any confidence be assumed to reflect a real difference in mass. Differences in size could reflect merely differences in coiling or even in water content and could be quite independent of the real variation in the content of nuclear material, including DNA. It was thought desirable for this reason to ascertain in the first place whether the size variations were associated with variation in dry mass. For this purpose measurements were made by interference microscopy of the dry mass of isolated 4*C* nuclei in different *Lathyrus* species. These values are plotted against the chromosome volumes of the different species as estimated from measurements of mitotic metaphases (Fig. 2*a*). The volumes were calculated assuming the chromatids to be cylindrical in form, an assumption which, although not necessarily strictly true, is a reasonable enough approximation. Fig. 2*a* shows a clear correlation between chromosome size and mass, and a considerable variation in nuclear dry mass between the related diploids is equally clear. That part of the variation in nuclear dry mass and in chromosome volume is associated with a variation in nuclear DNA is confirmed by nuclear DNA measurements made by Feulgen photometry. Values of 2*C* nuclear DNA are plotted in Fig. 2*b*, against chromosome volume for twelve *Lathyrus* species. Of particular interest is the nuclear DNA variation, by a factor of up to 3·5, between diploid species within this genus. The variation is equivalent to that found between 2*x* and 7*x* in a polyploid series. The evolution of these fourteen-chromosome

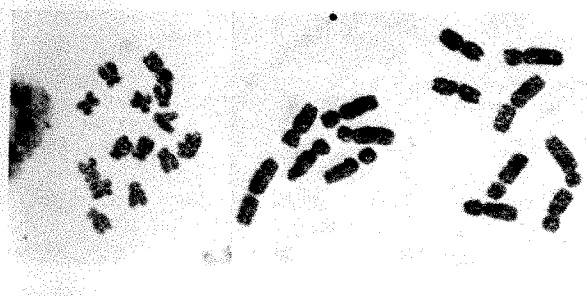


Fig. 1. Mitotic metaphase in (*a*) *Lathyrus angulatus* and (*b*) *L. hirsutus*. (× *c.* 1,200)

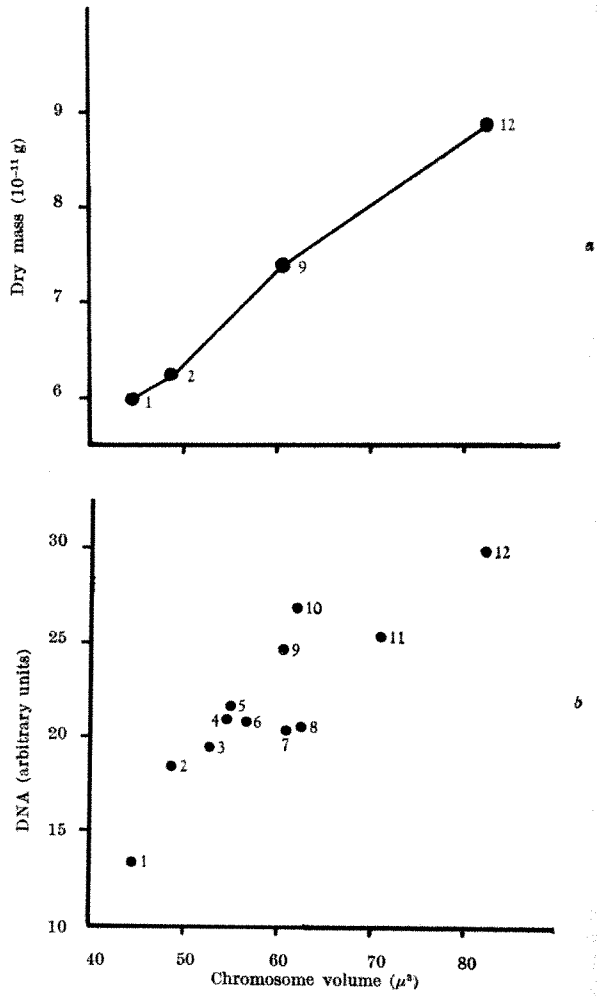


Fig. 2. (*a*) Dry mass of isolated 4*C* nuclei of *Lathyrus* species plotted against chromosome volume. (*b*) 2*C* nuclear DNA values plotted against chromosome volume. (1) *L. angulatus;* (2) *L. articulatus;* (3) *L. nissolia;* (4) *L. cicera;* (5) *L. annuus;* (6) *L. aphaca;* (7) *L. sphaericus;* (8) *L. ochrus;* (9) *L. odoratus;* (10) *L. tingitanus;* (11) *L. sativus;* (12) *L. hirsutus.* Dry mass estimated from five nuclei in each of at least two plants in each species. DNA measurements by Feulgen photometry in ten nuclei in each of at least two plants in each species. Procedure also applies to the data on *Vicia* and *Lolium*

Lathyrus diploids has certainly not proceeded independently of variation in the amount of nuclear material, including variation in nuclear DNA.

Vicia

Vicia species are nearly all diploids, some with fourteen chromosomes, some with twelve. As in *Lathyrus*, there is a large variation in chromosome size between the species. That these size differences reflect differences in dry mass and in nuclear DNA is confirmed, as in *Lathyrus*, by the interference and photometric measurements respectively, as shown in Figs. 3*a* and 3*b*. Again there is anything but constancy with respect to nuclear mass and DNA between the diploid species. A seven-fold variation in nuclear DNA distinguishes *Vicia sativa* from *Vicia faba*—a variation equivalent to that between $2x$ and $14x$.

Lolium

As in *Lathyrus* and *Vicia* there is a considerable, though lesser, degree of variation in nuclear DNA between the diploid species, all of which have fourteen chromosomes. DNA data for six species are given in Fig. 4. It will be seen that the amounts of nuclear DNA vary by about 30 per cent between species. It will also be observed that the species fall into two distinct groups, inbreeders with the greater amount of DNA which, as would be expected, have larger chromosomes, and outbreeding species with less nuclear DNA and with smaller chromosomes. Whether the relationship between the amount of nuclear DNA and the breeding system is widespread or not can only be determined by further surveys. Certainly the relationship is not universal because no such clear-cut correlation is found in *Lathyrus*. In the meantime we note once again in *Lolium* a large variation in nuclear DNA between related diploids, quite independently of variation in chromosome number.

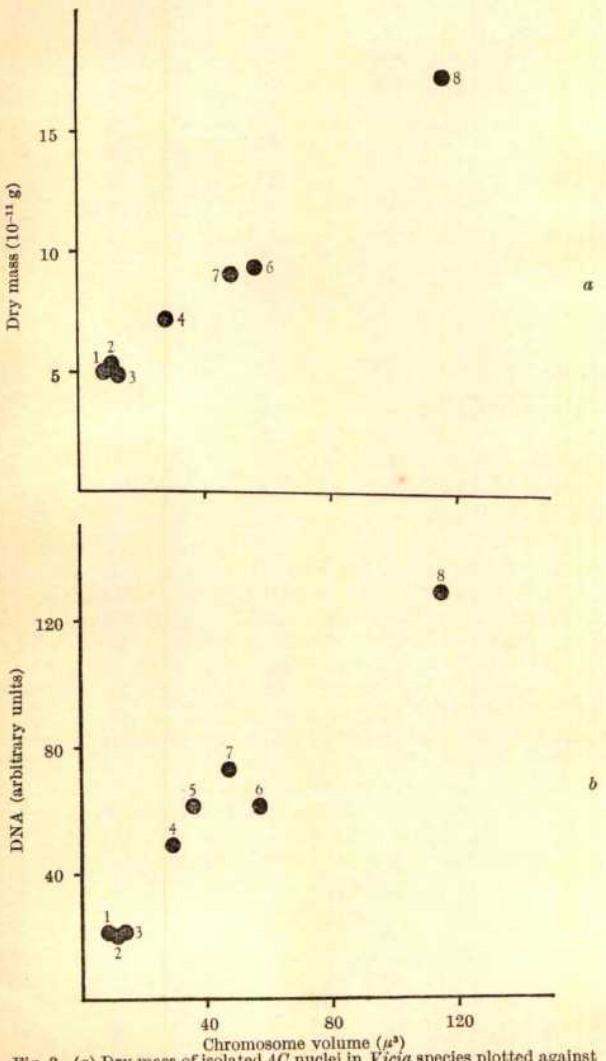


Fig. 3. (*a*) Dry mass of isolated 4*C* nuclei in *Vicia* species plotted against chromosome volume. (*b*) 2*C* nuclear DNA values plotted against chromosome volume. (1) *V. angustifolia;* (2) *V. picta;* (3) *V. sativa;* (4) *V. hirsuta;* (5) *V. narbonensis;* (6) *V. lutea;* (7) *V. sepium;* (8) *V. faba*

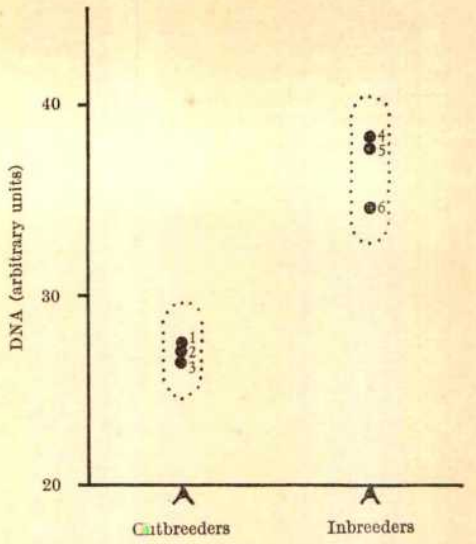


Fig. 4. Nuclear DNA amounts in *Lolium* species. (1) *L. perenne;* (2) *L. multiflorum;* (3) *L. rigidum;* (4) *L. remotum;* (5) *L. temulentum;* (6) *L. loliaceum*

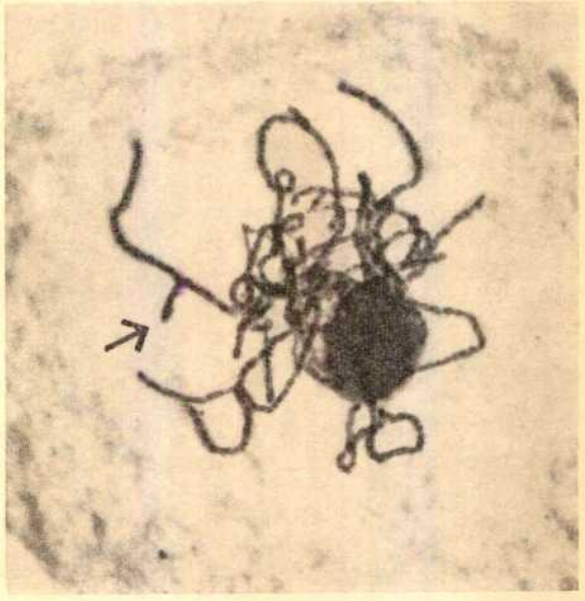


Fig. 5. Pachytene nucleus in a pollen mother cell of *L. perenne* × *L. temulentum*. The loop (arrowed) indicates chromosome duplication

Having established an inconstancy in nuclear DNA between related diploids in a number of genera, the question that immediately arises is by what means the variation originates. The two most likely possibilities are that increase in DNA may arise through (*a*) lengthwise duplication of chromosome segments, that is, an increase in chromosome length, or (*b*) increased polynemy (strandedness) of the chromosomes, that is, an increase in thickness. In genetic terms the distinction between the two possibilities is important. The first implies an increase in map distance and replication of some loci.

The second is achieved independently of change in map distance and by replication of all loci. If lengthwise duplication is responsible we should be able to detect this by the investigation of F_1 hybrids between species with large and small chromosomes and species with large and small amounts of nuclear DNA respectively. At pachytene chromosome duplication in these hybrids should result in loops of the unpaired extra chromosome segments. An investigation of this kind has been carried out in *Lolium* and is briefly described here.

Lolium perenne × *L. temulentum*

L. perenne has smaller chromosomes than *L. temulentum*[3] and, it will be recalled (Fig. 4), the lower nuclear DNA content. The amount of nuclear DNA in the F_1 hybrid is, as would be expected, intermediate between that of the parental species[4]. A pachytene nucleus in a pollen mother cell of the hybrid is shown in Fig. 5. The arrow marks a typical loop. Loops of this kind along with unpaired terminal segments are evidence for chromosome duplication. This evidence does not exclude the possibility of differences in polynemy between the chromosomes of the two species. It does show that in part at least the variation in chromosome size results from the duplication of chromosome segments.

It is evident from these data that a correspondence in chromosome number even between species of the same genus does not necessarily imply a correspondence in the quantity of nuclear material. As we have shown, the quantitative variation in nuclear material, including nuclear DNA, may be enormous. Evidence from *Lolium* shows that at least some of this variation is accounted for by chromosome duplication. Darlington has also pointed out to us that pairing of chromosomes found at meiosis in many haploids is evidence of duplication being widespread among Angiosperm chromosomes. Whether such duplication may entirely account for the nuclear variation in diploids is, however, questionable because of the extent of the variation. Polynemy could also be involved. As yet, however, there is no convincing evidence for it.

It is worth noting[5] that Angiosperm species in general have much higher amounts of nuclear DNA than mammals. So also have *Amphibia*. Whether this implies some difference in the organization or in the function of the nuclear DNA between the different groups remains to be seen.

One surprising feature of our results is the magnitude of the nuclear DNA variation between species of such close genetic relationship. The dipolids *Lolium perenne* and *Lolium temulentum*, for example, are morphologically much alike and, it will be recalled, hybridize quite readily. Moreover, the hybrid between them is fertile to the extent of setting about 12 per cent seed on backcrossing[6]. Evidently variation in the amount of nuclear DNA may not necessarily be directly reflected by variation in phenotype. It may be that much of the nuclear DNA is "uninformative".

Finally, and from a genetical standpoint, one obvious distinction between change in chromosome material associated with polyploidy as compared with that reported independently of polyploidy is that the former is achieved at the expense of increasing the number of linkage groups whereas the latter is achieved without changing the number of linkage groups. The distinction is of importance in relation to the release of variability at meiosis. In this respect alone the distinction may be of adaptive importance.

We thank the Agricultural Research Council for supporting much of this work. One of us (G. H. J.) holds an Agricultural Research Council postgraduate studentship.

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DIURNAL VARIATION IN ORGANISMIC RESPONSE TO VERY WEAK GAMMA RADIATION

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THERE is widespread agreement that natural background radiation contributes to the observed mutation rate in organisms but to our knowledge there is no evidence that it affects any other biological phenomenon of either animals or plants.

The question of a responsiveness to the natural background radiation is of interest for many reasons. One of these pertains to a possible influence of the radiation on the rates of such fundamental biological activities as, for example, cellular oxidations. The intensity of background radiation not only varies with specific locations over the Earth, but the range from weakest to strongest fields may involve an increase of about a hundred-fold. The average level of basal or standard metabolism or of "spontaneous" activity of an organism could (other things being equal) conceivably reflect differing background intensities. If no such general relationship could be demonstrated, two possibilities would still exist; either there is actually no effect or there is a masking, compensatory biological reaction. A second point of interest bears on a possible contribution of the radiation to the informational input for the geophysically related temporal and spatial organization of the activities of the organism, or the timing system of the biological clock.

It has been reported that the degree of darkening of fiddler crabs relative to appropriate controls is influenced by superimposing lead plates over a wooden cover under which crabs are otherwise shielded[1]. This is presumably a response to increased cosmic ray showers (Rossi effect). Whether increased showers darkened or lightened the crabs varied with a daily rhythm. Darkening occurred at a time of lower metabolic rate and lightening at a time of higher rate with a persistent 24-h rhythm[2,3].

More recently, the orientational responses of planarian worms in very weak caesium-137 gamma fields were investigated[4,5]. It was found that 'north-bound' worms showed what appeared to be a systematic annual variation in direction and amount of turning in response to a field about twice the background presented from the right. The variation included both clockwise and counter-clockwise turning, and paralleled in a striking manner a persistent annual variation in metabolism, or in response to photoperiod, reported for a widely diverse selection of animals and plants[6–10]. These observations suggest that responsiveness to very weak radiation may be related in some way to a geophysically correlated variation in metabolic rate. Not only have persistent annual variations in metabolism been established, but a persistent, poly-

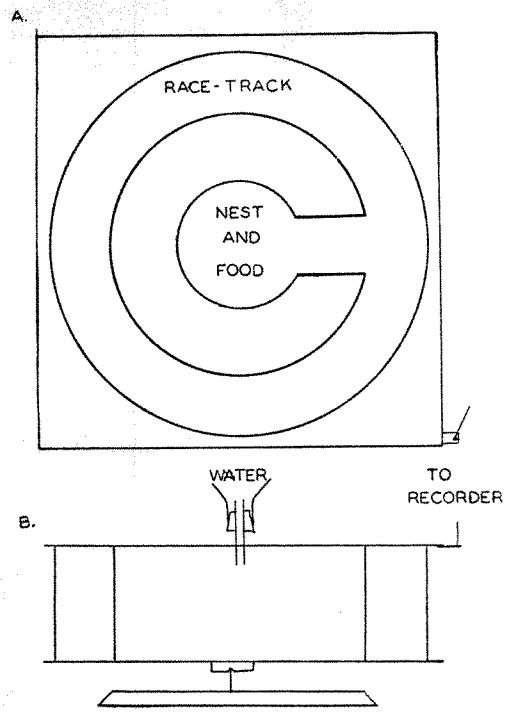


Fig. 1. *A*, Top view of horizontal "race-track" mouse cage. *B*, Side view of same, showing knife-blade pivot and continuous water-supply. For scale dimensions, the outer diameter of the race-track is 8·5 in.

phasic solar-daily pattern of metabolic variation has also been defined, which has a common form in widely different kinds of organism[11].

An experiment was designed to determine: (1) whether small changes in level of radiation have any measurable influence on the overall spontaneity of an organism; (2) whether the characteristic, fundamental 24-h pattern of metabolic variation is reflected in any comparable variation in responsiveness to a very weak radiation field. This article describes and discusses our findings.

The hourly spontaneous activity of four white mice was monitored quantitatively throughout a 3 month period under conditions of alternating periods of one and a half times and about eight times the level of the natural background radiation. To monitor the activity, rocking horizontal "racetrack" actographs were constructed of 0·25 in. screen. The general form of the "track" is shown in Fig. 1. The track was pivoted slightly eccentrically on a knife-edge support so adjusted that there was always tension on a recorder arm irrespective of the location of the mouse in the actograph. Very slight rocking movements produced as the mouse ran around the track were recorded mechanically through a spring-tension system by pen and ink on a moving drum. Two actographs, about 22 in. from centre to centre, were placed on the northern edge of a wooden table (*NE* and *NW*) and the other two were placed the same distance apart on the southern edge (*SE* and *SW*). The two pairs of actographs were separated by about 36 in. The table was situated near the centre of an otherwise unoccupied room illuminated for the duration of the experiment only through windows along the south and west sides. The mice were therefore subjected to day–night changes in illumination and temperature. Food and water were always available in excess. The activity of each mouse for each hour was registered on a 24 h clock driven drum which moved forward 1 cm/h while moving at right angles to the direction of rotation at about 1 cm/day. The latter movement permitted several days of continuous, uninterrupted recording on the same drum. The drum carried millimetre graph paper. In this way the duration of activity was recorded to the nearest 6 min.

A gamma-ray source was prepared which consisted of 24 μc. of caesium 137 deposited on filter paper sandwiched between 'Plexiglass' plates. The activity for all four mice was monitored under conditions in which for alternating periods (usually each of 72 h) the source was placed beneath the table top, first 8 in. below a point centred between the two mice at one end of the table and then below a point centred between the other two mice: when the position of the source was changed, this was done at about 10 a.m. When the γ-ray source was in place it was at an average distance of 13·5 in. from the closest two mice—thus raising the total ambient radiation to about eight times background ("irradiated" or "experimentals")—and at the same time at an average of about 41 in. from the two more distant mice—thus providing an intensity about 1·5 times background ("non-irradiated" or "controls").

A mouse was placed in each of the four actographs on June 18, 1964, and the gamma source was placed at once between the *NE-NW* mice. No data were used until the time of the changeover of the source to the southern end of the table on June 25. This initial period allowed the mice to become accustomed to the apparatus. Thereafter, all mice underwent fourteen periods as controls alternating with the same number of periods as experimentals. Recording finished on September 18.

For each of the twenty-eight periods the average hourly activity was determined for the experimental and control mice. In the course of the experiment one pair of mice acted as a synchronous control for the other pair and a consecutive, alternating control for itself. It was hoped that this method would take into account and compensate for differences in natural spontaneity among the individual four mice. By extending the experiment over a 3 month period, randomization was effected of differences between experimentals and controls which were not synchronous in time; that is, differences arising from variations in daily illumination, temperature, and geophysical phenomena correlated with the weather, time of month, etc. The position of the mice on the table and any differences between the cage recorders were controlled in that all four mice and cage recorders contributed equally to the experimental and control samples.

Each of the four mice showed its own specific mean 24-h pattern of activity for the 3 month period. All, however, showed predominantly nocturnal activity. There was no conspicuous difference between the mean specific detailed patterns for the same individual for the days on which it served as experimental and control. As the variations in environmental temperature and illumination were the same for all four mice, it was clear that the differences between the four mice in their mean daily patterns represented individual peculiarities in response to the same environmental variations. The mean daily pattern of activity for all the control mice is shown in Fig. 2 together with the mean pattern for all the experimental mice. Fig. 3*A* shows the mean daily pattern of

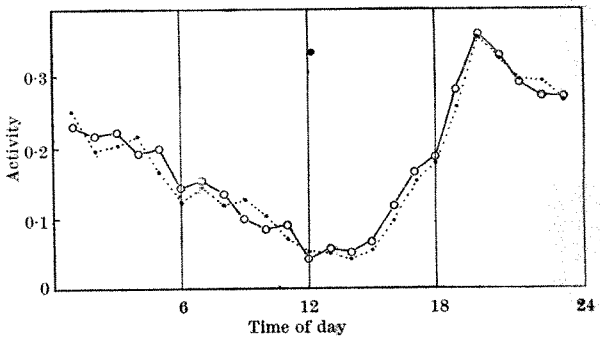


Fig. 2. Mean daily cycle of activity for all four mice during the 3-month investigation while in the higher (broken line) and lower (solid line) gamma fields

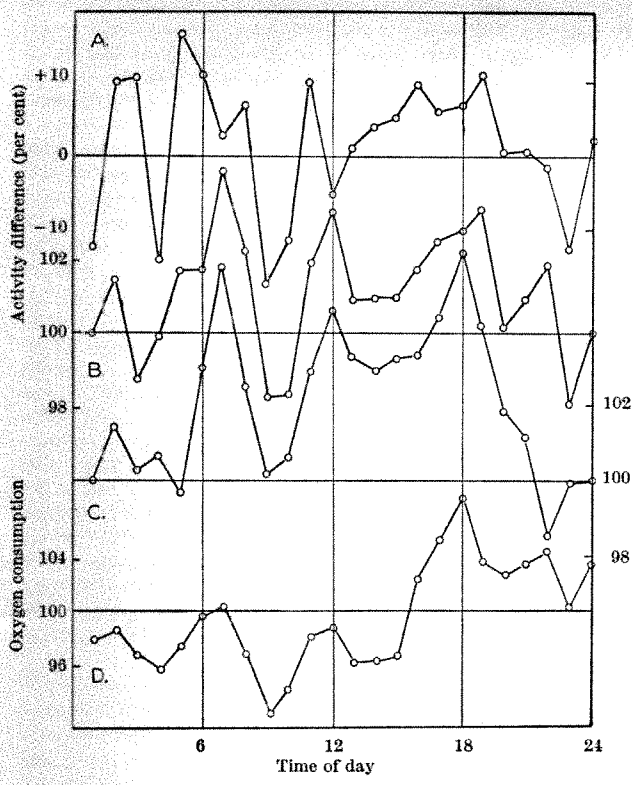


Fig. 3. *A*, Daily variation in activity of the control mice relative to that of mice in the experimentally elevated γ-radiation field compared with oxygen consumption of (*B*) potato, (*C*) carrot and (*D*) mealworm, showing a cycle form which persists in carefully controlled "constant" conditions in widely different kinds of living systems. The variations are expressed as percentage-deviations from either the daily mean rates or of the values from midnight to 1 a.m.

differences between the activity of the controls and experimentals.

The hourly differences are plotted as percentages of the daily mean for all mice. A positive value indicates that the experimental animals were less active than the controls. The mean period of activity per hour was 0·1749 h for the controls and 0·1714 h for the experimentals. Fig. 2 suggests that no statistically significant difference in overall spontaneous activity is likely to exist between the two. The range in the daily variation is very large in comparison with any possible mean influence of the increased radiation. Only further experiments can reveal whether the observed overall 2·0 per cent depression at the higher level of radiation compared with the lower level represents a real, reproducible difference. However, when the mean hourly differences (Fig. 3*A*) were considered a non-parametric χ^2 test showed these to differ from random expectation ($P < 0{\cdot}05$), favouring the side of depression. These hourly differences ranged from an apparent 16·9 per cent depression in activity in response to the higher field relative to the lower one at 5 a.m., to a 17·6 per cent increase at 9 a.m. The distribution of values about zero appeared more like asymmetrical bimodal than a random distribution.

The results of this experiment suggest that the increase or decrease of activity in response to the presence of the higher γ-radiation field depends on the time of day. It is probably not accidental that the apparent daily variation in the response of mice to the higher γ-radiation field should possess a pattern which resembles the persistent, basically polyphasic, daily variation in oxygen consumption which has been reported for many organisms. Examples of such organisms would include potatoes (Fig. 3*B*), beans[7], mealworm larvae[12] (Fig. 3*D*), carrots[11] (Fig. 3*C*), and young chick embryos[13], under constant illumination and at constant temperature. It has been suggested[6,11,14] that this polyphasic daily variation in metabolism is derived as a response of the living system to some mean geophysical cycle or cycles of factors other than those normally controlled in the laboratory.

The 24-h pattern of respiratory variation for potatoes (Fig. 3*B*) is for the 12-month period from February 1, 1964–January 31, 1965, including the period of the present investigation. This same specific daily pattern in the potato has been recorded for 9 consecutive years, with more than a million potato-hours of data. The coefficient of correlation between the consecutive corresponding mean hourly values for the metabolism of potatoes (Fig. 3*B*) and the apparent mouse response to γ-irradiation (Fig. 3*A*) was 0·561 ($t = 3{\cdot}16$; $P < 0{\cdot}005$). The daily variation in mouse response to the weak γ-radiation level is very similar to the geophysically-dependent variation in potato metabolism. Much of the remaining difference between these two daily patterns appears to be a consequence of unexplained variations about a common general cycle-form rather than about zero. This is suggested by the greatly increased similarity which at once becomes evident when 3-h moving means for the two are compared (Fig. 4*A*).

Some earlier results[16] from experiments with rats offered an opportunity to determine whether a deep-seated 24-h variation underlies the circadian rhythm. The mean activity for each clock hour of the day had been determined for a male white rate using hourly data from 58 consecutive days. This specific duration of the experiment was suitable for analysis because it allowed at one and the same time the randomization of a large-amplitude, rather regular, mean 25·25-h circadian cycle which scanned the solar day almost exactly three times and a mean lunar-day variation of rather large amplitude which scanned the day almost exactly twice. The residual 24-h variation, converted as were the mouse data to a 3-h moving mean of percentage deviations from mean hourly activity, is shown in Fig. 4*B*. A general similarity with the cycles of Fig. 4*A* is evident and the cycle shows the maximum at 6–7 a.m. and the broad evening maximum.

Rats and mice, unlike potatoes, beans, carrots, mealworm larvae, and young chick embryos, are organisms

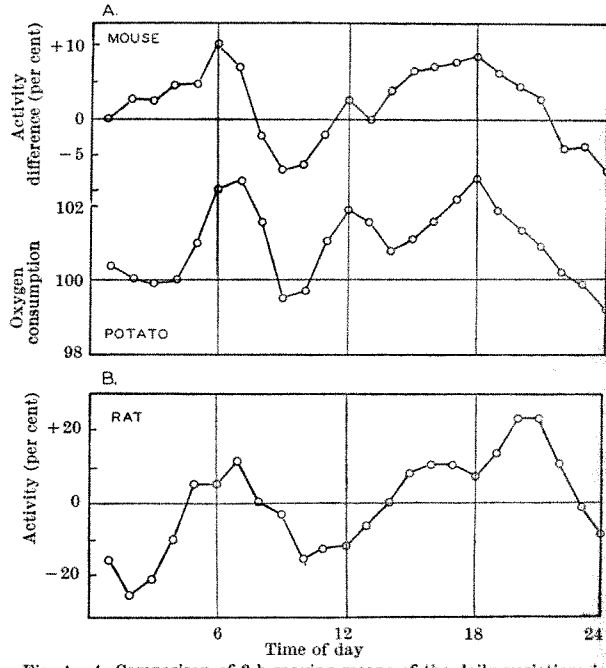


Fig. 4. *A*, Comparison of 3-h moving means of the daily variations in apparent response of the mice to the increased radiation and oxygen consumption in potatoes investigated during the same year. The two relationships were based on more than 4,000 mouse-hours and more than 100,000 potato-hours, respectively. *B*, Mean solar-day variation found to coexist with an overt 25·25 h circadian and a lunar-day variation in a male rat (ref. 16) during 1,392 consecutive hours of activity monitoring in constant low illumination. This was exposed as a residual variation after complete randomization of the other two components

with a conspicuous overt circadian periodicity. Although Fig. 4*A* strongly suggests that a 24-h component of some geophysical activity underlies the observed 24-h circadian variation, Fig. 4*B* suggests that this type of 24-h component underlies a circadian periodism even while, under conditions of constant illumination and temperature, its overt cycles regularly deviate from the 24-h length.

The fact that mice show mean deviations (Fig. 3*A*) for specific hours of the day ranging rather sharply from a stimulation of more than 15 per cent to a depression of the same order, together with the rather nice mean balance between the two over the course of a 24-h period, suggests that the overall relative stability observed under different levels of radiation exists only within the framework of an orderly mean 24-h pattern of variation in responsiveness. As with many other otherwise well regulated physiological phenomena, the overall constancy is invaded by a 24-h variation.

There appears to be a good correlation between the fundamental extrinsically derived, polyphasic 24-h variation in the consumption of oxygen and the response to very weak γ-radiation field, but the results in Fig. 2 show no comparable relationship between the large circadian variation in activity and the response to radiation, despite the far larger range of overall metabolic variation which must be involved. This suggests that variations in other still uncontrolled environmental factors rather than metabolic rate as such are determining the strength and direction of the influence of a very weak field of γ-radiation on the mice. Just as there seem to be specific times of day when a substantial response of one or another sign is present, so conditions may be found naturally, or be created experimentally, in which overall mean influence of small alterations in the radiation fields can be observed even with organisms such as mice.

It seems reasonable to presume that the general polyphasic pattern of daily variation in oxygen consumption found in several widely diverse animals and plants reflects a corresponding pattern of sensitivity to the ambient natural radiation level. This seems especially probable since both field strengths used here lie well within the range of intensities naturally occurring over the Earth's surface.

That sensitivity to background radiation contributes to the temporal organization of the living organism is suggested by the finding that background radiation shows a characteristic mean daily variation with a range of the order of 2 per cent. A statistically significant correlation continuing over several months has been reported between the day-to-day deviations from the mean daily cycle form of oxygen consumption in potatoes with the comparable deviations in the level of background ionizing radiation[15]. It was not established whether the correlation reflected a direct response to the radiation.

Perhaps it is equally significant that a mammal, here the mouse, has a more basic physiological 24-h variation underlying its circadian rhythmic system (Figs. 3*A* and 4*A*). The specific pattern of this variation seems to be shared with a very diverse selection of other animals and plants. It is possible that this variation or a related variation may exist for all terrestrial organisms.

The general similarity of the mean 24-h oscillatory pattern whether measured with actographs or respirometers in organisms with or without overt circadian rhythms points to the existence of a widespread, if not universal, deep-seated solar-day biological variation. Its mean accuracy is evident from its apparent phase-locked relationship to the hour-angle of the Sun. The persistence of this same specific relationship, indicated by the maxima at 6–7 a.m. and in the late afternoon, whether the ambient temperatures are those of a laboratory room or of an arbitrarily set constant-temperature water-bath implies complete independence of temperature. Such a variation could be one of the long-sought temperature- and drug-independent timing oscillations for the biological clocks.

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NEPHRECTOMY-POTENTIATED TISSUE CHANGES WITH PARTICULAR REFERENCE TO GLOMERULONEPHRITIS

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During the course of investigations of renal hyperplasia induced by excision of a kidney, it was noted that rats which had been given anti-normal rat serum rabbit serum (NRSRS) followed by unilateral nephrectomy, showed marked histopathological changes in the kidney which remained. The changes found were: marked swelling of capillary tufts with obliteration of Bowman's space, without evidence of inflammatory cell invasion; marked cortical degeneration; cerebral oedema; after 4 days, mononuclear cell infiltration of the bronchial perivascular areas. This article reports the findings of a series of experiments which extend and confirm these original observations.

Sprague–Dawley rats, each of about 200 g, were fed a standard rat diet and individually caged. The animals were randomly divided into groups as shown in Table 1.

The NRSRS was produced by intravenous injection of rabbits with three 0·1 ml. portions of Sprague–Dawley rat serum per week for 2 weeks. Four days after the final injection, the rabbits were bled by sterile cardiac puncture and the serum tested for anti-rat serum precipitins using a qualitative test. The sera were positive when tested with an antigen dilution (rat serum) of 1 : 10,000.

Table 1. Treatment Groups

(1) Rats receiving 3 ml. normal rabbit serum.
(2) Rats receiving 3 ml. normal rabbit serum and subjected to unilateral nephrectomy.
(3) Rats subjected to unilateral nephrectomy.
(4) Rats receiving 3 ml. of anti-normal rat serum rabbit serum.
(5) Rats receiving 6 ml. of anti-normal rat serum rabbit serum.
(6) Rats subjected to sham operation only.
(7) Rats receiving 3 ml. of anti-normal rat serum rabbit serum and also subjected to sham operation.
(8) Rats receiving 3 ml. of anti-normal rat serum rabbit serum and subjected to unilateral nephrectomy.

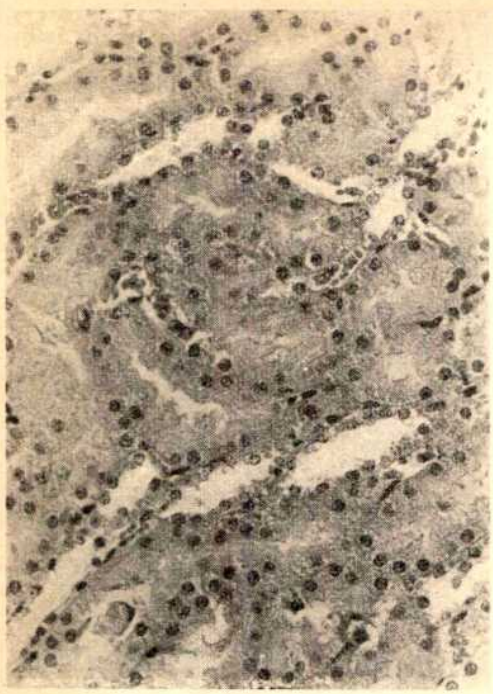


Fig. 1. Section of kidney 24 h after the final serum injection following nephrectomy. Note the cortical tubular degeneration present at this time. (Haematoxylin and eosin, × 220)



Fig. 2. Section of kidney 24 h after the final serum injection. Note the marked degeneration and necrosis of the cortical tubules. (Haematoxylin and eosin, × 50)

Twenty-four and two hours before nephrectomy, each rat received intraperitoneally 1·0 ml. of NRSRS. Under nitrous oxide–ether anaesthesia, the left kidney was removed through a mid-line abdominal incision. The renal vessels were carefully ligated to avoid excess bleeding and the abdominal wall was sutured in layers. Twelve hours after nephrectomy, a third and final injection of 1·0 ml. of NRSRS was given intraperitoneally. Control animals were on a similar time schedule. The rats were killed by decapitation at various time intervals after operation and kidneys, lungs and brains were removed. The tissues were fixed in neutral formalin. Paraffin sections 3–4μ thick were prepared and stained with haematoxylin and eosin, periodic acid–Schiff stain (McManus PAS technique) and the Heidenhain–Azan stain. Kidneys removed at operation were treated in a similar fashion.

The twelve animals that were given NRSRS and nephrectomized showed the following changes: in kidneys removed 12 h after nephrectomy no glomerular changes were noted, although appreciable tubular degeneration was present. The tubular cells were pale, many of the nuclei indistinct; the cell boundaries were often unclear. Twenty-four hours after kidney excision, the capillary tufts were oedematous, but little or no evidence of inflammatory cells could be discerned. There was considerable degeneration of the cortical tubules (Fig. 1), and the cortical vessels were distended. Cortical changes reached a maximum at 48 h. At this time many tubular cells had undergone marked degeneration and necrosis (Fig. 2). The areas affected were pale and cells in such regions had lost their outlines. In some cells, the nuclei were shrunken. At 4 days the cortex began to assume a more normal appearance; the epithelial cells had a recognizable outline and the congestion was reduced. The changes observed in this series are similar to those originally found, except that the glomerular changes tended to be less accentuated. Capillary swelling and mural oedema in afferent arterioles were also observed (Fig. 3). At 24 h, congestion and oedema were also noted in the brain; the perivascular and perineural spaces were widened and the capillaries were dilated (Fig. 4). After 3 days, focal perivascular and interstitial collections of large mononuclear cells, some of which were plasma cells, were noted in the lung associated with the larger pulmonary vessels (Fig. 5). It must be emphasized, however, that all these changes were only observed in rats which had received the serum and undergone nephrectomy. Control animals that had been inoculated only with NRSRS, normal rat serum only, unilaterally nephrectomized only, sham operated only, given twice the amount of NRSRS only, or given normal rabbit serum and

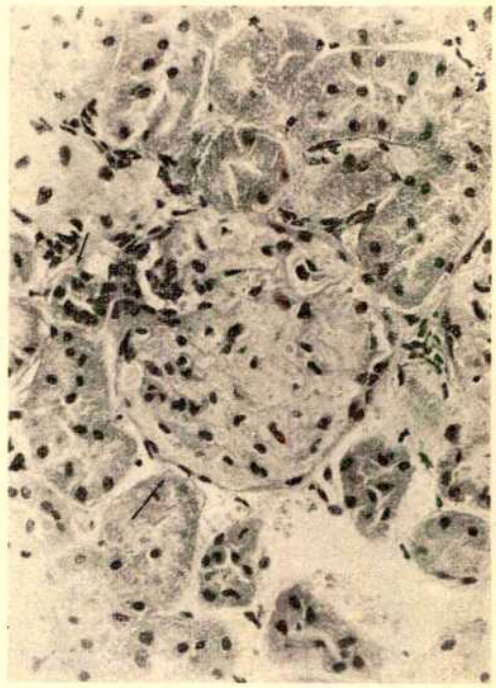


Fig. 3. Section of kidney 24 h after the final serum injection following nephrectomy. Observe the marked swelling of the vascular endothelium of the capillary tuft and afferent arteriole. (Haematoxylin and eosin, × 220)

subjected to unilateral nephrectomy, did not reveal any signs of tissue pathology. It was only when the NRSRS was combined with unilateral nephrectomy that histopathological changes appeared.

In order to substantiate further the histological observations described here, the experiments were repeated and, in addition, an intravenous injection of sterile Evans blue solution was used before nephrectomy. Three millilitres of sterile 2·5 per cent Evans blue in saline were injected into the tail vein of each animal at the time of the second injection of antiserum. The animals were killed after 12, 24, 48, 72 h, 4 and 5 days and the tissues observed grossly. Only in those rats subjected to unilateral nephrectomy and NRSRS were changes in vascular permeability found. At 24 h to 4 days the Evans blue dye was observed at the renal corticomedullary junction; staining was intense at 24 h but tending to be reduced at later periods. No dye was observed at 12 h or at 5 days. In addition, in three of the animals the dye was noted in the sclera and in the tracheal cartilages. We wish to emphasize that rats under other treatment régimes exhibited no extravasation of dye. The results are summarized in Table 2.

These results indicate that only the combination of nephrectomy and antiserum initiates these vascular changes. It may also be pointed out that the histopathological changes in the kidney closely parallel the transudative changes observed with the Evans blue technique.

From the results obtained we can assume that the occurrence of the pathological changes described arises from the simultaneous presence of two factors, one contributed by the injection of antibody directed against the normal host component and the other contributed by the unilateral nephrectomy. Although it has not yet been shown, it is reasonable to assume that the injection of anti-NRSRS results in the production of circulating antigen–antibody complexes, which are probably soluble, as such complexes could be assumed to be in antigen excess. (Since this article was originally prepared, we have obtained essentially identical results using washed rat serum-NRSRS precipitate as a substitute for the NRSRS.) The presence of these complexes by themselves

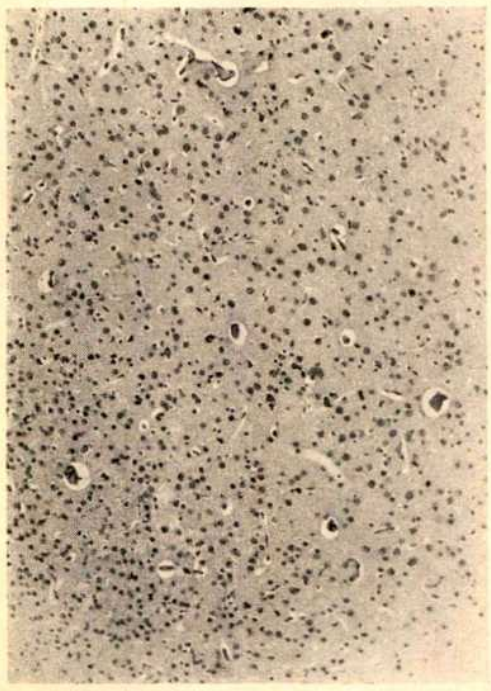


Fig. 4. Section of cerebral cortex 24 h after the final serum injection following nephrectomy. The widening of the perivascular and perineuronal spaces is illustrated. (Haematoxylin and eosin, × 50)

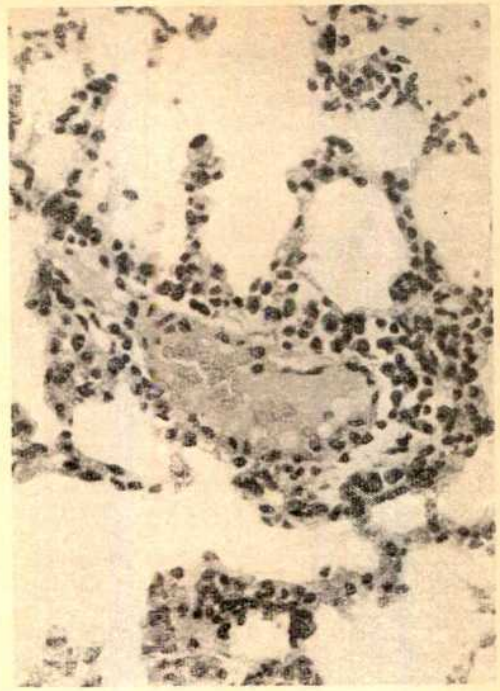


Fig. 5. Section of lung 4 days after the final serum injection following nephrectomy. A collection of mononuclear cells around a vessel is shown. (Haematoxylin and eosin, × 50)

is not sufficiently active to produce histopathological changes. The effect of the second factor may result from unilateral nephrectomy, for this procedure has been shown to have a direct effect on the production of hyperplasia in the contralateral kidney[1,2], and serum from a nephrectomized animal may be passively transferred and exert a hyperplastic effect[3]. Argyris and Trimble[4] have demonstrated that damage to the mouse kidney, such as by multiple needle insertion, results in the appearance of increased mitotic activity in the contralateral organ—which is another indication of humoral transfer.

The result of nephrectomy cannot be considered to be merely mechanical, for if the effect of nephrectomy were merely to change the ratio of kidney surface to circulating fluids, then it might be assumed that twice the concentration of serum antibodies should have the same effect on a non-nephrectomized animal. This was shown not to occur.

It may be postulated that the presence of these two factors in the host initiates a series of activities which produces changes in the vascular permeability which then

Table 2. EFFECT ON RAT OF UNILATERAL NEPHRECTOMY COMBINED WITH ADMINISTRATION OF ANTI-NORMAL RAT SERUM RABBIT SERUM

Treatment of rat	Histopathology noted in contralateral kidney	Changes noted in vascular permeability (Evans blue technique)
Normal rabbit serum 3 × 1 ml.	None	None
Normal rabbit serum plus nephrectomy	None	None
Sham operation	None	None
Sham operation anti-normal rat serum rabbit serum	None	Not done
Anti-normal rat serum rabbit serum 3 × 1 ml.	None	None
Anti-normal rat serum rabbit serum 3 × 2 ml.	None	Not done
Unilateral nephrectomy	None	None
Anti-normal rat serum rabbit serum 3 × 1 ml. plus unilateral nephrectomy	Kidney tubular degeneration glomerular swelling, capillary dilatation: cerebral oedema and mononuclear infiltration of lung	Dye noted in corticomedullary junction and medulla of sclera of 3/12 rats

results in histological renal alterations interpreted as glomerulonephritis.

These results also indicate that the effects of the changes are not localized to the kidney. It may well be that the action is on the vascular system in other areas of the body such that it changes the permeability of the vascular bed, and thus allows transudation to occur in many areas. An illustration of this may be the marked capillary dilatation and oedematous reaction in the brain. We believe the reaction in the lung is secondary and that the focal collection of large mononuclear cells may have a phagocytic action. It is noteworthy that the cells were not observed until three to four days after injection, well after the period of time when renal changes were noted.

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TRANSFER OF METABOLIC ENERGY TO SODIUM

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MANY living membranes can bring about a net flow of sodium from a dilute solution on one surface to a more concentrated solution on the other surface. Somewhere within this membrane the activity, or energy, of sodium must be reversibly raised to exceed that of the recipient solution in order that sodium will flow against the overall gradient.

It is not possible rigidly to quantify the activity or any other parameter of the energy of an individual ionic species. However, measurements can be made that will give comparative values in solutions that are nearly alike. Errors are lessened when estimates by one method are confirmed by a second method using different assumptions. *p*H measurement is an example of this type of measurement, and ΔpH is an estimate of a difference in hydrogen ion activity, a parameter of energy. In the course of an investigation of an analogous concept for sodium[1], observations on one series of salts stand out as unique in their implications, although not unique in the pattern for sodium salts. Because adenosine triphosphate (ATP) participates in sodium metabolism, its relationship to relative sodium activity deserves special mention.

ATP is a substituted tripolyphosphate with the fifth valence involved as an ester to ribose. Thus sodium in Na_4ATP may be expected to resemble sodium in salts of the sodium tripolyphosphate family. Sodium ATP differs from sodium pyrophosphate in that the pK_4 of sodium ATP is near *p*H 7·0 (*pK* is a function of ionic strength and of the specific cation). In essential metabolism ATP hydrolyses to the diphosphate (ADP), a substituted pyrophosphate, with the liberation of about 9,000 cal/mol. The ADP anion has a maximum charge of −3, and its 0·1 N sodium salt has a pK_3 at *p*H 6·51, compared with *p*H 6·68 for 0·1 N $Na_{3\cdot5}$ ATP. The terminal phosphorus which is split off appears as orthophosphate, either free, as an ester, or as an amide.

The activity per unit concentration or activity coefficient for sodium (γ^+_{Na}) may be expected to follow roughly an expression of the Debye–Hückel equation, for example,

$$\text{Log } \gamma^+_{Na} = -\frac{0{\cdot}523\sqrt{\mu}}{1+\sqrt{\mu}} \text{ at } 37^\circ \text{ C}$$

where μ is the ionic strength. For sodium salts at the same normality, μ increases with anion valence in the relationship $\mu = \frac{1}{2}$ (1 + anion valence) × normality. Hydrolysis of ATP^{-4} or of $ATP^{-3\cdot5}$ to anions of lower valence without a change in normality also results in a lowering of ionic strength. Sodium activity would be expected to rise.

Table 1. PERCENTAGE OF SODIUM BOUND AS ESTIMATED FROM CONDUCTANCE AND TRANSFERENCE

(*a*) ATP family

Normality	$Na_4P_2O_7$	Na_4ATP	$Na_{3\cdot5}ATP$	Na_3ADP	$Na_{2\cdot5}ADP$	NaH_2PO_4
0·2	29·8					
0·1	24·9	22·8	19·5	15·0	12·3	2·2
0·03	18·7	16·8	13·6	10·6	8·5	1·1
0·01	12·3	10·6	11·9	7·1	5·2	0·5
0·003	8·9	7·9	6·9	5·4	2·6	–

(*b*) Mixtures and complexes

Normality	$Na_{3\cdot5}$(ADP + P)	Na_2MgATP	NaMgADP
0·1	12·1	12·8	8·0
0·03	9·0	6·8	

Estimates of γ^+_{Na} in this series of salts using a sodium-sensitive glass electrode and a reference electrode with liquid junction[2] are shown in Fig. 1. It was assumed that $\gamma^+ = \gamma^- = \gamma^\pm$ in sodium chloride. The glass electrode was checked against a sodium amalgam electrode to eliminate the possibility of an artefact of the glass resulting from anion sensitivity. The curves for log γ^+_{Na} fall progressively below the Debye–Hückel limiting slope (*S*) in the order of increasing anion valence.

This separation of curves is due to ion binding which increases with anion charge. The ion binding is similar to that reported by Wall and Doremus[3] for the non-esterified pyrophosphate and tripolyphosphate. Application of their method[4], involving conductance and transference, gave the results shown in Table 1. The bound fraction is almost a linear function of anion charge at any normality, and is roughly a parabolic function of the square root of the normality at any valence. The data may be approximated by the empirical set of parabolas $B = 12{\cdot}5\ (V-1)\ N^{\frac{1}{2}}$ in which B is the bound fraction, V the numerical value of the anion valence and N the normality. By potentiometry and by conductance-

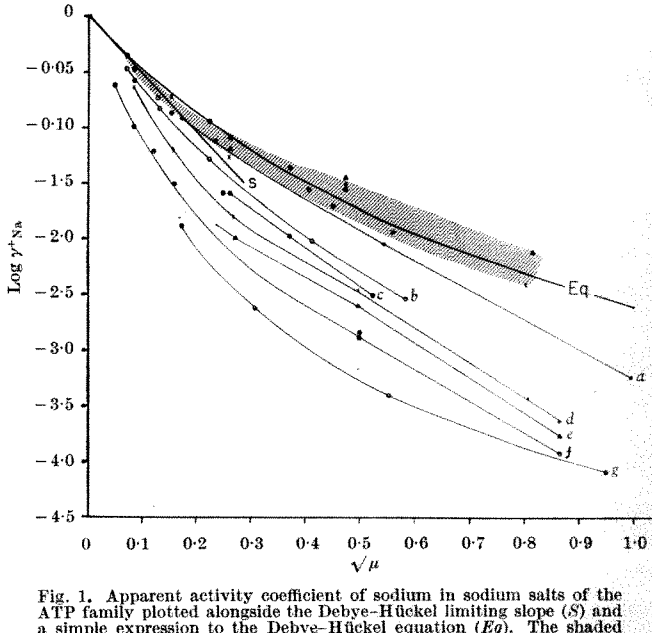


Fig. 1. Apparent activity coefficient of sodium in sodium salts of the ATP family plotted alongside the Debye–Hückel limiting slope (*S*) and a simple expression to the Debye–Hückel equation (*Eq*). The shaded area contains points from the same potentiometric data recalculated for the free fraction of sodium by conductance-transference or extrapolated by the equation for the bound fraction. *a*, NaH_2PO_4; *b*, $Na_{2\cdot5}ADP$; *c*, Na_3ADP; *d*, $Na_{3\cdot5}ATP$; *e*, Na_4ATP; *f*, $Na_4P_2O_7$; *g*, $Na_5P_3O_{10}$

transference, more sodium is bound by $P_2O_7^{-4}$ than by ATP^{-4}, perhaps because of the more concentrated charge of the $P_2O_7^{-4}$. (The data for non-esterified pyrophosphate may be fitted to the surface generated by arbitrarily assigning an extra half charge.) Recalculation of γ^+_{Na} based on the concentration of unbound sodium results in mapping of the curves into the shaded area in Fig. 1. This area contains the curve (*Eq*) of the Debye–Hückel equation given above and approaches the Debye–Hückel limiting slope (*S*) from above as expected for fully dissociated salts. Thus the magnitudes of the bound fractions as estimated by two methods are comparable, and relative values for γ^+_{Na} are rational. These data show that hydrolysis of ATP will release a significant portion of the bound sodium fraction.

The partial stoichiometric equation for the reaction involving the 3·5 salt may be written,

$$Na_{3\cdot5}ATP \rightleftharpoons Na_{3\cdot5}\,(ADP + P)$$

In Fig. 2, estimates of γ^+_{Na} of the left and right sides of the equation are plotted against the molarity of the sodium because that is a fixed quantity. Separation of these curves includes both the effect of a change in μ and a change in the bound fraction. At 0·1 N the rise in sodium activity is about 22 per cent, which would require about 110 cal/mol. This may be regarded as a transfer to sodium of a fraction of the energy liberated in hydrolysis.

Enzymatic hydrolysis probably involves the stable mono-magnesium complex. The corresponding salts are Na_2MgATP and NaMgADP, which are essentially 1:2 and 1:1 salts. The same mechanism holds. Estimates of γ^+_{Na} for these salts and for an equimolar mixture of NaMgADP with NaH_2PO_4 (the hydrolysis products) are shown in Fig. 3. Estimates of bound sodium are again given in Table 1.

This relationship uniquely fits the notion of the sodium pump. At a given temperature and normality, γ^+_{Na} may be affected only by a change in the medium or a change in anion distribution. The constant (0·523) in the Debye–Hückel equation includes the dielectric constant of the medium. It has been found that to change sodium activity reversibly by altering the medium, by adding uncharged or dipolar substances, requires large additions because the solvent water is 55 molar. A change by altering the distribution of anion charges is theoretically more suitable for the task. Hydrolysis of ATP is the most prominent instance of such change. (The addition of an equal number of moles of a strong monobasic acid to $Na_{3\cdot5}ATP$ would result in a similar reduction in ionic strength and in ion binding, but would result in a much lower *p*H. The pK_3 of sodium ATP is about *p*H 4·4 compared with the value of *p*H 6·2 on the right side of the equation.) The enzyme ATPase is present in cell membranes[5], and the hydrolysis of ATP is believed to occur there. This

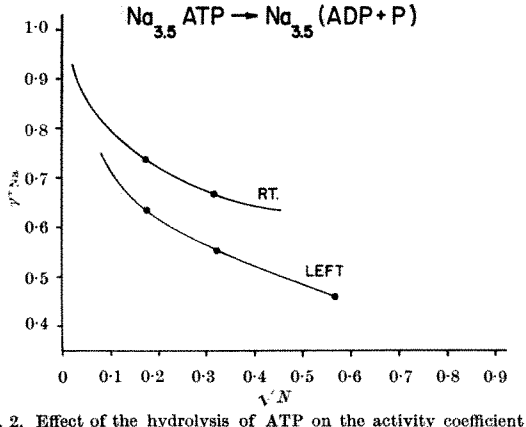


Fig. 2. Effect of the hydrolysis of ATP on the activity coefficient for sodium. The abscissa is the square root of the normality so as to include the effect of a change in ionic strength

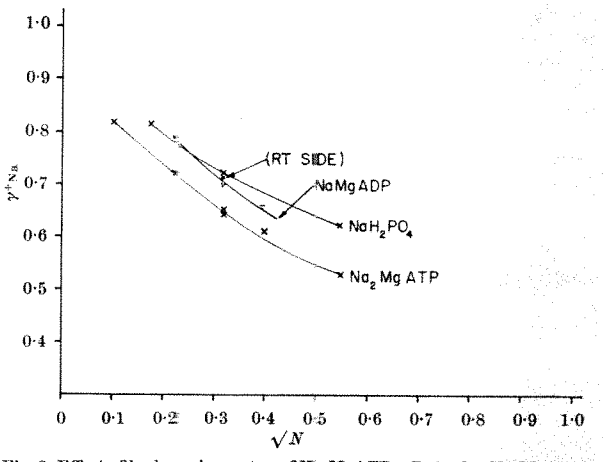


Fig. 3. Effect of hydrolysis on γ^+_{Na} of Na_2MgATP. Data for Na_2MgATP, NaMgADP, NaH_2PO_4 and Na_2(MgADP + P) are plotted against the square root of the normality to include the effect of ionic strength

enzymatic hydrolysis of ATP is affected by digitalis and by other agents which affect the transport of sodium across the membrane *in vivo*. Thus the rise in sodium activity described provides the most reasonable explanation for the rise in ion activity which is necessary to pump sodium against a gradient.

The transfer of energy to sodium is obligatory. The quantitation in a water medium is only approximate. Teleologically, the reaction appears wasteful. In a medium of lower dielectric constant, perhaps a cell membrane, the rise in γ^+_{Na} should be greater. Cation selectivity is unlikely unless it is conferred by the medium or by an intermediate. However, directional flow requires a gating device. Large gradients could also be created by gating between stages, for example, by means of the less polar end of the ATP molecule as it is oriented on the enzyme. (In this instance these anions would not move at random. Nevertheless the effect on sodium would be similar because γ^+_{Na} is still a function of the nearness of anion sites when these sites are fixed to a structural matrix[6]. The inorganic phosphorus or some intermediate would be free of the structure.)

There are other consequences. If sodium is the only permeable cation present, the Donnan potential[6] across a semi-permeable membrane is almost equal to $K\,\Delta\log Na$ activity. This is the nature of the electrode data presented. A rise in γ^+_{Na} and the accompanying rise in hydrogen ion activity necessarily affect the potential across a living membrane which is permeable to both species. Should this rise be a periodic or a triggered function, changes in membrane potential such as the electrocardiogram and the transmitted impulse would then be generated. Large electromotive forces, even that of the electric eel, could be created by a series of cells, each deriving electrical energy from this hydrolysis.

To sum up, by potentiometry, conductance and transference sodium activity rises with the exothermic hydrolysis of sodium adenosine triphosphate, or its mono-magnesium salt, because ionic strength and ion association are both lowered. This hydrolysis is associated with *in vivo* sodium transport against a gradient. The rise in sodium activity suggests a sodium pump analogous to the heat pump.

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LETTERS TO THE EDITOR

ASTRONOMY

Correlation between Peculiar Galaxies and Radio Sources

ARP has claimed[1] that more peculiar galaxies than can be statistically expected lie about midway along lines joining close pairs of radio sources. We have investigated the significance of these claims and, although we have found that the observed distribution might occur by chance in only one sky out of a hundred, we nevertheless believe that much of the correlation is caused by (i) observational selection; (ii) non-uniform large-scale distribution of galaxies and radio sources; (iii) normal clustering of galaxies, radio and peculiar included. The importance of the last two points is emphasized by the fact that bright radio sources occur preferentially in regions of the sky with a high density of galaxies[2].

We investigated the correlation between two classes of objects, population A (usually a class of peculiar galaxy) and population B (usually $3C$ radio sources) by plotting them on a sphere. The angular distances from each member of A to the two nearest members of B were measured. For purposes of comparison, a control population was constructed by moving population A one hour back in R.A. and the measurements were then repeated. Members of A either in or moving into the galactic zone of avoidance, $|b^{II}| < 15°$, were omitted and the survey was limited to the northern hemisphere. The positions of the objects were taken from the catalogues[3–5].

On the basis of Arp's claims we defined a "pair" to have an A to B separation less than 2·2° and a "triplet" to have an A to second-nearest B separation less than 4·5°. χ^2 tests were conducted on four-fold tables of real, comparison (control), "paired" and "non-paired", and Student's t tests on the mean of the distances from each A to the nearest B. These tested whether the number of real pairs was significantly different from the number of pairs in the control. When an entry in the table was less than 10, the χ^2 test was abandoned in favour of an exact probability test. To test for collinearity of the triplets, the internal angle θ between lines joining the peculiar galaxy to the two nearest radio sources was measured. For the most compact triplets a Student's t test was conducted on the means of $\theta°$ and $|90–\theta°|$ to test for significant deviations from 90° and 45° respectively. None was found.

In Table 1, the sixth column gives the probability from the χ^2 test that a correlation greater than or equal to that obtained is due to chance. An attempt to allow for effect (ii) altered this probability to that given in the tenth column.

The correction for effect (ii) was made by recalculating χ^2 with allowance for the hourly distributions in R.A. of both populations, for the real, and control results. The null hypothesis was made that the number of pairs in each hour R.A. is proportional to the product of the numbers of populations A and B in that hour. The numbers of paired and non-paired members of population A expected on this basis were then used to evaluate the corrected χ^2. These so derived give much less significant probabilities. Similar corrections produced similar results for Student's t-test.

In discussing the results it should be stated that we are not convinced that we have removed all effects arising from (ii) and have made no allowance for (i) and (iii); borderline significance (2–5 per cent) is thus not convincing.

The correlation between peculiar galaxies numbered 102–145 in Arp's catalogue and quasars is based on small numbers and is sensitive to the precise separation used to define a pair. There is a marginally significant correlation between all Arp's peculiar galaxies and radio sources, and a more significant one between Arp's special galaxies (Nos. 102–145) and radio sources (about the 1 per cent level). Unfortunately, we were unable to investigate the normal clustering effects fully but, from the photographs in Arp's catalogue, it appears that Arp's specials are more often in clusters than members of his other groups.

In conclusion (i) the evidence for correlation or non-correlation between quasars and peculiar galaxies is inconclusive; (ii) there is no evidence that triplets are approximately collinear; (iii) evidence for real correlation between peculiar galaxies and radio sources is weak. We will have over-estimated the significance of the deviation from chance by neglecting the internal clustering of each species, for example, peculiar galaxies are clustered; thus the effective population is smaller and the value of deduced χ^2 should be decreased.

Table 1

Population A	Nos. A	Population B	Nos. B	χ^2	Prob. (per cent)	Test 2	Prob. (per cent)	Correction for effect (ii)	Prob. (per cent)		
Quasars	78	*VV*1	83							Anti-correlated insign	*
Quasars	78	*VV*2	69	0·61	40·0					Insignificant	*
Quasars	78	3*CN*	12							Anti-correlated insign	*
Quasars	78	Quasars	78							Anti-correlated insign	*
Quasars	78	3*C* galaxies	103	0·7	40·0					Insignificant	*
Arp 102–45	33	3*C* (pairs)	250	2·46	15·0					Insignificant	
Arp 102–45	33	3*C* (triplets)	250	7·8	< 1·0					Significant	
Arp 102–45	33	Ditto *OQ*	180	–	–	Exact probability	0·8			Significant	*
All Arp	250	3*C* (pairs)	250	7·55	< 1·0			χ^2 Corrected	3·0	Borderline significance	
All Arp	250	Ditto *OQ*	173	6·1	1·0					Borderline significance	
All Arp	250	3*C* (triplets)	250	7·81	< 1·0					Borderline significance	
All Arp	250	Ditto *OQ*	173	9·8	0·1			χ^2 Corrected	2·0	Borderline significance	
Quasars	60	All Arp	250	2·46	15·0	Student's t	3·0	"t" Corrected	25·0	Insignificant	
Quasars	60	Arp 102–45	33	7·1	< 1·0	Exact probability	1·4			Unreliable	*
All Arp	250	Arp (pairs)	250	23·0	≪0·1			χ^2 Corrected)	1·5	Significant	†
All Arp	250	Arp (triplets)	250	21·4	≪0·1					Significant	†
3*C* galaxies	98	3*C* galaxies	98	2·2	15·0	Student's t	2·0	"t" Corrected	10·0	Anti-correlated insign	†*
	19 closest triplets (including quasars)					Student's t on $\theta°$	25·0	Student's t on $90–\theta$	50·0	Insignificant	
	27 closest triplets (omitting quasars)					Student's t on $\theta°$	90·0	Student's t on $90–\theta$	30·0	Insignificant	

*VV*1, Vorontsov-Velyaminov Tables 11–16 inclusive; *VV*2, Vorontsov-Velyaminov Tables 5–8 inclusive; *OQ*, omitting quasars; 3*C*, radio sources in ref. 5 (all, unless qualified); 3*CN*, 3*C* "*N*" type galaxies only.

* Pairs defined to have an angular separation less than 4·5° (so chosen in order to obtain sufficient numbers for tests).

† There is an automatic bias in our method when populations A and B are identical.

If there is a surviving correlation, a possible interpretation would be that clusters containing an Arp special galaxy are more likely to be of such an age or density that radio outbursts are more frequent than in average clusters. Some of the triplets are indeed in the directions of prominent clusters of galaxies. We thank Dr. Arp for his comments on this paper.

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PHYSICS

Far Infra-red Spectroscopy at High Pressures

PRESSURES sufficiently high to give significant distortion to crystal lattices or dense amorphous systems have been used in combination with spectroscopic observation for some years. The efforts of Drickamer *et al.*[1] have been conspicuously successful in this field but their spectroscopy has been in the ultra-violet, visible and near infra-red regions where the primary transitions are electronic. We have developed a new technique in which very high pressures are combined with far infra-red spectroscopy where absorption by lattice modes dominates, providing useful information about intermolecular interactions. Wilkinson and Sherman[2] have made a beginning in this field by making studies on alkali halides used as the pressure transmitting media in Drickamer's techniques. However, in this article we describe methods which are of general purpose in the sense that the transmission of the system allows features at any far infra-red wavelength to be studied. The new technique is complementary to X-ray studies under pressure in that information can be obtained about the changes of intermolecular forces with changing lattice parameters and crystal structure.

The main experimental problem in this research is to reconcile the conflicting requirements of high radiation transmission through the sample and maintenance of adequate mechanical strength to support the stresses used to create the pressures. The high radiation efficiency of interferometric spectroscopy is turned to advantage to obtain a workable solution. Two types of high pressure apparatus have been used—a six anvil cube type capable of containing pressures up to 35 kbars and a hydraulic type usable up to 12 kbars.

To demonstrate the effectiveness of the new techniques we have examined the effect of pressure on chlorobenzene. The reason for the choice is found in the results of some earlier far infra-red work where it was shown that liquid chlorobenzene has a well-marked isolated band which can be attributed to an intermolecular vibration[3]. The absorption resulting from this might well be expected to be strongly affected by changing the distances between molecules as is indeed the case. In addition to the study of a liquid derived from a dipolar molecule we have studied liquid carbon disulphide the constituent molecules of which have a large quadrupole moment.

Cube anvil device. This is an adaptation of the National Bureau of Standards (Washington) "six anvil high pressure apparatus"[4]. Two opposite anvils have $f/10$ apertures terminating in 0·1 in. diameter holes at the square faces. The latter are sealed by single crystal quartz disks with the optic axis perpendicular to the plane faces. This particular arrangement was found to give the greatest strength. The quartz windows were transparent at frequencies lower than 200 cm^{-1} except for an absorption band at 125 cm^{-1} The transmitted radiation was analysed by a Michelson interferometer[5]. With an acceptable signal to noise ratio, spectra from 10–200 cm^{-1} with resolution 4 cm^{-1} were obtained. Direct absolute intensity measurements could not be made in this device with the same precision as those in the hydraulic device because of variation in the movement of the anvils with increasing load. Reasonable corrections can be made, however, where thick samples and thin windows are used.

Absorption experiments were carried out under pressure on liquid and solid forms of chlorobenzene and carbon disulphide. The high pressure cell was constructed by enclosing the liquids inside a thin walled aluminium cylinder which was sealed at each end by the quartz windows, sample thicknesses being of the order of 0·5–1·5 mm. Pressure calibrations are made by a small manganin wire resistance gauge and by the resistance discontinuity in bismuth at 25·7 kbars. In order to study different solid phases in chlorobenzene a means of producing temperatures greater than 200° C was necessary. A graphite sleeve heater was incorporated, therefore, in the

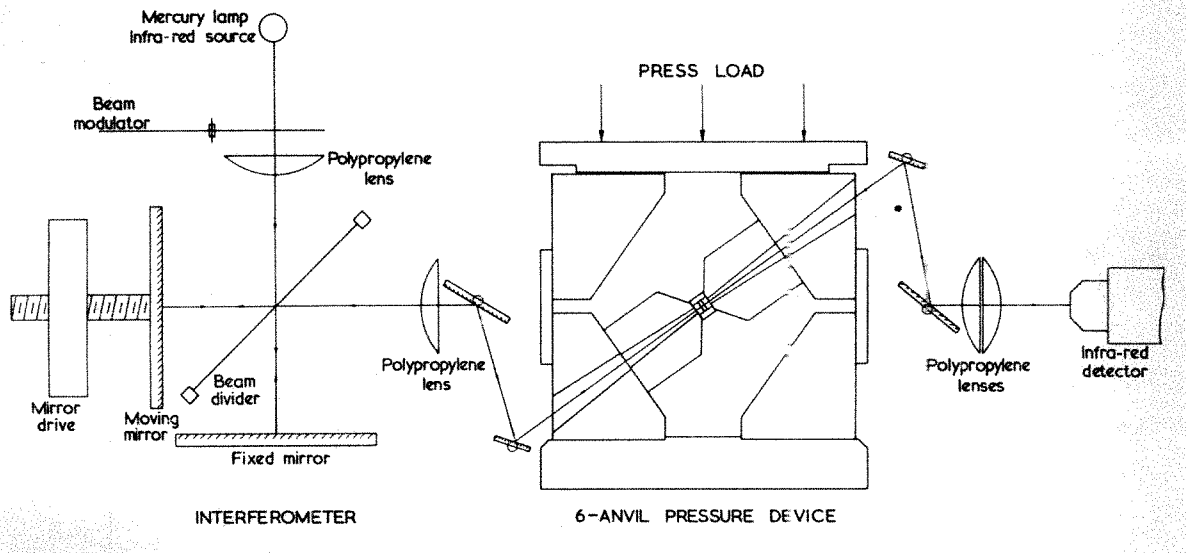


Fig. 1. High pressure six anvil cube device and Michelson interferometer

cube assembly and temperatures were measured by means of a thermocouple.

Hydrostatic piston and cylinder device. This apparatus is an adaptation of a similar one developed by Drickamer[6], although in this case larger apertures were used. The apparatus is not capable of reaching the pressures that can be obtained in the cube anvil device but has a number of advantages within its range: (1) a reduction in the time taken to obtain spectra of given resolution because larger apertures ($f/2$) and windows (0·25 in. diameter) are used, thus increasing the amount of transmitted radiation; (2) pressure measurement is more convenient in the larger sample volume because a manganin wire gauge can be used during a run; (3) the positions of the windows are continuously adjustable and thus the sample thickness can be varied over quite a wide range.

We have recently obtained some new results for the far infra-red absorption spectra of solid and liquid chlorobenzene and carbon disulphide up to pressures of 35 kbars. The absorption spectrum of liquid chlorobenzene was determined with the particular view of improving the understanding of the "pseudo-lattice" absorption mechanism found in many polar liquids. Fig. 2 illustrates values for α, the absorption coefficient as a function of frequency and applied hydrostatic pressure. The point at 29·7 cm^{-1} was determined at atmospheric pressure with a CN maser[2]. The internal consistency between different runs with the high-pressure hydraulic cell was ± 5 per cent. The significance of these results will be discussed on a later occasion.

Bridgman[7] has shown from volume pressure measurements that two solid forms of chlorobenzene are stable under a range of pressures at room temperature (20° C). The liquid freezes to solid I at -50° C under one atmosphere pressure and about 5 kbars pressure is necessary to retain the phase at 20° C. If the pressure is then raised to 15–20 kbars and the temperature raised to 250° C for a few minutes before returning to room temperature, a second solid form is obtained. The two spectra shown in Fig. 2 confirm the existence of these two solid forms.

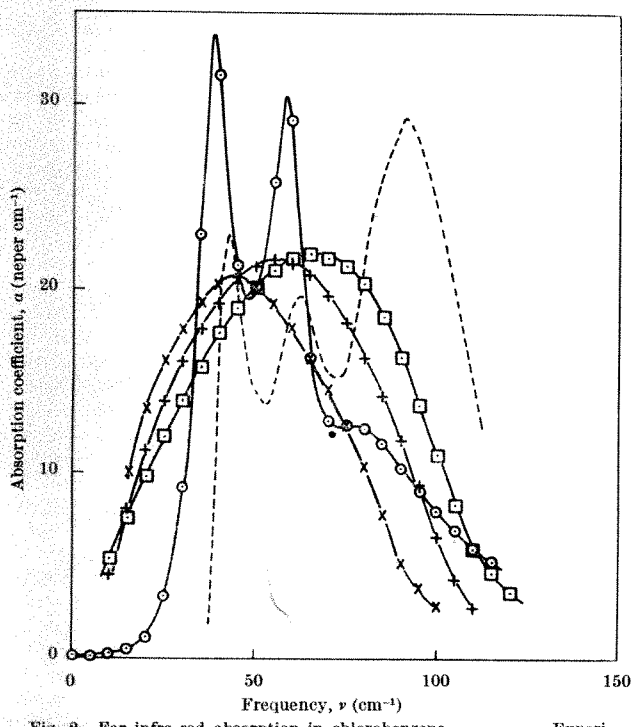


Fig. 2. Far infra-red absorption in chlorobenzene. ————, Experiments with hydraulic device; – – – – –, experiments with six anvil cube device. Liquid: ×, 0·6 kbars; +, 2·3 kbars; □, 4·4 kbars. Solid I, 5·0 kbars (○); solid II, 20 kbars (– – –). Temperature, 20° C

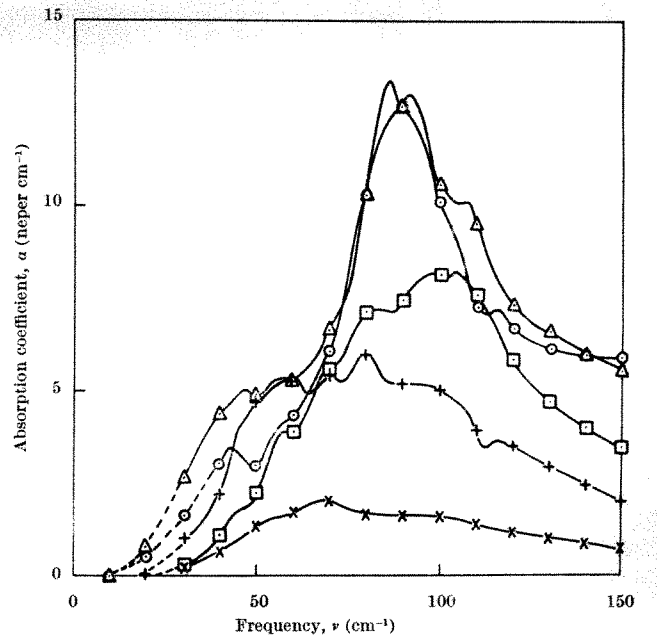


Fig. 3. Far infra-red absorption in carbon disulphide. ————, Experiments with six anvil cube device. Liquid: ×, 1 atm.; +, 3 kbars; □, 11·6 kbars. Solid: ○, 12·0 kbars; △, 12·7 kbars. Temperature, 20° C

Increasing the pressure to 35 kbars has only a small effect on the spectrum, the absorption features moving to slightly higher frequencies.

Carbon disulphide can be frozen at room temperature with a pressure of 12–13 kbars. It appears from the spectra shown in Fig. 3 that there is a very considerable increase in the total absorption up to and through this phase change, which cannot be simply explained by uncertainties in sample thickness and polycrystalline scattering. Even allowing for the 20 per cent greater compressibility of carbon disulphide pressure evidently has a much greater effect on the molecular interactions of quadrupolar carbon disulphide compared with dipolar chlorobenzene.

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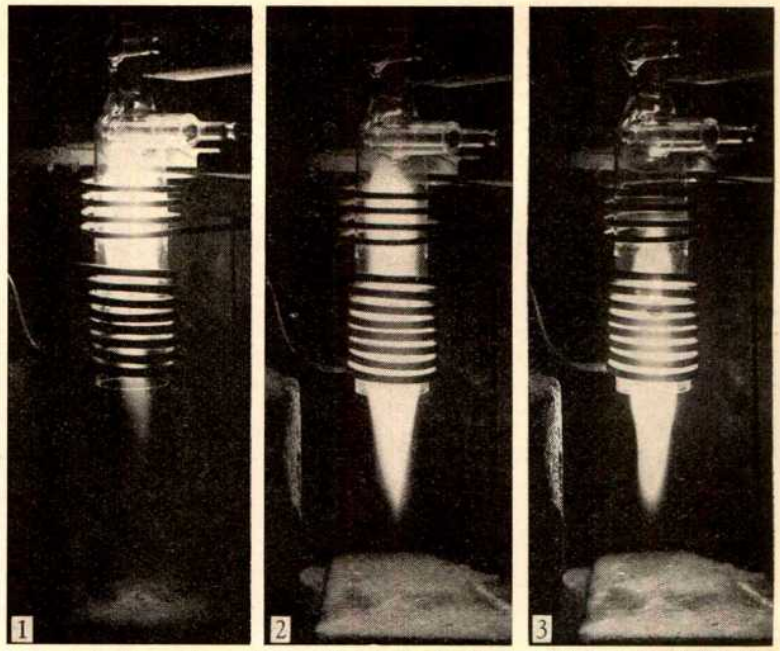
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Surface Wave Velocity Measurement

I have found[1] that surface wave velocities can be conveniently and accurately derived by measuring a critical angle at which, when an ultrasonic beam is reflected from a solid surface in a water bath, there is a violent change in the waveform of the reflected wave train. This occurs when the surface wave velocity and the wave velocity in the water are related by way of Snell's law for an angle of refraction of 90°. Although the indication is accurate and has proved valuable for elasticity measurement, the reason for this behaviour has not been entirely clear.

The transfer of the wave from the water, with acoustic impedance $1{\cdot}5 \times 10^5$ for a 1 cm² beam, is to a thin surface film of the solid—the surface wave only penetrates about one wavelength. At 5 Mc/s this would be about 0·5 mm and the acoustic impedance would be about 10^5, that is, lower than that of the water. In the neighbourhood of the angle at which the wave conversion occurs most efficiently, therefore, a phase change of about 180° occurs and this affects the reflected wave too. Because the waves used in this experiment are not plane waves, the incident beam can be considered to be partly of an angle of incidence greater than the mean angle and partly at a smaller angle. The extent of this effect can be deduced from the angle of lag of the radiation in the appropriate circumstances as calculated by, for example, Goodwin and myself[2]. It is thus possible, when such a beam encounters a solid surface in water, that one part of the beam produces a surface wave of one phase and the other another surface wave 180° out of the phase and that partial cancellation occurs. This leads to a reduction in the amplitude of the reflected wave and this is used as already outlined to determine surface wave velocity.



Figs. 1–3. Argon plasmas in 5 cm diameter torch. Fig. 1. High frequency (3·8 Mc/s). Fig. 2. Dual frequency (3·8 Mc/s and 280 kc/s). Fig. 3. Low frequency (280 Kc/s)

Devices utilizing this principle have been used[3] to investigate the elasticity of single crystals and that of elastically anisotropic polycrystalline aggregates both in bulk form and in sheets. Furthermore, the method of measurement has proved to be of value[4] in investigations of surface stresses (it enables third order elastic constants to be determined); there are also signs that the method can be used to show up incipient fatigue in a non-destructive manner before a crack actually develops.

In the past the two devices which have been developed to investigate this phenomenon have been rather complicated and difficult to construct, but new much simpler forms have now been evolved. In these a single transducer acting as combined transmitter/receiver produces a beam which is first reflected from the surface under examination, then again, but at normal incidence this time from an acoustic mirror (an air-backed thin film), then back to the specimen again and thence to the original ultrasonic transducer now operating as a receiving transducer. An ordinary ultrasonic flaw detector can often be used in this way.

It is believed that this simplification will encourage the use of these devices (one form[3] of which does not need a water bath) both in research and also in industry.

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Radiofrequency-induced Gas Plasma at 250–300 kc/s

INDUCED plasmas in static gases at atmospheric pressure were first reported by Babat[1]. A plasma torch based on inductive coupling between a radiofrequency field and a flowing, ionized gas was developed by Reed[2] and later by Reboux[3], Mironer and Hushfar[4] and Marynowski and Munroe[5]. In all cases, the frequencies used were in the megacycle range.

Because the "skin thickness" for radiofrequency heating increases with a decrease in frequency, the use of a lower frequency can be expected to produce a larger plasma with a more uniform temperature. Reed[6] states that attempts to initiate a plasma at 400 kc/s were unsuccessful, and similar results have been reported by Radyne, Ltd.[7], with a 100 kW, high impedance output generator. However, Reed[6] has pointed out that "starting a plasma is a separate problem from running it after it is started", and Dymshits and Koretskii[8] have recently described induced plasmas operating at frequencies as low as 290 kc/s. The latter authors gave only limited experimental details, but appear to have initiated the plasma by temporarily reducing the pressure.

We have used an alternative method of initiation in which a pilot plasma was established in the megacycle range and power was then coupled into this plasma from a second work coil energized at 280 kc/s. Experiments were carried out in a simple quartz-glass torch similar in principle to that described by Reed[6] but with an outside diameter of 5 cm and two tangential gas inlets. The two radiofrequency generators used were not designed for plasma work, but were readily available in the same laboratory and were of appropriate frequency ranges. They were: (1) Radyne *C155/SR*, nominal output power rating 15 kW, nominal frequency 4 Mc/s; work coil four turns, 6·5 cm internal diameter, 3·5 cm long. (2) Thermionic 500, nominal output power rating 20 kW, nominal frequency 375 kc/s. The output from this generator was stepped down by means of a 22 : 5 transformer in order to provide a suitably high inductance load with a practicable size of work coil. The optimum work coil was found by experiment to be eight turns, 5 cm internal diameter, 7·0 cm long. This was situated so that its upper end was 4–5 cm below the lower end of the Radyne work coil.

An argon plasma was initiated at a gas flow rate of about 60 l./min, by lowering a graphite rod into the upper (3·8 Mc/s) field and applying maximum power from generator No. 1. When the lower coil was energized (280 kc/s), the plasma spread down the torch into the region of the lower work-coil (Fig. 2) and increased in brightness. It was found possible to switch off the higher frequency output and maintain the plasma on the lower frequency alone (Fig. 3), but the power and diameter of the plasma then decreased. Attempts to initiate a plasma at 280 kc/s,

using both the graphite rod and Tesla discharge techniques, were unsuccessful.

Although no direct measurements of the power supplied to the plasma by each generator have yet been made, approximate values were obtained by a load substitution method: the power dissipated in a dummy load, a graphite cylinder of the dimensions required to produce the same load conditions as the plasma itself, was determined by measuring the equilibrium temperature of the graphite load in a slow stream of nitrogen and assuming that the power supplied was equal to the rate of loss of heat by radiation (convection losses are comparatively small). This method has been shown by Reed[9] to give results which agree well with those obtained by using the conventional water-cooled calorimeter. The output from generator No. 2 was about 13 kW with generator No. 1 on (Fig. 2) and 10 kW with generator No. 1 switched off. Similar measurements on generator No. 1 indicated an output power of approximately 7 kW.

There appears to be no reason why still lower frequencies should not be used provided that: (*a*) suitable means of initiation are incorporated, either by the use of a high-frequency pilot plasma as in the present work or possibly by pressure reduction as apparently used by Dymshits; (*b*) the size of the plasma torch is increased.

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RADIOBIOLOGY

Measurement of Polonium-210 in Human Blood

POLONIUM-210 is a naturally occurring alpha-emitting isotope of the uranium series derived from decay of its long-lived parent, lead-210. Polonium-210 is present in most plants and foodstuffs, as well as in human tissues[1–3]. Body burdens of this isotope are usually determined by measuring its activity in tissue samples or urine[2,4,5]. Tissue samples other than teeth are not readily available during life, however, and 24 h urine collections may be difficult to obtain and process, yet are often necessary to quantify urinary excretion. We have found that polonium-210 can be easily detected in 10 ml. human blood samples by a simple radiochemical and counting technique. The measurement of polonium-210 concentration in blood may thus be a practical method of estimating body burdens of this isotope, as well as of its parent lead-210[2]. As tobacco smoke has been shown to contain trace amounts of polonium-210[6], we have related blood concentrations to smoking habits in a group of middle-aged men.

Ten ml. samples of fresh clotted blood were weighed and digested in 30 ml. of hot concentrated hydrochloric acid, placed in plating cells with 0·5 N hydrochloric acid and 50 mg ascorbic acid, and the polonium plated on to silver planchets[4,7]. The planchets were counted for 48–72 h in low background gas-flow proportional counters[7]. Polonium-210 concentrations as low as 0·3 pc./kg blood could be reliably measured by this method. Blood samples were obtained from 100 healthy men between the ages of 45 and 54 years, all of whom resided in one suburb of Boston. Detailed smoking histories were obtained from these men, and they were divided into four groups: current cigarette smokers, current pipe and/or cigar smokers only, past cigarette smokers (had been regular smokers until a year or more previously), and individuals who had never smoked.

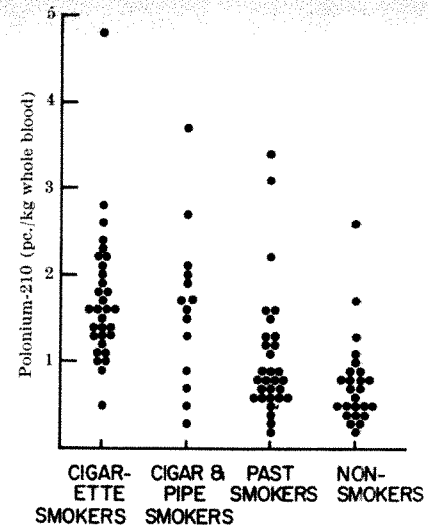


Fig. 1. Polonium-210 concentrations measured in 10 ml. blood samples from 100 healthy men 45–54 years of age

The results are presented in Fig. 1. The average polonium-210 concentration in smokers was 1·72 pc./kg blood, compared with 0·76 pc./kg in non-smokers. Although a significant difference existed between blood concentrations in cigarette smokers and non-smokers ($P < 0{\cdot}001$), a two to three-fold variation among individuals was present in each group. Such variation has also been observed among polonium-210 concentrations in pulmonary tissues of different individuals[7]. In addition, we have examined other body tissues including liver, spleen, kidney, urinary bladder and bone obtained at autopsy from ten cigarette smokers and four non-smokers. Average concentrations were very similar to those recently reported by Hill[1], but up to a three-fold variation in concentrations in each organ was observed among individuals.

The variation in blood concentrations may in part result from dietary factors. As Hill has pointed out, polonium-210 concentrations differ widely in various foodstuffs; beef and lamb kidney and certain shell fish, for example, may contain relatively large amounts of the isotope[1]. In a preliminary study of possible dietary effects, several blood samples were drawn from an 85 kg subject before and after a meal containing about 100 pc. of polonium-210 (200 g of beef kidney). Blood polonium-210 concentrations were 0·98 pc./kg before the meal and rose to a maximum of 1·56 pc./kg 12 h after the meal, which suggests that a significant fraction of the isotope present in food is absorbed into the blood.

In separate experiments in four heavy cigarette smokers, blood samples were obtained before and at regular intervals after sudden complete cessation of smoking. Blood polonium-210 concentrations fell an average of 27 per cent during the first week after smoking was stopped, but thereafter changed very little during the next 4–8 weeks.

In addition to the polonium-210 normally inhaled and ingested by the general population, it has been estimated that cigarette smokers retain an excess of about 1·5 pc./day per twenty cigarettes, much of which may be cleared from the lungs in the mucous sheet and swallowed[6,7]. The present results indicate that such small differences in chronic environmental exposure to polonium-210 can be detected in 10-ml. blood samples from exposed individuals. The values reported here should serve as a baseline to evaluate concentrations found in blood of individuals possibly exposed to high environmental levels of polonium-210 or its parent isotopes. In preliminary experiments, detectable concentrations of radium were found in 10-ml. blood samples from two normal individuals. Analysis of small blood samples, therefore, may also prove useful in estimating body burdens of alpha-emitting isotopes other than polonium-210.

We thank Dr. Edward P. Radford, jun., of the University of Cincinnati College of Medicine for confirming our counts on several of these samples in his laboratory. This work was supported by a grant from the Bureau of State Services, U.S. Public Health Service, and by a grant from the Rockefeller Foundation.

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Caesium-137 in Edible Freshwater Fish

KNOWLEDGE of radioactivity in fish is largely limited to determinations made of the strontium-90 occurring in certain ocean species and to some attempts at the evaluation of caesium-137 content (^{137}Cs) of the same or similar specimens. Häsanen and Miettinen[1] found concentrations of the order of nanocuries of caesium-137 per kilogram (nc./kg) in freshwater fish. These were taken from lakes in Finland during 1962 and 1963 in the course of an investigation into sources of caesium-137 other than caribou and reindeer meat in the Laplander's diet. This finding could have been predicted from the earlier work of Pendleton and Hanson[2], which demonstrated the rapid accumulation of caesium-137 by various aquatic forms through several trophic levels in a freshwater pond contaminated with caesium-137. Caesium-137 in these organisms was found to reach concentrations several orders of magnitude greater than those found in the surrounding water.

During the first 3 months of 1965, the concentrations of caesium-137 present in freshwater fish available commercially in the Chicago area were similar to those found earlier in Finland (Table 1). The fish (which were purchased at random in local food markets) were boned fillets (except for smelts) and ready to cook. Both the concentration of caesium-137 and of potassium-40 were determined by gamma-ray spectrometry in 1–2-kg samples of fresh fish using conventional low-background techniques. A number of varieties of ocean fish were also examined and showed appreciably lower concentrations of caesium-137 but similar potassium levels (Table 1). This inherent difference between salt and freshwater species as regards the content of caesium-137 had been previously noted by Häsänen and Miettinen[1].

The caesium-137 concentration present in fish depends primarily on three factors: (1) the concentration of the radionuclide in the water environment; (2) the accompanying level of potassium (ratio of caesium-137: potassium); (3) the position of the fish in question in the aquatic food chain (that is, the trophic level). Published values of caesium-137 in salt and freshwater are sparse. However, monthly measurements in Lake Huron showed average concentrations of 0·4 and 0·3 pc./l. during 1963 and 1964, respectively[3]. During this same interval the concentration in lakes and rivers in the vicinity of Chalk River, Ontario (presumably uncontaminated by reactor wastes), was three to four times greater than that in Lake Huron[3]. Surface waters of the Pacific Ocean sampled in mid-1953 indicated an average level of 0·6 pc. caesium-137/l. (ref. 4). Profiles of caesium-137 activity in ocean water have shown decreasing concentration with depth[5]. Judging by the limited data available, there did not appear to be appreciable differences between the concentration of caesium-137 in salt and freshwater. However, the possibility exists that closed lakes which do not possess large dilution capacity may contain higher levels of caesium-137 because they collect the run-off from large areas. Conversely, because of the large dilution effect possible in the oceans, the effective concentrations may be much lower than that observed in surface waters.

The potassium content of the oceans is ten to several hundred times greater than of freshwater. The value in the oceans, based on a large number of analyses, is fairly constant, and is given as 380 parts per million (p.p.m.)[6]. The few measurements taken in the Great Lakes show values of a few p.p.m., with smaller lakes and rivers in the area containing somewhat higher amounts[7]. Amounts in closed lakes in this region and further north are an order of magnitude higher, with values approaching marine concentration in some instances[7]. The concentrations of rubidium and stable caesium are several orders lower than that of potassium in both salt and freshwater and are almost insignificant[6,7].

The greater concentration of caesium-137 in freshwater fish, seen in Table 1, probably reflects, in the first place, the higher caesium-137 : potassium ratio present in freshwater[8]. The trophic level is also a factor—as shown by the higher caesium-137 concentration in carnivorous fish as opposed to plankton feeders and other non-carnivorous fish, in both salt and freshwater. The lower concentrations in lake perch and smelt may also result from younger age at capture as well as different feeding habits.

The question now arises as to what effect the presence of relatively high concentrations of caesium-137 in freshwater fish may have on the average intake by human beings

Table 1. CAESIUM-137 AND POTASSIUM IN SALT AND FRESHWATER FISH PURCHASED BETWEEN JANUARY AND APRIL 1965

Species*	Feeding habits	^{137}Cs (pc./kg wet weight)	Potassium (g/kg wet weight)	^{137}Cs pc/g K
	Freshwater fish			
Lake trout	Carnivorous †	2,210	2·49	887
,,	,,	3,874	3·50	1,107
,,	,,	2,923	2·90	1,008
Pike	,,	2,610	3·58	729
,,	,,	1,428	3·25	439
,,	,,	1,167	2·47	472
Whitefish	Plankton	1,316	3·57	369
,,	,,	328	3·51	93
,,	,,	265	3·65	73
Lake perch	,,	365	2·89	126
,,	,,	330	2·96	111
Smelts	,,	327	2·60	126
,,	,,	377	3·17	119
	Marine fish			
Flounder	Bottom feeder, molluscs, etc.	24	2·88	8·3
Halibut	Carnivorous	62	5·26	11·8
Ocean perch	Plankton, annelids	32	3·08	10·4
,, ,,	,, ,,	56	3·11	18·0
Ocean catfish	Carnivorous	43	4·04	10·6
Haddock	,,	66	4·02	16·4
Salmon	,,	71	3·51	20·2
Cod	,,	43	4·17	10·3
Red snapper	,,	12	1·76	6·8

* The common or commercial names of fish are used here instead of taxonomic names because of the difficulty in determining the latter with any precision with frozen, dressed fish.
† 'Carnivorous' is meant to imply feeding on fish and other animals smaller than the fish in question.

in their diet and on the resulting body burden. Measurements of caesium-137 in the diets of inhabitants of the Chicago region have been made as part of the "Tri-City Food Sampling Program"[9]. During 1964 the average adult intake of caesium-137 was found to be 200 pc./day or 45 pc./g potassium. Whole body measurements of human subjects in the Chicago area showed a relatively constant average level of 150 pc. caesium-137/g potassium during the latter half of 1964 (ref. 10). This concentration of caesium-137 in man was taken to be the equilibrium level attributed to an intake of 200 pc./day for several months. The composite diet in question contained 8 kg of fish and 73 kg of meat per annum. The fish actually sampled in the study were primarily saltwater varieties, and accordingly the daily intake of caesium-137 from fish was ~5 pc. If we consider that equal amounts of salt and freshwater fish are consumed each day, and take average values from Table 1, the daily intake of caesium-137 from fish becomes 15 pc. Restricting the fish consumption to freshwater varieties results in an average intake of 30 pc. caesium-137/day from fish. This will, at most, produce a 15 per cent increase in the average daily intake of this radionuclide. Those persons who consume larger quantities of fish than implied by the standard diet, or who substitute fish for meat, will have a correspondingly higher daily intake of caesium-137. An extreme, though still feasible, example would be one in which an individual, on an otherwise normal diet, ate a pound of carnivorous freshwater fish per day and little or no meat. This would lead to an intake of 1,200 pc./day and an equilibrium body burden of 900 pc./g potassium (according to the relationship that 200 pc./day intake will lead to an equilibrium burden of 150 pc./g potassium). For a 70 kg man (standard man), containing 140 g of potassium, the whole-body burden of caesium-137 would be 0·13 μc. The body burden of caesium-137 in Eskimo and Laplander populations, depending wholly or in part on caribou and reindeer as a source of fresh meat, ranged from a few tenths of a μc. to as high as 3·0 μc. during 1964 (refs. 11–13). Thus it would appear that individuals living in the United States and Canada (and probably in the mid-latitude regions of Europe and Asia also), who regularly consume as much as a pound of carnivorous freshwater fish in a day, could approach the caesium-137 body burden of Arctic and sub-Arctic peoples who depend on caribou or reindeer as a prime source of meat.

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A Liquid Scintillation Method for measuring Carbon-14 Uptake in Photosynthesis

THE accurate determination of the radioactivity of stock sodium carbonate solutions and the correction of the radioactivity of membrane filtered algae to zero thickness are two of the greatest barriers to accurate productivity measurements using carbon-14.

The purpose of this paper is to outline a method which has been very useful in a recently completed photosynthesis study, and which may offer a solution to these problems.

Goldman[1,2] has dealt successfully with these two problems by oxidizing all organic carbon to carbon dioxide, and measuring carbon-14 activity in the gas phase. This method is relatively time consuming, and it does not adapt itself readily to the rapid counting of large numbers of planchets.

Jitts and Scott[3] have used liquid scintillators to estimate zero thickness corrections by counting labelled plastic films on a Geiger–Müller counter, then dissolving the films in liquid scintillator solution to determine the activity accurately. In their calculations it is assumed that the quenching properties of plastic film are similar to those of the equivalent weight of algae.

The advantages of the method described here are: (1) All photosynthesis determinations may be corrected individually for self-absorption and counting efficiency. (2) The high efficiency of liquid scintillation counting makes more rapid and more accurate counts possible. (3) The method is adaptable to field use, and saves considerable time in handling papers and planchets. (4) The activity of stock solutions can be determined accurately and directly, using the same method.

The scintillator solution is made up as follows: 100 g naphthalene; 7 g 2,5-diphenyl oxazole (PPO); and 0·3 g dimethyl, 2,2,*p*-phenylene*bis* (5 phenyloxazole) (dimethyl-POPOP) are dissolved in 1 l. of reagent-grade dioxane. The scintillator solution may be used for counting aqueous carbon-14 samples of all sorts; it gives good counting efficiencies even when it contains almost 30 per cent water[4].

Determination of carbon-14 uptake by algae. After termination of a photosynthesis determination the algae are filtered on to a 'Millipore' membrane in the usual manner. The membrane and its contents are transferred directly to a liquid scintillator vial containing the scintillator solution; vigorous shaking will dissolve the damp 'Millipore' quickly. Dry 'Millipore' membrane is insoluble in this scintillator solution, and so membranes must be taken directly from the filtering apparatus while they are still wet. The membranes must be completely dissolved before counting commences or the results will be highly variable. A slight greenish tinge, which may cause colour quenching, will appear in the scintillator solution just after the membrane has been dissolved if the layer of phytoplankton is particularly heavy. This colour disappears within a few hours, and counting should be delayed until it disappears.

Samples must be counted at above 5° C, since the solution freezes at 3°–4° C. After counting, an appropriate volume (10–100 lambdas) of carbon-14 labelled liquid toluene standard is added to each sample, and all vials are re-counted. The vials must be shaken immediately before counting in both cases, as the algae (or remnants thereof) settle out within a few minutes. The addition of a thixotropic gel, such as 'Cab-O-Sil *M*-5' (Godfrey L. Cabot, Inc.), should eliminate this problem with very little loss of counting efficiency, if an automatic counter is to be used, or if samples are of very low activity. The counting efficiency for each vial is $E = (C' - C)/S$, where C' is the number of counts per minute measured after addition of the toluene standard, C is the number of counts per minute of the sample alone, S is the actual radioactivity of toluene standard added in disintegrations per minute, and E is efficiency of the counting apparatus (all counts cor-

rected for background). The correction for self-absorption will vary slightly from sample to sample. C/E will give corrected figures (d.p.m.) for carbon-14 uptake.

Activity of carbon-14-labelled sodium carbonate stock solutions. A small, accurate volume of stock labelled sodium carbonate solution may be pipetted directly into a vial of the scintillator solution and counted. The vials may be standardized and corrected for efficiency in the same manner as for algal samples. Ten to twenty-five lambdas of stock solution will normally be adequate, if the stock activities are of the order of 5–10 μc./ml.

The following results represent counts on planchets selected at random from a day's photosynthesis determinations on a small turbid pond. All determinations were carried out in 250-ml. reagent bottles suspended directly in the lake from a transparent float. 0·5 ml. of labelled sodium carbonate solution of approximately 5 μc. was added to each bottle. The incubation time was 4 h, from 10 a.m. to 2 p.m. Exactly 75 ml. from each bottle were fixed with 0·5 ml. of 40 per cent formalin and filtered on to a 'Millipore' *HA* membrane, 2 in. in diameter. Membranes were first dried and counted with a 'Tracerlab' *FD*-1 gas flow Geiger–Müller tube with a 'Mono-mol' resin window and a 'Tracerlab Compumatic II' scaler; then dampened and counted as described above in a 'Tracerlab' *LSC*–10 *B* liquid scintillation counter using the same scaler.

The efficiencies of both the gas flow and liquid scintillation method were calculated from the corrected liquid scintillation counts as determined by adding carbon-14-labelled toluene standard.

The counts and comparative efficiencies of the two methods are set out in Table 1.

Table 1

Depth (m)	Gas flow Geiger–Muller (d.p.m.)	Liquid scintillator (d.p.m.)	Actual carbon uptake by algae (d.p.m.)
Surface	4,416	10,283	28,638
1	3,767	11,735	32,671
2	1,416	3,814	10,615
2	1,478	4,384	12,205
5	244	726	2,022
5	248	748	2,082
Dark bottle	232	547	1,523

	Average percentage efficiency of counting	Standard deviation
Gas flow	13·09	1·47
Liquid scintillator	35·92	0 65

The high counting efficiency and low standard deviation, coupled with the individually determined efficiencies and reduced manipulation of filter membranes, make the liquid scintillation method desirable where primary production is very low or erratic.

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CHEMISTRY

Analysis of Volatile Substances in Samples containing Non-volatile Substances by Process Gas Chromatography

RECENTLY, we experienced difficulties with the automatic analysis by gas chromatography of a process stream consisting of a liquid mixture of organic chemicals. These arose because traces of non-volatile tars and polymers in the sample eventually accumulated in the entrance of the first column of the chromatograph. (This chromatograph normally uses two columns to perform the analysis and has a thermal conductivity detector. The first column is used to separate and backflush volatile heavy ends out of the analyser; the second column completes the separation of the substances to be analysed.) Eventually, after a series of analyses, the accumulation of non-volatile materials in the first column was sufficient to block it completely, thus putting the analyser out of commission. The analyser was, in fact, injecting 10-μl. samples every 15 min into a 0·125-in. bore column. The concentration of non-volatiles was about 0·5–1·0 per cent by weight. Blockage occurred after 7–10 days of continuous operation.

The problem described here rarely arises in gas chromatography in laboratories, as the frequency of analysis is rarely as high. Even if a column blocks up in the laboratory, it is usually an easy matter to change it. This state of affairs cannot be tolerated with a process instrument where a minimum of 6 months' continuous operation is almost mandatory. It was, in fact, essential that this problem be solved to achieve fully reliable on-line analysis. This has now been done and this communication describes the essence of the method.

I considered that it was necessary to remove the non-volatile materials from the first column by washing the column with a liquid solvent. An immediate objection to this is the damaging effect of the solvent to the column. This can be avoided by using an extra column which we call a "cleanse column" together with a column switching valve. The cleanse column is, in fact, an unpacked 0·125-in. bore tube about 1–2 ft. long and the system is operated as follows.

The sample is injected into the cleanse column. The non-volatile constituents of the sample stick to the walls of the cleanse column tube and the volatiles pass on to the analytical columns. The column switching valve is then shifted. This isolates the sample valve and cleanse column from the rest of the chromatograph. While the analysis proceeds, the cleanse column and sample valve passages are flushed out with a suitable solvent to remove the non-volatiles. Residual solvent is removed by purging the lines with pure dry instrument air.

Before a subsequent analysis is due to commence, the column switching valve is reversed, thus re-connecting the sample valve and cleanse column into the carrier gas supply. This is done during the time the first column is being backflushed, so that residual air is purged clear by the carrier gas flow and does not affect the analysis. The next analysis cycle can then be commenced.

The system uses some 40–60 ml. of solvent per analysis or 4–6 l. per day. A solvent recovery still is used in conjunction with the analyser. The solvent must, of course, readily dissolve the non-volatiles in the sample. The boiling-point of the solvent should be preferably some 20°–30° C above the analyser operating temperature. This is to prevent formation of vapour locks in the lines, thus preventing free flow of the solvent. The solvent should be sufficiently volatile, however, otherwise a prolonged air purge is required to remove remaining traces from the system before starting the next analysis. The technique described above has permitted non-interrupted operation of a process chromatograph for

almost 6 months at the time of writing, where previously the maximum period was 10 days.

To the best of my knowledge, this is the first example of the technique being used in on-line process analysis. It is understood that a similar technique has been tried in the laboratory with a process chromatograph for the removal of sugars[1] in the analysis of wines. This analyser was not, however, ever installed on a running process.

I thank those staff of Beckman Instruments, Inc., who encouraged me to try out the technique.

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Microwave Spectrum of Silyl Isocyanate

THE microwave spectra of silyl isothiocyanate[1] and silyl azide[2] have shown them to be symmetric and asymmetric tops respectively. We report here that silyl isocyanate has a spectrum which, although that of a symmetric top, has numerous very unusual features.

The spectrum consists of several series of lines with first order Stark shifts separated by about 5 Gc/s. Each series extends over about 2 Gc/s and is roughly divided into groups. The lower frequency groups of each series are clear cut, but beyond about the third they become more complicated and are difficult to distinguish. The lowest frequency group of each series was assigned to the ground vibrational state and gave a good fit when frequency was plotted against K^2. In particular the lines assigned to $K = 1$ show no splitting and the lines $K = 3$ show the characteristic intensity increase of molecules with three equivalent protons, this indicating a symmetric top.

The bulk of the remaining lines were assigned to molecules in excited states of a low-frequency vibration. Those groups attributed to the first excited state each possessed an l-type doublet and a central group, indicating a degenerate vibration, associated with bending of the heavy atom chain. The intensities of the groups suggested a vibration frequency of less than about 50 cm^{-1}, and the splitting of the l-type doublets, assuming the small amplitude approximation, yielded about 30 cm^{-1}. This figure should be compared with 54 cm^{-1} in silyl isothiocyanate[1] and 220 cm^{-1} in silyl acetylene[3,4]; apparently the present low value arises from a very low restoring force in the bending rather than the atomic masses.

Spectra were obtained of the most abundant and isotopically substituted species. Molecules containing silicon-29, silicon-30 and carbon-13 were studied in natural abundance, whereas artificial substitutions of oxygen-18, nitrogen-15 and deuterium were made using the reaction of cyanic acid and trisilyl amine[5]. In all symmetric cases the simplest analysis of the data yielded very large values of the centrifugal distortion constant D_{JK} (500–670 kc/s), while D_J was "normal" (about 1 kc/s). With more detailed treatment significant higher order constants were found in some cases. The partially deuterated, slightly asymmetric, molecules gave a straight line through the points $K = 0$, 2, 3 and the mean of $K = 1$ when frequency was plotted against K^2; the slope gave a value for D_{JK}. The points $K = 4$ and 5 were well off (14–90 Mc/s) the lines. All calculated constants are in Table 1.

An attempt was made to determine the "r_s" structure; this method attempts, with much success for "regular" molecules, to approximate the equilibrium structure to within a few thousandths of an Ångstrom unit[6]. All atoms except nitrogen were placed using Kraitchman's equations[7]; because nitrogen is near the centre of mass its true "r_s" position was difficult to determine. It was placed using, besides a simple first-moment method, a double first-moment procedure in which the shift in centre of mass on full deuteration was observed from the change in "r_s" positions of silicon. From this two simultaneous centre of mass equations were set up having as unknowns the nitrogen position and the height of the silyl pyramid. These first moment values agreed and were used in the derived structure. Table 2 contains this as well as those of related molecules. It is seen that the present N—C distance is much shorter than that of the other molecules, suggesting that the nitrogen and/or carbon positions may be incorrectly determined. While this is entirely possible, it is noteworthy that the present C—O distance is comparable with that of related molecules and that the S—O distance is 0·068 Å shorter than the sum of the Si–N distance in silyl isothiocyanate and the N–O distance in cyanic acid. Furthermore, the derived H–Si and D–Si parameters show no self-consistency and are very different from those of other silyl compounds.

Table 1. ROTATIONAL AND CENTRIFUGAL DISTORTION CONSTANTS OF SILYL ISOCYANATE

Species	Rotational constant(s) $B_0(C_0)$ (Mc/s)	D_J (kc/s)j	D_{JK} (kc/s)	Others (kc/s)
$^{28}SiH_3{}^{14}N^{13}C^{16}O$	2,517·888 ± 0 014	0 9 ± 0·2	641·0 ± 1·3	$H_{JJK} = (-5\ 9 \pm 1·3) \times 10^{-2}$
$^{29}SiH_3{}^{14}N^{12}C^{16}O$	2,484·455 ± 0 022	1 3 ± 0·3	613 7 ± 1 6	$H_{JKK} = (12 \pm 6) \times 10^{-2}$
$^{30}SiH_3{}^{14}N^{12}C^{16}O$	2,452 814 ± 0 025	1 3 ± 0 4	598·7 ± 0·7	
$^{28}SiH_3{}^{15}N^{12}C^{16}O$	2,517 743 ± 0 027	1·1 ± 0·3	674 6 ± 2·2	$H_{JJK} = (-8·5 \pm 2·4) \times 10^{-2}$
$^{29}SiH_3{}^{15}N^{12}C^{16}O$	2,484 489 ± 0 095	4·5 ± 1 4	648 ± 22	
$^{28}SiH_3{}^{14}N^{13}C^{18}O$	2,500 419 ± 0 04	1 (assumed)	627·9 ± 0 7	
$^{28}SiH_3{}^{14}N^{13}C^{18}O$	2,387 680 ± 0·016	−0·6 ± 0·3	600 2 ± 0·8	
$^{28}SiD_3{}^{14}N^{13}C^{16}O$	2,331·769 ± 0 021	0·9 ± 0 3	533·8 ± 0·5	
$^{29}SiD_3{}^{14}N^{13}C^{16}O$	2,305 948 ± 0·062	3·3 ± 1·0	518 3 ± 0 2	
$^{30}SiD_3{}^{14}N^{13}C^{16}O$	2,281·170 ± 0·01	1 (assumed)	507 0 ± 4·1	
$^{28}SiH_2D^{14}N^{13}C^{16}O$	2,461·94 2,438 43		637	
$^{28}SiHD_2{}^{14}N^{13}C^{16}O$	2,400 86 2,376·12		578	

Table 2. STRUCTURE OF SILYL ISOCYANATE AND RELATED MOLECULES

Molecule	Ref.	r(O–C) (Å)	r(C–N) (Å)	r(N–Si) (Å)	r(H–Si) (Å)	∠(H–Si–H)
SiH_3NCO	Present	1 179	1 150	1·699	1·506	110° 24′
SiD_3NCO	Present				1 514	112° 12′
SiH_3NCS	1		1·211	1·714	1·488	111° 22′
SiH_3Cl	8				1·483	110° 58′
SiD_3Cl	8				1·480	110° 52′
SiH_3CCH	9				1·488	110° 12′
HNCO	10	1 171	1·207			
CH_3NCO	11	1·18	1 19			
$(SiH_3)_3N$	12			1 74	1·54	

Thus silyl isocyanate, though a symmetric top, is an unusual one, in comparison with both chemically related molecules and other symmetric tops. Other isocyanates (cyanic acid, methyl isocyanate, germyl isocyanate[13]) and isoelectronic silyl azide all have bent heavy atom configurations. Nevertheless, the low frequency band, with its correspondingly low force constant, reflected in the very high values of D_{JK} (ref. 14), suggests that the force constraining silyl isocyanate to the symmetric configuration is rather weak. The vibration is probably of large amplitude, with considerable anharmonicity, which makes use of the small amplitude approximation dubious, particularly in treating l-type doublets and deriving structural parameters[15]. The latter is especially evident here. The K-irregularities of the partially deuterated species probably arise because the energies of the rotational levels involved are becoming comparable with those of levels in the first excited vibrational state, with possibilities of Fermi resonance.

From first order Stark splittings of the lines $K = 2$ and 3 of the transition $J = 4 \rightarrow 5$ a value of 2·15 ± 0·10 D was obtained for the dipole moment.

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New Metal-rich Phosphides of Niobium, Tantalum and Tungsten

PREVIOUS investigations[1–5] of the niobium–phosphorus and tantalum–phosphorus systems indicate that only two intermediate phases occur in each system, a monophosphide (which shows a varying amount of stacking disorder in the structure) and a diphosphide. These results are based on investigations of the products formed when the metals react with phosphorus at temperatures below 1,100° C. In the tungsten–phosphorus system, the occurrence of tungsten monophosphide and a high and a low temperature form of tungsten diphosphide has been observed[6–8]. No further intermediate phases appear to be formed by direct reaction between tungsten and phosphorus below 1,100° C. It has been reported, however, that tungsten phosphides containing less than 50 atom per cent phosphorus can be prepared by fused salt electrolysis. Thus, Hsu *et al.*[10] obtained a crystalline compound of composition W_3P by electrolysis of a fused sodium metaphosphate bath containing tungsten trioxide and sodium chloride at temperatures below 900° C. This compound was described as having the body-centred tetragonal iron triphosphide-type structure with cell dimensions $a = 9{\cdot}890$ Å; $c = 4{\cdot}808$ Å.

I have started a re-investigation of these systems and have extended the studies to include temperatures much higher than those previously covered. Some preliminary results are quoted here.

In the first stages of the re-investigation, phosphide samples were prepared by heating mixtures of red phosphorus and the appropriate metals in evacuated and sealed silica tubes. X-ray powder analyses of these products are in general agreement with the earlier work, while some new features were observed relating to the stacking-disorder phenomena in the niobium monophosphide and tantalum monophosphide structures[5]. These new observations will be discussed in a forthcoming paper to be published elsewhere. In agreement with Hulliger's recent report[9], niobium diphosphide and tantalum diphosphide crystallize with the niobium diarsenide-type structure. Tungsten diphosphide, originally reported to crystallize with molybdenum diphosphide-type structure[7], has recently been obtained with the niobium diarsenide-type structure[9]. In the present investigation the occurrence of both forms of tungsten diphosphide was confirmed; the molybdenum diphosphide-type phase forms when the reaction temperature exceeds about 700° C, while the niobium diarsenide-type occurs at lower temperatures. Heat-treatment of the high-temperature form at 600° C for two days did not yield any detectable amounts of the low-temperature form, which shows that the transformation is very sluggish. The two phases have the same ideal crystallographic formula, but small deviations from the ideal composition for one or both cannot be excluded.

In preliminary attempts to explore the systems at temperatures above 1,100° C, phosphide samples initially prepared in silica tubes were heated in an argon-arc furnace. The phosphorus losses on rapid heating were found to be surprisingly small for samples containing less than 50 atom per cent phosphorus, and X-ray examination of the products indicated that several new intermediate phases had been formed.

Rapidly arc-melted tungsten–phosphorus samples invariably consisted of three phases: α-tungsten, tungsten monophosphide and a further phase. The diffraction lines from this phase can be indexed on the basis of a body-centred tetragonal unit cell having dimensions $a = 9{\cdot}858$ Å; $c = 4{\cdot}800$ Å. Comparison with the diffraction pattern of Mo_3P (α-Zr_3S-type structure[11]) indicates a very close structural resemblance, and it seems highly probable that the two compounds are isostructural. The composition W_3P is therefore assigned to the new tungsten phosphide. It appears likely that this phase is identical with the W_3P phase obtained by Hsu *et al.* The unit cell dimensions agree fairly well and in the absence of single-crystal data it is very difficult to make an unambiguous distinction between the Fe_3P- and the α-V_3S-type structures, as explained in refs. 7 and 11.

The powder patterns of the arc-melted niobium–phosphorus and tantalum–phosphorus alloys showed greater complexity than those of the tungsten–phosphorus alloys. At about 25 atom per cent phosphorus, phases crystallize with the Ti_3P-type structure and accordingly seem to be Nb_3P and Ta_3P. These compounds are probably the most metal-rich in their respective systems. More phosphorus-rich preparations yielded very line-rich diffraction patterns (particularly in the case of the niobium phosphides) which could not be easily interpreted. However, well-developed single crystals were often formed in the alloys, and extensive X-ray investigations using single-crystal techniques have therefore been started. So far, a tantalum phosphide with the composition Ta_5P_4 has been characterized crystallographically by a combination of single-crystal and powder methods. The unit cell is orthorhombic and contains four formula units. The space group is most probably $P\,n\,n\,m$ and the intensity data indicate strongly that all atoms occupy $4g$ positions in this space group.

Since the technique of arc-melting produces alloys which are frequently grossly inhomogeneous, a phase-analysis of arc-melted alloys cannot be expected to furnish reliable information about the phase-relationships at high temperatures. A series of high-temperature annealing treatments under well-defined conditions has therefore been started. Detailed reports of the phase-analytical as well as the X-ray crystallographic data obtained will be published elsewhere. The crystallographic data obtained in the present preliminary investigation are collected in Table 1.

In view of the present results it might be asked whether the new phases obtained by arc-melting are in fact thermodynamically unstable at lower temperatures or whether other factors, such as activation energy barriers, prevent their formation below 1,100° C. A conclusive answer to this question cannot be reached in the absence of quantitative thermodynamic information. However, there are strong reasons to believe that W_3P is truly unstable at lower temperature. While this phase can

Table 1. CRYSTALLOGRAPHIC DATA ON NIOBIUM, TANTALUM AND TUNGSTEN PHOSPHIDES

Phosphide	Structure type	a (Å)	b (Å)	c (Å)	$\beta°$
Nb_3P	Ti_3P	10·128		5 091	
NbP	NbAs	3·334		11·377	
NbP_2	$NbAs_2$	8·8715	3·2663	7 5194	119·097°
Ta_3P	Ti_3P	10 154		5 012	
Ta_4P_4	(orthorhombic)	14 420	11·547	3·400	
TaP	NbAs	3·319		11 341	
TaP_2	$NbAs_2$	8·8608	3 2677	7 4882	119·308°
W_3P	α-V_2S (?)	9 858		4·800	
WP	MnP	5 732	3 249	6 222	
α-WP_2	$NbAs_2$	8 5022	3·1695	7 4660	119 367°
β-WP_2	MoP_2	3·166	11 161	4·973	

The unit cell dimensions were determined in Guinier-Hägg type focusing cameras using copper $K\alpha_1$ radiation with silicon ($a = 5{\cdot}4305$ Å) as the internal calibration standard.

For NbP_2, TaP_2 and α-WP_2 a least-squares refinement of the powder diffraction data indicated standard deviations for a and c of 0 0007 Å, for b 0 0002 Å and for β 0 005°. The relative accuracy of the unit cell dimensions for the remaining phases is estimated to be higher than 0 03 per cent.

obviously be retained from the high-temperature range owing to the rapid cooling process in the arc furnace, it disappears completely from W_3P-containing samples on annealing at temperatures between 800° and 1,000° C, and the only remaining phases are tungsten and tungsten monophosphide. Hsu *et al.* reported that their W_3P phase was unstable above 900° C. This suggests that the energetic conditions prevailing during electrolysis permit the formation of metastable W_3P, which can then be recovered only at moderately high temperatures without extensive decomposition.

The Nb_3P and Ta_3P phases remain unchanged after prolonged heat-treatment at 1,000° C, and it therefore appears likely that their formation at low temperatures is prevented by reaction kinetic factors only.

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Photochemistry of the *S*-Nitroso Derivatives of Hexane-1-thiol and Hexane-1,6-dithiol

S-NITROSOHEXANE-1-THIOL, $CH_3(CH_2)_5SNO$, and di-(*S*-nitroso-)hexane-1,6-dithiol, $ONS(CH_2)_6SNO$, were prepared by the method reported by Saville[1]. Their electronic spectra in hexane solution were measured and were found to be similar to the spectrum of *S*-nitrosotoluene-α-thiol[2]. The relevant data are presented in Table 1.

Table 1. ELECTRONIC SPECTRA OF THE *S*-NITROSO DERIVATIVES OF THIOLS

Thiol	λ_{max} (Å)	ε_{max} (M^{-1} cm^{-1})	λ_{max} (Å)	ε_{max} (M^{-1} cm^{-1})
Toluene-α-thiol	5600	26	3400	1030
Hexane-1-thiol	5500	21	3400	940
Hexane-1,6-dithiol	5500	42	3400	1830
Assignment to transitions.	$n \longrightarrow \pi^*$		$n_0 \longrightarrow \pi^*$	

As can be seen from the data in Table 1, the ε values for the di- and mono-*S*-nitrosated hexane thiols bear a ratio to each other of very nearly two as would be expected from their structures.

Irradiation at 3650 Å of the mono compound in methanol solution causes photolysis according to the stoichiometry:

$$2RSNO \xrightarrow{h\nu} RSSR + 2NO \quad (R = \text{hexane})$$

The reaction mechanism is similar to that reported for the photolysis of *S*-nitrosotoluene-α-thiol[2].

Irradiation of the di-nitrosated compound at 3650 Å caused photolysis, and the products were a wax-like solid and nitric oxide. The solid product had a melting point of 35° C and a molecular weight (Rast's method) of 285. Elemental analysis was consistent with the empirical formula $(CH_2)_6S_2$. From these data it would appear that the solid product was largely 1,2,9,10, tetrathia*cyclo*-hexadecane.

The mechanism of the photolysis probably includes the following steps:

$$ONS(CH_2)_6SNO \xrightarrow{h\nu} ONS(CH_2)_6S\cdot + NO$$

$$ONS(CH_2)_6S\cdot + ONS(CH_2)_6SNO \rightarrow ONS(CH_2)_6S_2(CH_2)_6SNO + NO$$

$$2ONS(CH_2)_6S\cdot \rightarrow S(CH_2)_6S_2(CH_2)_6S$$

$$ONS(CH_2)_6S_2(CH_2)_6SNO \xrightarrow{h\nu} ONS(CH_2)_6S_2(CH_2)_6S\cdot + NO$$

$$ONS(CH_2)_6S_2(CH_2)_6S\cdot \rightarrow S(CH_2)_6S_2(CH_2)_6S + NO$$

Work is proceeding on this and similar systems in order to elucidate a general mechanism for the photolysis of compounds containing two SNO groups.

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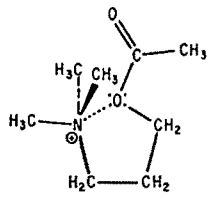
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Effects of Solvents on Acetylcholine Conformation

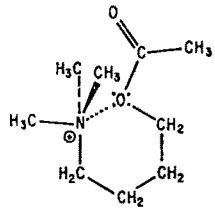
IN earlier communications[1-3] we reported the results of our investigations with acetylcholine and related substances. These led us to conclude that the acetyl group is activated primarily by an inductive influence of the quaternary nitrogen. This conclusion was based in large measure on the carbonyl stretching frequencies which were observed for acetylcholine and its homologues in ethanol, as well as on their rates of hydroxylaminolysis in water.

In a recent communication, Canepa and Mooney[4] challenged these data and conclusions. They reported the carbonyl stretching frequencies for acetylcholine in water and ethanol and called attention to the well-known influence of the solvent on the carbonyl stretching frequency. They suggested that the solvent hydrogen-bonding to the carbonyl group could explain the observed difference in the energies of absorption between acetylcholine and ethylacetate and, more particularly, they dismissed the possibility of an inductive onium effect. In a subsequent paper Canepa[5] proposed that the conformation of acetylcholine in the crystal and in water involved an interaction between the quaternary nitrogen and the unshared electrons on the ester oxygen. Although no data are offered which support his conclusions for the behaviour of acetylcholine in aqueous solution, he insisted that the bond angles encountered in the crystal allow an interaction between the positive-charged onium nitrogen and the ester oxygen.

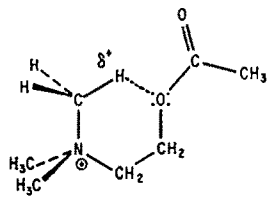
We have undertaken to examine some of the points raised by these publications and wish to report the results of our investigations of acetylcholine and related homo-



1



2



3

Figs 1–3

logues and derivatives in a number of solvents which support our conclusions of an inductive influence by the quaternary nitrogen on the carbonyl stretching frequency and which can account for the "active" acyl character of acetylcholine.

The sources, or the synthesis, of all the compounds examined have been reported in our previous publications[1,2] with the exception of *N*-acetoxytrimethylammonium bromide. This substance was prepared by the method of Geiger[6]. The determinations of the infra-red spectra were carried out using a Perkin–Elmer '221-*G*' spectrophotometer programmed for maximum resolution. Attenuated total reflectance (ATR) spectra were determined using a Perkin–Elmer ATR accessory with a KRS-crystal. Nuclear magnetic resonance studies were carried out with a 'Varian' spectrometer at 60 Mc/s in deuterium oxide.

Table 1. INFRA-RED SPECTRA BY TRANSMISSION

	DMSO ($\nu_{C=O}$)	Ethanol ($\nu_{C=O}$)
$CH_3-N^{\oplus}(CH_3)_2-O-C(=O)-CH_3, Br^{\ominus}$	1777 cm^{-1}	1783 cm^{-1}
$CH_3-N^{\oplus}(CH_3)_2-CH_2-O-C(=O)-CH_3, Cl^{\ominus}$	1772	1780
$CH_3-N^{\oplus}(CH_3)_2-CH_2-CH_2-O-C(=O)-CH_3, Cl^{\ominus}$	1744	1760
$CH_3-N^{\oplus}(CH_3)_2-CH_2-CH_2-CH_2-O-C(=O)-CH_3, I^{\ominus}$	1737	1752, 1732
$CH_3-N^{\oplus}(CH_3)_2-CH_2-CH_2-CH_2-CH_2-O-C(=O)-CH_3, I^{\ominus}$	1734	1748, 1728
$N(CH_3)_2-CH_2-CH_2-O-C(=O)-CH_3$	1733	1748, 1728
$CH_3-CH_2-O-C(=O)-CH_3$	1733	1748, 1728

The data given in Table 1 report the carbonyl stretching frequencies in dimethylsulphoxide for a number of esters related to acetylcholine. These results show quite clearly that, in a solvent with which no hydrogen bonding can take place, there exists a relationship between the stretching frequency for the carbonyl group and the position of quaternary ammonium group.

This conclusion is supported by results from experiments in which deuterium-labelled dimethylsulphoxide was used as a solvent. No shift was observed in the carbonyl absorption spectra of acetylcholine and its homologues, indicating no solvent proton interaction with this functional group of the molecule.

The closer the onium group is to the carbonyl group, the higher is the observed energy. More particularly there is an evident impact of the quaternary nitrogen in acetylcholine when one compares its carbonyl spectrum with that of dimethylaminoethylacetate.

Furthermore, this result firmly argues against an interaction between the quaternary nitrogen and the ester oxygen in that one would expect a greater effect on the carbonyl stretching frequency of 3-dimethylaminopropylacetate methiodide and even 4-dimethylaminobutyl acetate methiodide since the bond angles would allow an even closer approach than in acetylcholine (see Figs. 1 and 2).

The values for the $\nu_{C=O}$ of acetylcholine, dimethylaminoethylacetate, and ethylacetate in DMSO, and DMSO–water, ethanol, and ethanol–water are recorded in Table 2. It is evident from these data that, while a solvent effect may be readily observed, the $\nu_{C=O}$ for acetylcholine is clearly greater than the related dimethylaminoethylacetate. For example, in 50 per cent DMSO the $\nu_{C=O}$ for acetylcholine is 1,733 cm^{-1} and only 1,723 cm^{-1} for dimethylaminoethylacetate.

A more dramatic demonstration of the impact of the onium nitrogen on the carbonyl stretching frequency is shown in Table 3. The addition of hydrochloric acid to dimethylaminoacetate to neutralize the solution shifts the $\nu_{C=O}$ toward that of acetylcholine; making the solution somewhat acid increased this effect. Surely it is reasonable to assume that the proton reacts with the unpaired electrons on the nitrogen (it being the stronger base). The resultant onium character of the nitrogen behaves like the quaternary nitrogen in acetylcholine and influences the carbonyl stretching frequency in a like manner.

Table 3. EFFECT OF *p*H ON $\nu_{C=O}$

$CH_3-N(CH_3)_2-CH_2-CH_2-O-C(=O)-CH_3$	In 50% Ethanol	1723 cm^{-1}
	pH 7 - neutralized with HCl	1731 "
	At pH 2 with HCl	1735 "

There remains the remote possibility of a conformational interaction between the unshared electron pair on the ester oxygen and a proton from one of the methyl groups on the quaternary nitrogen (Fig. 3). The inductive influence of the onium nitrogen is marked and is evidenced by the shift in the infra-red C–H stretching frequency to 3,008–3,010 cm^{-1}.

An attempt was made to evaluate the contribution of this species to the conformation of acetylcholine in solution by examining the nuclear magnetic resonance spectra of this substance and related compounds. In short, no evidence for such interaction was observed. The methyl protons on the onium nitrogen exhibit a sharp absorption band at 6·8 τ like choline chloride and tetramethylammonium iodide. (We thank Dr. L. Dolby and C. Wilkins for carrying out these observations.)

Other evidence, which challenges the hypotheses of interaction between the unshared pairs of electrons on the

Table 2. INFRA-RED SPECTRA BY ATR

	DMSO ($\nu_{C=O}$)			Ethanol ($\nu_{C=O}$)		
	100%	80%	50%	100%	50%	H_2O
$CH_3-N^{\oplus}(CH_3)_2-CH_2-CH_2-O-C(=O)-CH_3, Cl^{\ominus}$	1742 cm^{-1}	1742 cm^{-1}	1733 cm^{-1}	1756 cm^{-1}	1737 cm^{-1}	1727 cm^{-1}
$N(CH_3)_2-CH_2-CH_2-O-C(=O)-CH_3$	1730	1729	1723	1744, 1727	1723	
$CH_3-CH_2-O-C(=O)-CH_3$	1730	1730		1744, 1727		

ester oxygen and the onium nitrogen, emerges from reactions involving carbonium ion intermediates β to a hydroxy group. These carbonium ions show no stabilization from a neighbouring hydroxyl group and these experiments argue against interaction of the type proposed[7].

Finally, in investigations of a cyclic conformation for choline sulphate, nuclear magnetic resonance studies revealed no methyl absorption splitting. This would have been expected from the restricted rotation of two of three methyl groups on the onium nitrogen. The single absorption for the methyl group in deuterium oxide and 90 per cent CD_3OD, 10 per cent deuterium oxide ruled out electrostatic interaction between the sulphate and the quaternary nitrogen. The fact that this more highly favoured six-membered ring conformation does not exist in solution emphasizes the unlikelihood of any interaction between the quaternary nitrogen and the ester oxygen[8].

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BIOCHEMISTRY

Solubility-composition Relations of Gluten Proteins

IT is thought that the tertiary structure of a globular protein in aqueous solution is quite largely conditioned by the interactions between the amino-acid residues and water. In general, the expected result is a folding of the polypeptide chain(s) such that the charged residues are at the surface of the molecule while the uncharged residues are packed in the central regions of the molecule. The structure is stabilized by hydrophobic interactions and intra-chain hydrogen bonding in the interior, and at the surface by hydrogen bonding with water[1]. The ability of this theory to account for the behaviour of some globular proteins has led to attempts to derive relationships between composition and structure that may have predictive value. Two examples are Fisher's "law"[2] and Hatch's[3] polar ratio. In 1908, proteins were classified by British and American committees according to their solubility[4]; there may therefore be a relation between this classification and the predictions obtained by applying the suggestions of Fisher and Hatch. Glutenin and gliadin, the exemplars of two of the nine solubility classes of simple proteins, were used in an attempt to predict their properties in solution from their amino-acid composition.

Fisher's limiting law has the basic proposition that polar residues have a very limited solubility in the interior of a soluble protein molecule and that non-polar residues have a very limited solubility in the outer shell of the molecule. For spherical molecules with a polar outer layer of constant thickness (4 Å), the volume of polar residues in the surface will be proportional to the square of the diameter, while that of the non-polar core will increase as the cube. Thus the ratio p between the volume of the external (polar) shell and the internal (non-polar) core will fall sharply with increased diameter. The ratio p has been calculated for some thirty proteins by Fisher and he has shown that the p values are usually greater than the limiting value p_s for a sphere of appropriate dimensions. In those instances in which p is appreciably less than p_s the proteins are known to polymerize readily, presumably as a result of hydrophobic interaction between exposed non-polar portions of their cores.

Fisher's method of calculation applied to the amino-acid compositions reported by Wu and Dimler[5] for gliadin and glutenin gives values for p of 0·195 and 0·25. Corresponding p_s values are given by ideal spherical proteins of molecular weight 2·05 million and 1·1 million. It would therefore be predicted that gliadin would only be stable in solution as a spherical molecule with a molecular weight of at least two million while glutenin could exist in solution as stable spherical molecules of half this weight. In fact, the molecular weight of gliadin is in the range of 40–50,000 (ref. 6) and it behaves in solution as a reversibly associating system[7]; glutenin appears to be heterogeneous with a particle size of 1–4 million. Viscosity data[8] indicate that at low ionic strengths it is stable in an expanded form which would require $p \gg p_s$ (p_s for $1 \times 10^6 = 0{\cdot}025$; for $4 \times 10^6 = 0{\cdot}15$).

Table 1. HATCH POLAR–APOLAR RATIO

Haemoglobin (human)	1·03 (extreme)
Lactic dehydrogenase	1·16
Structural protein (mitochondria)	1·18
Gliadin	1·21
Median of forty-eight proteins	1·45
Glutenin	1 47
Cytochrome *c* (horse)	2·09
Tropomyosin	2 99 (extreme)
Ratio if glutamine and asparagine residues are apolar	
Gliadin	0·17
Glutenin	0·33
Ratio if glutamine and asparagine residues are ambivalent	
Gliadin	0 33
Glutenin	0·61

Recently, Hatch[3] has calculated a polar : non-polar ratio for forty-eight proteins with "no obvious peculiarities of amino-acid composition, such as occur in collagen, for example". Whereas Fisher (following Nemethy and Scheraga[9]) regards glutamine residues as non-polar, Hatch includes them in his polar count. (If this is done in applying Fisher's law a minimum molecular weight for glutenin of 16,000 is found—which would require a remarkably high surface–volume ratio for stability at its observed molecular weight of several million.) The position of gliadin and glutenin in relation to the extremes and median of Hatch's values for forty-eight proteins is shown in Table 1. It will be seen that Hatch's ratio would not indicate any unusual properties of gliadin and glutenin either in solubility or other properties listed in his paper. Nevertheless, the proteins are unusual enough in their solubility properties to have been regarded as types of solubility classes. It is true that their compositions have "obvious peculiarities" (one-third glutamine, one-sixth proline) and perhaps this is one reason for the relative failure of Hatch's ratio as compared with Fisher's law in predicting their properties. If Nemethy and Scheraga[9] are followed in considering glutamine and asparagine residues essentially non-polar, ratios of 0·17 and 0·33 for gliadin and glutenin are found. (If they are placed in Hatch's class of ambivalent residues together with histidine, tyrosine and tryptophan the ratios are 0·33 and 0·61.) These values seem more in accord with the general character of proteins with low Hatch ratios.

The greater success of Fisher's law, when applied to gliadin and glutenin, than of Hatch's ratio suggests that glutamine residues should not be regarded as having any polar attributes that would enforce their presence at the protein surface. The greater effort of computation necessary in applying Fisher's law appears worthwhile, although in its application it may be necessary to assume that a local preponderance of non-polar residues in a polypeptide chain may enforce their presence at the

surface, thus giving an opportunity for hydrophobic interaction in molecules that otherwise appear able to adopt a purely polar exterior conformation. Future attempts to relate protein composition with protein properties in solution should consider those classes of proteins with more extreme solubility properties and include the seed proteins and the protamines and histones.

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Facilitated Diffusion in Mouse and Rat Erythrocytes

THE discussion of the effect of narcotics on the permeability of erythrocytes which appears in some text-books[1-3] is based on a report by Jacobs and Parpart[4]. These authors showed that *n*-butyl alcohol decreased the permeability of erythrocytes of certain species to glycerol and increased the permeability to glycerol in the erythrocytes of other species. In all cases reported, butanol increased the permeability to thiourea. Davson suggests in several of his writings (for example, ref. 2) that narcotics decrease the permeability "in certain special cases of erythrocyte permeability . . . [of] . . . those substances that apparently show an unusually rapid rate of penetration". Similar results were reported by Hunter *et al.*[5], and it was suggested that a carrier mechanism, or facilitated diffusion, was involved in these "special cases".

These data might suggest that glycerol crosses the membrane of some erythrocytes by facilitated diffusion and crosses others by simple diffusion but that thiourea always crosses by simple diffusion. That this might not always be the case is suggested by the results of Jacobs *et al.*[6]. They reported a series of quite obvious differences between the permeability of the red cells of rats and mice. One of these differences was the rather rapid rate of penetration of thiourea into mouse cells as compared with rat cells. Since these data suggest that the thiourea permeability of mouse erythrocytes might be another example of a "special case", the present experiments were performed to check this point.

Rat (*Rattus rattus* and *R. norvegicus*) and mouse (*Mus musculus*) blood were obtained by decapitation, the blood being collected in a large volume of isotonic, phosphate-buffered sodium chloride solution without anticoagulant. The cells were washed three times with this solution. Permeability measurements were made using a densitometer[7]. A portion of mouse cells was added to 0·3 M thiourea in water with and without butanol and the rate of haemolysis was measured at 0° C. Since the perm[illegible] of rat cells to thiourea is considerably lower, it w[illegible] to use volume-change measurements with [illegible] They were equilibrated in a solution of 0·[illegible] buffered, isotonic sodium chlorid[illegible] measured by adding a [illegible]

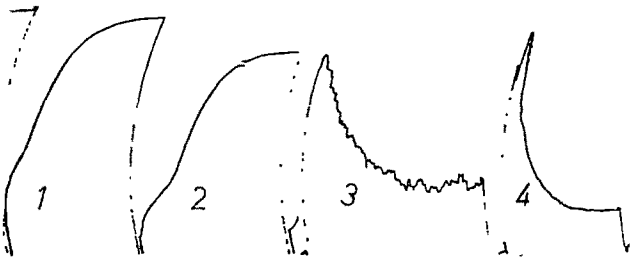


Fig. 1. Effect of butanol on the permeability of mouse and rat erythrocytes to thiourea. 1, Haemolysis of mouse cells in 0·3 M thiourea. 2, Haemolysis of mouse cells in 0·1 M butanol in 0·3 M thiourea. 3, Shrinking of rat cells, previously equilibrated in thiourea, in sodium chloride solution. 4, Shrinking of rat cells, previously equilibrated in thiourea, in 0·2 M butanol in sodium chloride solution

rat cells to thiourea in the presence of butanol has already been reported[4]. The decrease in the permeability of mouse cells to thiourea in the presence of butanol has not been reported, but is what would be expected if the permeability of these cells to this non-electrolyte represents another example of a "special case" of permeability.

Other data included by Jacobs *et al.*[6] suggest that in addition to glycerol[4], ethylene glycol and urea permeability in rat cells represent additional "special cases". To test this point the effect of butanol on the permeability of these cells to the three non-electrolytes was also investigated. Concentrations of 0·1 and 0·2 M butanol were investigated. Glycerol permeability was measured using the shrinking technique at 30° C. Ethylene glycol permeability was measured at 0° C by both the shrinking and haemolysis techniques, while haemolysis measurements at 0° C were used to investigate the permeability to urea. Relative times were calculated by dividing half the total time for the experimental curve by half the total time for the control curve. Thus, a relative time greater than unity indicates a decrease in permeability, while a relative time less than unity indicates an increase. The following relative times were obtained: glycerol, 6·3; ethylene glycol, shrinking, 3·7; haemolysis, 2·4; and urea, 1·4.

These data indicate that the permeability of at least mouse erythrocytes to thiourea is decreased by butanol just as this substance decreases the permeability of red cells of several species to glycerol. In addition, butanol decreases the permeability of rat cells to glycerol, ethylene glycol and urea. According to the interpretation suggested in ref. 5, facilitated diffusion appears to be involved in these four cases. Thus, to Fig. 8 of ref. 5 a right-handed symbol (facilitated diffusion-butanol inhibition) should be added to the urea group for the rat, to the ethylene glycol group for the rat, to the thiourea group for the mouse and to the glycerol group for the rat.

The experiments were performed in the Biology Department, University of the Andes, Bogotá, Colombia. We thank the Rockefeller Foundation for a grant in support of this work.

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Sulphydryl Reagents and Rh Activity of Erythrocyte Stroma

IT has recently been shown that the Rh activity of lyophilized human Rh-positive stroma could be abolished with certain sulphydryl reagents, that this could be prevented by the concurrent addition of excess thiol, and that the loss was reversible with one such reagent[1]. It was not established whether the antigenic activity of non-lyophilized erythrocytes shows such an effect. The resolution of this problem was hampered in the case of intact cells by the presence of soluble sulphydryl systems, including haemoglobin and glutathione[2], and in the case of non-lyophilized stroma both by the occlusion of these soluble components, and by the difficulty of quantitation of wet stroma. The results of the present experiments suggest that the loss of Rh activity of lyophilized stroma brought about by sulphydryl reagents is equally true of stroma which has not been lyophilized.

Two methods of obviating occlusion were used. The first may be termed a *p*H shuttle, and consisted of washing the intact cells in 0·9 per cent sodium chloride, rupturing in distilled water, adjusting the *p*H under continuous monitoring to 8·3 with trisodium phosphate, then adding citric acid to *p*H 5·5, followed by centrifugation. The residue was then taken up in 15–20 times its volume of distilled water and the process repeated. At the higher *p*H the suspended phase assumed approximately colloidal dimensions and occluded material was released. Five or six such shuttle steps were required and it was noted that some loss of Rh activity resulted. A second method was then used which did not appear to result in loss of activity. Rapid freezing at the temperature of solid carbon dioxide was substituted for the *p*H shuttle, and the material was washed with distilled water. The freeze-thaw procedure was repeated twice.

Using stroma washed with the freeze-thaw method a fine uniform suspension was made up volumetrically in distilled water, a measured portion removed, lyophilized and weighed, and the relation between dry weight and volume calculated. Twenty mg of the lyophilized suspension was matched with the corresponding volume of non-lyophilized stroma. The samples were washed with 0·1 M sodium phosphate buffer (*p*H 7·0), centrifuged and treated with 10^{-4} M *p*-chloromercuribenzoate (Nutritional Biochemicals) in the same buffer for 60 min at 22° C with agitation. A similar set was treated with phosphate buffer only. The experiment was repeated using 3×10^{-4} M reagent. The specimens were then centrifuged, washed twice with phosphate buffer, and 1 or 2 ml. human isoimmune anti-D suitably diluted in normal human serum were added. The dilution was standardized so that 20 mg of untreated O Rh positive lyophilized stroma could just remove all anti-D activity after 60 min incubation at 37° C, and subsequent centrifugation. Similarly for the present experiments the supernatants after centrifugation were studied for residual anti-D activity against trypsinized O positive erythrocytes, as previously described[1]. The results are summarized in Table 1. The partial loss of activity with the lower concentration of *p*-chloromercuribenzoate and the complete loss with the higher concentration were indistinguishable from non-lyophilized stroma. In each case it was noted when 0·1 M [illegible] (Nutritional Organic) was present [illegible] results of similar [illegible] (Nutritional [illegible]

Table 1. RH ACTIVITY AND *p*-CHLOROMERCURIBENZOATE

O positive stroma	Treated with 5 ml.	Resulting loss of Rh activity
Non-lyophilized	Phosphate buffer, *p*H 7	None
Non-lyophilized	10^{-4} M PCMB in phosphate	Partial
Non-lyophilized	3×10^{-4} M PCMB in phosphate	Complete
Non-lyophilized	3×10^{-4} M PCMB and 0·1 M 2-ME* in phosphate	None
Lyophilized	Phosphate buffer	None
Lyophilized	10^{-4} M PCMB in phosphate	Partial
Lyophilized	3×10^{-4} M PCMB in phosphate	Complete
Lyophilized	3×10^{-4} M PCMB and 0·1 M 2-ME in phosphate	None

Twenty-mg specimens were all from the same donor, and were incubated at 22° C for 60 min with agitation. After washing twice with phosphate, anti-D serum was added and incubated for 60 min at 37° C. After centrifugation, the supernatant was studied for residual anti-D activity.
* 2-mercaptoethanol.

Table 2. RH ACTIVITY AND *N*-ETHYLMALEIMIDE

O positive stroma	Treated with 5 ml.	Resulting loss of Rh activity
Non-lyophilized	Phosphate buffer	None
Non-lyophilized	10^{-3} M NEM in phosphate (at 22°)	Partial
Non-lyophilized	10^{-3} M NEM in phosphate (at 37°)	Complete
Non-lyophilized	10^{-3} M NEM and 0·1 M 2-ME in phosphate (at 37°)	None
Lyophilized	Phosphate buffer	None
Lyophilized	10^{-3} M NEM in phosphate (at 22°)	Partial
Lyophilized	10^{-3} M NEM in phosphate (at 37°)	Complete
Lyophilized	10^{-3} M NEM and 0·1 M 2-ME in phosphate (at 37°)	None

Procedure as in Table 1 with the indicated incubation temperatures.

on the lyophilization process itself. These results show that such sensitivity does not depend on lyophilization. Freezing of intact erythrocytes without drying is not associated with significant disruption under certain circumstances[4] and it would not be anticipated that freezing and thawing of stroma would result in changes comparable to lyophilization. The present investigation does not show whether the Rh antigen of the intact erythrocyte is capable of reacting with sulphydryl reagents.

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Copper Transport in Mammalian Tissues

RECENTLY, Neumann and Silverberg[1] presented results of experiments which were very similar to those which we had briefly reported earlier[2,3]. They concluded that amino-acids facilitate the transport of copper at the cellular level and presented conclusive evidence to support this. They further concluded that the facilitation of copper transport by amino-acids fulfils the criteria of an "active transport system", as it is dependent on temperature, concentration and oxygen, and operates against a chemical concentration gradient.

[illegible] believe that the latter conclusion cannot be based [illegible] evidence presented by Neumann and Silverberg. [illegible] two criteria, dependence on temperature and [illegible]ion, apply equally to the processes of simple [illegible]ion. Dependence on oxygen may [illegible]mical energy—but this is [illegible]en—the operation of

greater than 1·0 between the c.p.m./mg of tissue and the c.p.m./mg of medium during the incubation may simply be the result of a larger number of avid binding sites for copper in a unit weight of the slice as compared with the number in the unit weight of the tissue. Thus the possibility that the transfer of copper through the cell membrane occurs by diffusion or facilitated diffusion cannot be excluded on the basis of the results presented by Neumann and Silverberg.

In a paper quoted by Neumann and Silverberg, Ussing[4] states emphatically that the "definition (of active transport) excludes cases where a substance is concentrated in a cell by being bound to some cellular constituent as is the case with many dyes". He rightly regards it essential that the concentration of free, unbound ions should be known before one accepts that the maintenance of a chemical concentration gradient is meaningful in terms of the designation of a transport mechanism. Neumann and Silverberg did not measure the concentration of free Cu^{++} in the medium or the intracellular compartment.

It is for these reasons that we do not regard Neumann and Silverberg's claim that facilitation of copper transport by amino-acids fulfils the criteria of an "active transport" as having been proved. Their data and the results of similar experiments which we carried out do not exclude this possibility, but neither they nor we have as yet experimental proof of an active transport of copper.

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Adsorption of Lactate Dehydrogenase to the Particulate Fraction of Homogenized Skeletal Muscle

WE have recently conducted an investigation of the factors involved in the release or retention of lactate dehydrogenase (LDH) from the particulate fraction of chicken breast muscle[1]. By appropriate procedures, we could effect essentially a complete release or retention. The major factors were the *p*H and ionic strength of the homogenization media; high *p*H and high ionic strength caused a greater solubilization of the enzyme. The influence of other factors could be explained in terms of their effect on *p*H and ionic strength. Adjusting conditions for the retention of LDH activity in the particulate fraction does not, however, establish that this enzyme occurs in a particulate or bound form *in situ*. Other possibilities such as co-precipitation, adsorption, and physical entrapment must be considered.

We had shown earlier that co-precipitation was not an important factor in the removal of LDH from the soluble fraction[1]. This was accomplished by creating in the absence of any particulate material the same conditions that allow extensive retention of LDH in the particulate fraction of a muscle homogenate after centrifug

Two points argue against any extensive co
of entrapment as an explanation for the partic
of LDH in crude homogenates. These are the
ness of recovery of LDH in the particulate fra
centrifuged homogenate which can easily be

Initially, it appeared that adsorption of soluble LDH to the particulate fraction during homogenization was extremely unlikely. We based this on two criteria. One was the almost complete association of LDH activity in the particulate fraction that can be obtained under certain conditions and the other was our observation that there was increased retention in the particulate fraction with a decrease in concentration of tissue[1]. It was necessary, however, to examine these phenomena more closely because of the sensitivity to *p*H and ionic strength of the distribution of the LDH between soluble and non-soluble components. Essentially, what we attempted to do was to mix solubilized LDH with particulate fractions of muscle from which the LDH had been removed under conditions that would duplicate as closely as possible those occurring when muscle is homogenized. We were interested in seeing whether we could get re-adsorption, whether the extent of re-adsorption would be sufficient to account for what we observe in homogenized muscle, and whether re-adsorption follows the same type of binding pattern, that is, increases with decreasing tissue concentration, as in homogenized muscle. In Table 1 the percentage re-adsorption of solubilized LDH to the particulate fraction of muscle is shown as a function of tissue concentration. The extent of rebinding is sufficient to account for the amount of LDH associated with the particulate fraction when muscle is homogenized, and, further, the pattern of binding with tissue concentration is the same. In these experiments we kept the ratios of LDH activity, buffer concentration, and amount of particulates constant. This would mean that the concentration of buffer in the final homogenate would decrease with decreasing tissue concentration and would imply that the final buffer concentration in the homogenate is the determining factor in re-adsorption and not the ratio of buffer to LDH or binding sites on the non-soluble fraction of the muscle homogenate. The *p*H was maintained at 6·6, at which value essentially no LDH would be removed by precipitation[1], and it is similar to the *p*H of many samples of homogenized chicken breast muscle.

In an attempt to distinguish between an adsorption of soluble LDH during homogenization (which certainly appeared possible from the data of Table 1) and location of LDH with the muscle particulates *in situ*, experiments were carried out to determine the distribution of LDH in muscle cell homogenates by differential centrifugation in two situations. In one, an LDH-rich particulate fraction was prepared by homogenization in water and centrifugation, while in the other the LDH-rich particulate fraction was obtained by re-adsorption of solubilized LDH to muscle particulates from which the LDH had been previously removed by salt. All other conditions of the procedures were kept constant. Table 2 summarizes the data obtained in one experiment. Other experiments gave comparable results. No major differences were observed between the two procedures in the distribution patterns regarding LDH or protein. The LDH activities

Table 1. ADSORPTION OF LDH AS A FUNCTION OF TISSUE CONCENTRATION

Tissue concentration (per cent w/v)	LDH activity* (μmoles DPNH/min/g tissue) Supernatant	Residue	Total	LDH activity in supernatant fraction (per cent)
30	770	1,320	2,090	37
20	280	1,130	1,410	20
		100	2,135	1 6
			1,617	0·4
			1,854	0 2
			450	< 0 1

the 10 per cent (w/v)
740 μmoles DPNH
± 249.
sodium chloride.
ively dialysed

Table 2. DISTRIBUTION OF LDH IN VARIOUS SUBCELLULAR FRACTIONS FROM MUSCLE

Fraction	Specific activity of LDH (μmoles DPNH/min/mg protein)		Percentage of total LDH activity		Percentage of total protein	
	*A**	*B*†	*A*	*B*	*A*	*B*
Resuspended homogenate	6·7	5·7	—	—	—	—
S_1	3·0	3·0	4·4	5·9	10·9	12·0
R_1	6·9	5·8	34·6	39·5	36·0	41·0
R_2	8·1	6·9	46·4	36·2	41·0	31·0
R_3	7·9	4·6	6·2	7·5	5·6	10·0
R_4	3·4	3·8	0·3	0·3	0·6	0·6
S_4	11·8	14·7	7·7	5·9	4·8	4·4

* Sample *A* was prepared by homogenizing the muscle (10 per cent . w/v) in distilled water followed by centrifugation at 105,000*g* for 30 min. Almost all the LDH remains with the particulate fraction by this technique. The supernatant fraction was extensively dialysed against 15 mM histidine buffer, *p*H 7·0. Then enough solid sucrose was added to make it 0·88 M. The particulate fraction was then resuspended in this soluble fraction by homogenization in a Potter-type homogenizer. It was then centrifuged at 105,000*g* for 1 h. The supernatant fraction (S_1) was decanted. The residue was resuspended in 0·88 M sucrose, 15 mM histidine buffer, *p*H 7·0, and the following centrifugations were performed: 2,000*g* for 7 min (R_1); 15,000*g* for 10 min (R_2); 70,000*g* for 40 min (R_3); 105,000*g* for 60 min (R_4); the supernatant fraction from the final centrifugation was labelled S_4.

† Sample *B* was prepared by homogenization of a 10 per cent preparation in 150 mM sodium chloride followed by centrifugation at 105,000*g* for 1 h. This causes most of the LDH activity to go into the supernatant fraction. This fraction was dialysed extensively against 15 mM histidine, *p*H 7·0, and then enough sucrose was added to make it 0·88 M. The residue was washed twice by rehomogenization and centrifugation in 150 mM sodium chloride and 15 mM histidine, *p*H 7·0, respectively. It was then taken up in the dialysed supernatant fraction, rehomogenized and subjected to the same differential centrifugation as described for sample *A*.

LDH activity was determined by the procedure of Wu and Racker (ref. 6). Protein was determined by a biuret procedure (ref. 7).

The total LDH recovered was 78 per cent for both samples *A* and *B*. The protein recovered was 72 per cent in both cases.

of sample *B* were somewhat lower than those of *A*, which may possibly have been caused by the extended dialysis of the LDH of sample *B*. We conclude from this that the pattern of rebinding of soluble LDH to the non-soluble muscle fraction is similar to that obtained when the muscle is homogenized directly under conditions which lead to retention of the enzyme. In other words, not only the extent of retention but also the distribution of LDH among various subcellular fractions of muscle homogenized under conditions conducive to retention could possibly be attributed to adsorption of soluble enzyme to the particulate fraction after homogenization. The capacity of the muscle particulate fraction in binding LDH is considerably greater than the amount of LDH normally present in the tissue. We have prepared LDH-rich particulate fractions which have bound more than six times the quantity of LDH originally present.

We cannot say definitely at this time on the basis of our results whether LDH exists as a soluble cytoplasmic enzyme, is bound to subcellular fractions, or exists in an equilibrium between soluble and non-soluble phases. Several reports using a cytochemical approach have indicated that LDH is bound to the subcellular structures of muscle[2-4], and Amberson *et al.*[5] arrived at a similar conclusion by a different approach. In view of the high binding capacity of the particulate fraction of muscle, any investigation which purports to show the particulate nature of muscle LDH must take into account the possibility of adsorption of a soluble enzyme.

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[1] Hultin, H. O. [redacted] Table 1

L-Lysine Dehydrogenase Deficiency in a Patient with Congenital Lysine Intolerance

RECENTLY, a new metabolic defect, which was believed to arise from congenital lysine intolerance, was described in a 3-month-old baby girl[1]. An enzymatic impairment in the pathway for the degradation of lysine seemed the most probable explanation of the main finding, that is, high plasma lysine levels. Because the enzymes responsible for the metabolism of lysine in human beings had not previously been investigated, efforts were made to find an enzyme system capable of degrading lysine. This report presents evidence for the existence of a hitherto unknown enzyme, L-lysine: nicotinamide-adenine dinucleotide (NAD) - oxidoreductase (deaminating) (trivial name: L-lysine dehydrogenase) in human liver homogenates.

Fresh human liver was cut into small pieces, added to four parts of a 0·05 M *tris*-buffer (*p*H 8·8) and homogenized in a glass Potter homogenizer with a loose fitting glass pestle. The homogenate was centrifuged at 1,500*g* for 3 min and the clear supernatant used for enzymatic assay after dilution with nine parts of buffer. The standard incubation medium contained: 0·4 ml. 0·95 M L-lysine in 0·05 M *tris*-buffer (*p*H 8·8), 0·05 ml. of a 0·06 M NAD-solution, 0·02 ml. 0·05 M cysteine and 0·3 ml. of the 1 : 50 homogenate. The reagent blank contained buffer instead of substrate. After pre-incubation of the mixtures in a 37° C water bath for 10 min, the formation of $NADH_2$ was measured in an Eppendorf photometer at a wavelength of 366 nm at 37° C and recorded with a W+W recorder. Enzyme activities were calculated and expressed in μmoles lysine metabolized per minute. Experiments were also performed without cysteine at 25° C, as with increasing temperature the activity decreased.

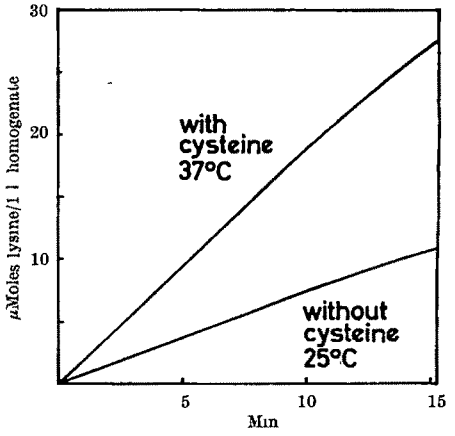


Fig. 1

As can be seen from Fig. 1, a linear relationship exists between the amount of lysine metabolized and time. The presence of cysteine results in a two- to three-fold increase in activity. These results strongly suggest that the proposed reaction[2,3]:

L-lysine + NAD + H_2O → α-keto-ε-amino caproic acid + $NADH_2$ + NH_3

is catalysed by a specific deaminating amino-acid de-[redacted]ase with NAD acting as hydrogen acceptor. [redacted]timal conditions had been established, liver [redacted] of the patient was assayed for lysine de-[redacted]e activity. The results are summarized in [redacted]d indicate a deficiency of L-lysine: NAD-

oxidoreductase. Because a defect of this enzyme results in clinical hyperlysinaemia it is suggested that in man the first step in lysine degradation is achieved by oxidative deamination.

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α-Glycerophosphate Dehydrogenase in Mouse Adrenal Cortex

IN the adrenal cortex of the mouse, non-NAD-linked α-glycerophosphate dehydrogenase can be demonstrated in the mitochondria and shows an interesting distribution which is different from that of the Krebs cycle enzymes.

Sections of adrenals from mature mice were cut and frozen-substituted using the method of Chang[1]. The distribution of mitochondrial non-NAD-linked α-glycerophosphate dehydrogenase was found by the histochemical method of Farber[2]. It was observed that this enzyme is confined to the zona glomerulosa of the adrenal gland (Fig. 1). Companion sections stained for succinate dehydrogenase activity showed the latter enzyme, and thus the activity of the Krebs cycle, to be more intense in the fasciculate and reticular zones and comparatively faint in the glomerular zone (Fig. 2).

It has been suggested that non-NAD-linked α-glycerophosphate dehydrogenase plays a part in the utilization of reduced NAD generated outside the mitochondria and that it is part of a system alternative to lactic dehydrogenase which maintains a high rate of glycolysis. It thus seems likely that the glomerular zone of the mouse adrenal cortex depends on glycolysis for a major part of its energy requirements compared with the fasciculate and reticular zones, which apparently depend more on the Krebs cycle activity as a source of energy.

It may be added here that the adipose tissue around the adrenal capsule also shows a high α-glycerophosphate dehydrogenase activity. A similar high enzyme activity has been reported by Farber[3] in the adipose tissue about the kidney.

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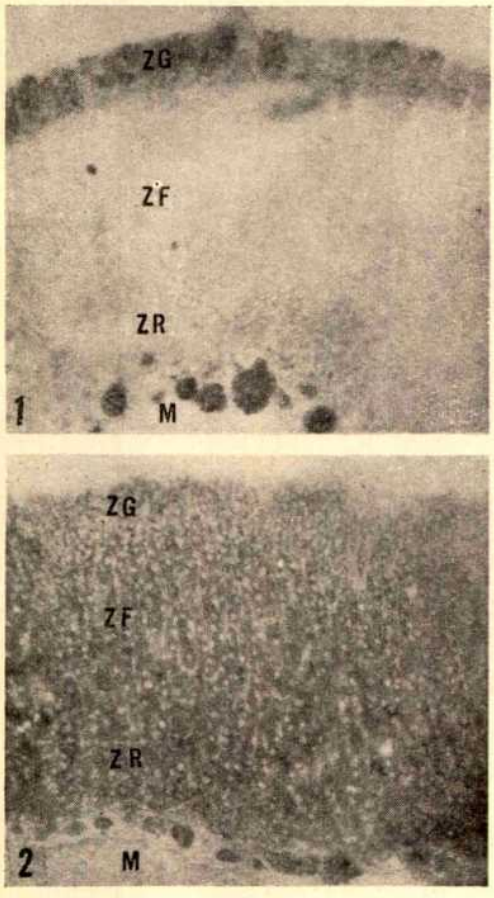


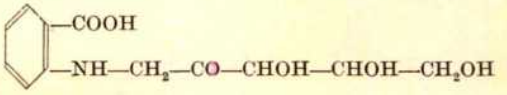
Fig. 1. Section through the adrenal cortex of mouse showing histochemical distribution of α-glycerophosphate dehydrogenase (×125). *ZG*, Zona glomerulosa; *ZF*, zona fasciculata; *ZR*, zona reticularis; *M*, medulla.

Fig. 2. Section through the adrenal cortex of mouse showing the histochemical distribution of succinate dehydrogenase (×125). Abbreviations as in Fig. 1.

Identification of 1-(o-Carboxyphenylamino)-1-deoxyribulose and Anthranilic Acid in Cultures of the Basidiomycete *Schizophyllum commune*

IN the course of investigation of indigo biosynthesis in mutant strains of *Schizophyllum commune*, these strains were found to produce a number of interesting compounds which were released into the medium. Both the pigmented strains (130 and 2456) and the non-pigmented strains (699 and 2417) were grown either in flasks on a shaker or in a carboy with aeration. A minimal medium was used containing in each litre of distilled water: 20 g glucose, 1·5 g $(NH_4)_2HPO_4$, 120 μg thiamine hydrochloride, 0·46 g KH_2PO_4, 1 g K_2HPO_4 and 0·5 g $MgSO_4.7H_2O$. After filtering through cheese cloth to remove the mycelium, the medium was brought to *p*H 4·0 with 0·1 N hydrochloric acid and extracted with an equal volume of ethyl acetate. A dark red material was obtained after drying the extract overnight over anhydrous sodium sulphate and concentrating it by distillation at 35° C under vacuum to about 3 ml.

Chromatography of the extract resulted in the detection of two unknown compounds which showed an intense blue fluorescence under ultra-violet light. Chromatography on Whatman No. 1 paper, using several solvents and location reagents (2 per cent dimethylaminobenzaldehyde (PABA) in 80 ml. acetone and 20 ml. hydrochloric acid, and dimethylaminocinnamaldehyde (DMCA) in 90 ml. acetone and 10 ml. hydrochloric acid, both applied by dipping) showed that one of the unknown compounds was identical to natural anthranilic acid (Table 1), while the results with the second unknown compound indicated it to be 1-(*o*-carboxyphenylamino)-1-deoxyribulose (CDR)* (Tables 2 and 3).



* Abbreviations used: CDRP, 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate; AA, anthranilic acid; PRA, *N*-(5′-phosphoribosyl) anthranilic acid; InGP, indolyl-3-glycerol phosphate; CDR, 1-(*o*-carboxyphenylamino)-1-deoxyribulose.

CDR was extracted from ethyl acetate with phosphate buffer at *p*H 7·7. Hydrolysis with 1 N sodium hydroxide at 100° C for 5 min[1] resulted in breakdown of CDR and appearance of anthranilic acid, due to cleavage of the molecule. This alkaline lability is expected from an Amadori compound[2].

The extract of *p*H 7·7 was also used in the preparation of 2,4-dinitrophenylhydrazone of CDR. Addition of 0·5 per cent 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid to the buffer extract resulted in the formation of an orange precipitate after standing overnight at room temperature. The ethanol-washed precipitate was used for ultra-violet absorption spectroscopy. At *p*H 7·7, the absorption spectrum (taken with a 'Beckman *DB*' spectrophotometer) was similar to those obtained from a hydrazone derivative of a synthetic CDR by Doy and Gibson[3] (Fig. 1).

Even though synthetic CDR was not available in this experiment, the colour reactions (especially with alkaline 2,3,5-triphenyl tetrazolium chloride), the behaviour during alkaline hydrolysis and the ultra-violet absorption spectrum clearly show that CDR is produced by pigment-producing strains of *S. commune*. Neither CDR nor anthranilic acid was found in strains that lacked the pigment. CDR was suggested by Yanofsky[4] as a possible intermediate in the tryptophan pathway and was later identified in a culture medium of mutant strains of *Aerobacter aerogenes*, *Escherichia coli*[3,5] and *Salmonella typhimurium*[5], all blocked at various stages in tryptophan synthesis. On the other hand, strains of *S. commune* that produce CDR and anthranilic acid do so on minimal medium without addition of any intermediate of tryptophan biosynthesis. It is possible that the presence of CDR in the culture medium of pigmented strains of *S. commune* results from a partial block between indolyl-3

Table 1. R_F VALUES, COLOUR REACTIONS, AND FLUORESCENCE OF THE FIRST UNKNOWN COMPOUND AND ANTHRANILIC ACID

Solvent	R_F of unknown	R_F of AA (standard)	PABA	DMCA	Fluorescence under ultra-violet light
MBBW*	0·87	0·87	Yellow	Brown → pink	Strong blue
IprBor†	0·75	0·75	,,	,,	,,
IprAm‡	0·31	0·32	,,	,,	,,
NaClA§	0·67	0·70	,,	,,	,,
BuA‖	0·86	0·88	,,	,,	,,
BuEt¶	0·90	0·90	,,	,,	,,
PyrAm**	0·70	0·70	,,	,,	,,
BuEtAm††	0·83	0·83	,,	,,	,,

* Methanol–*n*-butanol–benzene–water 2 : 1 : 1 : 1. Ascending.
† 20 per cent borate buffer, *p*H 8·8, 80 per cent isopropanol, saturated with sodium chloride. Descending.
‡ Isopropanol–ammonia (880)–water 20 : 2 : 1. Ascending.
§ Eight per cent w/v aqueous sodium chloride–glacial acetic acid 100 : 1. Descending.
‖ *n*-Butanol–acetic acid–water 120 : 30 : 50. Descending.
¶ *n*-Butanol–ethanol (70 per cent)–water 4 : 1 : 1. Ascending.
** Pyridine–ammonia (880) 4 : 1. Ascending.
†† *n*-Butanol–ethanol (70 per cent)–ammonia (880) 1 : 1 : 2. Ascending.

Table 2. R_F VALUES OF THE SECOND UNKNOWN COMPOUND AND REPORTED VALUES OF SYNTHETIC CDR

Solvent	R_F of unknown	R_F of synthetic CDR	Reference
MBBW	0·85	0·85	3
BuA*	0·75	0·68	5
MePr-PO_4†	0·77	0·76	5
Ethanol (75 per cent)	0·72	0·72	5

* Water saturated butanol–acetic acid 95 : 5. Descending.
† Methanol–propanol–0·1 M phosphate buffer *p*H 6·0 1 : 2 : 1. Ascending.

Table 3. COLOUR REACTIONS OF THE SECOND UNKNOWN COMPOUND

Colour reagent	Colour	Inference	Reference
Ammonium molybdate	Blue-green	Reducing sugar	9
Alkaline silver nitrate	Brown	Reducing sugar	10
2,4-dinitrophenyl-hydrazine	Orange	Ketose	11
Carbazol-cysteine	Pink	Ribulose or xylulose	12
Dimethylaminobenz-aldehyde, 60° C	Purplish	Deoxypentose or deoxypentoside	13
Dimethylamino-benzaldehyde	Yellow	Aromatic amines	14
Alkaline 2,3,5-triphenyl tetra-zolium chloride	Immediate intense red at room temperature	Amadori rearrange-ment products	10

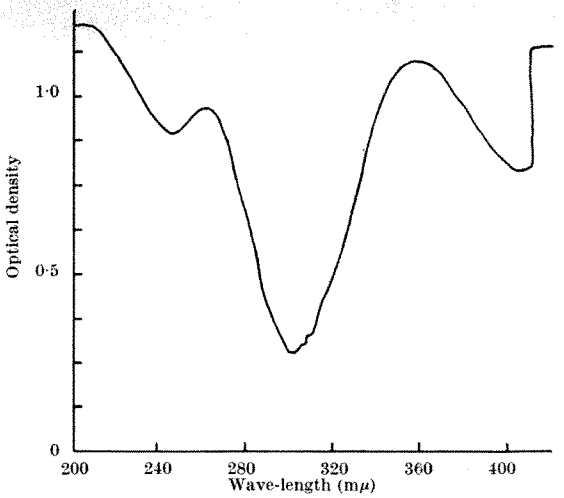


Fig. 1. Ultra-violet absorption spectrum of 2,4-dinitrophenylhydrazine of CDR at *p*H 7·7

glycerol-phosphate and 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate that brings about an accumulation of CDRP and *N*-(5′-phosphoribosyl) anthranilic acid. The lability of PRA is increased by acid and decreased by alkali[6]. In acid, PRA undergoes spontaneous hydrolysis to anthranilic acid and ribose-5-phosphate. Such a partial block will result in the accumulation of anthranilic acid and the non-phosphorylated form of CDRP, that is, CDR (ref. 7). The same pigmented strains that accumulated CDR and anthranilic acid were also found to accumulate indolyl-3-acetic acid[8]. It might indicate that anthranilic acid and CDR can participate in reactions other than those that lead to tryptophan synthesis.

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Early Effect of Ethionine on Liver Affinity for Iron

INCREASED affinity for iron by the liver was shown to occur *in vivo* and *in vitro* when ethionine was fed to animals from 1 to 3 weeks[1–3]. After 3 weeks of feeding ethionine there was a change in the affinity of the liver for iron, so that by 4 weeks there was no significant difference between the experimental and the control animals. By 6 weeks the affinity effect had been reversed by the liver so that the animals treated with ethionine had a decreased affinity when compared with the controls. The purpose of the present experiment was to determine the changes in the affinity before 1 week of ethionine treatment, as it is known that changes in the liver occur

Table 1. LIVER AFFINITY FOR IRON (C.P.M./G LIVER EXPRESSED AS A PERCENTAGE OF STANDARD)

Duration of experiment	No. of rats	Experimentals Mean	*S.D.*	Controls Mean	*S.D.*	Significant level
12 h	12	2·44	0·30	2·81	0·50	$P = 0·05$
1 day	9	3·34	0·58	4·13	0·99	$P < 0·05$
2 days	9	3·78	0·50	3·91	0·70	$P > 0·05$
3 days	10	4·86	0·54	4·24	0·56	$P < 0·05$

very rapidly after feeding ethionine[4,5]. The present experiments were designed to observe any possible changes in affinity shortly after the administration of ethionine and to correlate this with certain morphological and histochemical changes observed previously[5].

Male albino rats of the Sprague–Dawley strain were used, each weighing approximately 300 g. They were fed a synthetic diet containing 18 per cent casein[2] by way of tubes. The experimental animals received 0·5 per cent *d,l*-ethionine in the diets, and in the control animals the ethionine was omitted. Each animal in the first group received 3·5 g of the diet 12 h before killing. The other animals—those killed after 1, 2 and 3 days—received 10·5 g of diet per day in three divided feedings over 12 h periods during the day and they fasted for 12 h before being killed. Livers were removed, sliced, and incubated in 15 ml. of Ringer's solution containing 10 mc. iron-59 in the form of ferrous sulphate. The method of incubation was identical to that described previously[2]. Liver slices were then removed, washed in 15 ml. Ringer's solution for 0·5 h and then counted in a well-scintillation counter. The results were expressed as percentages of the standard, which was 2 ml. of the original incubating fluid counted for 1 min. The data were analysed using Student's *t* test for paired-comparisons. Because of the design of the experiment, comparisons could be made only between experimental and control values at any given interval, but not between different intervals.

The data shown in Table 1, and the mean difference in iron uptake between the experimental and control groups for each interval, are plotted in Fig. 1. Twelve h after ethionine feeding, the radioactive iron uptake in the liver was lower in the experimental than in the control animals. However, the difference in affinity was considered to be statistically marginal ($P = 0·05$). By 1 day, the affinity of the liver of ethionine fed animals for iron was significantly less than in the controls ($P < 0·05$). However, by 2 days there were no statistically significant differences between the experiments and controls ($P > 0·05$). By 3 days the livers of ethionine-fed animals showed an increase in the affinity for iron ($P < 0·05$). This increase is similar to that seen at subsequent periods up to 3 weeks[2,3].

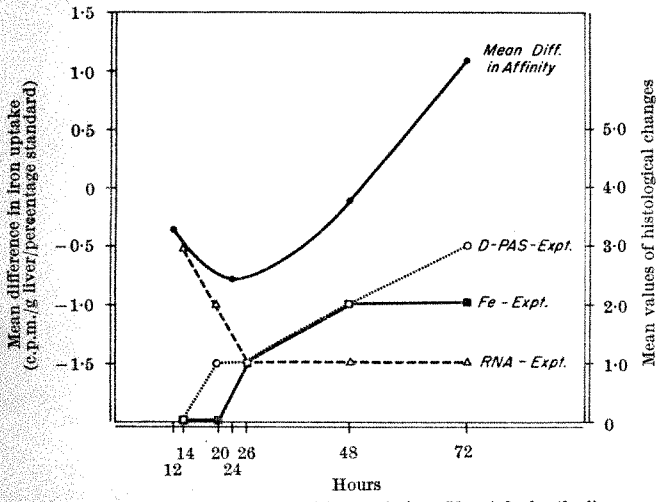


Fig. 1. Comparison of the mean difference in iron-59 uptake by the liver between experimental and control animals at different intervals, with changes in histochemically demonstrable RNA, DPAS-positive material and iron

An attempt was made to correlate the changes in the affinity of the liver for iron during the early stages of ethionine administration with the alterations in the histochemically demonstrable RNA, diastase resistant, periodic acid–Schiff (DPAS)-positive material and iron in the liver[5]. This was performed by assigning arbitrarily 1+ to each degree of change in RNA, DPAS-positive material, and iron observed previously[5] and plotting this against the presently observed changes in affinity (Fig. 1). It can be seen that at the times when there was maximal decrease of RNA and initial increase in DPAS-positive material and iron, the affinity for iron in the ethionine-fed animals was lower than in the controls. This occurred at 12–26 h. Afterwards there was increased affinity associated with further increase in both DPAS-positive material and iron, but no further changes in the histochemically demonstrable RNA.

The initial depression of liver affinity for iron corresponds to the reported rapid inhibition of protein synthesis[6,7]. It corresponds also with the maximum decrease of histologically demonstrable RNA in the liver[5] (Fig. 1). It is tempting to speculate that the decreased affinity for iron in the liver at an early interval of ethionine administration is associated with depressed synthesis of apoferritin along with the general inhibition of protein synthesis[6,7]. However, some of our recent data indicate that apoferritin synthesis may not necessarily parallel other protein synthesis[8].

On the basis of the intimate association of iron deposition and development of DPAS-positive material[5], it has been suggested that the DPAS-positive material that develops before iron deposition is associated with the increased affinity for iron[2]. However, the initial increase of DPAS-positive material and the initial appearance of histochemically demonstrable iron[5] corresponds to a decreased affinity of the liver for iron in the present experiment (Fig. 1). Furthermore, after prolonged ethionine feeding a decrease in liver iron[9] was noted at a time when affinity was decreasing[3], while a DPAS-positive material was still present in abundance. From this it would appear that other additional factors are at play or that the DPAS-positive material represents a situation with changing physical–chemical properties.

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Rapid Column Chromatography of Purine and Pyrimidine Bases on 'Ectaola' Cellulose at Room Temperature and Elevated Pressure

A NUMBER of methods have been published for the column chromatographic separation of the purine and pyrimidine bases[1]. The shortcomings of these are their slow rates of flow and the fact that the separations require up to three days. In view of the biochemical importance of these

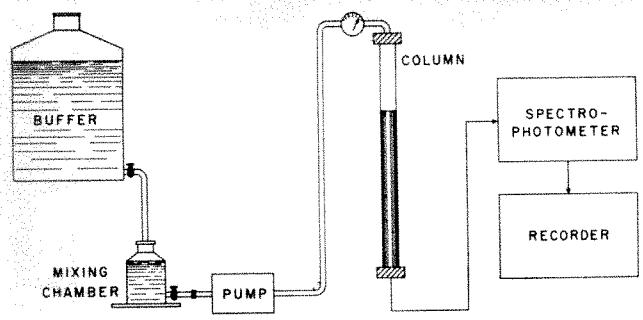


Fig. 1. Diagram of gradient elution system

heterocyclic components, faster techniques for their quantitative estimations are needed.

After testing various ion exchangers, namely, 'Dowex', 'Amberlite', and 'Sephadex', for their efficiency of separation, we found that 'Ectaola' cellulose achieved the most satisfactory results. 'Ectaola' has previously been used for column chromatography of nucleic acids[2] as well as for the thin-layer chromatography of nucleic acid derivatives[3]. A modification of these methods was used in the present work. It involves a gradient elution procedure at slightly elevated pressure and allows the separation of the five chemically most important bases in about 60 min at the 10^{-10} molar level. Less common bases and the nucleic acids may also be separated by this technique over longer periods of time.

In principle, the procedure uses a buffer gradient system of (1) 1 N sodium chloride in ammonium hydroxide and ammonium chloride at *p*H 10·60, and (2) distilled water adjusted with hydrochloric acid to a *p*H of 2·75. All liquids have sufficiently low density in the range of 240–290 mμ.

The major steps of the method are illustrated in Fig. 1. From the buffer reservoir containing a sodium chloride buffer, a continuous flow is supplied into the mixing chamber filled with buffer at *p*H 2·75. A magnetic stirrer provides effective mixing of the two buffers which are then pumped through the ion-exchange column at constant rates of flow, giving a linear gradient elution pattern. The absorption characteristics of the eluant are finally measured with a Gilford spectrophotometer (model 2000).

The ion-exchange column consists of a high-pressure nylon tube 65 cm in length and 0·5 cm in inner diameter, filled with a slurry of 200–325 mesh of 'Ectaola' (Brown Co., Berlin, New Hampshire) for the lower 45-cm section. The upper 20 cm of the column are only provided to hold sufficient ammonia for the column regeneration after each run. Care has to be taken that no air is trapped in the resin or introduced during the loading of the column.

Each analysis is preceded by the introduction of 8 ml. of ammonium hydroxide and ammonium chloride buffer *p*H 10·60 (ionic strength 0·2) which is followed by 10 ml. of the *p*H 2·75 buffer. This provides an increased hydroxyl ion retention by moving from top to bottom of the column[4].

For calibration a standard mixture of purine and pyrimidine bases (2·5 μmoles/ml.) dissolved in sodium hydroxide (*p*H 10·6) is added to the column. The sample is allowed to penetrate the top layer of the resin bed. Afterwards, the upper 20 cm of the column is filled with the *p*H 2·75 buffer; after all connexions are secured, the analysis is started. The tubing from the mixing chamber to the top of the nylon column holds exactly 20 ml. of *p*H 2·75 buffer. The salt gradient is established by a constant supply of the sodium chloride buffer (*p*H 10·60) to the mixing chamber containing exactly 125 ml. of *p*H 2·75 buffer. The pump is set at a flow rate of 96 ml./h; this results in a back pressure of about 35 lb./in.^{2}. A complete development of the five common nucleotide bases is accomplished in less than 90 min (Fig. 2). The spectrophotometric measurements are taken at 260 μm.

To locate the position of the individual base, each compound is run separately. It should be pointed out that the positions of uracil and thymine are almost identical (Fig. 2) when run separately. To achieve a separation between these two bases an extended equilibration of the column with ammonium hydroxide (6 h) and at a faster rate of flow (120 ml./h) is recommended (Fig. 3). To test application of the method outlined to biochemical research, the bases of hydrolysed DNA and RNA are recorded in Fig. 4. Aside from the common bases of both nucleic acids, three additional peaks appeared on the elution chromatogram; they are marked by the Roman numerals I–III. They correspond to the positions of methyl-cytosine (I), uric acid (II), and hypoxanthine (III). The areas under the peak can very easily be used for the quantitative determination of each base present in the sample. Because the Gilford spectrophotometer records the data in a linear fashion and the peaks are perfectly shaped, a simple digital integrator can simultaneously print out the area under the peaks as they emerge from the column. The recovery of each component is in the range 95–100 per cent.

In summary, a pressure-gradient-elution technique for rapid and quantitative separation of the purine and pyrimidine bases is presented. The separation of the bases can be achieved within 60 min at room temperature. The precision is better than 5 per cent for the individual

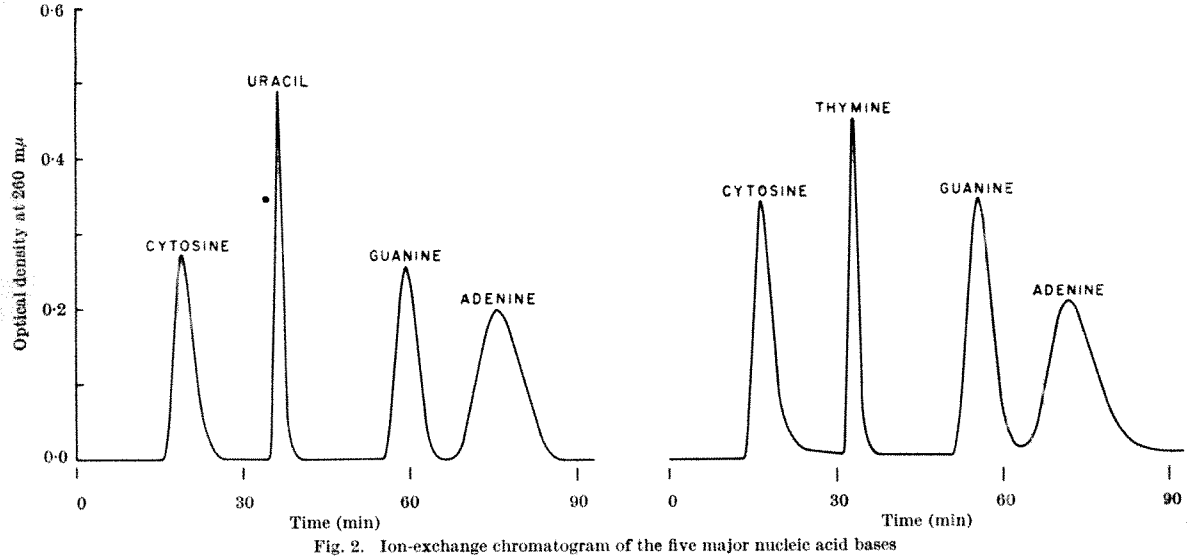


Fig. 2. Ion-exchange chromatogram of the five major nucleic acid bases

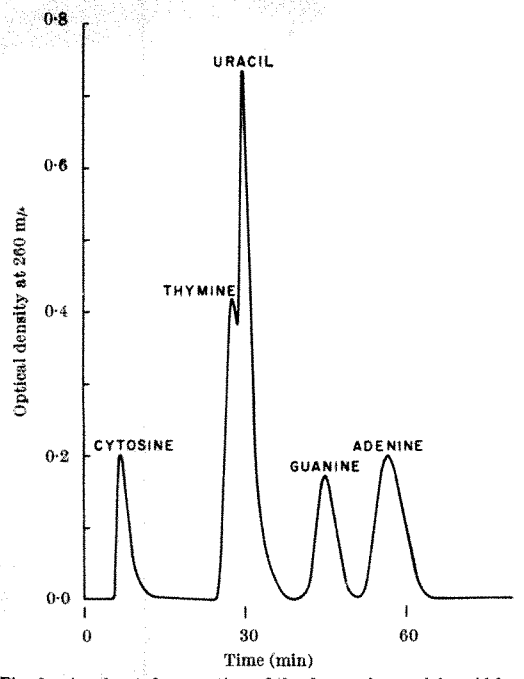


Fig. 3. Accelerated separation of the five major nucleic acid bases

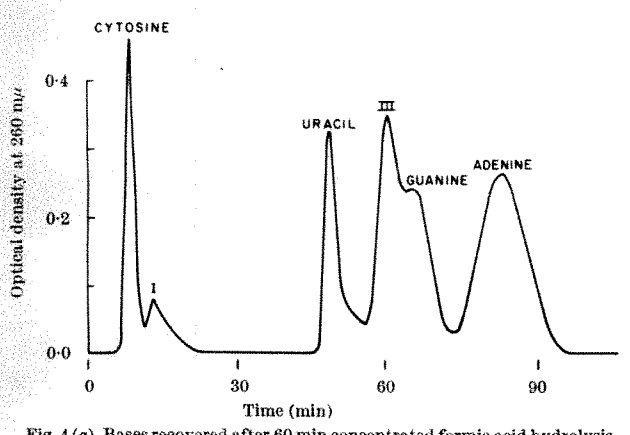


Fig. 4 (*a*). Bases recovered after 60 min concentrated formic acid hydrolysis of RNA at 175° C

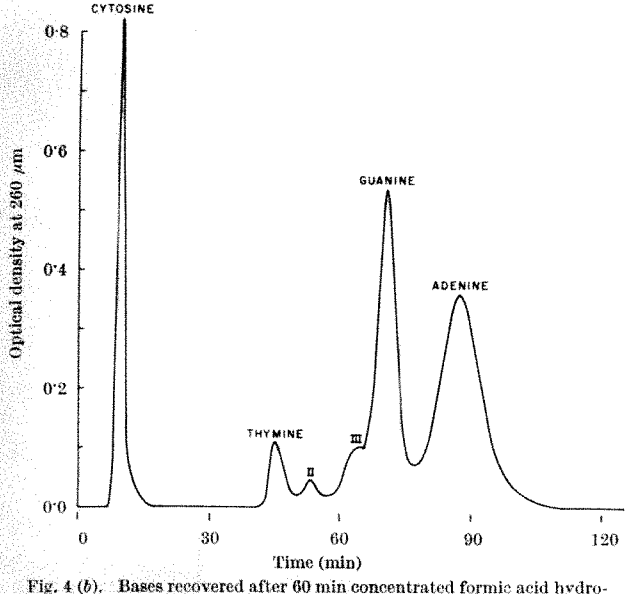


Fig. 4 (*b*). Bases recovered after 60 min concentrated formic acid hydrolysis of DNA at 175° C

base at the 10^{-10} molar level. The wide application of this technique to biochemistry and related sciences is obvious.

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Chemical Nature of the Pigment Spots of *Enoplus communis*

AT the anterior end of the free-living marine nematode *Enoplus communis* are situated two pigment spots, dark red-brown in life. Their superficial appearance is very reminiscent of eye-spots. Schultz[1] suggested that these pigment spots were excretory granules and were not in any way associated with photoreception.

Unlike the eye-spots of some marine nematodes, there is no lens structure or well-defined perimeter. The pigment is localized in two lateral pockets inside the oesophageal tissue, and having posterior to the pigment spots diffuse areas of granular pigment which extend back through the oesophageal tissue.

In an attempt to characterize the pigment, a number of solvolysis experiments were carried out, and specific bleaching reagents used. The pigments were fixed in formalin and were found to be readily soluble in concentrated sulphuric acid; they were, however, wholly insoluble in concentrated hydrochloric acid. The pigments disappeared in normal sodium and potassium hydroxides in a few minutes. They were quite unchanged in such organic solvents as diethyl-ether, chloroform, benzene, acetone and xylene. They did not dissolve in ethylene chlorohydrin, but this is only specific after separation from the protein component[2].

After dissolving the spots in concentrated sulphuric acid, there was a partial return of the colour on diluting with water[3].

Strong bleaching was found in concentrated and 50 per cent nitric acid and weak bleaching was recorded with solutions of hydrogen peroxide (6 per cent, w/v), potassium dichromate (2·5 per cent, w/v), and potassium permanganate (1 per cent, w/v)[4,5].

These results, together with the dark brown colour and granular consistency, strongly suggest that the "eye-spot" is melanin, and is therefore unlikely to be involved directly in photosensitivity.

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Occurrence of D-Lysergic Acid and 6-Methyl-ergol-8-ene-8-carboxylic Acid in Cultures of *Claviceps purpurea*

OVER the past few years we have been examining the composition of ergot alkaloids produced *in vitro* by strains of *Claviceps purpurea* isolated from sclerotia growing on grasses in England. It was noted that exhaustive chloroform extraction of culture filtrates adjusted to *p*H 8·5

removed only about 40 per cent of the total Ehrlich-positive material; the remaining material at *p*H 3·5 was readily extracted with *n*-butanol. Paper[1,2] and thin-layer[3] chromatographic analysis of the chloroform-soluble fraction showed chanoclavine to be the principal component present accompanied by small quantities of elymoclavine and the ergotoxine group. Chromatography of the butanol fraction revealed only one fluorescent, Ehrlich-positive spot which was inseparable from D-lysergic acid and 6-methylergol-8-ene-8-carboxylic acid, the isomer of lysergic acid recently obtained from cultures of *C. paspali*[4]. We now have re-examined culture filtrates of a strain of *C. purpurea* originally isolated from *Spartina townsendii* to investigate further the composition of the butanol fraction.

The fungus was grown in submerged culture on a rotary shaker (200 r.p.m., 10 cm eccentric throw) at 24° C in 500 ml. Erlenmeyer flasks containing 100 ml. of the following medium modified from Stoll *et al.*[5] (values in per cent): calcium nitrate, 0·1; epsom salt, 0·025; potassium dihydrogen phosphate, 0·025; potassium chloride, 0·0125; $FeSO_4.7H_2O$, 0·0033; $ZnSO_4.7H_2O$, 0·0027; sucrose, 10; asparagine, 1; cysteine hydrochloride, 0·001; yeast extract, 0·01; distilled water, *p*H 5·0. Maximum alkaloid production (100 μg/ml.) was reached within 10 days.

One litre of culture filtrate adjusted to *p*H 8·5 was extracted with chloroform (3 × 500 ml.) and then, at *p*H 3·5, with *n*-butanol (3 × 500 ml.). The residue obtained from the butanol extracts (142 mg) was applied to 20 g of 'Zeo-karb 225 (H^+)' ion exchange resin and, after washing with water, the fluorescent band was eluted with 1 M ammonium hydroxide to give 66 mg of an off-white solid. The ultra-violet absorption spectrum showed that the principal chromophore present in this material corresponded to that of the $\Delta^{8,9}$-acid (λ_{max}287 mμ) although some absorption in the region of lysergic acid (λ_{max}312 mμ) was also observed.

Following the procedure outlined by Kobel *et al.*[4], the crude material from the ion exchange column was esterified with methanolic hydrochloric acid and the resulting mixture reduced with lithium aluminium hydride in anhydrous tetrahydrofuran. In this way 15 mg of solid was obtained which on thin-layer chromatography in two systems[3,4] showed two Ehrlich-positive spots, one fluorescent and corresponding to lysergol standard, and the other non-fluorescent and corresponding to elymoclavine standard. Together with the other evidence presented, identification of these reduction products clearly established the presence of lysergic acid and 6-methylergol-8-ene-8-carboxylic acid in the original extract. This is the first time that the $\Delta^{8,9}$-acid has been demonstrated as a *C. purpurea* metabolite. D-Lysergic acid, in trace amounts, has previously been reported[6] in culture filtrates of mixed cultures of *C. purpurea* and a *Claviceps* sp. isolated from *Pennisetum typhoideum* but, to our knowledge, the present investigation gave the first identification of D-lysergic acid in pure cultures of *C. purpurea*.

We thank Dr. J. Rutschmann of Sandoz, Ltd., Basle, for kindly supplying a pure sample of the $\Delta^{8,9}$-acid.

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Nutritive Values of Shrimp Flour

THIS communication presents some new data and ideas concerning the chemical constituents and potential uses for shrimp of the south Atlantic Ocean, and points the way to further systematic investigations of the links in selected food chains, such as that of phytoplankton→zooplankton→fishery resources.

The shrimp, *Artemesia longinaris* Bate, which feeds on plankton, inhabits shallow waters from southern Brazil to Argentina; it enters the commercial catch off Mar del Plata, Argentina. Specimens were collected with a coarse plankton tow net from the Argentine R.V. *Zapiola* in March 1965 off Mar del Plata. The average wet weight of these animals was 2·73 g. The meat was separated by hand from the shells and heads of 138 individuals, and the components frozen and dried in a vacuum drying oven. Afterwards, the meat and shells were prepared for chemical analysis (Food and Drug Research Laboratories, Maspeth, Long Island, N.Y.) by grinding them separately to a fine meal in a Wiley cutting mill. The shrimp meal had a pleasing yellow colour, an attractive smell, and rich flavour. Such qualities could possibly help to make this material acceptable for protein enrichment of soups, bread, crackers and other foodstuffs.

The dry matter in the crude whole shrimp was about 32 per cent of the wet weight. The hard chitinous shells together with the heads and other attached matter amounted to 14 per cent of the original wet weight. Thus 18 per cent of the fresh shrimp could be converted to dry meat. The approximate composition of the dried shrimp meat and shells is given in Table 1. The calculated protein of the whole dried shrimp (65·6 per cent, or 21 per cent of the wet weight) is comparable with the protein content of lean beef, ox liver, salmon or turkey.

The amino-acid constituents of the meat and shell portions of the shrimp were determined by column chromatography, after acid hydrolysis, according to the general methods developed by Moore, Spackman and Stein[2]. It was found to be necessary to assay cystine microbiologically[3] owing to the fact that losses of this amino-acid were encountered in the ion-exchange procedure with the shrimp meat hydrolysate. Alkaline hydrolysis of portions of the samples was performed when the materials were prepared for tryptophan analysis[4].

Amino-acid profiles of shrimp meat and shells are shown in Table 2. The glycine and valine content of the shells was higher than in the meat, while cystine, histidine, methionine, and tryptophan were more abundant in the

Table 1. APPROXIMATE COMPOSITION OF SHRIMP MEAT AND SHELLS AFTER DRYING (ACCORDING TO METHODS GIVEN IN REF. 1)

Determination	Results (per cent)		
	Meat	Shells	Whole shrimp
Fat	6·98	18·70	12·0
Ash	15·72	34·42	23·4
Protein (N × 6·25)	78·94	48·29	65·6

Table 2. AMINO-ACID COMPOSITION OF DRIED MEAT AND SHELLS OF SHRIMP

Amino-acids	Percentage of each in the protein (N = 16 basis)	
	Dried meat	Dried shells
Alanine	4·97	6·35
Arginine	5·03	7·37
Aspartic acid	13·96	16·76
Cystine	2·80	0·42
Glutamic acid	15·51	14·31
Glycine	7·57	17·33
Histidine	3·13	0·51
Isoleucine	3·24	3·71
Leucine	6·65	6·35
Lysine	6·26	3·03
Methionine	3·01	0·48
Phenylalanine	3·77	4·58
Proline	4·04	7·53
Serine	3·62	4·16
Threonine	4·29	4·88
Tryptophan	0·66	(< 0·02)
Tyrosine	3·70	4·11
Valine	4·00	7·42
Total	96·21	109·30

meat. It is possible that the recovery of tryptophan was incomplete in the analyses. Comparison of the amino-acid profiles in the meat of *A. longinaris* Bate with that of the common canned shrimp[5] sold throughout the United States shows that they have a similar composition. In this respect it would also be of interest to make a similar analysis of the common krill (*Euphausia superba* Dana and other species) which is abundant in the Antarctic seas. In Antarctica, krill occupies the intermediary link in the food chain from primary phytoplankton to whales (or penguins). Because of the decline in whaling, it would be advantageous to focus special attention on the crustaceans formerly eaten by whales, with the view of utilizing this abundant supply of food for man. It has come to our attention[6] that in the Philippines shrimp shells are ground into a powder, mixed with flour and eggs, and fried to make a crisp delicacy. This would suggest that the chitinous materials could be put to some use when whole shrimp powder becomes available for human use. It is hoped that chemical and nutritional investigations on krill and other unconventional sources of marine foods will find application in the production of protein enrichments for the human diet.

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Electrophoretic Fractionation of Heat-denatured Eucollagen

IN the early stages of collagen fibrogenesis, soluble forms of collagen can be extracted with salt solutions; as the tissue develops, dilute acids and acidic buffers are needed. Eventually these reagents also become ineffective as a relatively insoluble form of collagen is elaborated. This transformation or "maturing" is believed to be caused by an increase in the number of intramolecular and intermolecular cross-links[1].

Soluble material can be obtained from this mature collagen by a preliminary treatment with cold alkali. As a result of this, it is possible to dissolve in this medium a large proportion of the collagen that was previously insoluble in citrate buffer (*p*H 3·7). Such alkali-treated collagen is called "eucollagen"[2]. This communication reports the preparation and electrophoretic analysis of eucollagen solutions prepared from the shin tendons of cattle.

The flexor tendons from the shin of a calf foetus, a 3–4-month-old calf and a 14-month-old steer were frozen, comminuted, immersed in liquid nitrogen for a few seconds and then shattered in a percussion mortar and pestle. The neutral salt-soluble collagen was removed by extraction in solution of sodium chloride. Each sample was extracted for 24 h in a cold solution of sodium chloride, after which the mixture was centrifuged at 1,500*g* for 30 min. The tissue residue was then removed, ground with sand in the cold, and then subjected to extraction procedures for two further periods—the first for 5 days, and the second for 24 h. The supernatant was removed by centrifugation at 1,500*g* for 30 min at each stage, and all extractions were carried out at 4° C.

The tissue residue that remained after the extraction with sodium chloride was then washed twice with cold water, and the acid-soluble collagen was extracted from it by two periods of treatment in citrate buffer (*p*H 3·7, 0·15 M). Each extraction lasted 3 days and was performed at 4° C. On each occasion the tissue residue was removed by centrifugation at 1,500*g* for 30 min.

After extraction of the acid-soluble collagen the tissue residue was washed twice with cold water and used for the preparation of eucollagen. The method used was essentially that described by Higgs and Reed[3]. Unless otherwise stated, the stages of preparation were carried out at room temperature. They were as follows: (*a*) the samples were allowed to stand overnight at 20° C in a saturated solution of sodium sulphate; (*b*) 5 days at 20° C in 5 per cent sodium hydroxide saturated with sodium sulphate; (*c*) three successive treatments, each for 1 h with saturated solutions of sodium chloride; (*d*) 1 h treatment with saturated solutions of sodium chloride containing sufficient acetic acid to bring the *p*H to 6·0; (*e*) 4 h standing in distilled water the *p*H of which was adjusted to 6·0; (*f*) two 12-h period of standing in distilled water *p*H 6·0 at 5° C; (*g*) four successive immersions each lasting 2 days, in 0·15 M citrate buffer (*p*H 3·7) at 8° C; (*h*) a fifth extraction in 0·15 M citrate buffer (*p*H 3·7) for 2 days at 8° C. These procedures provided a solution of eucollagen, which was then centrifuged for 30 min at 2,000*g* to remove any large debris, and finally used for electrophoretic analysis.

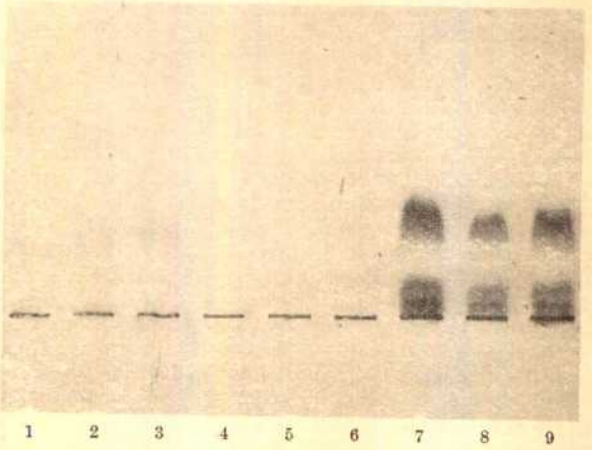


Fig. 1. Samples 1–3. Eucollagen prepared from the shin tendons of a 3–4-month-old calf. Samples 4–6. Eucollagen prepared from the shin tendons of a calf foetus. Samples 7–9. Eucollagen prepared from the shin tendons of a 14-month-old steer

In the electrophoretic study a discontinuous buffer system was used in the vertical apparatus described by Smithies[4]. A gel was prepared with 10 per cent starch and 5 per cent urea in a buffer which consisted of 0·1 M glycine brought to *p*H 3·0 (at 20° C) with lactic acid. This was poured into the end sections of the apparatus, and when the gel had set, a second gel which contained 12 per cent starch and 5 per cent urea in a buffer of 2 mM aluminium lactate (brought to *p*H 3·0 (at 20° C) with lactic acid) was poured into the centre section.

Before electrophoresis the eucollagen solution was dialysed for 12 h against the aluminium lactate buffer containing 0·5 M sucrose; and then incubated at 37° C for 30 min immediately before the application of the samples to the starch gel. Electrophoresis was carried out at 2° C for 7 h at 400 V, 25–30 m.amp.

The results are shown in Fig. 1. They demonstrate the separation achieved by this electrophoretic technique,

and that formation of insoluble collagen increases as the animal approaches maturity.

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Utilization of Adenine but not Nitrate as Nitrogen Source by *Prototheca zopfii*

CULLINMORE[1] has recently reported that *Prototheca zopfii* (Cambridge Collection strain No. 263/5) will utilize butyrate as the sole carbon source for growth, but only after a lag period of 3 days. It is presumed that this represents a period of enzyme induction. We have been investigating some aspects of the nitrogen metabolism of this strain of *Prototheca* and have observed that while adenine can be used as the sole nitrogen source there is again a lag period before growth commences. In this case, however, the lag is only about 30 h. (Fig. 1). The increase in dry weight obtained suggests that ring nitrogen of the adenine molecule is utilized in addition to the amino group.

Pure cultures of *Prototheca zopfii* were grown in 100-ml. Erlenmeyer flasks containing 20-ml. of the medium used previously[2], but with the ammonium nitrate omitted and with the addition of 1 per cent glucose and 10^{-5} M thiamine[3]. 140 mg nitrogen/l. was then supplied either as ammonium sulphate, potassium nitrate or adenine. After autoclaving, the *p*H of the medium was 6·1. The flasks were inoculated under aseptic conditions and were shaken in darkness at 25° C. The dry weight of algae in cultures was determined by means of sintered glass filter crucibles dried in an oven at 80° C.

Inorganic nitrogen assimilation by *Chlorella vulgaris* has been extensively studied and it was shown that the activity of the nitrate reductase is inhibited by ammonium assimilation[4] and further that the synthesis of the enzyme is repressed by ammonium[5]. We assumed that this situation might apply to *Prototheca zopfii*, which is generally considered to be a colourless *Chlorella*[6], and were interested to determine whether adenine as well as ammonium would repress the production of nitrate reductase. However, a preliminary experiment showed that the alga is unable to utilize nitrate as sole nitrogen source as is shown in Fig. 1. We are not fully convinced that this observation is previously unrecorded but in the investigations known to us the alga was grown in media which contained either ammonium chloride or ammonium sulphate as nitrogen source. This derives from Barker's[7] original work in which a medium containing ammonium chloride was used and the significant observation was made that growth was still dependent on the addition of small quantities of yeast extract owing to the requirement for thiamine[8].

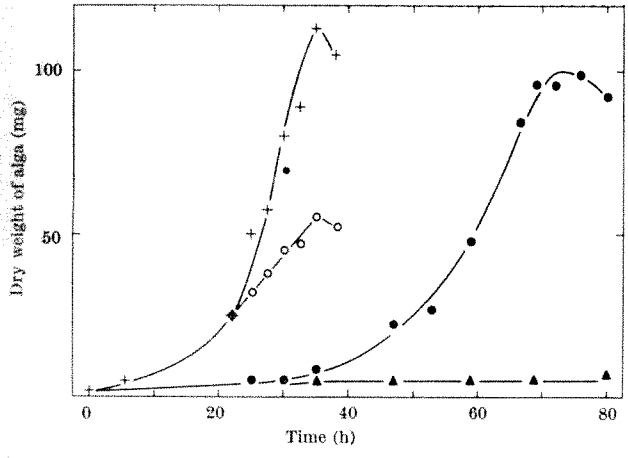


Fig. 1. Growth of *Prototheca zopfii* with various nitrogen sources in the medium. +, 140 mg N/l. as ammonium sulphate; ○, 28 mg N/l. as ammonium sulphate; ●, 140 mg N/l. as adenine; ▲, 140 mg N/l. as potassium nitrate

It has been suggested that the fact that *Prototheca zopfii* contains a definite plastid[9,10] implies that it arose from a mutation rendering it incapable of synthesizing chlorophyll[10]. At present it is not known whether the alga is unable to synthesize nitrate reductase, as in the case of the *Chlorella* mutant isolated by Shafer *et al.*[11], or whether nitrate assimilation is actually blocked at the stage of nitrite reductase. In *Ankistrodesmus*[12] the nitrate reductase requires as coenzyme reduced nicotinamide adenine dinucleotide ($NADH_2$) and the nitrite reductase reduced nicotinamide adenine dinucleotide phosphate ($NADPH_2$). The latter enzyme is particulate. Similarly, a nitrite reductase requiring ferredoxin has been associated with chloroplasts isolated from several higher plants[13]. Thus there seems to be a definite possibility that the inability of *Prototheca* to utilize nitrate is associated with the absence of a functional chloroplast. If this is indeed the case the biochemical differences between *Prototheca* and *Chlorella* are more complex than the inability to synthesize chlorophyll, and as a consequence carbohydrate by photosynthesis, would indicate. The assimilation of ammonium by yeast leads to a stimulation of the pentose phosphate pathway of glucose oxidation to satisfy the requirement of the glutamic dehydrogenase for $NADPH_2$ (ref. 14). Although it is quite clear that *Prototheca zopfii* is capable of degrading glucose by this pathway[15] a comparable coupling to nitrogen metabolism may not have developed.

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AGRICULTURE

Incidence of *Rhizoctonia* in a Cultivated and a Fallow Soil in Hong Kong

THE arable land available in Hong Kong is very limited and farming is so intensive that the annual production of 8–9 vegetable crops, often on the same land, is common. For many reasons, especially from an accumulation of soil-borne pathogens, this might be expected to have a deleterious effect on the soil.

A comparison[1] of the fungi present in a cultivated and in a fallow soil using Warcup's soil-plate method[2] indicated that at least two genera of fungi which are known to be causal agents of soil-borne diseases were more prevalent in the cultivated soil. *Pythium* sp. was only isolated from the cultivated soil. Whereas the occurrence of *Fusarium dimerum* was high in this soil, it was seldom found in the fallow soil.

Because it was rarely possible to isolate the common soil fungus *Rhizoctonia* by this method, a modification of the method devised by Papavizas and Davey[3] was used instead. Mature lima bean (*Phaseolus vulgaris* L.) stems were flushed in running tap water for 1–2 min, immersed in a 10 per cent solution of bleaching powder for 30 sec, and then washed in sterile water. The stem internodes were then cut into 1/8 in. segments, and buried in samples of freshly collected cultivated and fallow soils (stored in glass troughs). After incubation at 23°–24° C for 3 days, the stem segments were washed first in tap water to remove the attached soil particles and then in five changes of sterile water in a Griffin's electric shaker for 2 min. The stem segments were then damp-dried between sheets of sterile blotting paper and transferred to plates of 2 per cent water agar. Any fungal colonies growing out from the stem segment after 24 h incubation at 23°–25° C were sub-cultured on PDA plates[3]. In general, because only one small colony appeared after such a short incubation period, one isolation was made from each stem segment.

During June–August 1964, a total of 260 stem segments were plated out for the two soils. The results showed that, whereas 41 per cent of the lima bean stem segments taken from the cultivated soil were colonized by *Rhizoctonia*, only 2·7 per cent of the segments from the fallow soil were infected. It thus became clear that *Rhizoctonia* was far more abundant in the cultivated soil than in the fallow soil.

It is tempting to suggest that cultivation of the soil resulted in the build-up of *Rhizoctonia* but evidence to support this is lacking. Moreover, the pathogenicity of both *Rhizoctonia* and the other possible pathogenic genera isolated in the previous study[1] has not been established as yet. There were no serious outbreaks of any soil-borne disease in the intensively cultivated cabbage fields. The reasons for the absence of disease are not known; it is possible, however, that the micro-organism was not pathogenic, or that it was not sufficiently virulent. Unfavourable weather conditions or perhaps resistance of the crop may also have played a part.

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An Attempt to predetermine the Sex of Calves by Artificial Insemination with Spermatozoa separated by Sedimentation

BHATTACHARYA[1,2] has described a method for separating from rabbit semen two fractions of sperm with which he achieved a significant degree of predetermination of sex. He suggested that the separation depends on the tendency of the female producing *X*-sperm to sediment more rapidly than the male-producing *Y*-sperm, when the sperm are rendered immotile by cooling to 1° C and then allowed to sink under the influence of gravity for 12 h through a column of a viscous egg yolk medium. This communication describes briefly the results of experiments designed to examine the applicability of Bhattacharya's technique to cattle.

Specimens of about 1·5 ml. of semen from either Hereford or Friesian bulls were provided by the Cambridge Cattle Breeding Centre. After 24 h for treatment at Babraham to separate the fast and slowly sedimenting spermatozoa, samples from the top and bottom of the columns were given code numbers and returned to Cambridge. For the first series of separations an egg yolk/glycerine medium ('*A*') was used, in conjunction with the glass apparatus described by Bhattacharya[2]. Later separations were carried out in a milk/glycerol medium ('*B*'), with an apparatus consisting of a stack of stainless steel sheets drilled with holes to form columns which could be fractionated by sliding the sheets sideways. Between November 1964 and July 1965, 411 cows were inseminated with fractionated semen (24 h old), and 191 of them became pregnant, giving a conception rate of 46·5 per cent. A control group of inseminations using untreated semen from the same collections gave 192 pregnancies in 310 cows, a conception rate of 61·9 per cent. The 191 test pregnancies yielded 153 calves of known sex, of which eight were twins; in forty-two cases the sex of the calves was not reported, usually because of sale of the cows before calving. A comparison of the predicted and actual sexes of 139 of these calves is given in Table 1. Fourteen returns (eight males and six females) were excluded from the analysis, either because a non-standard technique was used (for example, medium '*A*', but the stainless steel apparatus), or because sperm counts made after the semen had been dispatched to the inseminators showed that there was doubt whether the samples were the best representative 'top' or 'bottom' fraction. Of the twins, there was one pair of males and one pair of mixed sex in each of the two categories, medium '*A*' predicted female, and medium '*B*' predicted male. In 136 calves of the control group the sex of which was returned, there were sixty-nine males and sixty-seven females.

Table 1. SUMMARY OF CALVING RESULTS

Technique	Predicted sex	Actual sex
Medium '*A*', glass apparatus	Male	12 M 18 F
	Female	32 M 31 F
Medium '*B*', stainless steel apparatus	Male	12 M 11 F
	Female	13 M 10 F
Medium '*B*', deep freeze method	Female	15 M 19 F
	Total	84 M 89 F

Another batch of thirty-seven heifers was inseminated in San Francisco in August and September 1965. The semen was allowed to sediment in glass tubes containing medium '*B*', and the bottom fraction was selected after the column had been quickly deep frozen. All the heifers became pregnant, but twenty of them had to be inseminated twice or more with the fractionated semen. The animals were slaughtered after 35–60 days of pregnancy, except for one which was allowed a normal delivery. The sex of the foetus was determined from a macroscopic inspection of the primary sex organs, except for three rejected cases where development was incomplete. As can be seen in Table 1, the sex distribution was again not very far from equality.

Although in these experiments the predictions were not successful, subsequent work has indicated that the techniques used here for differential sedimentation may not be entirely free from disturbing factors, and by applying slightly modified methods Schilling[3,4] has apparently obtained greater success in predetermining the sex of calves. Further biological tests of the significance of the variation in sedimentation velocity among spermatozoa are clearly needed.

We acknowledge the help of Mr. H. J. Ballinger, who collected and analysed many of the data.

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Observations on the Parthenocarpic Response of Blueberry to Gibberellic Acid

IT was previously noted[1,2] that the lowbush blueberry, *Vaccinium angustifolium* Ait., will respond to gibberellic acid (GA) placed on the open flower, by the formation of parthenocarpic fruit. It has not been possible to effect the same response in the field on any broad scale. The reasons for this are not understood, but some evidence has been obtained to suggest that certain clones are more receptive to GA than others. The period of receptivity to the growth substance is also well defined and the parthenocarpic fruits seem to swell up more slowly than do seeded berries.

Because in a practical field operation there is no possibility of excluding pollinating insects, only a limited experiment could be established to determine whether application of GA curtails normal fertilization or whether the opposite effect occurs.

Vegetative cuttings in the greenhouse were induced to flower by subjecting them to a regimen of 8 h of light per day[3]. When flowering became general in eleven clones, freshly opened flowers were selected, unopened buds and older flowers were excised, and one of the following treatments was administered: (1) treatment with water (controls); (2) hand pollination; (3) treatment with GA in water (50 p.p.m.); (4) sprayed with 50 p.p.m. GA, and 24 and 48 h later hand pollinated; (5) hand pollinated and 24 and 48 h later sprayed with GA at 50 p.p.m.

Table 1. RELATIONSHIP BETWEEN SEED CONTENT AND PHYSIOLOGICAL AGE

	Early	Late
No. of berries	121	40
Percentage of seedless	2·5	62·5
No. of seeds	3,462	153
Mean seeds	28·6 (29·3)	3·8 (10·2)*

* Bracketed numbers include only fruits with seeds.

Table 2. RELATIONSHIP BETWEEN SEED CONTENT AND TREATMENT

Treatment	Fruits	Seeds	Distribution of berries with respect to seed content 0	10	11–30	+30	Mean No. seeds per fruit
(1)	2		2	—	—	—	—
(2)	50	1,412	—	10	20	20	28·2
(3)	23	50	16	5	2	—	2·5
(4)	28	907	—	5	11	12	32·4
(5)	58	1,238	10	11	20	17	21·3

When the fruits matured, increased in size and changed colour, they were harvested and seed counts were made weekly (June 4–July 17). These data are presented as early fruit (harvested before June 15) and late fruit (harvested June 16–July 17) in Table 1. The seed content of fruits harvested from the various treatments is shown as Table 2.

The early fruit was heavily seeded, while the berries maturing later generally contained fewer seeds. The data of Table 1 indicate that only three berries that formed parthenocarpically had matured sufficiently for the early harvesting. None the less, treatment with GA resulted in a greater set response (84·3 per cent) than did hand pollination (62·3 per cent). Between the time when the set was estimated (14 days after treatment) and the date of harvesting, 58 per cent of the GA-induced fruits were lost. When the flowers were sprayed with GA and pollinated 24 or 48 h later, no parthenocarpic fruits were formed but ten seedless berries resulted from the application of GA subsequent to hand pollination.

The results of this work support the finding[4] that physiological maturity in the blueberry is expedited by increased fertilizations. Furthermore it would seem that the stimulus to form fruits parthenocarpically from GA either is not quantitatively sufficient to permit ripening within the season, or incompletely substitutes for the natural stimulation to form berries that follows fertilization. These possibilities are indicated both by the lateness of maturing of the seedless fruits and by the high wastage of set berries.

GA can thus induce parthenocarpic response in the blueberry; however, it seems clear that the response that follows the application of this compound does not duplicate (at least in its entirety) the natural "berry-building" processes that stem from pollination and fertilization. Also the force of the response to GA depends on the clone treated.

These data support other observations that suggest a considerable variability in the setting of fruits in *V. angustifolium*. One clone at Tower Hill, New Brunswick, has never set fruit. Another produces large fruits, usually with a low seed count (4·2 seeds/fruit, this experiment). Some clones are natural parthenocarps.

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BIOLOGY

Influence of Oestrogen on the Loss of the Zona Pellucida in the Rat

IN the rat, the fertilized egg normally loses its zona pellucida about 10 h after it reaches the uterus. Blastocysts flushed from the uterine cavity before 12 a.m. on the fifth day after the onset of a positive smearing reaction are still surrounded by their zonae, but those flushed in the early afternoon of the fifth day have lost their zonae[1–3]. Implantation begins some 6 h after the loss of the zona (110–114 h after ovulation)[1].

When the ovaries are removed before noon on the fourth day of pregnancy, the blastocysts do not implant, but the zonae disappear at the normal time—that is on the afternoon of day 5. Blastocysts of ovariectomized animals treated with 5 mg progesterone also lose their zonae at this time and do not seem to differ from those of normal or untreated ovariectomized animals.

If the ovaries are removed on day 2 of pregnancy, however, and the rat is treated with 5 mg progesterone per day, the zona pellucida persists until the eighth day of pregnancy. In this period the blastocyst assumes a spherical shape owing to the enlargement of the embryonic cavity. On day 8 these blastocysts rupture their zonae but do not implant until oestrogen is given together with the progesterone. Unless oestrogen is supplied, the ruptured zona persists undissolved in the uterine cavity. The dose of oestrogen (17-*b*-oestradiol) required to bring about the dissolution of the zona is less than that (0·05 mg) required for implantation.

These results show that oestrogen is necessary for the dissolution of the zona pellucida in the rat. The loss of the zona pellucida at the normal time in animals ovariectomized on day 4 of pregnancy suggests that small quantities of this hormone are produced before the fourth day of pregnancy. The results also suggest that delayed implantation is not related to the persistence of the zona pellucida.

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Chromosome Abnormality in Cattle

RECENTLY, a chromosome abnormality found in three cases of lymphatic leukaemia in cattle was described[1]. This communication reports my latest findings concerning this abnormality. It appears that in Sweden cattle chromosomes have only once before been investigated using modern techniques. On that occasion Melander[2] found the chromosome number in animals of the Swedish breed *SLB* to be 60; all autosomes were characterized by having strictly terminal centromeres. The existence of telocentric chromosomes has now been verified in several groups of animals including cattle[3–6].

In order to obtain further information on the chromosomes of *Bos taurus* in Sweden, animals belonging to the Swedish breeds of *SLB* and *SRB* from mid and southern Sweden were studied. Using a modified form of the blood tissue-culture technique[7], counts were soon made of the chromosome numbers of 1,134 animals. Eighty-nine per cent appeared to have 60 chromosomes, a figure which agreed with previous counts (Figs. 1 and 2). One hundred and twenty-two animals were found to possess a complement of 59 chromosomes of the type mentioned above[1], and a number of bulls were included in this group (Fig. 3). In four bulls, only 58 chromosomes were found per cell (Fig. 4). All the animals which were investigated appeared normal and some of the bulls have since been used for artificial insemination purposes. Until now only animals of the *SRB* breed have been found with this chromosome abnormality.

It would appear that the chromosome abnormality found in these cattle is a case of intraspecific chromosomal polymorphism. This type of variation—centric (chromosome) fusion, or translocation of the Robertsonian type—is relatively common in invertebrates[8–11]. It may exist in the lizard *Gerrhonotus scincicauda*[12], and it has also been described in some mammals.

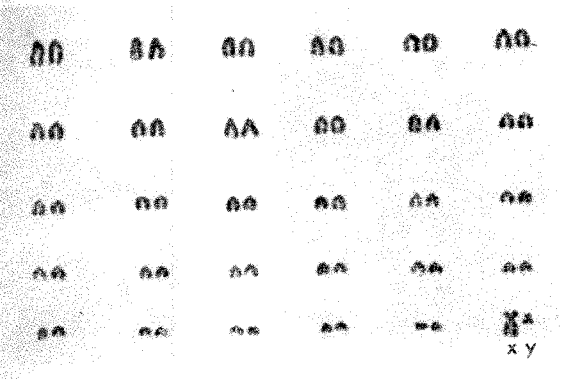


Fig. 1. $2n = 60$, male. The autosomes have been arranged as nearly as possible according to decreasing size. (× 900)



Fig. 2. $2n = 60$, female. The autosomes have been arranged according to decreasing size. (× 900)

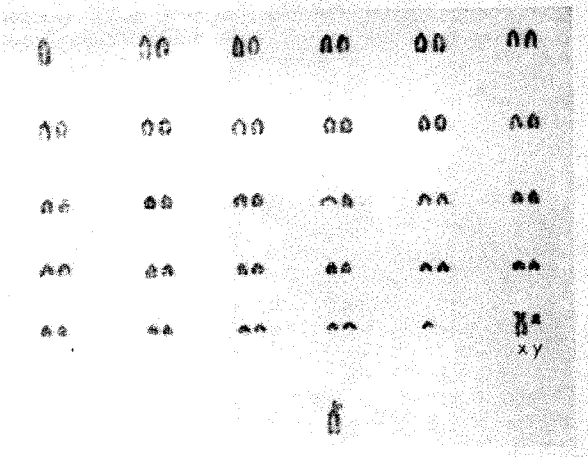


Fig. 3. $2n = 59$, male. Note that two telocentric chromosomes are replaced by a long subtelocentric chromosome. (× 900)

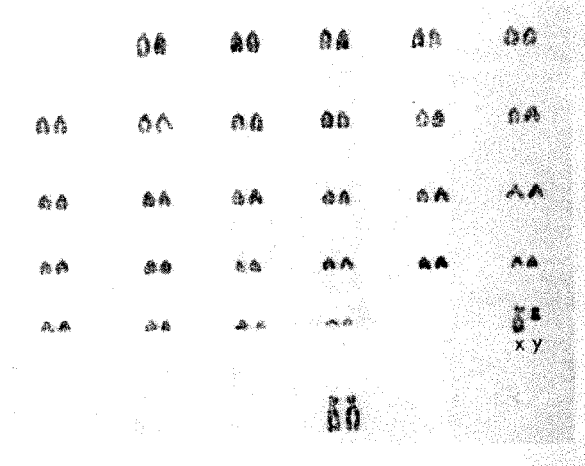


Fig. 4. $2n = 58$, male. Instead of two telocentric chromosome pairs, a subtelocentric chromosome pair is present. (× 900)

The first example of the abnormality in mammals was described in *Sorex araneus*. Besides an XY_1Y_2 sex chromosome system in the male[13], extensive studies have revealed two races, one monomorphic and one polymorphic. The number of chromosomes varies between 21 and 31, but the number of chromosome arms remains constant[14–17].

Though the European genus *Mus* seems to be cytogenetically uniform, the African sub-genus *Leggada* contains both inter- and intra-specific variation of the centric fusion type. Recently, Matthey[18] found chromosome numbers 31, 32, 33 and 34 in the species *M. minutoides musculoides*. This constitutes a typical Robertsonian system. Another species, *M. triton*, appeared to have a rather more complicated translocation system than the Robertsonian pattern[19]. In *M. minutoides minutoides* from South Africa the complements consisting of 18 and 19 chromosomes were observed. Some animals with 18 chromosomes had an $N.F. = 36$, thus all chromosomes were bi-armed. In the same population individuals were found with an $N.F. = 35$. This reduction resulted from a pericentric autosomal inversion. Cytologically, this species only differed from another species or sub-species from the same area, *M. minutoides indutus* ($2n = 36$), by translocations of the Robertsonian type. Furthermore, Matthey suggests that "this mixed population is the last phase of a previous polymorphic system splitting in two species in

statu nascendi". On this basis it is possible to get sympatric speciation resulting from chromosomal mutations. This theory contradicts one proposed by Mayr[20], who claims that groups require to be geographically isolated from each other before speciation can occur.

Intra-specific chromosome polymorphism was reported previously in *Gerbillus pyramidum*[21] and *Acomys cahirinus*[22]. However, hybridization experiments revealed that there are reproductive barriers between the different cytogenetic types, which indicates that an advanced stage in speciation has been reached by both groups[23].

In a study of some species of *Acomys*[23], Matthey observed two chromosome numbers in the members of a race of *A. minous*. A metacentric chromosome was found to be the cause of the difference; this would appear to represent a more complicated type of polymorphism than that explained by the Robertsonian type.

Centric fusion of telocentric chromosomes is a common characteristic of neoplastic tissues[24] and is frequently found in cells in tissue culture[25-26]. As outlined by White[8], and also pointed out by Ohno *et al.*[25], a translocation of this type is of adaptive significance because new gene complexes are formed with different recombination values relative to the position of other gene loci. Little or no loss of genetic material is thought to occur and the number of chromosome arms is constant as is the DNA content per nucleus.

The Robertsonian type of chromosome translocation which we have described in cattle is the first example of this type of rearrangement observed in domestic animals.

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Cuticular Structure in the Phalangida

APART from passing references such as that of Kastner[1] to the cuticle of *Trogulus*, there is no information about the cuticular structure of the Phalangida. Histological investigations of the cuticle in three species of the Palpatores revealed a marked difference between the cuticle of the dyspnoic *Nemastoma lugubre* (Mull) and the two eupnoic species *Leiobunum rotundum* (Lat.) and *Phalangium opilio* Lin.

In the two eupnoic species the situation is closely comparable with that of insects[2] and scorpions[3]. In cuticle with an overall thickness of 14μ there is an endo-cuticular component 9μ thick and an exo-cuticle 4μ thick. The boundary between these two components is sharp and comprises an area which has little affinity for aniline blue, orange *G* or acid fuchsin. It is perhaps comparable with the amber, or quinone tanned, exo-cuticle of scorpions[3,4]. The endocuticle differs from the relatively homogeneous exo-cuticle in its laminate structure. These laminae have a strong affinity for aniline blue and a slight residual affinity for acid fuchsin. In addition to these two major components there is, on the sclerites, an epi-cuticular layer corresponding to the cuticulin of the epi-cuticle in scorpions. This is bordered externally by further thin epi-cuticular components.

In the arthrodial membranes the exo-cuticle is absent and they comprise a 2μ heterogeneous epi-cuticular component overlying the strongly laminate endocuticle which is uniformly stained by aniline blue.

In contrast the thick cuticle of *Nemastoma lugubre* comprises numerous laminae which are closely and regularly packed in the external 3·5μ but less so in the internal 19μ. In neither of these regions do the laminae have any marked affinity for the foregoing stains. The most internal laminae do, however, have a slight positive reaction with acid fuchsin. Throughout the body the external closely packed laminate region contains the melanin which gives the species its characteristic colour. This is only lacking in the region of the two white spots.

In general the staining reactions of the arthrodial membranes, in which the epi-cuticular component is acid fuchsin positive and the endo-cuticular component aniline blue positive, compares with the condition in other arthropods. In view of the fact that the endocuticle of *Phalangium* and *Liobunum* is also aniline blue positive with a residual affinity for acid fuchsin, it would seem that sclerotization only affects the exo-cuticle. The limitation of the acid fuchsin positivity in *Nemastoma* to the innermost lamina suggests that in this genus which, together with the Cyphophthalamae, Laniatores and Trogulidae, has long been known from superficial inspection to have a harder cuticle, this hardening is associated with the sclerotization of the entire thickness of the cuticle. It is further seen that in all three species the endo-cuticular component of the sclerites with its loosely laminated structure is more closely similar to the structure of the endo-cuticular component of the arthrodial membrane than is the case in scorpions where the laminae of the arthrodial membrane are more marked in adult animals than are those of the endocuticle.

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'Wavy–Fused' Mutants in the Medaka, *Oryzias latipes*

AIDA[1] first reported the occurrence of *wavy* and *fused* vertebral mutations in the medaka. These characters are simple recessive to normal and are independent of all known genetic characters and sex. The vertebral column of the *wavy* fish curves dorso-ventrally (Fig. 1 *B*) and some centra of the *fused* fish are fused (Fig. 1 *C*). Fig. 1 *AD* show the backbones of normal and *wavy–fused* fish. Recently, Takeuchi[2] also reported a *wavy* mutant of the medaka which had appeared in the breeds of Yamamoto in Nagoya University and this mutant was used in the present experiment. Although the *wavy* mutant is a simple recessive, the way in which it expresses itself suggests the presence of modifiers of the principal gene. The *fused* mutant used here originally appeared in Aida's breed and has been kept in the laboratory of Yamamoto (Nagoya). A detailed

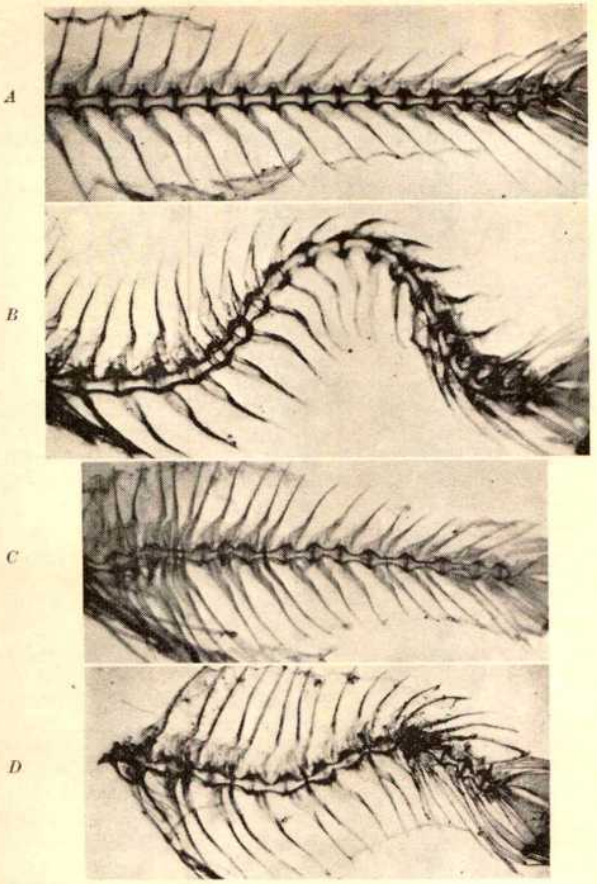


Fig. 1

genetic investigation of the *fused* mutants was performed by Yamamoto *et al.*[3].

Fishes were treated with 2 per cent potassium hydroxide for several hours and stained by alizarin *S* before they were examined under the microscope. The F_1 offspring of the male *wavy* mated to female *fused* was normal. Of 1,294 F_2 offspring, 885 were normal, 314 *fused*, 85 *wavy* and 10 *wavy–fused*. The ratio of normal plus *wavy* to *fused* plus *wavy–fused* is about 3 : 1 with a P value of 0·95. However, the ratio of normal plus *fused* to *wavy* plus *wavy–fused* is far from three to one ($P \ll 0{\cdot}01$). The result agrees with the earlier findings[2]. If the *wavy* and *fused* genes are on homologous chromosomes, the appearance of *wavy–fused* fish must arise from recombination, and the recombination fraction calculated from the number of F_2-offspring by the "maximum likelihood method"[4] is about 40 per cent. The value of the recombination fraction is unusually high and the foregoing hypothesis may not be true. The separation in F_2-offspring is better interpreted as follows: the *fused* and the main *wavy* genes are not on the homologous chromosomes. The relatively small numbers of *wavy* and *wavy–fused* offspring may arise from modifiers of *wavy* gene and the weakness of *wavy–fused* fish.

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Control of Lateral Bud Inhibition, Flower Emergence and Dormancy in the Blackcurrant

IN south-east England vegetative growth in the blackcurrant starts in April and ceases in July or early August, by which time shoots are about 60 cm long, bearing from twenty to thirty buds which usually start to initiate flowers from late June onwards. Normally, they do not break until the following spring.

In the field, premature bud break during the summer can be observed in shoots with damaged growing points, or occasionally after extensive leaf fall caused by an early and severe attack of *Pseudopeziza ribis*. This suggested that apical dominance and/or the presence of leaves may contribute to the maintenance of buds in a quiescent state during the summer.

To investigate the role of these two factors, experiments were carried out on the variety 'Wellington *XXX*' during 1964 and 1965.

Treatments consisted of either complete defoliation of whole bushes or the removal of the apical 2 in. of each shoot at weekly intervals beginning on June 1 and June 15, respectively. In untreated control bushes extension growth, assessed by weekly measurement, had ceased by July 15.

The results of these two experiments are summarized in Table 1 from which it will be seen that both treatments cause buds to break. Defoliation, however, is conspicuously more effective than decapitation. In the earlier defoliations bud break was distributed throughout the length of the shoots; following later treatments, however, there was a tendency for it to be restricted to the upper region only. By contrast, decapitation allowed the top two or three buds only to break and these were situated immediately below the pruning cut. Decapitation was effective in inducing bud break only up to the time of cessation of extension growth whereas defoliation caused bud break for a further five weeks.

During the period of extension growth, therefore, natural inhibition appears to be caused by a combination of apical dominance and leaf inhibition. After extension growth ceases buds seem to be inhibited by effects emanating from the leaves rather than the apex. From the end of August, when lateral buds appear to have entered full winter dormancy, leaf removal failed to induce bud break. Once this stage has been reached under normal field conditions, an adequate period of chilling at temperatures below 45° F (ref. 1) is required to ensure bud break in the following spring.

These results suggest that, apart from the apical dominance effect, growth inhibitors may be synthesized in the leaves and passed back into the buds, thus playing the principal part in preventing their growth. The presence of substantial amounts of inhibitor was demonstrated by El-Antably[2] in short-day grown blackcurrants.

One of the striking results of the defoliation experiment was the fact that leaf removal allowed the newly initiated flowers to emerge within a few weeks of their formation. Clearly there is no specific inhibition of the flower buds; their failure to emerge in the first season would seem to

Table 1. NUMBER OF BUDS BREAKING PER SHOOT

Treatment date	Apex removed, leaves present (mean of 3 bushes)	Apex intact, leaves removed (mean of 4 bushes)
June 1	—†	8·25‡
8	—	12·25§
15	2·96	12·50§
22*	1·29	11·00§
29	0·45	13·75§
July 6	0·09	11·75§
13	0·03	20·50§
20†	0·00	6·75§
27	0·00	4·25§
Aug. 3	0·00	5·25§
10	0·00	2·00§
17	0·00	0·25§
24	0·00	0·00§

* Flower initiation in untreated bushes.
† Cessation of extension growth in untreated bushes.
‡ Regeneration of vegetative growth only.
§ Regeneration of vegetative growth + flowers.

be linked directly with the general inhibition of lateral buds.

In the south-east of England, flower initiation in 'Wellington *XXX*' takes place just before the cessation of extension growth (ref. 3 and compare with ref. 4). In sectioned buds this was seen to have occurred by June 22 in 1964, that is 23 days before the cessation of extension growth. The first defoliation treatment (June 1) induced vegetative growth only, whereas defoliation on or after June 8 resulted in the emergence of flowers also. These observations suggest that the first irreversible steps towards flower initiation had already occurred in the buds 2 weeks before the first initials could be detected. This conclusion was supported by data obtained in 1965 when plants in the field were maintained in an actively growing condition by artificial illumination or gibberellic acid applied some weeks before extension growth of untreated bushes had ceased. Where the treatments were applied more than 2 weeks before the microscopically detectable onset of flower initiation (early July), the plants continued to grow vegetatively and flower initials were not observed when the buds were examined several weeks later. Buds of untreated control plants carried advanced initials at this time. Where treatments were applied less than two weeks before flower initiation was observed, flowers developed in the lower buds of treated plants despite the continuation of extension growth.

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Biological Activity of Volatile Fungal Metabolites

A RECENT survey has measured the effects of volatile metabolites from sixty-two fungal cultures on chosen characters of growth and reproduction of five assay species, namely: *Rhizopus sexualis* (Smith) Callen, *Chaetomium globosum* Kunze ex Fr., *Stereum hirsutum* (Willd.) Fr., *Aspergillus niger* van Tiegh., and *Botrytis cinerea* Pers. ex Fr. The sixty-two species tested consisted of thirteen Phycomycetes, thirteen Ascomycetes, six Basidiomycetes and thirty Fungi Imperfecti, representing a wide range of habit. These species were tested using the simple apparatus shown in Fig. 1; each test consisted of a comparison of three replicate cultures, each containing the assay fungus on the upper agar plate and the test fungus on the lower plate, with three replicate controls each containing the assay fungus on the top plate and sterile agar on the lower plate.

In these conditions the gases from forty-one of the sixty-two species had a significant effect on the rate of increase of colony diameter of at least one of the assay species. Table 1 summarizes these observations.

The relatively crude measurements have not revealed any interactions affecting rate of increase of diameters of colonies of *R. sexualis*, but two test species (*Saccharomyces cerevisiae*, *Schizosaccharomyces octosporus*) produced at least twice the least significant inhibition of all the other four assay species; one (*Fomes annosus*) produced more than ten times the least significant inhibition of *C. globosum* and of *A. niger*, and almost twice the least significant inhibition of *S. hirsutum* and of *B. cinerea*. There were many more inhibitory than stimulatory reactions, and most of the stimulation was very slight.

Table 1

No. of species having a significant effect ($P = 0{\cdot}05$)	*Rhizopus sexualis*	*Chaetomium globosum*	*Stereum hirsutum*	*Aspergillus niger*	*Botrytis cinerea*
Inhibitory	0	11	15	4	19
Stimulatory	0	2	3	7	5
No measured effect	62	49	44	52	38

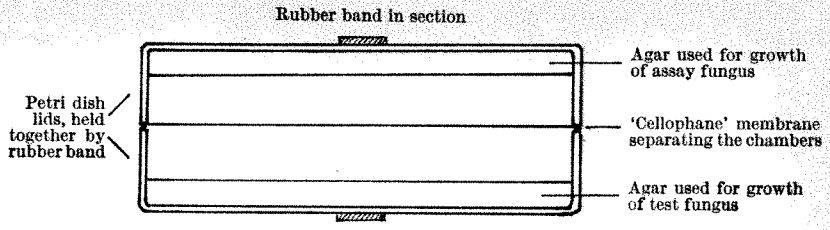


Fig. 1. Assembly used for the tests of effects of volatile metabolites

The survey has also examined the effects on zygospore production by *R. sexualis*, perithecial formation by *C. globosum*, and conidial formation by *A. niger* and *B. cinerea*. The effects on these very different reproductive processes varied, as was to be expected. Sixteen species significantly stimulated zygospore production, six species inhibited it significantly ($P = 0{\cdot}05$). Empirical observation indicated that two species (*Saccharomyces cerevisiae*, *Ceratocystis coerulescens*) inhibited perithecial production by *C. globosum* by more than 50 per cent, and three species (*Mucor plumbeus*, *Ceratocystis coerulescens* and *Fomes annosus*) stimulated zygospore production in *R. sexualis* but inhibited perithecial production in *C. globosum*. Eight species inhibited conidial production by *A. niger* and/or *B. cinerea* by more than 25 per cent.

This work has been carried out to give a preliminary indication of the likely frequency of occurrence of such metabolites, in expansion of earlier work in these laboratories[1] and by Lösel[2]. These published results are comparisons of means of observations of three replicate dishes in a single series of experiments. The main stimulatory and inhibitory effects have been confirmed by other experiments, but many of the differences are too small to justify discussion without further information. The general pattern, however, suggests that the occurrence may be widespread. No consistent relationship appeared in the range of effects on the measured characters of growth or of reproduction, and the distribution of activity showed no consistent relationship to any standard taxonomic system. The survey is being continued by investigations of the distribution of occurrence of such metabolites, of their effects on fungi and on green plants, and of their chemical structure and mode of action. Individual cases are being examined in detail; for example, active constituents of gas mixtures from cultures of *Saccharomyces cerevisiae* and of *Fomes annosus* have been identified by gas chromatography and biological tests of authentic pure compounds, and full descriptions are being published elsewhere (for example, ref. 3).

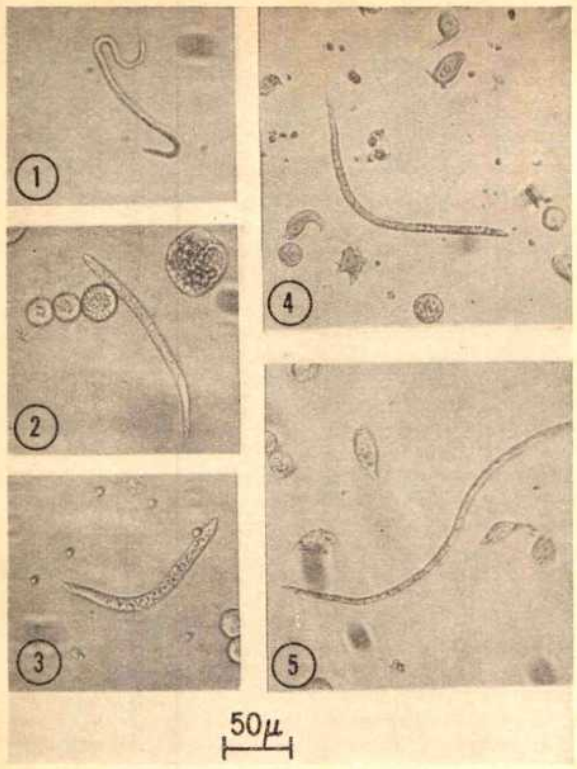
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In vitro Development of Microfilariae of *Macacanema formosana* in Mosquito Cell Cultures

EARL[1] reported the maintenance of microfilariae in mixture 199 plus dog serum for 61 days. Sawyer and Weinstein[2] and Weinstein[3] reported development of the microfilariae of *Dirofilaria immitis* and *Wuchereria bancrofti*, respectively, to late first-stage ("sausage-form") larvae. This communication describes the *in vitro* development of



Figs. 1–5. Larval stages of *Macacanema formosana* showing development occurring in the presence of *Aedes aegypti* cells in culture. Fig. 1. Microfilaria from blood of monkey. Fig. 2. "Pre-sausage" form days 3–4 in culture. Fig. 3. "Sausage" stage day 5 in culture. Fig. 4. Second stage larva days 6–9 in culture. Fig. 5. Maximum stage reached in culture days 14–17

Macacanema formosana microfilariae through second- and possibly to third-stage larvae in the presence of *Aedes aegypti* cells in culture.

The *A. aegypti* cells are those of Grace[4] and have been maintained in serial culture for 3 years. They have been adapted by Suitor[5] to a growth medium consisting of 0·5 per cent *Philosamia cynthia* haemolymph, treated according to Grace[6], plus 10 per cent foetal bovine serum in Grace's[7] insect cell culture medium (GMA). The cells are cultured in 2-oz. glass or plastic screw-capped bottles at 28° C and have a generation time of approximately 42 h.

Microfilariae were obtained from the blood of a Taiwan monkey, *Macaca cyclopsis*. Five-ml. samples of blood containing 70–80 microfilariae/mm³ were drawn and placed in tubes containing heparin. Blood was held for 12–16 h at approximately 30° C until utilized. To separate microfilariae from the blood, 1 ml. samples were diluted in 10 ml. of 0·02 per cent saponin in Ringer's saline, *p*H 7·2. Complete lysis of the red cells occurred on incubation with intermittent shaking at 37° C for 15 min. The samples were washed by centrifuging at 1,500 r.p.m. for 4 min, discarding the supernates, and resuspending the pellets in 5 ml. of GMA. After the washing was repeated, samples were recentrifuged, as above, the supernates discarded, and the pellets resuspended in complete insect cell growth medium with or without *A. aegypti* cells. The growth media were not changed for the duration of the experiment.

Preliminary results indicated that development of the microfilariae failed to take place at 28° C, but did take place at 22° C. Since the insect cells in culture were able to multiply at 22° C, the following results were all obtained from experiments carried out at that temperature.

Development of the microfilariae was observed daily by direct-light and phase-contrast microscopy. Measurements were made with a calibrated ocular micrometer.

M. formosana microfilariae were 145–200μ by 3·0–3·8μ when isolated from the monkey blood (Fig. 1).

Microfilariae in complete insect cell medium without cells became sluggish within 2 days. They grew to 190–230μ by 6–7·5μ in approximately 6 days, after which many of them became vacuolated. Most of the microfilariae were dead by the seventh day.

In the insect cell medium with growing and multiplying cells microfilariae developed in the following manner:

On the third and fourth days (Fig. 2), the larvae became shorter and broader. Their movement was sluggish and their interior somewhat granular in appearance. A distinct tail was present.

On the fifth day (Fig. 3), the larvae reached the "sausage" stage. Movement during this period was minimal. "Sausage" stage larvae measured 120–128μ by approximately 9·6μ. Internal granulation was heavy.

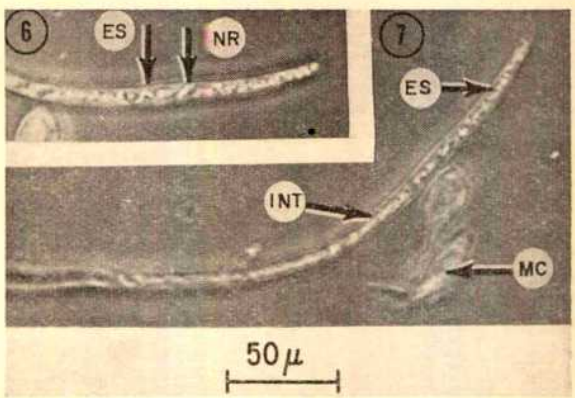
On the sixth to ninth days (Fig. 4), the larvae grew rapidly in length and narrowed slightly. Ninth day larvae measured 230–270μ by 6–8μ. The interior of the larvae cleared partially and flexion movements increased during this time. Phase-contrast microscopy revealed internal development.

On the fourteenth to seventeenth days (Fig. 5), the larvae reached the maximum stage of development attained in this experiment. They measured 290–303μ by 6–9·6μ. These larvae were active and some made "probing" motions with the anterior end.

On the nineteenth day (Figs. 6 and 7), observations of larvae by phase-contrast microscopy showed the presence of a mouth, oesophagus, nerve ring, and intestine. The external cuticle of the larvae had separated but had not been shed. Larvae survived up to 33 days but developed no further. At the time of death, the larvae were heavily vacuolated and in a state of degeneration.

The development of internal organization (oesophagus, nerve ring, and intestine) in the *M. formosana* larvae after 5 days *in vitro* indicated that the larvae had reached second stage. However, no moult was observed. By the nineteenth day the mouth had opened to the exterior of the larvae and the oesophagus, intestine, and nerve ring had become more distinct. The larvae at this stage had moulted but still retained the external cuticle, and so the tail and the anal plug were still present. Although it cannot be stated that the "infective" stage was reached, definite development of second and possibly third stage larvae occurred. Attempts to test the infectivity of the larvae are planned.

No apparent reaction occurred between *M. formosana* larvae and the insect cells nor was multiplication of the cells impaired by the presence of the larvae. In several experiments where cell development was slowed during the initial days of incubation, apparently because of the lack of adequate amounts of growth-promoting substances in the medium, larval development was also retarded. This suggests that conditions necessary for cell develop-



Figs. 6–7. Phase-contrast microscopy of *M. formosana* larvae showing development attained in 19 days in the presence of *A. aegypti* cells in culture. *ES*, Oesophagus; *INT*, intestine; *NR*, nerve ring; *MC*, mosquito cell

ment are similar or identical to those necessary for development of the filarial larvae.

The life cycles of many parasites involve insects as intermediate hosts. An *in vitro* method for investigating this phase of development of a parasite, offering conditions closely approximating the environment in the insect, is highly desirable. Insect cells in culture may provide this method.

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Types of Group Selection

THE terms group selection and intergroup selection[1,2] have been used in referring to a variety of phenomena including recently the evolution of behavioural social systems involved in population control[3,4]. Since the phenomenon of group selection promises to receive greater attention from students of evolution in the future, I feel that a clarification of terms at this time will help to prevent confusion. The "groups" under consideration may be quite different from each other and the action of natural selection on them will depend on the nature of the group.

At least two extreme types of group can be recognized: the family or lineage group and the population. Intermediate types occur, especially in species in which culture is important, but only the extreme types will be discussed here. The lineage group is a sub-group within a population, and is defined on the basis of kinship. A population in the ecological sense, although it may also have a common lineage, is generally defined from other populations spatially, irrespective of lineage. Theoretically the two concepts can overlap, but in practice this is not embarrassing. The term kin selection may be applied to lineage groups (following Smith[5] in part). The evolution of parental care, whether behavioural, morphological or physiological, may be attributed to kin selection.

The simplest lineage group is composed of one or more parents and their offspring. In the typical case the family group disperses before the first generation has reached maturity. In some social insects, a few species of birds, and in some other animals, the young remain with the parents and help to raise subsequent broods, as in some cases of "helpers at the nest"[6]. Among birds, in the Mexican jay (*Aphelocoma ultramarina*)[7], fairy wren (*Malurus cyaneus*)[8], Australian magpies (*Gymnorhina dorsalis*[9] and *G. tibicen*)[10], and some other species[11], territories may be defended by social groups larger than pairs, in which incipient communal behaviour may occur; but the most extreme development of this evolutionary potentiality in birds appears to be the situation found in certain anis (Crotophaginae) in which all phases of breeding may be communal[11]. In the latter cases, the extent to which inbreeding determines the genetic composition of successful groups is not known but deserves study.

Assuming lineage to be important in the latter cases, we can then recognize a continuum among various species in the importance and complexity of the family or lineage group and in the number of generations in which it may stay together as a unit. The existence of such a continuum is provided for in the expression "inclusive fitness" in Hamilton's[12] model for kin selection. It is possible at all positions on this continuum to recognize the existence of competition between lineage groups. This may be done whether the unit is a pair of titmice and their brood and the competition influences the evolution of clutch size[13] (simple extreme in Table 1) or if the unit is a flock of anis (*Crotophaga* spp.) and the competition determines the social organization and behaviour of the flock and species (complex extreme in Table 1). The critical point in all these cases is that the group is characterized (to varying degrees) by a common lineage, which is characteristically maintained by social bonds within a population and with no important ecogeographic isolation from other such groups. The evolution of the ability to form such social bonds is also provided for in Hamilton's[12] model.

Table 1. CHARACTERISTICS OF TWO TYPES OF GROUP SELECTION

	Kin selection		Interpopulation selection	
	Simple extreme	Complex extreme	Slight ecogeographic separation	Large ecogeographic separation
Lineage relationship	Simple family	Communal group	Irrelevant	Irrelevant
Duration of group	One generation	Indefinite	Indefinite	Indefinite
Gene exchange between groups	Unrestricted	Partially restricted by social behaviour	Partially restricted by geography	Nearly absent
Competition between groups	Sympatric	Sympatric	Allopatric	Allopatric

Smith[5] has referred to one extreme of this continuum as "group selection" and to the other extreme as "kin selection". In my opinion, because these cases may be recognized as lying along a continuum (provided for in Hamilton's[12] model) they should all be interpreted as representing one phenomenon, which may conveniently be called kin selection, or perhaps lineage selection. To use the term "group selection" for any of these cases is confusing, as Wynne-Edwards[3,4] has used "group selection" and "intergroup selection" in explicit reference to intraspecific interpopulation competition, which is quite a different phenomenon. In order to avoid confusion I propose the following definitions: selection between lineage groups within populations will be called kin selection; selection between spatially defined (allopatric) populations of a species will be called interpopulation selection (see Table 1).

Whether the term group selection will eventually prove useful as a generic term to include both kin selection and interpopulation selection remains to be seen. The differences between the two are significant. Kin selection depends on sympatric competition between lineage groups within a population and can be treated theoretically in a manner comparable to individual selection[12], since the basic time unit is most frequently the generation and the lineage groups are not prevented from interbreeding except partially in the complex cases, as in species with communal territories. Interpopulation selection depends primarily on allopatric competition; the basic time units are the time constants for extinction and colonization, and it may require a different type of mathematical expression, such as game theory[14].

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ENTOMOLOGY

Apolysis in Arthropod Moulting Cycles

FOR many years comparisons of moulting cycles in the Arthropoda have suffered from lack of a generally accepted terminology. The most ambiguous word, especially for Insecta, has been "moult", which has been used to denote two completely different events in the cycle.

The colloquial meaning of "moult", like that of "la mue" and "der Häutungsakt", has always been the visible shedding of the old shell, or exoskeleton, at ecdysis. When Snodgrass[1] wrote, "*Moulting*, with arthropods, is the separation of the old cuticle from the new cuticle formed beneath it; *ecdysis* is the emergence of the insect from the moulted skin", he was attempting to wrest the term moult away from its colloquial sense to apply it to an earlier, preparative event in the cycle: an event that had already been sharply distinguished from ecdysis in Crustacea by Drach[2] and in Insecta by Hinton[3].

Unfortunately, although the distinction between the two events was recognized to be of great physiological importance[4], and the term ecdysis was generally accepted for shell-shedding, the earlier event never received a specific name[5].

We now propose *apolysis* for the freeing of the epidermal cells from the old exoskeleton in any arthropod. It can be clearly seen in sections (Fig. 1), and is most like D_1 among the stages described by Drach[2] for Crustacea and "moulting" as defined by Snodgrass[1] for Insecta. Together with the secretion of at least some layers of the new cuticle and digestion of the old, apolysis is an essential preparation for the ecdysis which usually, though not necessarily, follows.

Apolysis is derived from the Greek 'απόλυσις, meaning "a freeing from" or, more particularly, "the loosing of a bandage"; it stands in contradistinction to ecdysis (ἔκδυσις), which means the actual "sloughing" of the skin. We are indebted to Prof. D. E. Eichholz for suggesting the new term.

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Light-on and Light-off Effects on the Circadian Flight Activity in the Mosquito *Anopheles gambiae*

A PRELIMINARY investigation of the flight activity of female *Anopheles* (*Cellia*) *gambiae* Giles (Lagos strain) fed on sugar has been undertaken using a modification of the recording technique described by Jones[1]. Groups of five or ten recently emerged adults were placed in a 'Perspex' walled chamber (*c.* 1·5 l.). This was placed in a sound-proof box in a constant temperature room, maintained at 24°–26° C. The mosquitoes were supplied with a small quantity of 15 per cent glucose solution with a cotton wool "wick", which also served to maintain a relative humidity in the chamber of 65–85 per cent. Artificial lighting (150–200 lux) was used during both the rearing and experimental periods, and the light régimes were controlled with a simple time switch. Flight activity was recorded acoustically for periods of up to 1 week, and a direct record of the activity was made with a pen marker on a kymograph with a continuous paper roll attachment. A similar recording "channel", without mosquitoes, was used to record any unwanted acoustic or electronic interference which was loud enough to operate the pen markers.

Groups of females subjected to alternating periods of 12 h light and 12 h dark had periods of intense activity

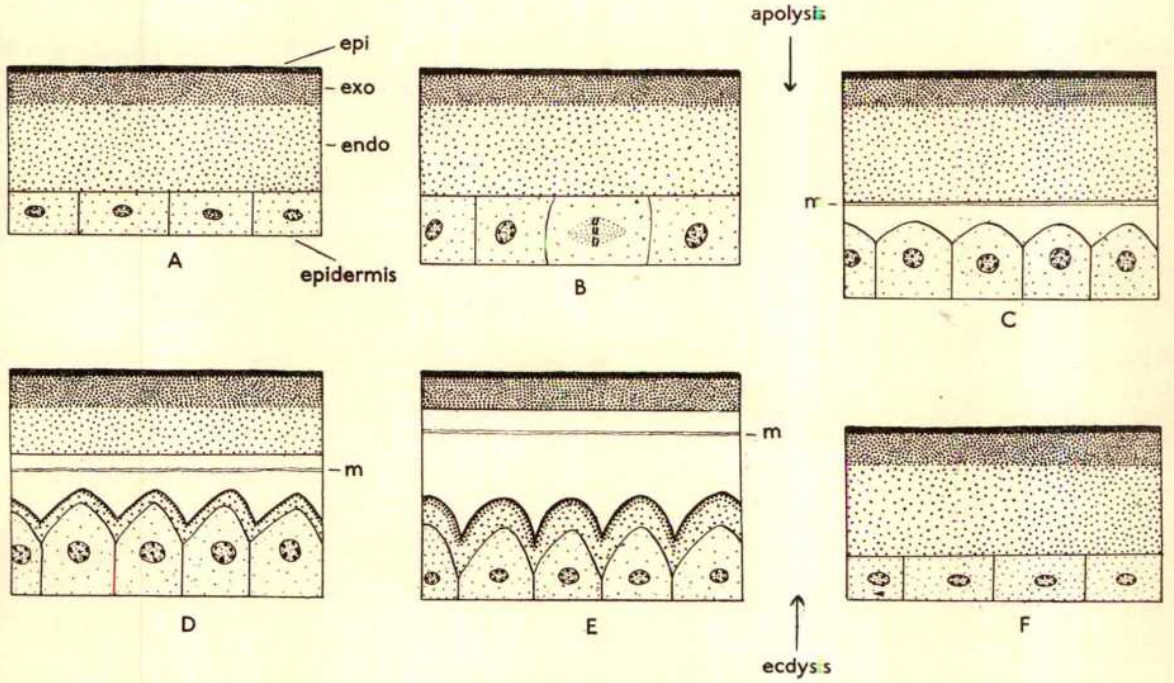


Fig. 1. (*A–F*) The successive changes in the form of the integument of an arthropod during and after the moulting cycle. *endo*, endocuticle; *epi*, epicuticle; *exo*, exocuticle; *m*, moulting membrane or ecdysial membrane

lasting about 20–30 min following both the light-on and the light-off signals. They were inactive during the remainder of the light period, but were usually active in "bursts" throughout the dark period. A 2 h dark period included in the first 9 h of a 12 h light period produced no response. If the light was switched off 3 h early, there was no immediate increase in activity, but the insects became active 1–2 h earlier than usual. If the light was switched off 6 h late, the onset of activity was delayed until the light was switched off.

There was no obvious cyclical pattern of activity in mosquitoes kept in constant light throughout their life, but cyclical activity with a period of about 23 h followed a change to constant dark. The active part of the cycle lasted about 12 h and the first period of activity started with the light-off signal. It is not clear from these findings whether the change resulted from the synchronization of the individual cycles which were previously out of phase, or whether the cycle was started by the change from light to dark.

The light-on and light-off responses differed in their respective latencies. Light-on activity usually started within a few seconds of the signal, whereas the onset of light-off activity was delayed usually for 1–5 min.

It would seem from these results that the pattern of flight activity is controlled by an endogenous rhythm similar to that which controls oviposition and sugar feeding in other mosquitoes[2–4]. The cycle has a free-running period in the dark of less than 24 h and light appears to have an inhibitory effect on activity. Thus, in alternating light and dark, activity is synchronized with the external régime by the delaying effect of light. Eyles and Bishop[5] have observed that egress of *Anopheles quadrimaculatus* from their resting places at sunset can be prevented by artificial illumination. The cycle is not immediately responsive to light-off signals given 3 h or more earlier than usual. The latency of the light-off response and the similarity with the results of other work[6] suggest that a hormonal mechanism is involved[7] and that light acts by inhibiting secretion. The speed of the light-on response suggests that it is mediated directly by the nervous system and may be a "startle" reaction to the sudden switching on of the light. Pre-adaptation of the insect to the dark may be an important constituent of this reaction.

We thank Mr. G. Davidson and Miss W. Wall, of the Ross Institute of Tropical Hygiene, for supplying the mosquito eggs.

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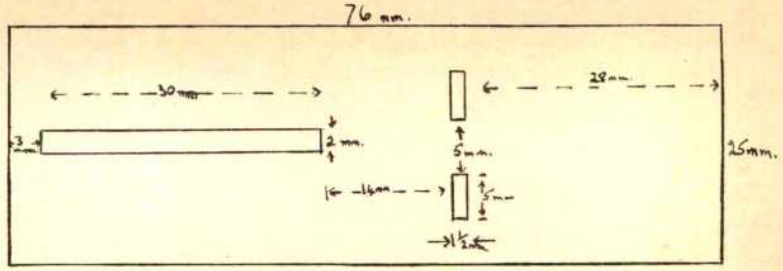


Fig. 1

HAEMATOLOGY

Haptoglobin Types in Dried Bloodstains

THE determination of haptoglobin types in dried bloodstains has until recently met with very limited success. The method for determining haptoglobin types using starch gel[1–4] is the most satisfactory technique available for serum samples. Direct transposition of this technique to bloodstains has been tried by many research workers[5–7] both in starch and acrylamide gels. The patterns obtained from stain material differ considerably from those shown by fresh serum samples. It is extremely difficult to interpret the results as they can be obscured by the excess haemoglobin present and it can only be done if the stain is very fresh (that is, not more than about 3 days old). The problem of excess haemoglobin can be overcome by subjecting the sample to a short preliminary electrophoresis in starch gel at *p*H 6·5 (tank buffer: 0·04 M disodium hydrogen phosphate adjusted to *p*H 6·5 with *ortho*-phosphoric acid; gel buffer: 20 ml. tank buffer diluted to 150 ml.). At this *p*H, haemoglobin moves towards the cathode while the haptoglobin/haemoglobin complex moves towards the anode. After 30 min electrophoresis at 6 V/cm, a block of gel about 1 cm² is removed on the anodic side of the origin and inserted into a gel at *p*H 8·6 with a buffer system according to Poulik[4]. However, we required a method which was accurate and would allow the determination of haptoglobin types in stains at least one month old. These conditions have been met by using an immuno-electrophoresis method.

Immuno-electrophoresis was carried out using the buffer system described by Hirschfeld for *Gc* groups[8] using 1 per cent 'Ionagar'. (Tank buffer, 1·38 g barbituric acid, 8·7 g sodium barbitone, 0·384 g calcium lactate, 2 l. water. Gel buffer, 0·553 g barbituric acid, 3·503 g sodium barbiturate, 0·512 g calcium lactate, 1 l. water *p*H 8·6.)

The gels were cut into the pattern shown in Fig. 1.

It was found that antigen slots give better definition of precipitate arcs than the more usual circular wells. It also enabled sufficient antigen to be inserted into the gel while keeping the origin narrow. Enough stain material to fill the antigen slot was soaked in a minimum of gel buffer for 30 min–1 h before inserting it into the gel. Electrophoresis was carried out at 6 V/cm for 4·5–5 h at 4° C. Anti-haptoglobin serum was used to fill the central trough and diffusion was allowed to proceed for 24–36 h at 4° C. Most batches of antisera were diluted 1 : 4 with gel buffer. The haptoglobin types could be determined by the differing position, length and shape of the precipitate arcs, as shown in Fig. 2.

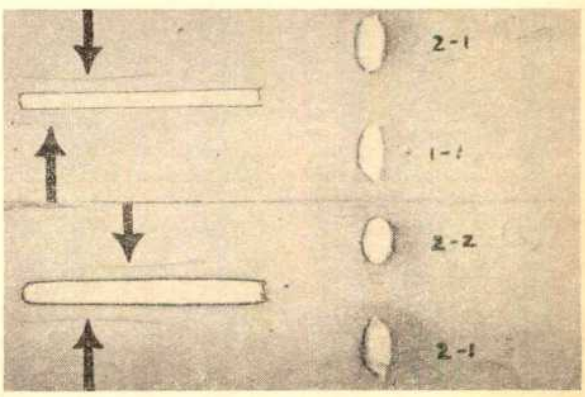


Fig. 2

The accurate interpretation of these arcs takes considerable practice. The most important parameter is the position of the peak of the arc. These are indicated by arrows in Fig. 2. The interpretation of the length and shape of the arc will follow with practice if attention is centred on the peak of the arc. In many cases it is possible to read the precipitate arcs in the wet gel. We have, however, found it better to reserve final judgment until saline washing (24 h), drying and staining the gel in amido black are completed. Weak precipitates can be seen clearly after this procedure. As the position of the arcs shifts progressively towards the cathode with age of the stain components, stains of a similar age and of known haptoglobin types should be run as controls at the same time as the suspect material. This should be done on each run as slight variation of movement can occur from one run to the next depending on the operator.

Stains up to 3 months old have been correctly typed. The type of cloth which forms the substrate of the stains seems to have only a quantitative effect; more material is required (in area of cloth) if the substrate is cotton than if it is wool. The intensity of the bloodstaining also has considerable bearing on this, of course. Experience is required in estimating the correct amount of material to use.

Many hundreds of these determinations have now been made in this Laboratory both on test and case material and we are satisfied that this is a method which can be used in the routine examination of bloodstains in forensic laboratories.

All this work has been performed with Shandon immunoelectrophoresis equipment and Dutch Red Cross antisera.

After this work was completed a report by Hallermann, Kiwi and Sturner[9] came to our notice which describes work on similar lines. Hallermann *et al.* suggested the use of a modified gel buffer containing glycerine. Its composition is: Solution *A*, 7·505 g glycine, 3·85 g sodium chloride, 1 l. water. Solution *B*, 0·1 M sodium hydroxide. 62·5 ml. of solution *A* are mixed with 37·5 ml. of solution *B* giving a buffer of *p*H 10·0, $\mu = 0{\cdot}15$. This is diluted to $\mu = 0{\cdot}05$ and mixed with an equal volume of barbituric lactate gel buffer mentioned earlier. The tank buffer remains the same as before. We have tried this and find that on some occasions the separation is marginally better, while on other occasions no difference can be detected.

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Morphological Variations in the Eosinophil Granule revealed by Cytochemical Methods

MORPHOLOGICAL variations in the human eosinophil granule under electron microscopic examination have been recently recorded[1]. Furthermore, other variations in the eosinophil granule have been described before[1]. In fact, it seems probable that there are two or more different populations of granules.

This concept is substantiated by my own findings[2,3]. The regularity and morphological features of sudanophilia and peroxidase activity in human peripheral blood eosinophils were studied in an attempt to provide normal data for succeeding investigations. Material was obtained from 104 normal adults of both sexes. The reactions of eosinophils have been observed using Sheehan and Storey's[4] and Washburn's[5] methods, for sudanophilia and peroxidase activity, respectively.

Both sudanophilia and peroxidase activity were similar in appearance and intensity in all the granules of 83 per cent (82·8 per cent, sudanophilia, 83·1 per cent, peroxidase activity) of the eosinophils. However, in 17 per cent the positive granules showed wide variations in number, size, shape, colour, and distribution. In many cases several varieties of granules occurred in one eosinophil.

Thus, sudanophilia parallels peroxidase activity in human peripheral blood eosinophils and both may exhibit different features in the granules of one eosinophil.

Sufficient material now exists to support a working hypothesis on identification and characterization of distinct eosinophil granules with evolutionary and functional criteria.

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Coagulant Activity of Tiger Snake (*Notechis scutatus scutatus*) Venom

TIGER snake venom has the ability to clot blood. Holden[1] and Blömback[2] have presented evidence that prothrombin, Factor V and calcium ions are required for the venom to convert fibrinogen to fibrin. We have studied the action of this venom on several purified clotting factors. As the crude venom was used in these experiments, the results obtained may arise from more than one active constituent present in the venom.

In preliminary experiments with plasma systems, it was confirmed that the venom exerts its optimal coagulant activity only in the presence of prothrombin, Factor V, a divalent metal (calcium, barium, strontium or manganese), and a phospholipid emulsion which is able to increase the rate of activation of prothrombin. The venom had very little coagulant activity on purified human fibrinogen (Kabi Pharmaceuticals, Ltd.). Furthermore, the venom neither activated nor destroyed Factor X. When purified bovine Factor X (ref. 3), or Factor X in human serum, was incubated at 37° C with tiger snake venom (Koch-Light Laboratories, Ltd.), phospholipid and calcium ions, no activated Factor X was produced; if, however, after 30 min Russell's viper venom was added, it converted all the Factor X to the active form (X*a*). This is interesting in itself, as no other venom has yet been shown to be capable of activating prothrombin and incapable of activating Factor X (refs. 4 and 5).

Blömback[2] has proposed that the coagulant substance of tiger snake venom acts on Factor V, and as a result Factor V acquires the ability to activate prothrombin. In his experiments, incubation of a partially purified Factor V preparation with the venom produced a prothrombin converting activity which increased with time. However, we have repeated these experiments with pure bovine Factor V (ref. 6), and have found that incubation of Factor V with the venom, with or without phospholipid ('Inosithin') and calcium ions, produced no progressive increase in coagulant activity, but rather a gradual inactivation occurred.

The rate of activation of purified bovine prothrombin by the venom in purified systems was studied, with conditions as described elsewhere[7]. The effect of adding Factor V, phospholipid (phosphatidic acid/phosphatidylcholine, 1 : 1) and calcium ions to prothrombin and the

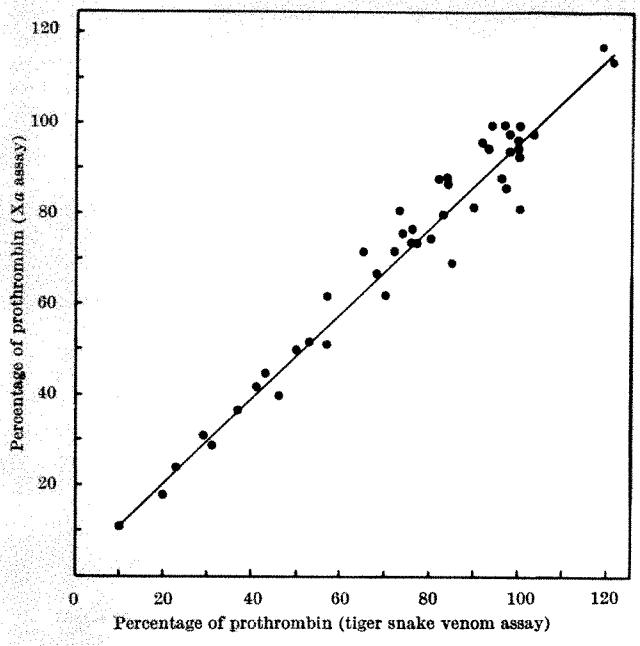


Fig. 1. Correlation between tiger snake venom assay and Xa assay of prothrombin. Regression line of y on x

venom in various combinations is shown in Table 1. The results are expressed as relative rates of prothrombin activation, and are compared with those obtained with activated Factor X (Xa). It can be seen that the venom and Xa have very similar properties; however, in the absence of calcium, Factor V increases the rate of prothrombin activation by the venom, but not by Xa.

Table 1. A COMPARISON OF THE RELATIVE RATES OF PROTHROMBIN ACTIVATION BY TIGER SNAKE VENOM (TSV) AND Xa IN THE PRESENCE OF FACTOR V, PHOSPHOLIPID AND CALCIUM IONS IN VARIOUS COMBINATIONS

Mixtures	Relative rates With TSV	With Xa
Prothrombin	0·033	0·36
Prothrombin + phospholipid	< 0·10	< 0·27
Prothrombin + Ca	1·0	1·0
Prothrombin + V + phospholipid	4·5	—
Prothrombin + V	22·3	0·87
Prothrombin + phospholipid + Ca	27·0	50·1
Prothrombin + V + Ca	145·8	311·0
Prothrombin + V + phospholipid + Ca	4,687·5	1,610·0

It is known that Xa is adsorbed on to some phospholipids in the presence of a divalent metal, and this is also true for the venom coagulant activity. As shown in Table 2, Xa and the venom activity are adsorbed on to phosphatidic acid/phosphatidylcholine (1 : 1) in the presence of calcium, barium or strontium ions, but there is little adsorption in the presence of magnesium. Moreover, both the venom activity and Xa show little ability to form a complex with an emulsion of pure phosphatidylcholine, which suggests that a negative surface charge on the phospholipid micelles is required for the formation of such complexes[6].

Table 2. ADSORPTION OF TIGER SNAKE VENOM (TSV) AND Xa ON TO EMULSIONS OF PHOSPHATIDIC ACID/PHOSPHATIDYLCHOLINE (PA/PC) AND PHOSPHATIDYLCHOLINE (PC) IN THE PRESENCE OF DIVALENT METALS

Phospholipid	Divalent metal	Activity adsorbed (per cent) TSV	Xa
PA/PC	None	< 3	0·02
PA/PC	Ba	105	63
PA/PC	Sr	29	13
PA/PC	Mg	6	0·16
PA/PC	Ca	100	100
PC	**Ca**	**7**	**8·7**

In view of these findings, we felt that the venom could be used in a one stage assay of prothrombin. Various modifications of the original one-stage prothrombin assay of Quick[8] have already been introduced in order to increase the specificity of the test. Spaet and Cintron[9] have devised a prothrombin assay in which Xa is added and converts prothrombin to thrombin in the presence of a high concentration of Factor V, fibrinogen, phospholipid and calcium ions. However, the test requires purified Xa, and it occurred to us that a similar assay could be designed in which tiger snake venom would be used instead of Xa.

0·1 ml. of the citrated plasma to be tested, diluted 1/10 in buffered saline (*p*H 7·5), was added at 37° C to 0·1 ml. of oxalated barium sulphate absorbed bovine plasma. Then 0·1 ml. of 0·025 M calcium chloride was added, followed quickly by 0·1 ml. of a mixture of 'Inosithin' (Associated Concentrates, Ltd.) 1·4 mg/ml. and tiger snake venom (60–120 μg/ml.) or Xa. Serial dilutions of normal citrated human plasma in buffered saline between 1/10 (100 per cent) and 1/1,000 (1 per cent) were assayed and a log dose was plotted against log clotting time. In a typical assay with the venom, the 1/10 (100 per cent) and 1/100 (10 per cent) dilutions of the normal plasma gave clotting times of 11 and 31 sec respectively, and the assay gave a blank clotting time of 80–90 sec. A significant correlation was obtained between the results given by the venom assay and those given by the assay with Xa (Fig. 1) in a study of forty-five different plasmas (correlation coefficient: 0·96).

A comparison was also made between the venom assay and the two-stage prothrombin assay[10]. Plasma samples sent to the laboratory for a routine two-stage prothrombin test which gave a "prothrombin" estimation of less than 50 per cent were also assayed by the venom test. As shown in Fig. 2, there is a considerable discrepancy between the estimations given by the two assays.

The essence of the two-stage test is such that an abnormal level of plasma antithrombin activity might affect the result of the assay. Therefore, each plasma included in this study was assayed for its thrombin inhibitor(s) activity[10]. The plasmas which have a thrombin inhibitor(s) activity higher or lower than 115 per cent of normal have been indicated in Fig. 2, in order to illustrate the major source of discrepancy between the assays which is associated with a rise of plasma thrombin inhibitor(s), as well as the frequency of occurrence of these abnormal levels in the group studied.

The most likely explanation of this discrepancy is that the two-stage test gives unduly low prothrombin estimations when there is a high titre of plasma thrombin inhibitor(s). In cases of obstructive jaundice, there is often a rise in antithrombin III (ref. 11), and this may occur with or without vitamin K deficiency. Therefore,

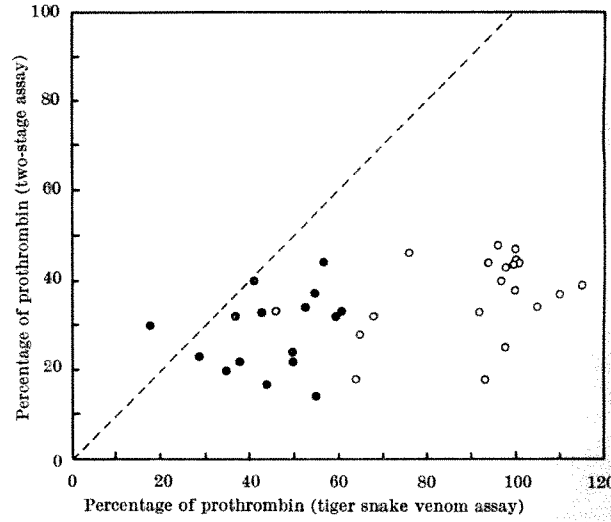


Fig. 2. Correlation between the two-stage prothrombin test and the prothrombin assay with tiger snake venom. ○, Thrombin inhibitor ⩾ 115 per cent; ●, thrombin inhibitor < 115 per cent

the inability to correct a low two-stage test by the parenteral administration of vitamin K might not necessarily indicate a severe parenchymal liver lesion.

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IMMUNOLOGY

Immunofluorescent Staining of Globule Leucocytes in the Colon of the Sheep

THE nature and fate of the globule leucocyte are of particular interest in relation to the immune mechanisms of the gut since it has been shown to increase during helminth infection[1] and appears to have certain affinities to plasma cells and Russell's body cells which have been shown to contain antibody[2].

Four previously worm-free sheep were experimentally infected with 1,500 infective larvae of *Oesophagostomum columbianum* and killed after ten weeks of infection. Tissue samples were taken from the colon and caecum of each animal and fixed in Helly's and Carnoy's fluids and cold acetone, embedded in paraffin wax and sectioned at 4μ according to the method of Culling[3].

Fluorescein isothiocyanate-conjugated rabbit anti-guinea-pig and rabbit anti-sheep globulin were obtained from Difco, Detroit. Rabbit anti-human serum was prepared according to the technique of Kabat and Mayer[4] and conjugated together with normal rabbit and human sera using fluorescein isothiocyanate following the technique of McDevitt *et al.*[5].

Sections of sheep colon were stained using rabbit anti-sheep globulin to detect globulins within cells; the rabbit anti-guinea-pig globulin and anti-human serum were used to test the specificity of this reaction. Further controls were made using conjugated normal rabbit and sheep globulin and unconjugated rabbit and sheep serum to test for non-specific attachment and for fluorescence of the serum proteins themselves. Phosphate buffered saline controls were used to test for fluorescence of the unstained sections. A slide of the aqueous mountant alone was also tested for fluorescence.

All the histological sections were first taken to water in the usual way and rinsed in phosphate-buffered saline, *p*H 7·2, for 15 min. Each slide was then flooded with the test fluorescent stain or control solution and left for 1 h. The slides were all rinsed using phosphate-buffered saline twice for 15 min and once for 1 h; these were then mounted using aqueous mountant and scanned.

All microscopy was done using a Leitz 'Ortholux' microscope with transmitted ultra-violet and white light with a dark ground condenser D1.20 A and an Fl. Oel. 95/1.32 objective with an Oel. 100/Fl. Oel. 95 × D1.20 stop inserted. Photographs of all the sections were taken with a Leitz 'Orthomat' photographic attachment using Ilford Pan *F* 50 *ASA* film.

Only in the sections stained with conjugated rabbit anti-sheep globulin was any fluorescent staining noted. All the globules in the globule leucocytes fluoresced (Fig. 1) and the plasma cells showed a similar fluorescence. In many of the plasma cells large fluorescent globules were shown which were of a similar size to those in the globule leucocytes. No fluorescence above a faint non-specific level was noted on any of the control sections (Fig. 2).



Fig. 1. Globule leucocyte stained with fluorescein isothiocyanate-labelled rabbit anti-sheep serum

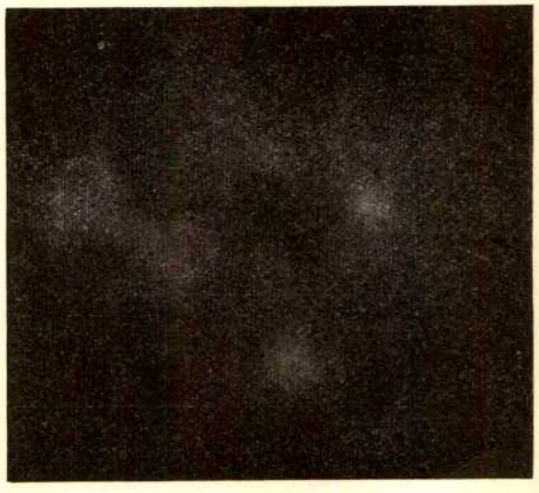


Fig. 2. Globule leucocyte from a control slide

It can thus be concluded that the cytoplasm of the globule leucocyte contains condensations of globulin. The similarities between the staining, the nuclear structure and the presence of globules of similar size and shape in the plasma cells and the globule leucocyte suggest that there is some relationship between these cells.

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PATHOLOGY

Susceptibility of Old World Monkeys to Yaba Virus

Bearcroft and Jamieson[1] reported an outbreak of a tumour epidemic in the laboratories of the West African Council for Medical Research in Yaba, Nigeria, among imported rhesus monkeys. Andrewes *et al.*[2,3] showed that this epidemic resulted from a virus of the pox group. Sproul *et al.*[4] classified the tumour as a histiocytoma. Earlier, we reported[5] that Asiatic monkeys (*Macaca mulatta*, *Macaca irus* and *Macaca speciosa*) are susceptible to the virus, and that a tumour developed, following accidental inoculation, in a laboratory worker as well as in five cancer patients. By contrast, African monkeys (*Cercopithecus aethiops*, *Cercopithecus aethiops pygerythrus*, *Cercopithecus mona* and *Cercocebus fuliginosus*) imported from east and west equatorial Africa are resistant. The hypothesis was advanced that this virus may be endemic in certain areas of Africa and that most African monkeys develop immunity at an early age.

As one approach to investigating this hypothesis, a number of young monkeys from African species born in our colony or in zoos in the United States were inoculated subcutaneously with dilution series of Yaba virus suspensions. Animals from the same species imported from Africa were also inoculated. Each virus suspension used in this investigation was also inoculated into at least two *Macaca speciosa* imported from Asia. "Takes" were defined as production of lesions at least 0·5 cm in diameter which persisted for at least 3 weeks, which showed characteristic histological features[4,5] and, when homogenized and injected subcutaneously into *Macaca mulatta*, produced similar lesions.

Table 1

Species	Takes/No. inoculated		
	African import	U.S. born	Asiatic import
Sooty mangabey (*Cercocebus fuliginosus*)	0/4	2/2	
Vervet monkey (*Cercopithecus aethiops pygerythrus*)	0/4	1/1	
Anubis baboon (*Papio anubis*)	0/3	1/1	
Patas monkey (*Erythrocebus patas*)	0/1	1/1	
Stumptail macaque (*Macaca speciosa*)		1/1	10/10

Table 1 summarizes the results. All African monkeys born in the United States and all Asiatic monkeys developed tumours with 10^{-2}–10^{-4} as the maximal effective dilutions of the virus; none of the imported African monkeys showed any reactions. These results seem to support the hypothesis that African monkeys acquire immunity to Yaba virus in their natural habitat; resistance does not seem to be species specific. It must be kept in mind, however, that in this investigation most of the monkeys born in the United States were young (several months beyond weaning), while most of the imported monkeys appeared somewhat older, although their exact age was unknown. Another weakness of this experiment is the small number of African monkeys born in the United States which were available for investigation.

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Electrophoretic Heterogeneity of Erythrocyte and Leucocyte Glucose-6-phosphate Dehydrogenase in Italians from Various Ethnic Groups

PREVIOUS investigations of drug-induced and fava bean-induced haemolytic anaemia in subjects with a deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) have demonstrated that they are similar both in their clinical characteristics and in the mechanism of genetic transmission of the enzyme defect. Nevertheless, a considerable amount of evidence has accumulated to indicate that this enzyme defect reflects heterogeneous genetic mechanisms in various ethnic groups. It has been demonstrated that the enzyme deficiency may be complete or intermediate, that it may affect erythrocytes alone or involve other tissues[1–3], and that the enzyme protein may differ in its characteristics in cells from subjects of different ethnic groups[3–7]—including its electrophoretic behaviour.

These investigations were almost exclusively undertaken with the erythrocyte enzyme. It is well known that in affected Caucasian male subjects the leucocyte G-6-PD is deficient while it is normal, or almost normal, in Negro subjects of both sexes[3,8]. The enzyme activity is very low (rarely more than 2 per cent of the controls and thus not suitable for assay) in the erythrocytes from sensitive Sardinian subjects which we have extensively studied[6,9–11], and usually in sensitive Caucasian subjects[3,8,12]. Accordingly the leucocyte enzyme is especially suitable for an investigation of the properties of the mutant enzyme in this ethnic group.

In this investigation, a careful study has been made of the properties of leucocyte G-6-PD and a comparison has been made with the erythrocyte enzyme of sensitive subjects from several ethnic groups. The present research is concerned with investigations of a group of twenty-nine subjects from three Sardinian families, from a Sicilian family and from a north Italian family. Our results indicate alterations in electrophoretic patterns of leucocyte G-6-PD from the sensitive subjects, and heterogeneity in the characteristics of the enzyme among the families from Sardinia, Sicily and north Italy.

The families studied were identified when the propositus was admitted to hospital with a haemolytic crisis. The propositi are: case Nos. 5, 11, 12 and 20 for a fava bean-induced haemolytic episode; case No. 24 for a rifocin-induced haemolytic episode; case No. 29 for a pyramidone-induced haemolytic crisis.

Crude samples of the enzymes were prepared as previously described[13]. The modification of the Tsao technique[14] for descending starch-gel electrophoresis which we used has already been described[15], as have the procedures for the G-6-PD and 6-phosphogluconate dehydrogenase (6-PGD) assays used to determine the protein and haemoglobin content[13].

Fig. 1 shows an example of the electrophoretic migration of erythrocyte (case Nos. 9 and 16) and of the leucocyte (case Nos. 3, 12 and 24) G-6-PD from normal and mutant subjects. Two bands were frequently present in the preparations of the leucocyte enzyme, both for male and female normal and mutant subjects. The faster moving band is more intensively stained and results from G-6-PD activity, while the slower one is much less apparent and can be attributed to 6-P-GD. We were able to show that this slower band was in fact 6-P-GD and not a G-6-PD isoenzyme in the following ways: (1) The colour was developed in the usual manner, the slice was then carefully washed and the blue tetrazole stain developed in the presence of 6-phosphogluconate as substrate. The new stain appeared in the same band as the slower component and gave added intensity to the latter. (2) When two symmetrical gel slices were separately stained for G-6-PD and for 6-P-GD, there was a perfect correspondence between the 6-P-GD band and the slower band which appeared in the G-6-PD slice.

The electrophoretic behaviour and activity of the erythrocyte and leucocyte enzymes from twenty-nine subjects are given in Table 1. The results can be summarized as follows:

(1) The subjects from the three Sardinian families showed a strikingly similar behaviour in the hemizygote subjects (case Nos. 3, 4, 5, 6, 7, 11, 12 and 20) and in the homozygotes (case No. 2), with the complete erythrocyte defect and with leucocyte G-6-PD markedly diminished (the activity was about 50 per cent of that of the controls) and altered in its electrophoretic behaviour (the rate of flow was slower). In the female heterozygotes (case Nos. 9, 10, 16 and 19), the erythrocyte G-6-PD showed a lowered activity and a slower electrophoretic mobility, while the leucocyte enzyme was normal. Case No. 17 could be classified as an abnormal homozygote and was of special interest because of its features: it exhibited a very low, but still detectable, level of activity and we were able to demonstrate (by the activity level and the electrophoretic run) the defect both in the red and in the white blood cells. Our results clearly indicate that in the Sardinian mutant subjects the leucocyte enzyme defect has an unusual genetic trait and this was also the case for the erythrocyte enzyme.

(2) The north Italian family can be considered an interesting variant. As a matter of fact, both in the male (case No. 24) and in the female mutants (case Nos. 23 and 26) the erythrocyte enzyme shows an intermediate activity and normal electrophoretic behaviour. The leucocyte enzyme has a normal activity but slower electrophoretic mobility. It is also different from what has been described for the Barbieri family[5], and for some intermediate Greek[12], German and Irish[8] mutant subjects.

(3) The only alteration which we could detect in the Sicilian family was the markedly slower electrophoretic migration of the leucocyte G-6-PD. It is apparent from the anamnesis that both the mother and a son had had haemolytic episodes following the ingestion of peas and drugs.

Table 1. ACTIVITY LEVEL AND ELECTROPHORETIC MOBILITY OF G-6-PD FROM ERYTHROCYTES AND LEUCOCYTES OF NORMAL AND MUTANT SUBJECTS

Case No.	Subject	Erythrocytes: Specific activity*	Erythrocytes: Percentage of electrophoresis†	Leucocytes: Specific activity*	Leucocytes: Percentage of electrophoresis†
	Normal	15·2 ± 1·2	100	132·6 ± 11·8	100
	L. family (Sardinian)				
(1)	Pietro father	14·6	100	140·3	100
(2)	Assunta mother	N.D.‡	–	74·5	92
(3)	Aldo son	,,	–	62·9	87
(4)	Carlo son	,,	–	70·8	91
(5)	Pino son	,,	–	63·0	90
(6)	Michele son	,,	–	51·7	93
(7)	Piero son	,,	–	64·2	88
(8)	Lina daughter	13·8	100	151·4	100
(9)	Natalia daughter	7·3	69	129·3	100
(10)	Maria daughter	7·0	78	138·5	100
(11)	Ignazio son of Maria	N.D.‡	–	52·6	93
	T. family (Sardinian)				
(12)	Mario father	N.D.‡	–	71·2	89
(13)	Irene mother	15·0	100	132·4	100
(14)	Pino son	13·8	100	116·5	100
(15)	Anna daughter	14·7	100	141·6	100
(16)	Rosa daughter	7·2	71	122·8	100
(17)	M. Teresa daughter	2·2	77	76·3	90
	B. family (Sardinian)				
(18)	Luigi father	15·6	100	147·6	100
(19)	Clara mother	7·2	81	131·4	100
(20)	Andrea son	N.D.‡	–	62·3	91
(21)	Paolo son	14·0	100	125·8	100
	B. family (north Italy)				
(22)	Carlo father	13·8	100	153·0	100
(23)	Maria mother	5·0	100	134·6	91
(24)	Alberto son	8·2	100	143·2	81
(25)	Giovanni Carlo's father	15·8	100	118·4	100
(26)	Assunta Maria's mother	6·8	100	130·6	78
	P. family (Sicily)				
(27)	Leonardo father	16·1	100	137·4	100
(28)	Amalia mother	13·8	100	141·0	77
(29)	Placido son	13·5	100	132·5	76

* A unit of activity of G-6-PD is defined as the amount of enzyme required to give an increase in optical density at 340 mμ of 1·0 per min. The erythrocyte specific activity is referred to 1 g of haemoglobin and that for leucocytes to 1 g of protein.

† The electrophoretic mobility of the enzyme is expressed as a percentage of the mobility of the controls. The values related to case Nos. 23, 24 and 26 are the average of three determinations carried out at various times.

‡ Not detected.

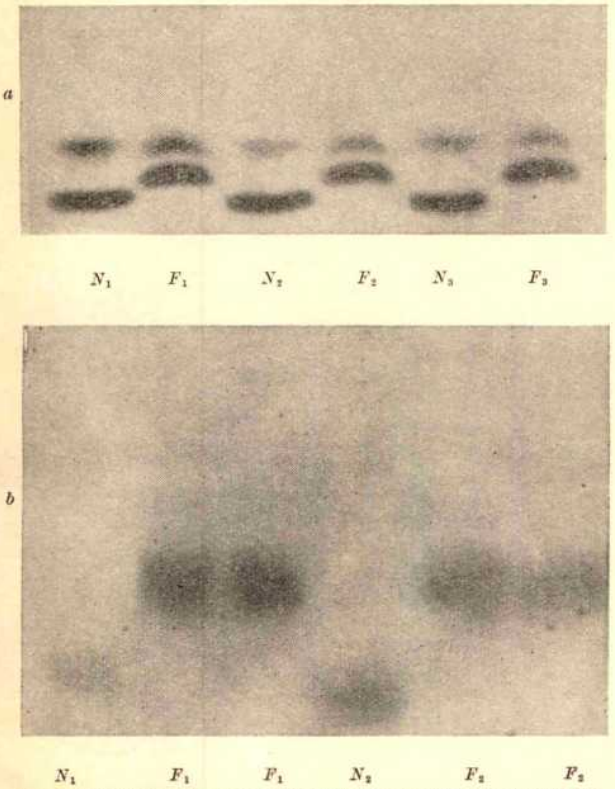


Fig. 1. Electrophoretic mobility of erythrocytes and leucocytes G-6-PD in starch gel of normal and mutant subjects. *a*, The leucocyte enzyme: F_1, F_3 and F_3 refer to case Nos. 3, 12 and 24; *b*, erythrocyte enzyme: F_1 and F_2 refer to case Nos. 9 and 16. Channels indicated as N refer to control enzymes

These results suggest a heterogeneity of G-6-PD deficiency in various Mediterranean ethnic groups and offer some evidence for a definite alteration in the electrophoretic pattern of the leucocyte G-6-PD from sensitive subjects.

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Uptake of Iodine-131-labelled Atabrine by Ehrlich Ascites Tumour and by Sarcoma S-180 *BALB*

ACKERMAN[1,2] reported a selective incorporation of iodine-131-labelled atabrine by lung and subcutaneous Novikoff hepatoma and Walker carcinosarcoma-256 tumours. In order to provide more information concerning the usefulness of this labelled compound for the localization and diagnosis of malignant tumours, we investigated its uptake by Ehrlich ascites tumours and sarcoma *S*-180 *BALB*.

A group of *BALB* mice was inoculated with Ehrlich ascites tumour cells. Eight days later they were injected subcutaneously with 10 μc. of iodine-131-labelled atabrine (specific activity, 0·12 mc./mg)[3]. Groups consisting of five animals each were killed 1, 2 and 5 days after the injection. The intraperitoneal exudate was immediately withdrawn with a syringe and centrifuged at 3,000 r.p.m. for 15 min. Both centrifugates and supernatants were counted for radioactivity with a scintillation counter; in addition, the radioactivity in the liver, spleen and leg muscles was measured. A control group of healthy animals injected with the same dose of iodine-131-labelled atabrine was killed at the same time intervals; the intraperitoneal cavity was washed with distilled water and the water counted for radioactivity under the same conditions. As in the experimental group, the radioactivity retained by the liver, spleen and muscles of control animals was measured. Table 1 gives values corresponding to the specific activities found (c.p.m./g tissue).

A similar injection test was performed on a group of five *BALB* mice with sarcoma *S*-180 *BALB*. These tumours were produced by inoculating tumour-cells 20 days beforehand. The animals were killed 5 days after the intraperitoneal injection of the iodine-131-labelled atabrine. The subcutaneous tumours were excised and also the adjacent and abdominal muscles. The radioactivity was counted in each case (Table 1).

Table 1. VALUES OF THE SPECIFIC ACTIVITY (C.P.M./G TISSUE)

	1 day	2 days	5 days
Ehrlich ascites tumour			
Iodine-131-labelled atabrine (injected subcutaneously)			
Cells (centrifugate)*	135 ± 87†	104 ± 19	107 ± 71
Exudate			
Liquid (supernatant)*	300 ± 215	81 ± 43	119 ± 39
Spleen	332 ± 150	382 ± 125	81 ± 35
Liver	273 ± 136	317 ± 125	263 ± 98
Muscle	159 ± 106	72 ± 21	12 ± 7
Control			
Intraperitoneal washing*	93 ± 15	38 ± 21	13 ± 10
Spleen	380 ± 265	425 ± 180	138 ± 98
Liver	426 ± 105	466 ± 137	764 ± 365
Muscles	112 ± 39	98 ± 54	68 ± 29
Sarcoma, *S*-180 *BALB*			
iodine-131-labelled atabrine (injected intraperitoneally)			
Sarcoma			94 ± 30
Muscles (leg)			89 ± 44
Muscles (abdomen)			97 ± 39

* c.p.m./ml.
† Standard deviation.

The experimental results revealed that the activity incorporated by the Ehrlich ascites tumour cells remained almost unchanged throughout the experiment; the control group showed no significant activity. The pattern of uptake and elimination by the liver and spleen was similar in both groups and the observed variations between them could be related to the influence of increased fluid content in the body of tumour-bearing animals. The most significant finding was the retention of iodine-131-labelled atabrine by the tumour cells, whereas in the other tissues of normal and ascitic animals the radioactivity decreases considerably. The liver, however, is the exception to the rule. This organ has previously been shown to be responsible for the elimination of this labelled compound through the gastrointestinal tract[3].

The sarcoma *S*-180 *BALB* showed slightly greater activity than the surrounding leg muscles.

These findings seem to corroborate the preferential uptake of certain aminoacridine compounds by the tumour tissue observed by Ackerman.

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Interaction of Poly-2-vinylpyridine-1-oxide with Silicic Acid

THE ability of poly-2-vinylpyridine-1-oxide (P204) to prevent the pathogenic action of quartz particles in the lungs of experimental animals and the cytotoxic effect in cell cultures has been repeatedly demonstrated[1]. Its mode of action is uncertain. Apparently it does not act by coating the particles because it is more effective if the P204 is added to the culture or is injected intraperitoneally into the animal than if the dust is pre-treated with it. Weiss and Harvey[2] suggested that P204 may be oxidized in the tissues to poly-2-vinyl-6-hydroxypyridine-1-oxide which might interact with silicic acid formed by dissolution of the quartz. They demonstrated that stable cationic silicon complexes are formed between 2-hydroxypyridine-1-oxide and *ortho*silicic acid. We have found that interaction occurs between poly-2-vinylpyridine-1-oxide itself and silicic acid by hydrogen bond formation.

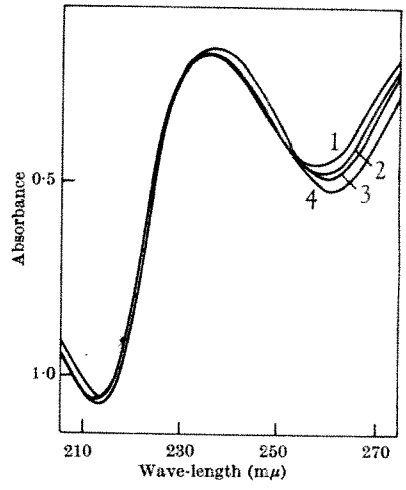


Fig. 1

Poly-2-vinylpyridine-1-oxide in chloroform has an electronic absorption spectrum with a peak at λ_{max} = 272·5 mμ (see Fig. 1 and Table 1). In ethanol this peak is shifted to λ_{max} = 268·5 mμ and there is an additional peak at λ_{max} = 220 mμ, the latter being obliterated by chloroform. In water P204 gives peaks at 213 mμ and 260·5 mμ. These values indicate that polyvinylpyridine oxide forms hydrogen bonds with water and with ethanol. When the oxide is dissolved in freshly prepared 0·01 M aqueous silicic acid, the peaks are at 213 mμ and 259 mμ. The shift of the longer wavelength peak indicates that the oxide hydrogen bonds more strongly with silicic acid than with

Table 1. ABSORPTION SPECTRA OF POLY-2-VINYLPYRIDINE-1-OXIDE

Solvent	λ_{max} (mμ)	ε_{max}
Chloroform	272·5	6,636
Ethyl alcohol	268·5	6,486
Water	260·5	5,300
Aqueous silicic acid*	259·0	4,550

* When the solution stood for 24 h, λ_{max} shifted to 257·0 (ε_{max} = 4,500).

water. If this solution is allowed to stand for 24 h, the peak shifts further to 257 mμ. It is probable that this does not arise from polymerization of the silicic acid, but from unfolding of the polymer chains which makes more groups available. If the silicic acid is allowed to stand for 24 h before admixture with the polymer, the absorption peak is at 259·5 mμ.

It would seem that the action of polyvinylpyridine oxide in preventing the pathogenic action of quartz may result from the direct action of the polymer itself, without its previous oxidation, in mopping up *ortho*silicic acid as it is formed in macrophages. These observations support the view that the pathological changes in silicosis are initiated by *ortho*silicic acid resulting from the dissolution of silica particles in the cytoplasm of phagocytes.

The P204 was prepared by oxidizing polyvinylpyridine (Midland Tar Co., Ltd.) with hydrogen peroxide in acetic acid. This report results from research sponsored by the Asbestosis Research Council.

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Some Effects of Certain Naturally Occurring Oestrogens on the Anterior Pituitary Glands of Rats

THE earliest investigations of the effects of oestrogens on the cytology of the anterior pituitary gland were made using oestrone[1–3] which was first isolated in crystalline form in 1929. Diethylstilboestrol, a synthetic substance with oestrogenic activity, has been used in most of the more recent work[4,5]. In this communication the cytological effects of chronic administration of the naturally occurring oestrogens—oestrone, equilenin, 16-epioestriol and oestriol—on the anterior pituitary gland of ovariectomized rats are compared: the action of the last three has not been previously investigated. Measurements of the DNA content of individual nuclei from pituitary glands of rats treated with the oestrogens and of controls were made using Feulgen micro-spectrophotometry.

Sixty-three female Sprague–Dawley rats were divided into seven equal groups. Six groups were ovariectomized at 90–100 days of age: one group was maintained as ovariectomized controls, a second group was injected subcutaneously with 1 ml. corn oil daily, and the third, fourth, fifth and sixth groups were given daily 0·05 mg of oestrone, equilenin, 16-epioestriol and oestriol respectively, each dissolved in 1 ml. of corn oil. The seventh group was maintained as intact controls. The rats from groups 3, 4, 5 and 6 were killed at 3, 6 and 14 months after starting oestrogen treatment, together with the appropriate controls. The pituitary glands and the uteri were weighed. Each pituitary was cut sagittally with a razor blade, just beyond the lateral edge of the posterior lobe. Prints of nuclei were taken on coverslips from the cut surface: these were freeze substituted, hydrated and then stained under rigorously controlled conditions with the Feulgen procedure[6]. The photometric absorption by single nuclei at 546μ was measured using a Deeley type integrating microdensitometer[7,8] and this gave a measure of their DNA content in arbitrary units. A search was specially made in each print for abnormal nuclei. The larger piece of pituitary was fixed either in 10 per cent formolsublimate or, more satisfactorily, in a fixative containing chrome-alum[9]. Sections (3μ) were cut coronally at intervals through each pituitary, and were stained with the periodic acid–Schiff (PAS)-trichrome method and by a modification of an aldehyde thionin–PAS–trichrome method[10].

In each case the effects of naturally occurring oestrogens were evident after 3 months of treatment and further treatment usually resulted only in an accentuation of these effects. After ovariectomy the gonadotrophs became degranulated, enlarged and vacuolated, having the typical appearance of "castration cells"[11,12]. Treatment with any of the four oestrogens almost suppressed this change at 3 and 6 months and in the 14 month group no "castration cells" were seen. In the case of oestrone treatment for 3, 6 and 14 months the PAS-positive granules of most of the gonadotrophs consisted of a few coarse clumps capping the nucleus. With 16-epioestriol and equilenin this appearance of the PAS-positive granules was manifest at 14 months, and with oestriol, although the "castration cell" vacuoles were absent the gonadotrophs were very abundant and appeared normal with well granulated cytoplasm at 3, 6 and 14 months.

Table 1. PITUITARY GLAND WEIGHT (MG) OF OVARIECTOMIZED RATS TREATED WITH OESTROGENS AND OF CONTROLS

Treatment	Pituitary gland wt.* (mg) at 3 months	6 months	14 months
Intact	15·2	14·4	—
Ovariectomized	13·5	15·5	12·5
Ovariectomized + oil	14·7	14·8	16·0
Ovariectomized + oestriol	14·0	20·6	18·2
Ovariectomized + 16-epioestriol	15·9	17·9	25·0
Ovariectomized + equilenin	15·1	23·0	—
Ovariectomized + oestrone	19·5	24·2	82·3 (range 36·2–175)

* Each value is the mean for three animals.

Oestrone induced so massive an increase in the number of acidophils that the glands assumed an abnormal and tumorous macroscopic appearance after 14 months of treatment. The weight of the pituitaries was greatly increased in rats treated with oestrone (Table 1). In areas of acidophil proliferation the cells had enlarged nuclei with an "empty" appearance, the chromatin forming a rim against the nuclear membrane; there was a large central nucleolus. During the normal oestrous cycle there is an increase in the number of mitotic figures in the acidophils at oestrus when increased levels of oestrogen are produced[13]. We found that only pituitaries treated with oestrone had abundant mitotic figures in the acidophils. Table 2 shows the distribution of nuclei from 14-month treated rats according to their content of DNA measured in arbitrary units with the microspectrophotometer. Only oestrone treatment had the effect of greatly increasing the proportion of nuclei with amounts of DNA greater than that of diploid nuclei ($2N$); some nuclei had amounts greater than that at the tetraploid mode ($4N$). In equilenin and 16-epioestriol treated rats, in intact controls, and in ovariectomized controls given oil, there were a few nuclei with DNA contents above the diploid amount. No nuclei with more DNA than the diploid amount could be

Table 2. DISTRIBUTION OF NUCLEI FROM 14-MONTH TREATED RATS ACCORDING TO THEIR CONTENT OF DNA MEASURED IN ARBITRARY UNITS

Treatment	Total No. of nuclei measured	DNA value: $2N$* No. of nuclei	$2N$* Percentage of total	Between $2N$ and $4N$ No. of nuclei	Between $2N$ and $4N$ Percentage of total	$4N$ No. of nuclei	$4N$ Percentage of total	More than $4N$ No. of nuclei	More than $4N$ Percentage of total
Intact	31	28	90·3	—	—		9·7	—	—
Ovariectomized + oil	36	33	91·7	2	5·5		2·8	—	—
Ovariectomized + oestriol	76†	76	100	—	—		—	—	—
Ovariectomized + 16-epioestriol	103‡	96	94·1	1			4·9	—	—
Ovariectomized + equilenin	89†	85	95·4	1			2·4	1	1·1
Ovariectomized + oestrone	164‡	105	64	18			7·7	12	7·3

* N, Amount of DNA in a haploid set of chromosomes. † From two rats. ‡ From

Table 3. UTERUS WEIGHT (MG) OF OVARIECTOMIZED RATS TREATED WITH OESTROGENS AND OF CONTROLS

Treatment	Uterus wt.* (mg) at 3 months	6 months	14 months
Intact	646	500	—
Ovariectomized	131	117	135
Ovariectomized + oil	124	129	226
Ovariectomized + oestriol	640	477	718
Ovariectomized + 16-epioestriol	501	494	928
Ovariectomized + equilenin	506	616	—
Ovariectomized + oestrone	705	561	764

* Each value is the mean for three animals.

found with oestriol treatment. Treatment with each of the oestrogens resulted in the maintenance of the weight of the uteri, which was greatly reduced by ovariectomy and by ovariectomy with corn oil (Table 3).

Under the conditions of our experiment only oestrone treatment induced (1) a great increase in the numbers of acidophils, (2) the presence of numerous mitotic figures in the acidophils, (3) the presence of abundant nuclei with contents of DNA greater than the normal presumed diploid amount ($2N$) and even exceeding the tetraploid ($4N$) amount, and (4) a great increase in the weight of the pituitary gland. The presence of many mitotic figures and many nuclei with a DNA content between the $2N$ and $4N$ amounts in the oestrone treated rats indicates that the latter are probably interphase nuclei which are synthesizing DNA in preparation for cell division. The occurrence of relatively numerous nuclei with contents of DNA well above the $4N$ value suggests that the increase in the number of acidophils is not due to a continuous, but otherwise normal, stimulation by oestrogen as suggested by Clifton and Meyer[5], but that the cells are abnormally stimulated by oestrone treatment, and that some of them have assumed characteristics usually possessed only by malignant cells.

All four of the naturally occurring oestrogens showed oestrogenic activity by preventing the development of "castration cells" in the pituitary gland after ovariectomy; oestrone was the most effective and oestriol the least. Fieser and Fieser[14], however, report that the oestrogenic potency of oestrogens administered subcutaneously as determined by "bioassay in rats" is such that oestrone is greater than oestriol and oestriol is greater than equilenin; by oral administration oestriol is more active than oestrone. Furthermore, Loraine *et al.*[15] found that 16-epioestriol is less active than oestriol when assessed by the Allen and Doisy[16] vaginal smear test. In our experiment all the oestrogens maintained the weight of the uteri which fell drastically after ovariectomy (Table 3). Lauson *et al.*, however, found that in assays based on increase in uterine weight in intact immature rats oestriol is more potent than oestrone[17].

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Effect of Thorazine (Chlorpromazine) on Collagen Metabolism

IN the course of an investigation of the effect of partial body X-irradiation on collagen metabolism we used thorazine (chlorpromazine) as an aid in immobilizing rats[1]. We found quite unexpectedly that the incorporation of carbon-14-labelled amino-acids into collagen was markedly suppressed during the first month, and considerably enhanced 2–4 months after the injection of the drugs. The effect of thorazine on collagen metabolism was observed in all tissues, but was most pronounced in bone collagen. Because of the involvement of bone collagen, we feel that our findings should be brought to the attention of clinicians who may be treating fracture cases with, among other drugs, thorazine, a drug which may impair the healing process.

Granulomata were induced in rats by implanting polyethylene sponges in the flanks of 50 day old Sprague–Dawley rats, by a method similar to that described by Boucek and Noble[2]. The sponges were collected after 7 days, sliced, and incubated with constant shaking in 5 ml. of Puck's medium *N*-16 containing 0·39 μc./ml. of 2-carbon-14-glycine and 5 per cent newborn calf serum in an atmosphere of 95 per cent oxygen and 5 per cent carbon dioxide. After the incubation was over, total collagen was extracted from the sponges using boiling water and the collagen thus extracted was purified by precipitation with trichloroacetic acid and acetone[3]. The purified material was dissolved in 2·0 ml. of water and a 0·1 ml. portion plated on planchets for counting in a windowless gas-flow counting system. Another portion was used for the spectrophotometric determination of the collagen content at 280 mμ using a solution of commercial gelatin as a standard. In order to correct for differences in the cellularity of different granulomata, the specific activity of collagen (c.p.m./mg) was normalized on the basis of the DNA content of the sponge, determined by the method of Webb and Levy[4].

Two types of experiments were carried out with the *in vitro* system described: (1) incubation of granulomata removed from granuloma-bearing rats injected intramuscularly 1, 24, 48 and 72 h earlier with 3·75 mg thorazine per 100 g body-weight (Table 1) and (2) incubation of granulomata from uninjected rats after addition of various amounts of thorazine to the incubation mixture (Table 2).

The data shown in Table 1 indicate that there is a significant increase in carbon-14 incorporation 72 h after the injection of thorazine. In addition, there appeared to be a slight depression of isotope incorporation 24 and 48 h after the drug was injected, which manifested itself after 3 h of incubation, although this is not highly significant. The increase in isotope incorporation on the third day after injection resembles changes seen in the intact animal long after thorazine injection. At present we cannot

Table 1. EFFECT OF INJECTION OF THORAZINE ON THE *in vitro* SYNTHESIS OF COLLAGEN

Time after injection of thorazine (h)	Incubation time (h) 1	2	3
1	183	495	788
	195	680	
24	228	246	378
	286	485	
48	254	157	545
	311	328	585
72	455	990	1,590
	545		1,687
Uninjected control	296 (270–324)	452 (282–642)	880 (650–1,135)

The figures in columns 2–4 represent carbon-14 activities expressed as c.p.m./mg of collagen per μg of DNA per g dry weight. With the exception of the control values, each activity value represents the result obtained with two granulomata from one animal, the granulomata having been incubated separately, but then combined for the isolation of collagen. In the case of the uninjected control rats the single, centred figures in columns 2–4 represent average carbon-14 activities of collagen obtained from experiments with four rats, each bearing two granulomata. As in the case of the thorazine-injected rats, the granulomata were incubated separately and then combined for isolation of collagen. The figures in parentheses represent the ranges of values obtained.

Table 2. EFFECT OF THE ADDITION OF THORAZINE TO THE INCUBATION MIXTURE

Thorazine added (μg)	Carbon-14 activity
None	1,252 ± 500*
125	2,372 ± 800
250	1,590 ± 600
500	1,443 ± 200
1,250	835 ± 150
2,500	425 ± 170

Carbon-14 activity expressed as c.p.m./mg collagen/μg DNA/mg dry weight.
* Standard deviation (based on three separate experiments).

explain this change in collagen metabolism due to the injection of thorazine.

The results shown in Table 2 demonstrate that thorazine, when supplied in sufficiently high doses (2,500 μg), has a pronounced inhibitory effect on the incorporation of carbon-14-glycine into collagen. There is no clear evidence at any time of an *in vitro* stimulation of the incorporation of labelled glycine into collagen by thorazine, as seen in the *in vivo* experiments. It is not possible to compare the drug concentrations prevailing in these *in vitro* experiments with the level of thorazine present in the granulomata after injection of thorazine, since we did not investigate the distribution of the drug in the injected rats. If one assumes a random distribution of the drug in the rat, there would be approximately 2 μg in the granuloma, a level considerably below that achieved at the lowest concentration of thorazine in the *in vitro* experiments. Since the stimulation of the incorporation of carbon-14-glycine into collagen after injection of the drug was not observed until the third day after the injection, it is conceivable that the effect of thorazine on collagen metabolism is secondary to some other effect of the drug, perhaps on the endocrines, such as on pituitary[5] or adrenal glands[6].

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PHARMACOLOGY

Effects of Actinomycin *D* and Puromycin on Chromosomes of Cultured Human Leucocytes

ACTINOMYCIN *D* is a potent antibiotic with cytotoxic properties in tissue cultures[1-3] which has been used in the treatment of human neoplasms[4-9]. The action of actinomycin may depend on its capacity to bind with DNA, thus inhibiting RNA synthesis[10]. It has also been suggested that it is the synthesis of the DNA-primed RNA which is inhibited[11]. Puromycin, also an antibiotic, is believed to inhibit protein synthesis in a wide variety of organisms and tissues by causing premature release of incomplete polypeptide chains from the ribosomal system[12-20].

The purpose of the experiment described here was to investigate the comparative effects of actinomycin *D* and puromycin on mitotic activity and chromosome morphology in cultured human leucocytes. Venous blood was drawn from healthy adult donors, and a modified version of the Moorhead technique for leucocyte culture was used. The blood samples were divided into 5 ml. amounts, to which 0·05 ml. of phytohaemagglutinin (Difco, form *M*) was added to facilitate the agglutination of red cells. The plasma obtained from 5 ml. of blood was pipetted into 5 ml. of synthetic tissue culture medium (*TC* 199, Difco) containing 0·05 ml. of phytohaemagglutinin. Graded concentrations of actinomycin *D* and puromycin dihydrochloride were added to separate cultures. Each antibiotic was dissolved in *TC* 199 in the following gradation of concentrations: 0·01, 0·05, 0·1, 1·0, 2·0, 2·5 and 3·0 μg/ml. The actinomycin *D* was obtained sterile. The puromycin had to be passed through a Swinny filter because the commercial preparation was not sterile. Two drops of colchicine ($1{\cdot}0 \times 10^{-6}$ M) were added to each culture 4 h before collection. The cells were fixed in glacial acetic acid : methanol (1 : 3). Slides were flamed over an alcohol lamp and stained with Giemsa. Metaphases were examined for abnormalities in cultures ranging from low to high antibiotic concentrations. The mitotic index was determined by counting the number of metaphases per 1,000 cells.

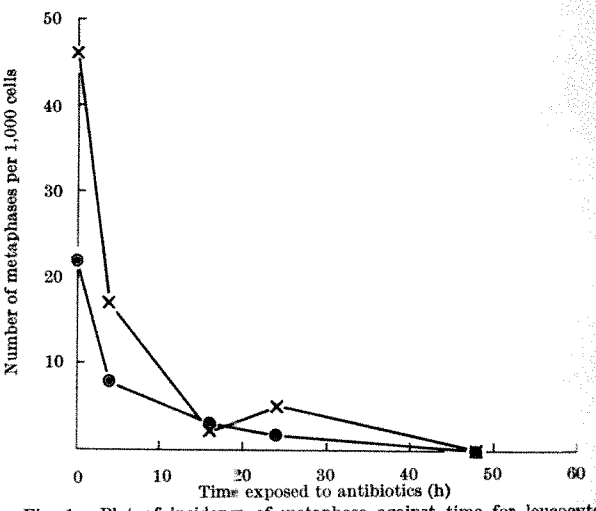


Fig. 1. Plot of incidence of metaphase against time for leucocytes exposed to 0·02 μg/ml. actinomycin *D* (○), and 3·0 μg/ml. puromycin (×)

There was complete inhibition of mitotic activity in cultures containing more than 0·02 μg/ml. of actinomycin *D*. The first noticeable effect after administering increasing amounts of the antibiotic to the cultures was a decrease in the mitotic index. It was not possible to analyse twenty-five metaphases in those cultures with more than 0·015 μg per ml. because very few metaphases were found in the higher concentrations.

No metaphases were found in cultures containing more than 3·0 μg/ml. of puromycin. Twenty-five metaphases were examined in the cultures containing 0·01, 0·05, 0·1, 1·0 and 2·0 μg/ml. of puromycin. Less than twenty-five metaphases were found in the cultures of 2·5 and 3·0 μg per ml., and so fewer than twenty-five were analysed.

Fewer cells were found in the cultures containing the higher concentrations of puromycin and actinomycin *D*. These cells tended to be in clusters, and some of them had a granular appearance. This may have been due to a toxic action on the leucocytes by both antibiotics. No morphological alterations of the chromosomes were observed, such as breaks, or the translocation-like cross-configurations produced in leucocyte cultures with mitomycin *C* (ref. 21).

To study the effects of 0·02 μg/ml. actinomycin *D* on leucocytes for varying periods of exposure up to 72 h, the appropriate amount of antibiotic was added 72, 48, 24, 16 and 4 h before collecting the cells. The same procedure was followed using 3·0 μg/ml. of puromycin. The results are shown in Fig. 1. Inhibition of all mitotic activity was most evident after the antibiotics had been in the culture for more than 24 h. No metaphases were found in the cultures exposed to the antibiotics for 48 or more hours.

The actinomycin *D* used in this experiment was obtained from Merck, Sharp and Dohme, Rahway, New Jersey; the puromycin from Nutritional Biochemical Corp., Cleveland, Ohio.

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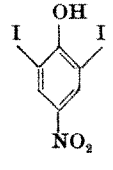
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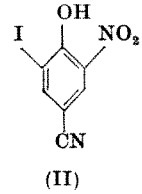
4-Cyano-2-iodo-6-nitrophenol: a New Fasciolicide

ALTHOUGH some drugs are effective against the adult liver fluke *Fasciola hepatica* in sheep and cattle, none is yet available that provides adequate control of the immature fluke at a tolerated dose rate.

For several years we have sought novel fasciolicides[1]. An interesting level of activity against the mature fluke found for the known anthelmintic 2,6-di-iodo-4-nitrophenol (disophenol) (I)[2] led us to synthesize and test a wide range of substituted phenols and their derivatives:



(I)



(II)

One of these, 4-cyano-2-iodo-6-nitrophenol (first synthesized by Mr. D. R. Broad) ('M & B 10,755') (II), showed high activity against both the adult and immature flukes and was selected for fuller evaluation[3].

'M & B 10,755' is a yellow, almost odourless, crystalline solid, m.p. 137°–138° C. It is sparingly soluble in water, but moderately soluble in most common organic solvents. The phenol is a fairly strong acid (pK_a 2·85 in water) and forms stable salts with inorganic and organic bases. The *N*-methylglucamine salt ('M & B 10,755*A*') is a yellow solid, which is freely soluble in water (65–70 per cent w/v at room temperature) giving a substantially neutral yellow solution.

The acute toxicity of 'M & B 10,755' in experimentally infected (*Fasciola hepatica*) rabbits, sheep and calves (Table 1) was established by oral dosing with a suspension of 'M & B 10,755' in tap water, and subcutaneous or intramuscular injection of a 57 per cent w/v aqueous solution of the *N*-methylglucamine salt, 'M & B 10,755*A*', containing 34 per cent w/v 'M & B 10,755'. Deaths resulting from toxicity occurred within 24 h of dosing. In the surviving sheep treated with 40 mg/kg, panting, respiration and increased body temperature were recorded. A transient local reaction followed treatment by injection and in some of the sheep and calves treated at doses above 20 mg/kg a sterile local reaction was found at the injection site. The results in breeding sheep treated every 28 days with 20 or 10 mg/kg by subcutaneous injection, starting before tupping, showed no adverse effect on oestrus or reproduction.

Table 1. ACUTE ORAL AND PARENTERAL MAXIMUM TOLERATED DOSE OF 'M & B 10,755' IN RABBITS, SHEEP AND CALVES AND THE MINIMUM EFFECTIVE DOSE AGAINST MATURE *Fasciola hepatica*

Experimental host	Maximum tolerated dose (mg/kg)		Minimum effective dose against the mature *F. hepatica* (mg/kg)	
	Oral	Subcutaneous/ intramuscular	Oral	Subcutaneous/ intramuscular
Rabbit	100	70	10	10
Sheep	75	40	50	8
Calf	75	40	50	8

In activity tests against mature *Fasciola hepatica* in rabbits, sheep and calves the compound was administered by oral dosing or parenterally, and the results (Table 1) show that in sheep and calves the compound is more potent by the latter route.

Dosing by the subcutaneous route was selected for testing against parasites of different ages. Sheep and calves experimentally infected with a single dose of 300 metacercariae produced from a laboratory-maintained strain of *Limnaea truncatula* were treated after 4, 6, 8 or 10 weeks. The effect of treatment was assessed by counting the number of mature and immature flukes found in the liver, bile ducts and gall bladder at post-mortem examination carried out 12 to 14 weeks post-infection. The results are summarized in Table 2. These show that treatment with 'M & B 10,755' at 20 or 34 mg/kg bodyweight as early as 4 weeks after infection produced a reduction in the number of flukes, and after 6 weeks 20 mg/kg eliminated the infection in the sheep. Against

Table 2. RESULTS OF THE TREATMENT WITH 'M & B 10,755' OF 4-, 6-, 8- AND 10-WEEK OLD *Fasciola hepatica* INFECTIONS IN EXPERIMENTALLY INFECTED SHEEP AND CALVES

Interval (weeks) between infestation and treatment	Dose (mg/kg) of 'M & B 10,755'*	Sheep			Calves		
		No. treated	Mean No. of parasites recovered	Percentage of Reduction	No. treated	Mean No. of parasites	Percentage of reduction
4	34	5	12·2	88			
	20	6	32	68·5	3	20	76
6	20	5	0	100	3	9	89
	10	5	29·2	70			
8	10	5	3·8	96	4	6	93
10	10	3	0	100	3	0	100
	5	4	36	64	3	20·7	75
Untreated		15	101·5	—	7	91·1	—

* Administered by subcutaneous injection using a 57 per cent w/v solution of 'M & B 10,755*A*' (34 per cent w/v 'M & B 10,755').

the 10 week old infection the effective dose in both species of animal was between 5 and 10 mg/kg body weight. These data suggest that routine treatment with 'M & B 10,755' may prevent the acute disease as well as effectively control the chronic disease, produced by the mature (older than 8 weeks) fluke.

Field experiments, further to investigate the potential of the compound in the control of fascioliasis in ruminants, are in progress.

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Citrinin—a Fungistatic Antibiotic and Narrowing Factor

DURING growth, fungi produce substances which have morphogenetic effects when applied to apices of actively growing hyphae. Factors which induce vacuolation, branching and other effects have been described[1]. The application of drops of culture filtrates to the margins of fungal colonies growing on agar is a suitable method for the demonstration of these morphogenetic factors, owing to the fact that developmental and ageing effects are most readily detected in this juvenile region. During a survey of culture filtrates from a large number of fungi which were being tested in this manner, one of the culture filtrates produced in the tips of the test fungus an abrupt and immediate change such that the subsequent growth of the axis was of considerably smaller diameter. The effect persisted after the treatment for more than 1 h and was followed by a gradual recovery until after 1·5–2 h the apex was again producing a hyphal tube of about the original diameter. No cytoplasmic change was evident in the treated tips. Branching was occasionally induced near the point of abrupt narrowing but was not invariably associated with the effect. The phenomenon of narrowing is of particular interest in relation to morphogenesis of the fungal colony as characteristically those hyphae produced behind the margin in the still developing but older regions of the colony are narrower than those at the extending margins. An understanding of the mechanism of hyphal narrowing could enlarge our knowledge of the process of ageing in fungi, and could also provide information helpful in elucidating the normal processes of hyphal extension growth.

The culture filtrate effective in producing narrowing in this survey was found to have become unintentionally contaminated with a species of *Penicillium*. Filtrates from pure cultures of this fungus produced the same effect, whereas those of the intended inoculum did not. After further investigation of the narrowing factor, the contaminant was identified on morphological criteria as *P. citrinum* Thom. This identification was later supported by an antibacterial spectrum test[2]. Before the morphological identification of the producer fungus the narrowing factor was isolated as a yellow crystalline compound. This later proved to be citrinin. *P. citrinum*, *IMI* 24307, was obtained from the Commonwealth Mycological Institute Culture Collection, Kew, Surrey, and when grown under conditions similar to those used for the Belfast strain it gave a similar narrowing effect and similar yields of citrinin.

In most of our studies the fungus was cultured in a liquid medium containing 7·0 g glucose; 0·5 g $MgSO_4.7H_2O$; 0·2 g potassium dihydrogen phosphate; 0·1 g ammonium nitrate; distilled water to 1 l. Then 100 ml. quantities in 500 ml. flasks were inoculated and incubated under static conditions. As measured by our biological assay of narrowing, best yields of the factor were obtained at 30° C after seven or more days. The yields of citrinin under these conditions were much lower than those reported by Hetherington and Raistrick[3] under their conditions. These workers were able to precipitate citrinin directly from their culture filtrates by adding concentrated hydrochloric acid, and they obtained yields of 1·5–2·0 g/l. Acidification of our culture filtrates gave no detectable precipitation. We first obtained citrinin by acidification of the culture filtrate and extraction with diethyl ether. The ethereal layer was separated, concentrated and extracted with a saturated solution of sodium bicarbonate. The sodium bicarbonate phase was acidified and extracted with ether. The ethereal phase contained the narrowing factor, and after removal of the ether by evaporation the solid residues were dissolved in hot ethanol and the long yellow needles allowed to crystallize out at low temperature. These crystals in aqueous solution were active in causing narrowing at a concentration of 1–2 p.p.m. At high concentrations the hyphae were killed. After characterization of the narrowing factor a simpler isolation method was adopted whereby the reduced volume of the initial acidic ether extract was shaken with a small volume of a saturated solution of sodium bicarbonate. On acidification of the aqueous phase, the solution became so saturated with citrinin as to result in a heavy precipitation of microcrystals. These were filtered, washed, dried and recrystallized from ethanol. The crystals obtained in this way from the Belfast strain of *P. citrinum* melted at 166°–168° C with obvious decomposition. Analysis of the carbon and hydrogen content closely agreed with that reported for citrinin[5]. Further proof of identification was afforded by infra-red and nuclear magnetic resonance spectra. The infra-red spectrum agreed with that published for citrinin by Kováč *et al.*[4].

The standard test fungus used in our survey and in subsequent investigations of citrinin as a narrowing factor was *Aspergillus niger* v. Tiegh. grown as previously described[5]. Other fungi tested in the same way and found to respond to citrinin solution by narrowing were *Pythium mamillatum*, *P. proliferum*, *P. ultimum*, *Phytophthora parasitica*, *Absidia glauca*, *Mortierella* sp., *Mucor hiemalis*, *M. plumbeus*, *Rhizopus stolonifer*, *Neocosmospora vasinfecta*, *Neurospora crassa*, *Serdaria fimicola*, *Trichoderma viride* and *Armillaria mellea* as well as the producer strain of *Penicillium citrinum*. Thus representatives of each major group of the fungi show the response, as does the organism which produced the factor. The production of citrinin is not confined to *P. citrinum*. Other producer species are *P. velutinum*[6], *P. steckii*[7], *P. notatum*[8], *P. lividum*, *P. phaeojanthinellum*, *P. chrzaszczi*, *P. implicatum*, *P. citreosulphureum*, *Aspergillus terreus*[9] and *A. candidus*[10]. It is not possible at this stage to know what significance citrinin might have as a general fungal morphogen as there is no record of any search for citrinin in genera outside the Aspergillales.

In addition to the investigation of the action of citrinin on hyphal tips we are undertaking a study of its effects on the germination of fungal spores. Citrinin has been tested in 1·5–50 p.p.m. solutions on sporangiospores of *Rhizopus stolonifer*. In a concentration of 1·5 p.p.m. the spores germinated much more slowly than in controls and the germ tubes were more branched. However, the hyphae were of regular diameter. At concentrations of 6·25 and 12·5 p.p.m. there was some distortion of growth with a very irregular hyphal diameter and a sinuous out-

line. There was a tendency for the average germ tube length to decrease with an increasing concentration of citrinin. At 25 p.p.m. the spores swelled considerably and produced papillae but no germ tubes. There was no indication of germination at 50 p.p.m. After a further incubation period of 12 days the spore suspensions were re-examined, and in solutions of 12·5–50 p.p.m. no further development had occurred since the 24 h period. At the end of 13 days the spores and germlings were found to be still viable and capable of normal growth on transfer to a suitable medium.

Citrinin, which was isolated in 1931 (ref. 3) and first described as an antibacterial antibiotic in 1941 (ref. 11), has been thoroughly investigated for activity against bacteria, and has also been tested against bacteriophage[12], sarcoma tissue[13], amoebae[14], paramoecia[15], animal cells[16], and higher plant cells[17]. Considering that it is a fungal product it is surprising that it has not been more thoroughly investigated in relation to its effects in fungi. The only two records which we have been able to find relate to the fact that it was found to inhibit the formation of adaptive enzymes in the metabolism of phenolic compounds by spores of soil fungi[18], and that some plant pathogenic fungi show growth inhibition when inoculated to nutrient agar containing 500–1,000 p.p.m. citrinin[19]. Other fungi were little affected, and at concentrations lower than 500 p.p.m. citrinin had no significant effect on any of the fungi. These media containing citrinin, however, appear to have been heat sterilized and it is known that citrinin is thermolabile. Our own results indicate that for at least one fungus the minimum fungistatic concentration is of the same order as the published minimum inhibitory concentration for *Staphylococcus aureus* (30–40 p.p.m.).

Because of its toxicity to animal tissues, citrinin appears to be unsuitable for chemotherapy as applied orally or by injection. However, citrinin has been reported to be effective as an ointment or powder for surface application in skin affections[20,21], and it may have no adverse effect when applied to the cornea or mucous membranes[22]. Because many fungal infections of animals are superficial, citrinin may have possible applications in the control of such diseases.

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HISTOCHEMISTRY

Enzyme Histochemistry of Developing Human Muscle

This communication reports an attempt to establish the sequence of events in the process of differentiation of developing human muscle into the two types of fibre.

By histochemical methods it is possible to recognize two basic types of muscle fibre in human muscle[1–3]. It was suggested that these fibres be called type I (rich in reduced nicotinamide–adenine dinucleotide (NADH)–diaphorase and poor in phosphorylase) and type II (rich in phosphorylase and poor in NADH–diaphorase), respectively. Subsequent authors have shown that type II fibres are also rich in mitochondrial α-glycerophosphate dehydrogenase[4] and in myosin ATPase[5].

In an earlier report on developing muscle[6] it was noted that, in the muscle of the newly born infant, as in certain newborn animals, the fibres are already fully differentiated, as in the adult, into the two basic fibre types, which are present in about equal numbers.

Twenty-one muscle specimens from twelve newly born full-term human infants and forty muscles from twenty-one newly born premature human infants (with birth weight less than 2,500 g) were obtained at autopsy. All had died within 48 h of birth.

Various muscles were dissected from seventeen human foetuses, ranging in gestation from 12 to 26 weeks. These were obtained mainly from therapeutic abortions, but a small number resulted from spontaneous abortions.

In addition to routine histological stains (haematoxylin and eosin, Van Gieson, and periodic acid–Schiff), the NADH–diaphorase[7], phosphorylase[8] and myosin adenosine triphosphatase[9] reactions were carried out. In the newborn full-term infants all muscles showed a differentiation into two types, as in the mature adult muscle (Fig. 1). The newborn premature infants also showed a subdivision of the muscles into two fibre types, and, with the exception of one infant of 26 weeks gestation (weighing 840 g), the distribution of the two types of fibre was approximately equal. The one exceptional infant had a disproportionately large number of type II fibres (poor in NADH–diaphorase) and a very small proportion of type I fibres.

Of the seventeen foetuses, five showed a very similar pattern. These were the five largest in the series and ranged in gestation from approximately 20 weeks to 26 weeks. In all their skeletal muscles there was a differentiation into two fibre types, but there was a disproportion between the two types. The vast majority belonged to type II and only a small proportion were of type I (Fig. 2).

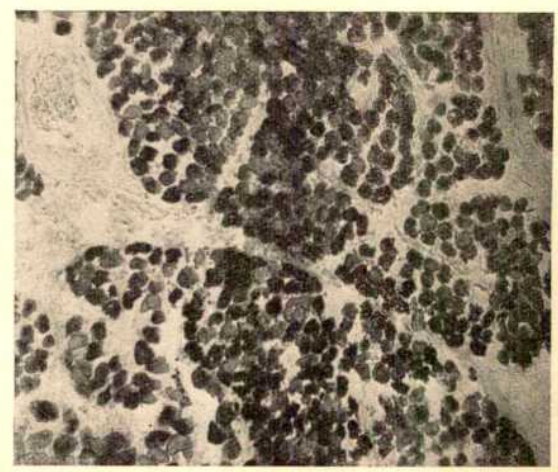


Fig. 1. Section of quadriceps of full-term new-born infant. (Phosphorylase reaction. × 100)

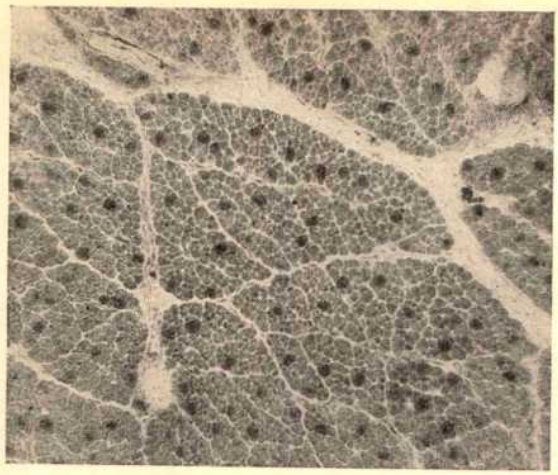


Fig. 2. Section of triceps of human foetus after 23 weeks' gestation. (NADH-diaphorase reaction. × 100)

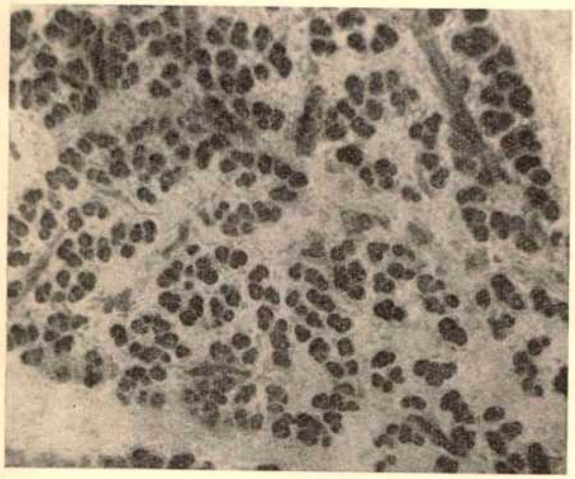


Fig. 3. Section of glutei of human foetus after 13 weeks' gestation. (ATPase reaction. × 100)

The proportion of type I fibres ranged from about 3–10 per cent of the total. These type I fibres were in the upper range of fibre diameter.

The remaining twelve foetuses, ranging in gestation from 12 to 18 weeks, did not show this pattern of differentiation. In some of the muscles all the fibres reacted uniformly for a particular enzyme system. In others there was some variation in enzyme activity of the fibres. However, this variation did not follow any consistent pattern and frequently clusters of either strongly or weakly reacting fibres occurred together (Fig. 3). Despite the variation in enzyme activity, these fibres could not be correlated enzymatically with the type I and type II fibres of mature muscles, as some fibres rich in ATPase were also rich in NADH–diaphorase and phosphorylase, and conversely.

Human foetal muscle can thus be divided into three phases of development by histochemical enzyme stains. In phase 1, approximately 12–18 weeks' gestation, there may be some variation in enzyme content of different fibres but this is not consistent and there is no clear cut sub-division into type I and type II fibres. In phase 2, approximately 20–26 week's gestation, there is a clear cut sub-division into type I and type II fibres, but type I form only a small proportion of the total and are larger in diameter. In this phase the type I fibres correspond in appearance and distribution to the so-called *b* fibres described by Wohlfart[10] in the developing muscle. In phase 3, from about 30 weeks' gestation onwards, there is a sub-division into type I and type II fibres, giving a checkerboard pattern, as in mature adult muscle, and with an approximate equal distribution of the two types.

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Staining and Typing of Langerhans' Cells in the Skin of the Black Guinea-pig

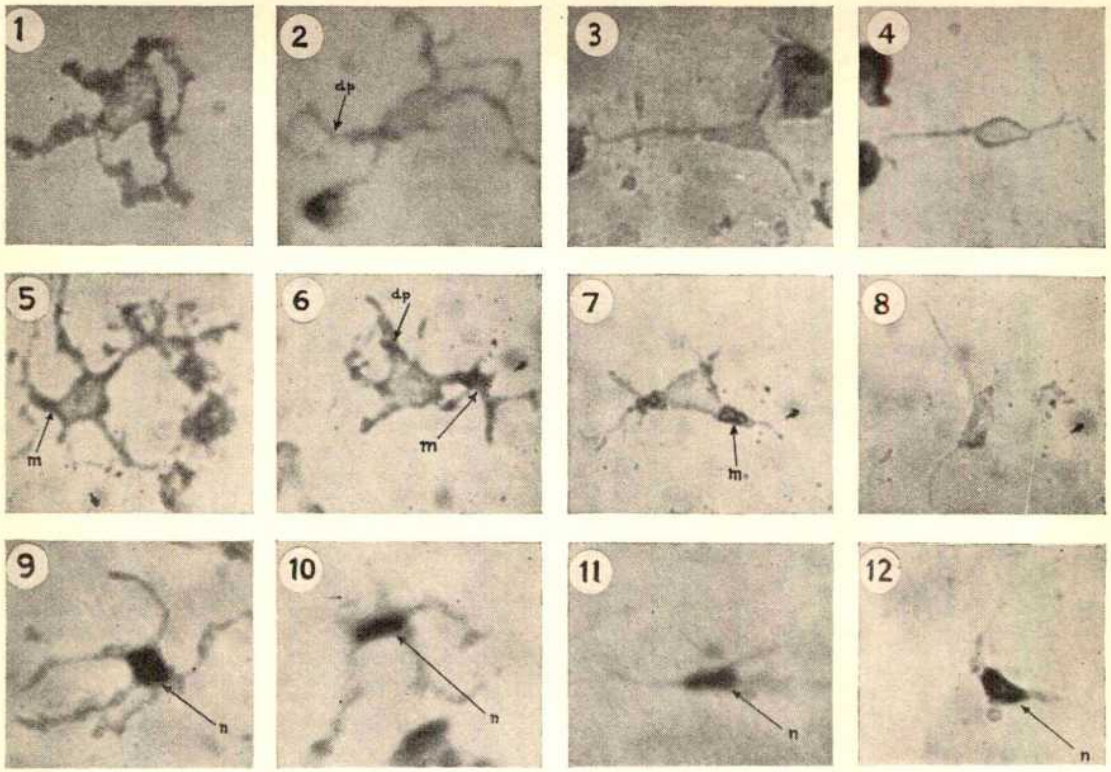
RECENTLY I described[1] some cytomorphic forms of the epidermal Langerhans' cells[2–4] in the black guinea-pig. This communication reports a method of classifying these cells.

A saline suspension of the digest of the pure epidermis from the ear of the guinea-pig, prepared according to the method devised by Shukla, Karkun and Mukerji[5], is mounted on albuminized slides and treated with (*a*) a solution of 3,4-dihydroxyphenylalanine (DOPA) according to Laidlaw and Blackberg[6], (*b*) Masson-Fontana stain for melanin according to Pearse[7] and (*c*) Harris's haematoxylin and eosin and Mayer's haem-alum stain for nucleus as described by McManus and Mowry[8]. The Langerhans' cells, one of the constituents of the epidermal digest, become stained by this process and can be observed under a bright field microscope. The cells possessing five, four, three and two dendritic processes are identified and classified as Type I, II, III and IV, respectively. A summary of the characteristic features of each type of Langerhans' cells is given in Table 1.

The Langerhans' cells of Types I–IV show a gradual reduction in the size, number of dendritic processes and the cytoplasmic contents (Figs. 1–12). These cells demonstrate a degenerative change in the internal structure.

Table 1. CHARACTERISTIC FEATURES OF TYPES I–IV LANGERHANS' CELLS

Characters examined	Langerhans' cell types I	II	III	IV
Nucleus				
Shape	Egg shaped	Oval	Triangular	Fusiform
Size (length × breadth in μ)	7 × 5	7 × 4	6 × 4	6 × 3
Consistency	Vesicular	Vesicular	Shrunken	Shrunken and crenated
Stainability with haematoxylin	Basophilic	Basophilic	Deeply basophilic	Deeply basophilic
Cytoplasm				
Quantity	Ample	Reduced	Thin rim around nucleus	Scanty
Stainability with eosin	Slight	Slight	Weakly positive	Positive
	±	±	+	+ +
Location of melanin	Periphery of the body and dendritic processes	Dendritic processes	Absent	Absent
DOPA reaction	Positive at periphery of the body and dendritic processes	Positive at dendritic processes	Negative	Negative



Figs. 1–4. Types I–IV Langerhans' cells after DOPA reaction. It is weakly positive in Type I, weaker in Type II and negative in Types III and IV

Figs. 5–8. Types I–IV Langerhans' cells after Masson-Fontana stain, which demonstrates melanin (*m*) in the dendritic processes (*dp*) and periphery of the cell in Type I, in the dendritic processes in Type II, at the roots of dendritic processes in Type III. Type IV cell does not show melanin granules

Figs. 9–12. Types I–IV Langerhans' cells after Mayer's haem-alum stain. The large vesicular nucleus (*n*) in Type I is gradually reduced in size in Types II, III and IV

The well defined oval, vesicular, basophilic nucleus in Type I gradually give way to the triangular form in Type III (Fig. 11) which in turn is transformed to the fusiform and deep and diffusely staining nucleus in Type IV (Fig. 12). The sparse peripheral melanin in Type I (Fig. 5) is gradually reduced in quantity in Type II (Fig. 6) and Type III (Fig. 7) and becomes obliterated in Type IV (Fig. 8). Again, cells of Types I–IV show a progressively weak DOPA reaction which indicates that there is a gradual regression in their capability to synthesize melanin (Figs. 1–4).

The changes observed in the Langerhans' cells of Types I–IV are reminiscent of the ischaemic degeneration of the pyramidal cells of the cortex[9], when the cells gradually disintegrate and disappear. Billingham and Medawar[4] postulated a similar type of degenerative extinction for the Langerhans' cells. The observations made here support his view.

I thank R. K. Saraut for his technical assistance in this work.

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PHYSIOLOGY

Evidence for the Discharge of Cholinesterase into Canine Eccrine Sweat

AN outstanding feature of the eccrine sweat glands in the paw of the dog is that pseudo-cholinesterase is abundant in their secretory cells[1–3]. During an earlier investigation[3] it was noticed that the inside of the lumen of the sweat ducts in the toe-pads of the dog often gave histochemical reaction for pseudo-cholinesterase. This finding led us to assume that the cholinesterase in the gland cells might be discharged into sweat. The work reported here provides evidence in favour of this assumption.

Thirty-one experiments were performed on seven puppies aged 2–10 months and on one adult dog aged about 2 years, all of which retained good ability to sweat on the foot pads spontaneously as well as in response to local application of sudorific agents such as acetylcholine or acetyl-β-methylcholine (mecholyl).

The sweat droplets, which were produced on the toe-pads or the central pads either spontaneously or by local injection of mecholyl at concentrations of 10^{-4}–10^{-5} g/ml., were collected with a fine glass capillary tube under a stereomicroscope. Each sample of sweat (about 0·1–0·2 mg) was added to 2 ml. of an incubation solution of the type used in Gomori's modification[4] of Koelle's histochemical method for cholinesterase[5–7]. Acetylthiocholine and butyrylthiocholine were used as substrate for the enzyme. Within half an hour of incubation at 37° C with either substrate, the solution exhibited a slight but definite white cloudiness, which became increasingly intense as the incubation was prolonged. This turbidity was found to result from the formation of fine rod-like or needle-shaped crystals in the incubation solution. If before

incubation eserine salicylate was added to the solution at a final concentration of 10^{-5} M, no such sedimentation was observed, which indicates that this reaction probably results from cholinesterase activity in the sweat and that the precipitated crystals are copper-thiocholine.

In another series of experiments, the sweat which was secreted spontaneously or by stimulation with mecholyl was blotted on to a piece of absorbent paper. After it had been dried for a few hours in the refrigerator, the paper was treated according to the histochemical technique used to demonstrate the presence of cholinesterase in tissue sections[4]. Following incubation for about 4–5 h at 37° C with either acetylthiocholine or butyrylthiocholine, the paper was dipped into a solution of 0·2 M ammonium sulphide. A marked dark brown colour (indicating the production of copper sulphide) developed on the paper at the sites where the sweat was absorbed (Fig. 1 *A* and *B*). Incubation with butyrylthiocholine always produced stronger staining than with acetylthiocholine, and both reactions were found to be completely inhibited by 10^{-5} M eserine salicylate, and further by diisopropylfluorophosphate (DFP) at concentrations as low as 10^{-8} M.

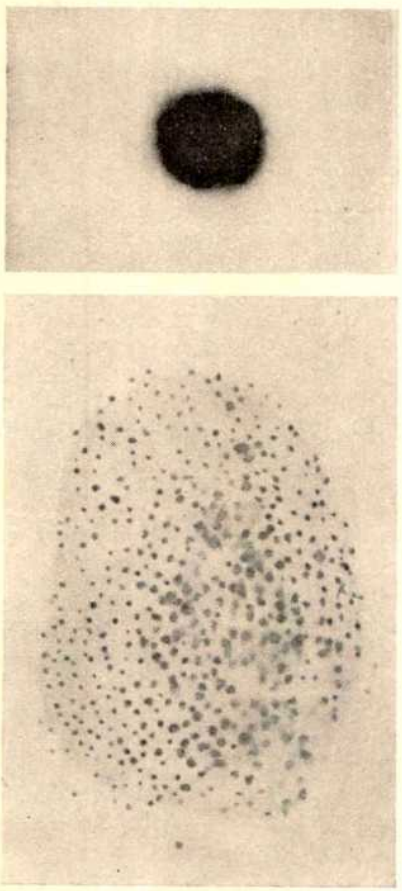


Fig. 1. Staining reaction caused by cholinesterase contained in the canine eccrine sweat. *A*, The sweat collected with a glass capillary tube was absorbed on to a piece of blotting paper and incubated with butyrylthiocholine for 4 h. The region where sweat was absorbed showed strong cholinesterase reaction. *B*, A piece of smooth-surfaced absorbent paper, which had been pressed for about 20 sec to a toe-pad showing profuse sweating, was incubated with butyrylthiocholine for 5 h. Note many stained dots revealed at the sites where the sweat droplets were absorbed

As a control experiment, normal saline was placed on the foot pad showing no sweating for a certain period of time, and tested in a similar manner to the other experiments. But neither sedimentation nor staining reaction was obtained with either substrate.

From these findings it is evident that the eccrine sweat from the paw of the dog contains cholinesterase and that it is almost exclusively of the pseudo-type, which is identical in its properties with the cholinesterase detected in the glandular tissues[3]. Furthermore, the cholinesterase activity in the sweat gland coil was histochemically found to be reduced after profuse sweating. All these findings, when taken together with the previous observation that the lumen of the sweat duct was often plugged with a substance reactive for pseudo-cholinesterase[3], suggest that the intracellular cholinesterase in the canine eccrine sweat glands is actually discharged into the glandular lumen in association with the production of sweat.

In this connexion it will be recalled that the pancreas of the dog also characteristically contains pseudo-cholinesterase in the alveolar cells and that it is discharged into the pancreatic juice[6]. The physiological significance of the presence of pseudo-cholinesterase in the dog's eccrine sweat glands as well as of its discharge into the sweat remains uncertain and requires further investigation.

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Enzymatic Activity of L-Glutamic Dehydrogenase in Liver Mitochondria of Adult and Foetal Rats

CONSIDERABLE differences in the radioactivity of carbon dioxide expired by liver slices of mother and foetal rats, after incubation with 1-^{14}C-L-glutamate, indicated a lower enzymatic activity of the L-glutamic-dehydrogenase (GDH) of the foetal liver compared with the adult liver[1]. The low activity of a large number of enzymes in foetal organs is known, but the oxidative enzymes generally are an exception, and usually show a higher activity in foetal than in maternal organs (for a comprehensive survey, see ref. 2). An increase in the activity of GDH was observed during foetal development in the central nervous system of pigs and monkeys[3].

We examined livers newly removed from healthy gravid rats and their foetuses, from the seventeenth to the twenty-second day of pregnancy. The tissue was broken down in a glass–'Teflon' homogenizer in ten volumes of 0·25 M sucrose, and afterwards centrifuged for 10 min at 600*g*. The mitochondrial fraction was obtained from the residue by centrifuging twice for 10 min at 10,000*g*. We added one volume per cent of 'Triton X 100' to the last fraction. Activity was determined by a modification of the method of Strecker[4] in a Zeiss spectrophotometer at 340 nm and 25° C in the following medium: 2·35 ml. 0·2 M phosphate buffer, *p*H 8·3, 0·5 ml. 10^{-3} M nicotinamide-adenine dinucleotide (NAD), 0·1 ml. 10^{-2} M potassium-L-glutamate and 0·05 ml. mitochondrial suspension. Activity was measured in ΔE_{340}/min mg protein, and the amount of protein in the mitochondrial suspension was determined after Lowry[5].

Table 1. ACTIVITY OF L-GLUTAMIC DEHYDROGENASE (ΔE_{340}/MIN MG PROTEIN) IN MITOCHONDRIA OF MATERNAL AND FOETAL RAT LIVERS

Day of pregnancy	Mothers: Activity and standard deviation	Mothers: No. of experiments	Foetuses: Activity and standard deviation	Foetuses: No. of experiments
17	355 ± 50·7	7	105 ± 37·6	5
18	431 ± 63·2	5	157 ± 36·1	4
19	366 ± 55·0	17	172 ± 55·1	9
20	430 ± 36·7	12	211 ± 87·5	9
21	392 ± 28·6	12	224 ± 49·5	8
22	394 ± 44·8	9	321 ± 76·8	9

The results are given in Table 1. On the twenty-first day the activity of GDH in the foetal livers was still considerably lower than in the maternal ones; approximately similar values were obtained only for the twenty-second day. The oxidation of L-glutamate is thus inhibited, both with GDH and by way of transamination, since a level of transaminase activity comparable to that of the adult is reached only at birth. However, this means that more glutamate is available for protein synthesis—an important reason for the relative resistance of the foetus to a maternal lack of protein, which has been shown experimentally[6]. The low activity of GDH in foetal liver mitochondria accords with the high respiratory quotient of foetal livers[7], which indicates an almost exclusive oxidation of carbon hydrates.

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Adrenergic Beta-receptor Blockade and Anaerobic Metabolism

IT is known that adrenaline accelerates glycogenolysis in liver and in muscles, which leads to an elevation of the concentration of blood sugar and blood lactate[1]. During work (and especially with high work loads) there is a substantial increase in the concentration of blood lactate, which is mainly produced in the working muscles[2]. This can in part arise from increased adrenergic activity during work. The purpose of the present investigation was to test this assumption by means of an adrenergic beta-blocking agent.

In four cases, the arterial lactate concentration was studied at rest, during work and 3 min after work. They were determined again after oral administration of 15–20 mg propranolol, an adrenergic beta-receptor blocking agent. At rest, the mean lactate concentration was 0·69 mmole/l. and this remained unchanged after propranolol. During heavy work, the mean lactate concentration was 3·00 mmole/l. and 3 min afterwards was 2·71 mmole/l. Both these figures were lower after propranolol (mean = 2·16 mmole/l. and 1·77 mmole/l., respectively). The decrease was significant ($P < 0·01$) during the blockade for eleven out of the twelve samples. It seems that the effect of adrenergic activity on the metabolism, especially during and shortly after muscular work, is at least to some extent an effect of beta-receptors.

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Patterns of Spontaneous Rhythmic Activity within Various Thalamic Nuclei

THE generation of spontaneous rhythmic activity has been studied by simultaneous recording with four microelectrodes (4 M sodium chloride, 1–3 M Ω), either arranged in a square with sides of 1·2–4·4 mm, or in a straight line with spacings of 1·2–3 mm.

With adult cats, which had been lightly anaesthetized with 40 mg sodium pentobarbitone/kg body-weight, spontaneous periods of rhythmic activity (spindles) were recorded from various thalamic nuclei, as described earlier[1]. The activity consisted of groups of cell discharges

Fig. 1. *A*, Generalized thalamic spindle. Rhythmic activity obtained by four microelectrodes (1–4), 1·5 mm apart, placed along a frontal line, and 7·25 mm under the dorsal surface of the thalamus. The vertical lines of the upper four traces indicate the spikes in the corresponding microelectrode records. *B*, Localized thalamic spindle. Recording as in *A*, with a period of rhythmic activity in trace 1 only, followed by local activity in trace 3 only. The start of a generalized spindle can be seen on the right-hand side. *C*, Duration of spindle activity recorded from the same four electrodes as in *A* and *B*, plotted as thick horizontal bars. Thin lines facilitate a comparison of the onset and stop of spindle activity at the different electrode locations. *D*, *E* and *F*, Three examples of generalized spindles. Time from an arbitrary 0-point along the ordinate, the time between successive bursts of spike discharges along the abscissa. Electrode location given by filled circle in insets. In *D*, the spindle frequency is similar in all locations, although the onset varies. In *E* and *F* the spindle frequency is different at various electrode locations, and sometimes alters during the spindle

followed by a positive wave, the sequence repeating itself from three to about twenty times (Fig. 1 *A* and *B*).

In most experiments, spindle activity was obtained from all electrode tracks, irrespective of their location in the thalamus. Contrary to the location of the slow 8-sec waves recorded by Morison *et al.*[2], spontaneous thalamic spindles occurred more frequently in laterally and ventrally situated nuclei than in more medial and dorsal groups.

Fully developed spindle sequences often occurred in one electrode location without any concomitant activity at the others (localized spindles, Fig. 1*B*). They could occur at all electrode sites.

However, rhythmic activity frequently occurred almost simultaneously in all four electrode locations (general thalamic spindles, Fig. 1*A* and *C*). Although an approximate simultaneity was recorded, the start of the spindles obtained by the various electrodes differed from some milliseconds to a second (Fig. 1*C*). In each spindle, the particular electrode position in which the rhythmic activity first appeared was determined. Such "leading" was observed at all thalamic locations, but the tendency to lead was greater in the lateral nuclei than in the medial ones. Furthermore, the internal spindle frequency and the rate of spindle occurrence was often higher in lateral than in medial thalamic nuclei (Fig. 1*D*, *E* and *F*), and higher in ventral than in dorsal locations. Typical spindle activity could be recorded from n. ventralis postero-lateralis and the peri-cruciate cortex after removal by suction of the medial 3 mm of the thalamus. Cell groups, separated by 1·2–2 mm, frequently discharged in a phase-locked manner, indicating driving of one cell group by the other, or of both by a common pacemaker.

The evidence reported does not support the idea of one or more medial thalamic nuclei serving as an indispensable and common pacemaker area for the rhythmic thalamic and cortical 10/sec activity. On the contrary, the possibility of rhythmic activity starting from virtually all thalamic nuclei leads to a concept of many facultative pacemaker areas within the thalamic complex. Given certain conditions, rhythmic activity may spread from one thalamic nucleus to others by projection axons, thus giving a general thalamic spindle which may influence much of the cerebral cortex. When rhythmic activity remains local, the spindle activity is found in one thalamic group and in its restricted cortical projection area only.

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Synaptic Delay and Conduction Time in Brain during Exposure to Simulated High Altitudes

RELATIVELY few techniques are available for the investigation of the long-term effects of physiological variables on conduction times and synaptic delay in the brain. The lateral olfactory tract (LOT) and prepyriform cortex provide a system whereby these parameters may be measured in rats chronically implanted with electrodes[1]. Fibres of the LOT synapse with dendrites of the prepyriform pyramidal cells[2]. When the LOT is electrically stimulated by a single shock, both the presynaptic LOT potential and the postsynaptic prepyriform response may be recorded from electrodes placed in the prepyriform cortex (Fig. 1). As the LOT potential first approaches and then invades the recording area, the adjacent cortex acts first as a current source and then as a sink; hence the presynaptic potential is biphasic—first positive, then negative. The time from stimulus artefact to negative crest of the presynaptic potential is a measure of conduction time along the LOT and the time from the negative peak to beginning of the prepyriform response is a measure of synaptic delay (Fig. 1).

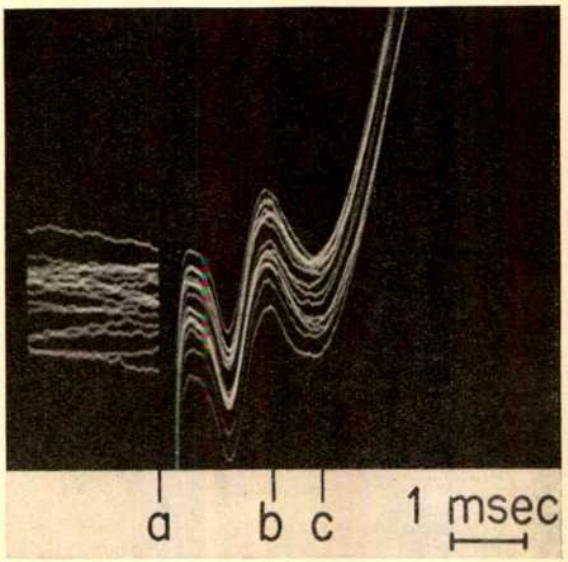


Fig. 1. Pre- and post-synaptic potentials recorded in the prepyriform cortex after electrical stimulation of the lateral olfactory tract (LOT). The initial low amplitude biphasic (positive-negative) response after the stimulus artefact is the potential of the LOT. Only the beginning of the prepyriform response is shown. The time from stimulus artefact to negative crest of the LOT potential (*a*–*b*) measures conduction time along the tract. The time from crest of the LOT potential to beginning of the prepyriform response (*b*–*c*) measures synaptic delay between fibres of the LOT and prepyriform pyramidal cells. Latency of the prepyriform response is the time from *a* to *c*

The susceptibility of synaptic transmission and conduction rate in the brain to the deleterious effects of short-term exposure to high altitudes has been demonstrated[1], but the effects of exposure for several days have not previously been investigated. Therefore, in the present experiments, the effect of exposure for 3 days to simulated altitudes of 3,800 m (12,500 ft., 475 mm of mercury pressure) and 5,490 m (18,000 ft., 380 mm of mercury pressure) on conduction time along the LOT and synaptic delay between tract and cortex were investigated in twelve rats chronically implanted with electrodes. Control pressures were 755 mm of mercury. General procedures for implantation of electrodes, electrical stimulation and recording, and simulation of high altitudes have been described previously[3]. Electrodes were bipolar (No. 26 wire, 1 mm tip separation). Rectangular pulses (0·03 msec duration, at twice the threshold intensity—usually 500 μamps) were delivered to the electrode in the LOT by means of a 'Tektronix' pulse generator and stimulus isolation unit. Responses were amplified with a 'Grass' Model *P5* alternating current pre-amplifier and were photographed with an oscilloscope camera. Experimentation was begun 1 month after implantation, when electrical activity had stabilized. Six different rats were exposed to each altitude.

Pre-exposure control means and standard errors were as follows: 0·94 ± 0·02 msec for conduction time along

the LOT; 0·60 ± 0·01 msec for synaptic delay; and 1·54 ± 0·04 msec for total latency. During exposure for 3 days to either altitude, conduction time, synaptic delay and total latency were significantly (t test, $P < 0.05$) prolonged after 2, 24, 48 and 72 h at altitude (Fig. 2). A greater percentage increase in synaptic delay than in conduction time was observed at both altitudes: synaptic delay was increased 24–32 per cent and conduction time 11–17 per cent at 5,490 m; synaptic delay was increased 15–23 per cent and conduction time 5–9 per cent at 3,800 m. The degree of change in total latency was intermediate between the changes in synaptic delay and conduction time. None of the indices measured showed any improvement with time at altitude; rather, the degree of change was slightly greater toward the end of the 3 days. After return to sea level pressures, conduction time and synaptic delay returned to normal more rapidly after the stay at the lower of the two simulated altitudes: values did not differ significantly from control levels 1 h after return from simulated 3,800 m altitude, but were still significantly prolonged 1 h after return from 5,490 m. All values were within 2 per cent of pre-exposure control levels 24 h after arrival at sea level pressure.

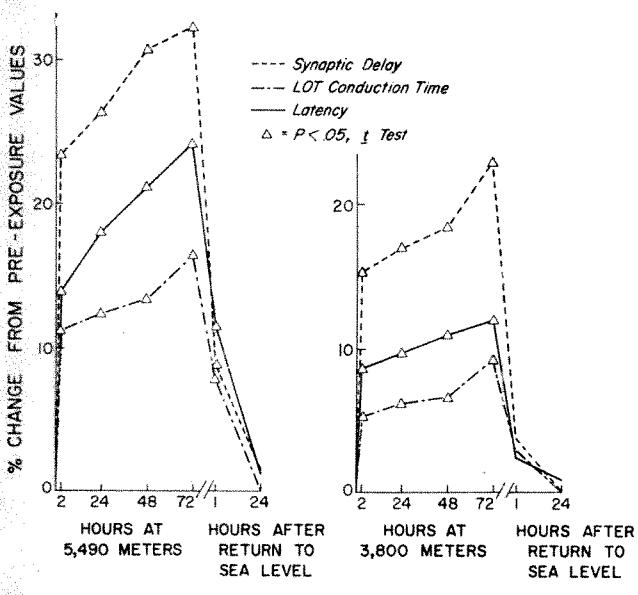


Fig. 2. Effects of exposure for 3 days to two simulated high altitudes and of return to sea level pressures on synaptic delay between fibres of the lateral olfactory tract (LOT) and prepyriform pyramidal cells, on latency of the prepyriform response, and on conduction time along the LOT. Values are expressed as percentage change from pre-exposure control values

Most of the previous investigations of hypoxia and synaptic transmission have been concerned with the effects on autonomic or spinal synapses of stagnant hypoxia due to circulatory arrest with a resultant accumulation of carbon dioxide, and have emphasized that peripheral synapses are relatively resistant to the effects of this type of hypoxia[3]. The present results show that the hypoxia of high-altitude exposure prolongs synaptic delay and conduction time in a brain system, even at the relatively moderate altitude of 3,800 m. Also, synaptic delay is altered to a greater degree than is conduction time at both altitudes, in agreement with previous observations on the effects of 2-h altitude exposure[1]. No doubt these types of changes contribute to the disturbances of central nervous function observed at altitude[3]. An important additional finding of the present experiments is that the prolongation in synaptic delay and in conduction time did not improve during the 3 day exposure to either altitude, but in fact tended to become even greater with time at altitude. However, despite the rather marked changes at altitude, the return to normal values occurred rapidly after descent to sea level pressures.

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VIROLOGY

Isolation and Analysis of Adenovirus 12 Sub-units from Adenovirus Type 12-infected Cells

THE isolation of adenovirus 12 group-specific ("A") and type-specific ("C") antigens has been described by Huebner *et al.*[1] The method used for isolation of the sub-units was step by step elution from columns of diethylaminoethyl cellulose (DEAE). Because the amount of material available at a given time varied, reabsorption was less important in this procedure than with gradient elution procedures. Eluent changes were regulated by a series of tests using cell protein alone in order to eliminate the possibility of widening or tailing of bands. Virus-antigen mixtures were equilibrated with 0·01 M phosphate buffer at 6·8 *p*H before application to the column of DEAE, which was prepared in 0·01 M phosphate buffer at *p*H of 7·2. All operations were carried out in a cold room. Sodium chloride solutions were prepared in 0·01 M phosphate buffer at 6·8 *p*H so that there was a constant *p*H but an increase in salt concentration during elution. An investigation of the effectiveness of changes in salt concentration versus changes in *p*H with cell protein showed that a constant *p*H was desirable and that changes in salt fronts were predictable. It was necessary to re-cycle eluates once or twice over fresh columns in order to obtain purified material in single peaks. A critical aspect of the procedure was the resin exchange capacity of 0·56 m. equiv./g.

A model 130 'Spectrochrom' analyser (Beckman Instruments, Inc.) was used in co-operation with the Dairy Products Laboratory, Department of Agriculture. The adenovirus 12 sub-units isolated by triple cycling from DEAE columns, as already described, were each fractionated on 'Sephadex *G*-200' prepared with phosphate-buffered saline and eluted under constant *p*H of 7·6 and constant conductivity. A precision dual-beam spectrophotometer was incorporated into the 'Spectrochrom' analyser which made it possible to scan the 'Sephadex *G*-200' column effluent at any three wavelengths. One hundred drops were collected in each tube and simultaneously monitored at 220, 280 and 540 mμ. The flow rate was about 12·5 c.c./h. Seventy-five samples were collected. Each peak was analysed by complement-fixation tests of pooled, concentrated eluents; randomized samples of pooled, concentrated eluents were used throughout the separation with the 'Spectrochrom' as controls (see legends on Figs. 1 and 2). By means of this molecular sieve operation, and using known standards which included serum proteins, milk proteins and certain enzymes, it was possible to determine the molecular weight (calculated at the peak) of the materials in question[2].

Figs. 1 and 2 give some results of 'Spectrochrom' studies of adenovirus type 12 "A" and "C" antigens. Fig. 1

shows the 'Spectrochrom' analysis of separation on 'Sephadex *G*-200' of adenovirus type 12 "A" antigen (group) which had been purified by triple cycling on DEAE anionic exchange cellulose. By direct calculation, the molecular weight of adenovirus type 12 "A" antigen was found to be 190,000. Analysis by complement fixation confirmed the biological specificity of this group-specific antigen in that neither the type-specific nor the virus-induced antigens associated with cells could be demonstrated (see legend, Fig. 1).

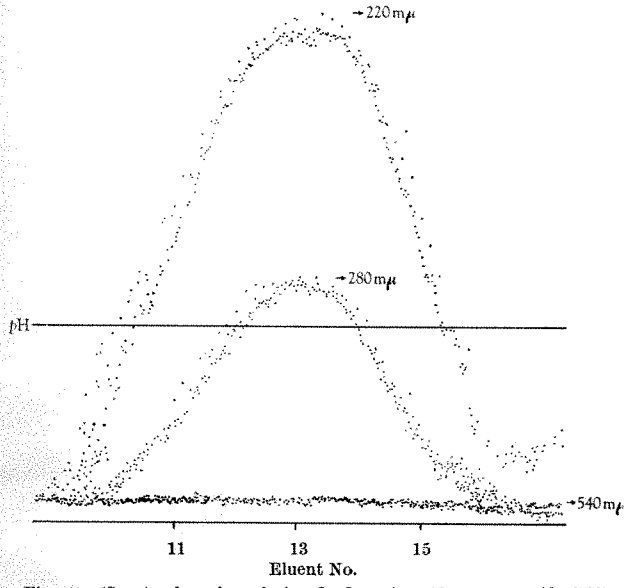


Fig. 1. 'Spectrochrom' analysis of adenovirus 12 group-specific "A" antigen at three wavelengths
Complement-fixation antigen titre

	A*	C†	T'‡	T"§
Starting material	1 : 64	0	0	0
Peak	1 : 16	0	0	0
Remaining eluent	0	0	0	0

* Adenovirus 12 rabbit "A" antisera, 1–50 dilution (4 U.). † Adenovirus 12 rabbit "C" antisera, 1–50 dilution (4 U.). ‡ Adenovirus 12 hamster tumour antisera, Laboratory for Infectious Diseases (LID) pool 13, 1–20 dilution, broad-reacting. § Adenovirus 12 hamster tumour antisera, LID Vargosko pool 2, 1–20 dilution, narrow-reacting

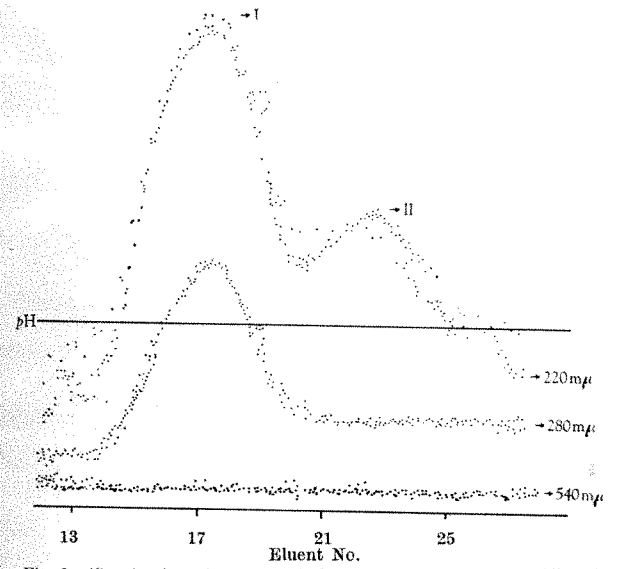


Fig. 2. 'Spectrochrom' analysis of adenovirus 12 type-specific "C" antigen at three different wavelengths
Complement-fixation antigen titre

	A*	C†	T'‡	T"§	T'"‖
Starting material	0	1 : 16	1 : 4	0	0
Peak I	0	0	0	0	0
Peak II	0	1 : 8	1 : 4	0	0
Remaining eluent	0	0	0	0	0

* Adenovirus 12 rabbit "A" antisera, 1–50 dilution (4 U.). † Adenovirus 12 rabbit "C" antisera, 1–50 dilution (4 U.). ‡ Adenovirus 12 hamster tumour antisera, LID pool 13, 1–20 dilution, broad-reacting. § Adenovirus 12 hamster tumour antisera, LID Vargosko pool 2, 1–20 dilution, narrow-reacting. ‖ Adenovirus 31 hamster tumour antisera, LID pool *F*3079, 1–40 dilution

Fig. 2 shows the separation obtained for DEAE triple-cycled purified adenovirus 12 "C" (type-specific) antigen. The calculated molecular weight of "C" antigen (peak II) was 63,000. Complement-fixation tests of the purified type-specific antigen (see legend, Fig. 2) also confirmed our previous observation[1] of the presence of antibody to adenovirus 12 type-specific ("C") antigen in broad reacting hamster tumour antisera. Complement fixation tests were negative with narrow reacting hamster tumour antisera which gave specific measurements for the presence of virus-induced antigenic material associated with cells. By means of electron microscope analysis, Valentine and Pereira[3] had been able to conclude that the molecular weight of the adenovirus type 5 "A" antigen was 210,000, while that for the "C" antigen was 70,000. Another component (Fig. 2), however, was eluted before and in connexion with "C"; the molecular weight of this component (peak I) was calculated to be 137,000. It is not known at this time what this component might be, as no complement fixation reactions were seen with broad and narrow reacting hamster tumour antisera or with group specific antisera (see legend, Fig. 2).

By the use of a 0·25 cm cuvette path length, the 540 mμ wavelength was monitored in order to rule out any interference that might arise from coloured material. It can be seen in Figs. 1 and 2 that the group specific adenovirus 12 antigen consisted of 220 mμ material with one-quarter 280 mμ material, whereas the type-specific adenovirus 12 antigen consisted mainly of 220 mμ material.

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A Method for the Rapid Assay of Newcastle Disease Virus

METHODS at present available for the detection and quantification of infectious Newcastle disease virus (NDV) particles include plaque assays[1,2], the development of haemagglutinating activity and/or death of fowl embryos[3], the appearance of cytopathic effects[4], and the enumeration of virus infected cells by fluorescent antibody staining[5]. With the exception of the last technique, all these methods require a minimum of 3 days for adequate evaluation. The adsorption of erythrocytes to infected cell monolayers as a means of detecting certain non-cytopathic myxoviruses[6] suggested to us a method for titrating NDV. This communication reports an assay for infectious

NDV based on the ability of this virus to initiate foci of haemadsorbing cells. Results from such a titration can be accurately determined 24 h after inoculation of the virus on to susceptible monolayers. The rapidity and reproducibility of this assay technique have made it exceptionally useful in our studies on the synthesis and properties of NDV.

The *HEp*-2 cells used in this study were propagated in Eagle's minimal essential medium containing 10 per cent inactivated calf serum. Two strains of NDV, California 11914 and *OS* (obtained from D. A. Wolff, Ohio State University), were used in this investigation and yielded identical results. The assay was accomplished as follows: confluent *HEp*-2 cell monolayers in 5·0 cm Petri dishes were inoculated with 0·2 ml. of virus at an appropriate dilution, and adsorption was allowed to proceed at 37° C for 30 min with occasional redistribution of virus-containing fluids. Residual unattached virus was removed without further washing, and 5·0 ml. of fresh growth medium were added to each dish after which incubation at 37° C was continued. Twenty-four hours after virus inoculation, the growth medium was replaced with 3·0 ml. of a chilled suspension of 0·5 per cent chicken erythrocytes in 0·15 M sodium chloride. After the erythrocytes had settled on to the monolayer (this usually required 20 min), unadsorbed red cells were removed by washing the monolayers several times with chilled saline. The haemadsorbing foci could be seen with a dissecting microscope with an opal glass stage at × 10–20 magnification. The appearance of foci under these conditions is shown in Fig. 1. A direct relationship was found to exist between the relative virus concentration and the number of haemadsorption foci (Fig. 2). This suggested that each focus resulted from the action of a single infectious particle. Virus titres were expressed in terms of haemadsorption focal units (HFU), as suggested by Watkins for herpes simplex virus[7].

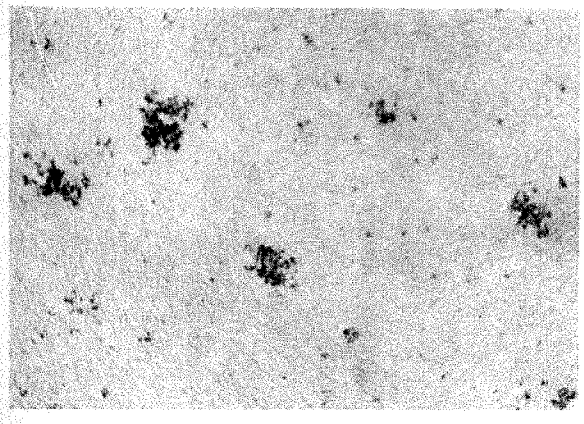


Fig. 1. Haemadsorption foci produced by NDV-infected *HEp*-2 cells (× *c.* 12)

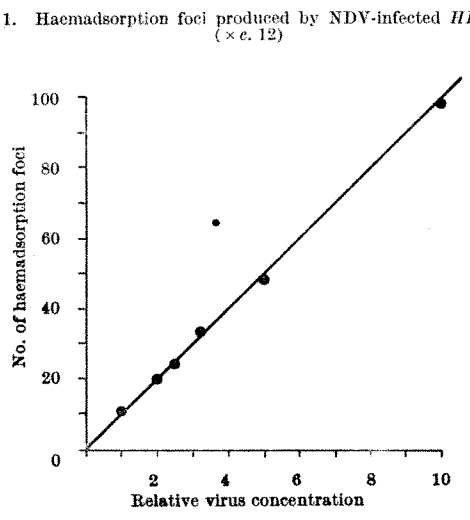


Fig. 2. Relationship between the relative virus concentration and the number of haemadsorption foci appearing in *HEp*-2 cell monolayers 24 h after virus inoculation

The adsorption of the virus to cell monolayers in Petri dishes was measured by the subsequent appearance of haemadsorption foci following various intervals of contact between virus and cells. The adsorption of NDV to *HEp*-2 monolayers was found to be maximal 30–40 min after inoculation and was in good agreement with the findings of Rubin *et al.*[8], who studied the adsorption of NDV to chick embryo cell monolayers.

Experiments with antiserum prepared against the *OS* strain of NDV have shown that *in vitro* incubation of NDV with antiserum for 30 min at 37° C completely abolished the ability of the virus to form haemadsorption foci. The addition of antiserum to the medium bathing infected monolayers during the incubation period also inhibited haemadsorption. Antiserum added for 30 min to monolayers 24 h after virus inoculation (at which time haemadsorption foci were fully developed in control monolayers) resulted in a loss of the erythrocyte-binding capacity of infected cells. Marcus[9] has observed a similar effect with NDV-infected HeLa cells.

The assay system described here offers distinct advantages over previously described methods for quantification of NDV infectivity. The need to maintain embryonated eggs or the preparation of primary chick embryo monolayers is circumvented, as a serially passed line of cells can be used in this assay. Agar overlays, staining and the use of antisera are unnecessary. Perhaps the main advantage of our system is that accurate results are available considerably earlier than they are with conventional plaque assay methods. The properties of this system indicate that it may be of value, not only in experimental studies with NDV, but also in clinical situations where time is an important factor.

This work was supported by the National Institutes of Health and the Van Wert County United Health Foundation Fund.

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SOIL SCIENCE

A Chemical Index of Soil Nitrogen Availability

MANY laboratory methods of obtaining an index of the availability of soil nitrogen to plants have been proposed, but biological procedures involving estimation of the amount of nitrogen mineralized when soil is incubated under aerobic conditions at 28°–35° C for 1–4 weeks have been generally preferred[1]. However, recent work has shown that the results of these incubation procedures are greatly affected by the method and duration of storage of the soil sample before analysis. This has led to a renewal of interest in chemical methods, because it seems likely that the results of chemical methods will be less affected by preliminary handling and storage of the soil

sample than the results of incubation methods. A chemical approach to the problem of obtaining an index of soil nitrogen availability is also encouraged by the experience that chemical methods of analysis are usually simpler and more rapid and precise than biological techniques. Studies in our laboratory of chemical methods of obtaining an index of the availability of soil nitrogen to plants have led to development of the following procedure which appears suitable for routine analysis of soils.

Five grams of < 2 mm soil is placed in a 125-ml. Erlenmeyer flask, 30 ml. of water is added, and the soil–water mixture boiled under reflux for 60 min, using a rheostat-controlled electric hot-plate. Twenty millilitres of 10 per cent (w/v) solution of potassium sulphate is added to the cooled soil–water mixture, the flask swirled for a few seconds to mix the contents, and the mixture filtered under suction (Whatman No. 42 filter paper). A 20-ml. portion of the filtrate is pipetted into a 50-ml. Kjeldahl flask designed for use with the steam distillation apparatus as described by Bremner and Keeney[2], and treated with 2 ml. of concentrated sulphuric acid. The flask is then heated on a micro-Kjeldahl digestion rack until the water has evaporated and the digest is cleared, and digestion is continued for 30 min after clearing. After completion of digestion, the flask is allowed to cool, about 10 ml. of water is added (slowly, and with shaking), and the flask cooled under a cold water tap. Five millilitres of boric acid indicator solution[2] is added to a 50-ml. Erlenmeyer flask marked to indicate a volume of 35 ml., and the flask placed under the condenser of the steam distillation apparatus. The cooled Kjeldahl flask is connected to the distillation apparatus, 10 ml. of 10 N sodium hydroxide placed in the funnel of the apparatus, and the alkali slowly added to the Kjeldahl flask by raising the funnel plug. Steam distillation is initiated by closing the stopcock on the steam bypass tube of the distillation apparatus, and the distillation stopped by opening this stopcock when the distillate has reached the 35 ml. mark on the receiver flask (the rate of distillation is about 7·5 ml./min, and the period of distillation about 4 min). The ammonium–nitrogen in the distillate is then determined by titration with 0·005 N sulphuric acid from a microburette graduated at 0·01 ml. intervals.

The procedure described and other laboratory methods of obtaining an index of soil nitrogen availability were evaluated by applying them to twenty-five surface soils having widely different nitrogen contents and by comparing the results with those obtained in a greenhouse study of the availability of the nitrogen in these soils to ryegrass. The laboratory procedures were applied to soil samples which had been air-dried, crushed to pass a 2 mm sieve, and stored for 2 months in tightly sealed glass bottles. The incubation methods evaluated included the technique described by Bremner[1] which involves an estimation of the (exchangeable ammonium + nitrate + nitrite)-nitrogen produced by incubation of soil under aerobic conditions at 30° C for 14 days, and a modification of the incubation procedure of Waring and Bremner[3], which involves an estimation of the ammonium–nitrogen produced by incubation of soil under waterlogged conditions at 40° C for 7 days (this modification was used because it was found to give better results than the original procedure involving incubation at 30° C for 14 days). The chemical methods evaluated included the alkaline permanganate distillation procedure of Subbiah and Asija[4], the sodium hydroxide microdiffusion method of Cornfield[5], total soil nitrogen as determined by Kjeldahl analysis, and the procedure proposed here.

The relationships between the results of the laboratory methods and nitrogen uptake by ryegrass are given in Table 1. It can be seen that the results of the aerobic and waterlogged incubation methods and of the chemical procedure proposed were more highly related to nitrogen uptake by ryegrass than were the results of the other methods tested.

Table 1. RELATIONSHIPS OF RESULTS OF VARIOUS LABORATORY METHODS AND NITROGEN UPTAKE BY RYEGRASS (TWENTY-FIVE SOILS)

Method	Correlation coefficient (r)*
Incubation	
Aerobic	0·71
Waterlogged	0 73
Chemical:	
Proposed	0 77
Subbiah and Asija	0·43
Cornfield	0 45
Total soil nitrogen	0·52

*$r > 0{\cdot}40$, $P = 0\ 05$; $r > 0{\cdot}51$, $P = 0\ 01$; $r > 0{\cdot}62$, $P = 0{\cdot}001$.

The chemical method described was developed from investigations of a procedure proposed by Livens[6] involving Kjeldahl estimation of the nitrogen extracted from soil by boiling water. We confirmed Livens's finding that his procedure provides a good index of the availability of soil nitrogen, but found that difficulties encountered in filtration of boiling water extracts of soils made this method impractical for routine analysis of soils. The method proposed has the advantages that it requires only a small sample of soil and yields an extract which is readily filtered and analysed. The extracts obtained by this method contain more nitrogen than those obtained by Livens's method, because the procedure adopted extracts exchangeable ammonium as well as nitrate and organic nitrogen. The Kjeldahl technique used for analysis of these extracts recovers the ammonium and organic nitrogen present, but excludes most of the nitrate. Besides being simple and precise (standard deviation, about 1 p.p.m.), the procedure proposed has an important advantage over incubation methods in that its results are not affected by air-drying or by air-dry storage of soil samples before analysis. For example, by this procedure, the averages of the values obtained of the extractable nitrogen for twenty-five soils were 48 p.p.m. using field-moist samples, 49 p.p.m. using freshly air-dried samples, and 43 p.p.m. using samples which had been air-dried and stored for 2 months before analysis.

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Unusual Sorption of Caesium by Vermiculite

SEVERAL recent reports[1–5] show that micas and vermiculite exhibit a strong affinity for caesium in dilute solutions. These sorptions, however, have been carried out in the presence of free electrolyte and the quantities sorbed were measured after equilibrium was reached. This communication describes an unusual time course of sorption of caesium by vermiculite in the absence of free electrolyte.

Montana vermiculite was suspended in 1 N sodium chloride solution and stirred in a Waring blender. The suspension was then washed free of excess sodium chloride and the particles smaller than 2μ were separated by sedimentation. Portions of the suspension containing particles smaller than 2μ were saturated with potassium or calcium by five centrifuge washings with 1 N solution of the chloride of the cation. Next excess electrolyte was removed and the colloid was suspended in water. Then, portions containing about 0·1 g vermiculite were withdrawn and centrifuged. The colloid was then suspended in 50 ml. of 10^{-4} N caesium chloride solution labelled with caesium-137. After various periods of time a 3-ml. portion was withdrawn from the mixture, centrifuged and the caesium-137 activity in the supernatant liquid determined by a scintillation counter. The vermiculite used in this

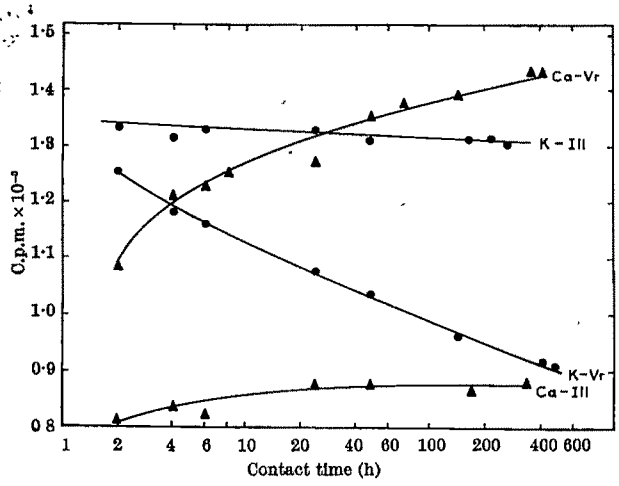


Fig. 1. Sorption of caesium by calcium- or potassium-saturated illite (Ill) and vermiculite (Vr) as a function of time. Caesium sorbed was determined by the difference between the activity of the original and the final solution in contact with the mineral

experiment consisted of about 90 per cent vermiculite and about 10 per cent interstratified vermiculite–biotite and had an exchange capacity of 140 m.equiv./100 g as determined by the method of Sawhney *et al.*[6]. A 2–0·2μ fraction of Fithian illite was similarly treated. Sorbed caesium was calculated from the difference between the activity of the caesium solution added and that of the supernatant liquid.

The results (Fig. 1) show that a slight increase in the amount of caesium sorbed by calcium-saturated illite occurred during the first few hours and it then remained constant, while the amount of caesium sorbed by potassium-saturated illite decreased slightly with time. On the other hand, the amount of caesium sorbed by calcium-saturated vermiculite continued to increase with time, while that sorbed by potassium-saturated vermiculite continued to decrease with time. Caesium sorbed by potassium-saturated vermiculite decreased from 82 per cent of added caesium at the end of 2 h to only 59 per cent at the end of 480 h. Further, in neither vermiculite was equilibrium reached even after 500 h. In contrast to this, when free electrolyte was present, equilibrium was reached in less than 24 h (ref. 1). The decrease in caesium sorbed by potassium-saturated vermiculite occurred at 5°, 25° and 80° C. Experiments showed that small amounts of potassium displaced from clay by caesium did not interfere in the determination of caesium-137 activity.

Attempts were next made to find an explanation for this unusual behaviour of potassium-saturated vermiculite. From the decrease in caesium sorbed with time, it is obvious that a maximum of caesium is sorbed very quickly, perhaps instantly, by a potassium-saturated vermiculite. This may change the mineral lattice so that caesium is extruded out of the interlayer edge sites where it is sorbed from dilute solutions[1].

A possibility suggested by J. A. Kittrick is that if potassium-saturated vermiculite in water is partially expanded by some water molecules in the interlayers, then the sorption of a less hydrated ion, caesium, at the interlayer edges of some crystals would bring the layers closer together. This would increase the attraction between the layers and would collapse the lattice. Since potassium would be the dominant interlayer cation and caesium would be only on the interlayer edges of these platelets, their *c*-axis spacing would be 10 Å. And, since caesium has a larger diameter, it will not fit into the collapsed 10 Å spacing, and thus would be extruded out because of the steric effect.

To test this hypothesis, the *c*-axis spacing of potassium-saturated vermiculite to which 10^{-4} N caesium chloride had been added, and caesium-saturated vermiculite was determined by X-ray diffraction. Portions from above vermiculite suspensions were centrifuged, and the colloid was suspended in minimum water. These thick suspensions were transferred to glass slides and X-rayed when wet, just short of flowing. A 'Norelco' X-ray diffraction unit with a flat specimen holder and a wide-range goniometer was used.

Wet potassium-saturated vermiculite had a 23 Å, a 11·5 Å and a 10 Å spacing; the potassium-saturated vermiculite was, thus, found to be partially expanded. No change was detected in these spacings when 10^{-4} N caesium chloride was added to potassium-saturated vermiculite. This would be expected, since the sample is still saturated primarily with potassium, and only a small fraction of it has caesium on its interlayer edges. On the other hand, wet caesium-saturated vermiculite had a collapsed 10·8 Å *c*-axis spacing as against a 12 Å observed by Barshad[7]. This supports the postulate that the caesium sorption, by a potassium-saturated and partially expanded vermiculite, brings the layers closer and may, thus, increase the attraction between the layers and collapse the lattice.

The results obtained support the hypothesis that caesium, being a larger ion than potassium, is extruded from the potassium-saturated and collapsed 10 Å lattice of vermiculite, and hence a decrease in sorbed caesium is observed.

This investigation was supported in part by a contract with the U.S. Atomic Energy Commission.

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GENERAL

"Significant" and "Highly Significant"

THE proposal by Dr. Miller[1] for quantitatively defined terms for various degrees of probability seems to me to be excellent. I would like to suggest, however, that the word "conclusive" for the case where $P < 0{\cdot}001$ is undesirable. The term "conclusive" suggests that no further work need be done and that no alternative interpretation is possible. In fact, an industrious person who does a few thousand experiments on the effects of a similar number of different chemicals on a particular biological process will expect to find a few of these apparently giving effects at the $P < 0{\cdot}001$ level owing to random chance even if none of the chemicals has any real effect at all.

It is almost certain that this occurs from time to time in the pharmaceutical field and little harm is done, at least in informed circles, by the statement that a particular result gives the probability specified, because it will be realized that much work may still be needed before the result can be regarded as genuinely established. If the term "conclusive" were used in the scientific literature at this stage, however, it could have a most undesirable result. When used in the advertising sense to the general public the word "conclusive" really cannot be expected to mean anything except conclusive. The word "decisive", though this case is not quite so serious, does seem also to suffer from the same disadvantage.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Thursday, August 25

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Discussion Meeting on "Should Britain have a Single or Dual-Standard Colour Television Service?"

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RADIOCHEMISTRY TECHNICIAN/SENIOR TECHNICIAN (preferably familiar with the techniques of working with radioactive isotopes)—The Director, Scottish Research Reactor Centre, East Kilbride, near Glasgow (August 26).

ASSISTANT EXPERIMENTAL OFFICER, SOIL CHEMIST (with a pass degree in science with chemistry as a major subject, H.N.C. in chemistry or an equivalent qualification) to undertake work involving the analysis of soil and plant materials—The Secretary, Hill Farming Research Organization, 29 Lauder Road, Edinburgh, 9 (August 27).

TEMPORARY LECTURER or TEMPORARY ASSISTANT LECTURER (suitably qualified graduate) IN GEOLOGY IN THE FACULTY OF SCIENCE, to give lectures and practical classes in metamorphic petrology to second- and third-year undergraduates, and to assist with some practical classes in mineralogy and crystallography—The Registrar, The University, Manchester, 13, quoting Ref. 183/66/Na (August 27).

ASSISTANT LECTURER or LECTURER (with a special interest in Eastern Europe (excluding the U.S.S.R.) and some knowledge of an Eastern European language, or prepared to learn one) IN GEOGRAPHY—The Registrar, University of Nottingham, University Park, Nottingham (August 29).

BIOCHEMIST (honours graduate in chemistry or biochemistry) IN THE CHEMICAL PATHOLOGY DEPARTMENT, Queen Elizabeth Hospital for Children, London, E.2—The Group Secretary, Administrative Offices, Hackney Hospital, London, E.9 (August 31).

BIOCHEMIST (with at least an honours B.Sc. in biochemistry, or, if medically qualified, extensive biochemical experience) IN THE DEPARTMENT OF ANATOMY, Queen's College, to join a team of biologists investigating cellular and subcellular effects of high pressure oxygen on organs in culture—The Secretary, University of St. Andrews, Queen's College, Dundee (August 31).

LECTURER IN VETERINARY PARASITOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/187/N (August 31).

LECTURER or ASSISTANT LECTURER IN PHYSICS—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (August 31).

LECTURER or ASSISTANT LECTURER (with a specialized knowledge in physical oceanography, particularly hydrology) IN THE DEPARTMENT OF OCEANOGRAPHY—The Deputy Secretary, The University, Southampton (August 31).

RESEARCH ASSISTANT (with an honours degree in physics or metallurgy and a knowledge of electronics) IN THE DEPARTMENT OF GEOLOGY, to collaborate in continuing electron microprobe studies of minerals with emphasis on solid solution relationships of sulphide, oxide, metal and silicate phases, and to be responsible for the operation and maintenance of microanalytical equipment—The Secretary, University College, Gower Street, London, W.C.1 (August 31).

SENIOR LECTURER/LECTURER IN PHARMACY at Rhodes University, Grahamstown, South Africa—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (South Africa and London, August 31).

LECTURER or ASSISTANT LECTURER (with an honours degree in engineering or mathematics and some experience in structural and/or machine vibration problems) in the Institute of Sound and Vibration Research—The Deputy Secretary, The University, Southampton (September 1).

LECTURER or DEMONSTRATOR (with interests in the genetics and biochemistry of micro-organisms) IN THE DEPARTMENT OF GENETICS—Dr. Sydney Smith, Department of Zoology, University of Cambridge, Downing Street, Cambridge (September 1).

LECTURER (with good academic qualifications and postgraduate experience, a special interest in the analysis and design of power generation and distribution systems, and preferably familiarity with modern methods of analysis and simulation of systems using computer techniques) IN ELECTRICAL ENGINEERING—The Registrar, University College of Swansea, Singleton Park, Swansea (September 1).

SHELL-MEX and B.P. LECTURER (with a degree in medicine, physiology, psychology or engineering and experience in some aspect of aeronautical vibration and noise problems) IN THE INSTITUTE OF SOUND AND VIBRATION RESEARCH—The Deputy Secretary, The University, Southampton (September 1).

EXPERIMENTAL OFFICER IN THE DEPARTMENT OF GEOLOGY to act as curator of the teaching and research collections of rocks, minerals and fossils, to be responsible for some museum-type display work, and to supervise the departmental library and map collection—The Registrar, The University, Manchester, 13, quoting Ref. 184/66/Na (September 3).

TUTOR (preferably with a higher qualification in medicine) IN MEDICINE—The Registrar, The University, Manchester, 13, quoting Ref. 188/66 (September 3).

LECTURER or ASSISTANT LECTURER (preferably with interests in either physical or economic geography, particularly of the developing countries) IN THE DEPARTMENT OF GEOGRAPHY, Fourah Bay College, The University College of Sierra Leone—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 5).

LECTURER (suitably qualified in sociology or social anthropology, with a higher degree and some experience of lecturing or tutoring at the undergraduate level) IN SOCIOLOGY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 5).

SUPERINTENDENT (with appropriate qualifications, for example, in veterinary science or pathology) OF THE ANIMAL HOLDING AND BREEDING UNIT, to assist in planning the proposed unit and to supervise it when established—Mr. G. R. Anderson, Assistant Registrary, The Old Schools, Cambridge (September 5).

LECTURER or ASSISTANT LECTURER IN PSYCHOLOGY at Queen's College, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (September 8).

ASSISTANT EXPERIMENTAL OFFICER (with O.N.C. in appropriate subjects, or equivalent, or expecting to obtain O.N.C. this summer, and preferably ex-Service candidate) IN THE INDUSTRIAL DIVISION OF THE DEPARTMENT OF INDUSTRIAL AND FORENSIC SCIENCE, Ministry of Commerce—The Secretary, Civil Service Commission, Stormont, Belfast 4, Northern Ireland (September 9).

EXPERIMENTAL OFFICER (Analytical Chemistry) (with an honours degree in chemistry or a qualification in geochemistry) IN THE DEPARTMENT OF GEOLOGY, University of Ibadan, Nigeria, to work in the geochemical laboratory on the chemical analysis of silicate rocks and minerals—The Inter-University Council, 33 Bedford Place, London, W.C.1 (September 9).

VETERINARY PATHOLOGIST (M.R.C.V.S. or its equivalent with postgraduate training and practical experience in veterinary pathology) in the Ministry of Agriculture's Veterinary Research Laboratories, Stormont, Belfast, Northern Ireland, to collaborate in the routine diagnostic and research activities of the laboratories and may be required to undertake lecturing duties in the Faculty of Agriculture in Queen's University—The Secretary, Civil Service Commission, Stormont, Belfast, Northern Ireland (September 9).

LECTURER (graduate in science, already with, or wishing to have, experience and research opportunities in virology including clinical virology) IN THE DEPARTMENT OF BACTERIOLOGY—The Secretary, The University, Aberdeen (September 10).

RESEARCH FELLOW (with a good honours degree or equivalent qualification in engineering or physics) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING for the fundamental study of high voltage power cables including heat transfer and cryogenics—The Deputy Secretary, The University, Southampton (September 10).

UNIVERSITY DEMONSTRATOR (with an honours degree in engineering, agricultural engineering, or appropriate postgraduate training) IN AGRICULTURAL ENGINEERING, to teach and conduct original investigations on farm machinery and implements and their uses—The Secretary, School of Agriculture, University of Cambridge, Downing Street, Cambridge (September 12).

CHAIR OF MATHEMATICAL STATISTICS—The Registrar, University of Liverpool, Liverpool (September 17).

LABORATORY SUPERINTENDENT and ADMINISTRATOR (with experience in administration and preferably a knowledge of laboratory organization and professional qualifications)—Prof. S. V. Perry, Department of Biochemistry, University of Birmingham, Birmingham 15 (September 19).

UNIVERSITY LECTURER (preferably with an interest in experimental taxonomy) IN BOTANY and CURATOR OF THE CLARIDGE DRUCE HERBARIUM—The Administrator, Botany School, University of Oxford, South Parks Road, Oxford (September 20).

EXPERIMENTAL OFFICER/ASSISTANT EXPERIMENTAL OFFICER (candidates over 22 should have a degree in mathematics or physics) IN THE UNIT OF STRUCTURAL CHEMISTRY, to assist with computational aspects of X-ray crystallography—The Secretary, Agricultural Research Council, 15 Regent Street, London, S.W.1 (September 30).

LECTURER (preferably with special interests in physical chemistry) IN CHEMISTRY at the University of Otago, Dunedin, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, September 30).

LECTURER/SENIOR LECTURER and a LECTURER IN CIVIL ENGINEERING at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

DIRECTOR OF THE FITZWILLIAM MUSEUM—The Registrary, University Registry, The Old Schools, Cambridge, marking envelope "Confidential" (October 10).

DIRECTOR OF THE NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING—The Secretary, National Institute for Research in Dairying, University of Reading, Shinfield, Reading, Berkshire (October 15).

CHAIR OF GENERAL PHILOSOPHY (History of Philosophy and Systematic Philosophy) IN THE FACULTY OF PHILOSOPHY of the University of Berne, Switzerland—Director of Public Education of the Canton of Berne, Munsterplatz, 3000 Berne, Switzerland (October 31).

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER IN THE DEPARTMENT OF GEOLOGY, for mineral analysis by X-ray spectrometry, to take charge of an analytical service and initiate developments using X-ray fluorescence and electron-microprobe techniques—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

GRADUATE BIOCHEMIST or CHEMIST (wishing to proceed to a higher degree) for research on soluble collagens of the human intervertebral discs, using recently developed chemical and physical techniques—The Registrar, Bradford Institute of Technology, Bradford, 7.

GRADUATE BIOCHEMIST or ORGANIC CHEMIST at the Chester Beatty Research Institute, to assist in research into the nature and consequences of the binding of chemical carcinogens to nucleic acids and proteins in vivo using radioactive techniques—The Secretary, Institute of Cancer Research, Royal Cancer Hospital, 34 Sumner Place, London, S.W.7, quoting Ref. 301/B/261.

HISTOPATHOLOGIST (with a medical or veterinary degree, postgraduate experience in histopathology and preferably general research and/or a specialized knowledge of histochemistry) IN THE DEPARTMENT OF EXPERIMENTAL PATHOLOGY, Chester Beatty Research Institute, Fulham Road, S.W.3—The Secretary, Institute of Cancer Research, Royal Cancer Hospital, 34 Sumner Place, London, S.W.7, quoting Ref. 300/G/25.

LECTURER (with particular interests in transition metal chemistry, and an active interest in research) IN INORGANIC CHEMISTRY—The Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1, marking envelope "Lecturer—Inorganic Chemistry".

PHYSICIST, BASIC GRADE (honours graduate, male or female, in physics) in a newly established medical physics department serving Cornwall and South-West Devon, based in new premises at Plymouth General Hospital—The Hospital Secretary, Plymouth General Hospital, North Friary House, Greenbank Terrace, Plymouth, Devon.

PHYSIOLOGIST/BIO-PHYSICIST to study physiological response to controlled changes in environmental conditions—D. C. Page, Head of Personnel Services, The Electricity Council, Research Centre, Capenhurst, Chester, quoting Ref. N/28.

POSTDOCTORAL RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY for research on solid state Raman spectra, using the new Cary 81 Laser/arc Raman spectrometer, in the general field of inorganic chemistry—Prof. I. R. Beattie, University of Southampton, Southampton.

POSTDOCTORAL RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY, to work with Dr. B. Capon on an investigation of bifunctional catalysis—The Registrar, The University, Leicester.

RESEARCH ASSISTANTS (with a degree, or equivalent, in engineering, and prepared to carry out research in electrical engineering, under guidance) IN THE DEPARTMENT OF ELECTRICAL AND CONTROL ENGINEERING, Liverpool Regional College of Technology, Byron Street, Liverpool, 3—The Director of Education, Education Offices, 14 Sir Thomas Street, Liverpool, 1.

RESEARCH PHYSICIST (preferably with a higher degree and relative experience) IN THE PHYSICS DEPARTMENT, to undertake an investigation of

electron scattering using 400 kV electron diffraction camera with a recently constructed energy analysis facility—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

SPECTROGRAPHIC TECHNICIANS IN THE DEPARTMENT OF MINES AND TECHNICAL SURVEYS, to determine trace elements in rocks, minerals, ores, and related materials of interest to Research Scientists engaged in geological, geochemical and mineralogical studies—The Civil Service Commission of Canada, Ottawa 5, Canada.

TECHNICIAN or SENIOR TECHNICIAN (with a special knowledge of electronic equipment as used in physiology and biochemistry) IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY—The Acting Bursar (Personnel), University of Reading, Reading, Berkshire.

TECHNICIANS and SENIOR TECHNICIANS IN THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY to assist with laboratory classes and to help with research work—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

TECHNICIANS/JUNIOR TECHNICIANS (2) (preferably with experience in sterile techniques and/or tissue culture) IN THE CYTOGENETICS SECTION OF THE NUFFIELD UNIT OF MEDICAL GENETICS, where a study of human chromosomes in relation to abnormal development, including cancer, is being carried out—The Registrar, The University, Liverpool, 3, quoting Ref. 193/N.

TECHNICIAN (student or junior status, preferably with previous experience in immunology, biochemistry or microbiology) IN THE DEPARTMENT OF IMMUNOLOGY, to work on cellular aspects of immunology—The Secretary, The Wright-Fleming Institute of Microbiology, St. Mary's Hospital Medical School, Paddington, London, W.2.

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PREPRINTS GALORE

THOSE brave spirits in the United States who would launch a formal scheme for circulating preprints of scientific papers are mostly at fault by being overzealous (see *Nature*, **211**, 333; 1966). But may they not also be unreasonably innocent of practical problems? Certainly the experience of the Information Exchange Groups being run from the National Institutes of Health in the United States, and referred to on page 904 of this issue, should be a stern warning to the would-be managers of the Physics Information Exchange (PIE). The memoranda circulated by Dr. Albritton, who administers the torrents of preprints now flowing in and out of biological laboratories throughout the world, are as clear a proof as anybody could expect that the benefits expected from the information exchange groups are likely to prove illusory.

To say this is not to imply that the IEG system is without value of any kind. On the contrary, as Professor Michael Woodruff has made plain (*Nature*, **211**, 560; 1966), individual scientists can profit greatly from this kind of service. But there are others who find the arrival of bundles of preprints with successive deliveries of mail a far from welcome phenomenon. For one thing, there are frequently too many of them for busy people to be able to digest. And though there is always the chance of coming across something of great value and immediate interest, the depressing truth is that the gold is accompanied by a great bulk of unwanted dross. One result is that some members of the Information Exchange Groups have decided they can afford to wait until research reports eventually appear in print. Really hot news, after all, has its own ways of getting into circulation. (It will be fascinating to see how long it is before authors start circulating to colleagues who are particularly concerned documents which will no doubt be known as pre-preprints.) The usefulness of the IEG system is bound, of course, to vary from one field to another, and even from one laboratory to another, but that is only to be expected. It is nevertheless worth recording simply because the sponsors of these print systems always suppose that what they have to offer will be of unalloyed benefit at least to those who are recipients—as distinct from providers—of information. Even that assumption is not as safe as it may seem at first sight.

None of this, however, is a good reason for complaining at the magnanimity of the National Institutes of Health. If the IEG system were simply an adjunct to the existing means of communication between professional scientists, it would not matter if some of those on the mailing lists found their presence there of little value. But things are not, unfortunately, as simple as this. The IEG scheme, like the PIE that may follow, is not a part of the world's natural resources which can be used or ignored as circumstances suggest. For one thing, it involves the deliberate spending of substantial sums of money, so that it is proper and indeed essential to ask whether the same resources might not be spent more profitably in other ways. But these schemes are also important—and suspect—because, if they continue, they cannot fail to have an important influence on the character of the scientific literature and, therefore, on the character of science itself.

There is enough in Dr. Albritton's memoranda to show that the cost of organized preprint circulation may be very much more than has previously been calculated. He estimates that the cost of a fully developed system would be $5 million a year, though that is probably an optimistic calculation. The cost of the IEG circulation is at present thought to be roughly $100 for each member—or enough to supply each of them with several free subscriptions to orthodox journals. Is the benefit really worth that cost? And if the National Institutes of Health are really anxious to help the flow of information between scientists, might they not do better by helping to provide some of the common services that could make the orthodox channels of communication more efficient, more effective, and more speedy? Questions like these have received only the most cursory attention, no doubt because they are difficult to answer. But to flood the world with preprints is the most facile of all solutions.

The effects of the preprint circulation schemes on the balance of the literature as a whole can be inferred without too much difficulty from Dr. Albritton's breezy account of the practical problems of preprint circulation. The fact that the delay in what is called the "print-shop" can be as much as two months, with an extra month or so for those who do not type their manuscripts properly, will not surprise the organizers of conventional journals. There is no doubt a Parkinsonian law to the effect that the demand by those with something to say on any uncontrolled channel of communication is certain to increase to the point of saturation. The difficulty, of course, is that IEG cannot take steps to choke off the demand without violating the basic principle that it must be indiscriminate and undiscriminating. (Even so, it would surely have been well within the spirit of the law if Dr. Albritton had restrained himself from issuing his tactless document with the slogan "Why not send it to us NOW?") The truth is, of course, that there is a sharp and irreconcilable conflict between the laudable wish of the preprint circulators to have everybody on their mailing lists, and their fond pretence that they are not engaged in the publication business. No amount of fine print among the rules of membership can alter that. So long as the circulation of preprints is sufficiently wide, within the scientific community, for scientists to be able to claim priority for their discoveries—and to make fools of themselves as well—then the system has all the properties of a channel of publication, and is intended to have them. Its defects are inaccessibility,

impermanence, illiteracy, uneven quality, and lack of considered judgment. Preprint publication differs from the more conventional kind, however, in that authors expect to publish twice, and that disputes over issues such as priority must be settled by reference to the chairman of the group concerned, and not by looking in the library. It would be good to know how the IEG chairmen would seek to deal with offenders who claim that they habitually throw their preprints away. It is proper to record that PIE is potentially more dangerous than IEG, because the names of those with access to the system would not be known to the organizers. It is probable that both systems, in their present forms, are more of a threat to the continuing health of scientific research than a fair wind in its favour.

But if not preprints, then what? The defects of IEG and PIE are no excuse for hiding from the problems which stimulated their emergence. One of these is the speed—or lack of speed—which attends publication in the orthodox journals. It is entirely proper that scientists should wish to see their work published as quickly as possible. The wish to establish priority is, or should be, only a small part of the reason for asking that publication should not be long delayed. It is much more important that the exchange of ideas is an essential part of science, and that the rapid exchange of ideas makes science more alive. Obviously there is a lot which orthodox journals should and could do to make publication faster, by better organization, the use of new printing processes and even of the telephone. Any way in which computers and data processing systems can help to make the literature more manageable and more accessible is obviously a deserving cause. It would be entirely in order if the institutions in the United States now being asked to lavish support on preprint circulation were to spend a fraction of the sum required on helping the journals to become more efficient.

There remain a number of nasty qualitative problems. Journals seek to be discriminating, and in part their reputations depend on how well they succeed. There is evidence that, in the past, the influence of the continuing criticism by the journals of what is eventually published in the literature has itself been an important formative influence in science. It has encouraged thoroughness and measured judgment. It has discouraged triviality and repetitive work. But the question now arises of whether the literature is not ripe for some much more drastic transformation. Much of it is hard to read. Much of it is less effective a means of communicating ideas and information than it could and should be. And, of course, there is too much of it. These characteristics of the existing arrangements for publication need careful attention by the scientific professions as a whole. If the National Institutes of Health are as well disposed towards the cause of effective publication as they seem to be, they could do a lot to help. The energy they choose to dissipate in Dr. Albritton's print-shop will be much less valuable.

CENTRES OF EXCELLENCE

OLD soldiers never die, and it is much the same with laboratories. Professors Richard Post and Marshall Rosenbluth, two members of the University of California, have drawn attention to an interesting and important problem by speaking out in defence of the Culham Laboratory they have been visiting in Britain. Quite properly, they say that the laboratory is a splendid place "unexcelled in the world". They go on to hope that the kind of basic research carried out at Culham will not be restricted as a result of the British Government's wish to use its substantial effort in research and development as a means of helping the economy move forward faster. What they do not say is that the Culham Laboratory was formed at a time when there were fond hopes of turning thermonuclear research to most spectacular practical results, and that its present concern for more academic work —which includes the development of the first ESRO satellite at a cost of £3 million pounds—reflects the recognition there has been that plasma physics is not child's play. The laboratory has done well to survive, let alone to become a centre of excellence.

It remains a real question to ask how Britain should make full use of Culham and of other laboratories which pursue academic—in the sense of uncommercial —ends but which are not incorporated into universities. Professors Post and Rosenbluth are probably right in saying that too crude an attempt to divert academically minded men into other pursuits would persuade a great many of them to emigrate, but in itself that is not an overwhelming argument. If the British Government is persuaded that academic plasma physicists contribute nothing to the economy, in its present mood it would probably be glad to say goodbye to those who do not wish to change their ways. The difficulty, and the real cause for anxiety, is that activities which seem purposeless on a short time-scale can be enormously important in the long run. This is where too crude a decision could be dangerous and there is a possibility that, in its anxiety to balance the books, the British Government will make the pendulum swing too far against research as such.

It is inevitable that laboratories such as Culham will be the first to feel this pressure. It will be interesting to see how they stand up to it. Trying to define how the investment made in them may eventually be returned should be a healthy exercise. To argue that a pool of skill must be maintained is sensible, the not overwhelming by itself. (Undertaking contract work for ESRO, in which Britain is the principal partner, is not a convincing reason for survival.) The ideal would be if these laboratories could find some way of being much more closely integrated with universities, for then they would be able to claim with justice that their activities were at least helpful in educating students. The schemes which exist for taking postgraduate students at these public laboratories are not a sufficient answer to this need.

NEWS AND VIEWS

Public Exploitation

BRITAIN'S National Research and Development Corporation expects to be committed up to its present borrowing limit of £25 million by 1968, according to the annual report for 1965–1966 published last week. The Managing Director, Mr. J. D. Duckworth, is hopeful, however, that Parliament will extend this limit by another £25 million. "If we don't get more," he said, "we must start cutting back on our commitments quite soon until our present investments start paying back." Despite the Government's decision to decrease research expenditure, Mr. Duckworth does not feel that the present financial difficulties will affect the position of this corporation; the sums involved are relatively small and would be invested in development and industrial application, not research. "It is in our area that Britain's economic future depends", he added. Informal discussions take place continually between the NRDC and the Ministry of Technology, but more formal negotiations with the Minister will probably begin in the near future so that a new Act can be presented to Parliament during the next session.

The NRDC was established under the Development of Inventions Act of 1948 to develop inventions derived from publicly supported research and inventions from other sources which lack a sponsor. The original Act was amended in 1954, 1958 and 1965; the 1965 Act increased the borrowing powers of the NRDC from £10 to £25 million. The corporation is designed to act as a link between research and industry, but is increasingly participating in joint ventures with industries both to speed up development of important innovations and to spread the cost of taking risks. Within the past year it has also tried to encourage innovation by sharing risks with the user as well as the developer of new products, although the NRDC only provides some capital and is repayed if profitability or productivity increases. Mr. Duckworth stated that at present only 10 per cent of the corporation's funds are being used in this new venture, but he expects that the proportion will greatly increase in the near future.

The increased activity of the NRDC is shown in the figure of £865,000 for receipts in the nine months to the end of March from licensing, dividends, sale of equipment, and development levies. Nearly £4 million has been spent, and of this more than £3 million on projects. In 1965 the total spent on projects was a mere £500,000.

During the last nine months the NRDC has taken on twenty-eight new projects, bringing its total to 121; 155 others are at present under consideration. The corporation continues to support hovercraft development and is supporting a hoverferry, the *CC6*, being developed by Britten–Norman, Ltd. This is designed to carry four to five cars and between thirty and forty passengers. Support for fuel cells, started in 1953, continues. According to Mr. Duckworth, the cells are not yet economically feasible, but the United States is interested and American licences have led to an exchange of information that should strengthen the technical position of the British effort.

New projects include the development of synthetic insecticides related to natural pyrethrum, which combine the lack of toxicity of the natural pyrethrins with significantly lower costs. There is a metal polish additive which may be useful in making such metals as copper, zinc, steel and aluminium more resistant to tarnishing. NRDC is also supporting projects in such areas as teaching machines, minimal farm cultivation, and a multi-gas burner, and is anxious to support computer-controlled automation studies.

Culham Despondent

Two visiting scientists at Culham Laboratory of the United Kingdom Atomic Energy Authority are pessimistic about its economic future. The two American physicists, Prof. Marshall Rosenbluth of the University of California and Prof. Richard Post of the Lawrence Radiation Laboratory, in a letter to the London *Times* on August 23, stated that they have "heard rumours that due to the present financial crisis, scientific research activities not involving an immediate financial return may be curtailed in Britain". They go on specifically to praise the Culham Laboratory as unexcelled in the field of plasma physics. "A laboratory such as Culham is a precious national asset, difficult to come by".

Prof. Rosenbluth has said on the telephone that Culham scientists are more worried about money than they have been during his frequent visits in the past, but added that he has no specific knowledge to justify his pessimism or that of his colleagues at the laboratory. However, he and Prof. Post state that if there is a cut-back on research funds, they "believe the majority of the creative leaders would be inclined to join the 'brain drain' and come to the United States".

The Ministry of Technology was not prepared to comment on the general situation of government aid for research which is closely tied to the economic situation in Britain as a whole; it does admit, however, that generally it is becoming more difficult to get money. The UKAEA, the public corporation directly responsible for Culham, says that it, like other public organizations, had been asked to save £2 million pounds in the next year, but that as yet no decisions had been taken as to where these cuts should be made. While it was very possible that some cuts would be made at Culham, there was no general policy to reduce the proportion spent on basic research.

Earthquake in Turkey

ON Friday, August 19, 1966, an earthquake of approximate intensity 9 on the Modified Mercalli Scale occurred in Eastern Turkey from an epicentre near latitude 39⅓° N., longitude 41½° E., between Varto and Hinis in Mus Province. This is some 50 miles south-south-east of Erzerum and between Erzerum and the western end of Lake Van. The area is remote and mountainous, and it is believed that more than 2,000 people died and that many more were injured. The shock was felt in the Soviet Union. Aftershocks are continuing and at least one of these was felt as far west as Istanbul, though no damage was done there.

Turkey is a country of which every part is liable to earthquakes, the most stable area being just inland, near the centre of the south coast (in Asia Minor).

The earthquakes have their foci at depths ranging from the surface to some 100 km, though normally the depth is about 13 km. The deeper foci lie roughly on an east–west line starting from near the centre of the west coast of Turkey-in-Asia. The shocks vary greatly in magnitude; there are about eight shocks a year of magnitude 5 or greater on the Richter logarithmic scale. The earthquakes often begin from old foci, occasionally from new ones, and many of these are not near towns. An earthquake at Erzincan on December 26–27, 1939, killed 23,148 people and injured 8,029. 16,629 buildings were completely destroyed (see *Nature*, **145**, 13; 1940).

Most Turkish earthquakes are of tectonic origin, due to "Alpine" orogenic pressure. Slipping occurs when this pressure exceeds that required for movement, usually along one or more of the deep ancient geological faults which abound. The first point which yields becomes an earthquake focus. One such structural line runs through Mürefte, Izmit, Bolu and Gerede, and, although it is a line of earthquake epicentres, it also has such an influence on relief that it is both a very densely populated zone and a corridor for communications. Two other active faults are the Kayas and Akpinar faults.

The general geological structure of Turkey awaits clarification. Three ideas at present hold the arena: (*a*) that it merely consists of strong transverse anticlines and synclines, sometimes faulted along the edges; (*b*) that it consists of a "*Zwischengebirge*" between the Pontine and Taurus Mountains; (*c*) that at Kirsehir is a Pontine nappe and that the Anatolian thrust at its base is essentially a boundary between the Pontic (Black Sea) and Taurus (Mediterranean) pre-Tertiary rocks which differ in facies.

On account of the enhanced interest at the University of Istanbul, Turkey is now better provided with seismograph stations than formally, and some advanced seismological work is being undertaken. N. Canitez and S. B. Ucer have recently recomputed all Turkish epicentres between January 1947 and December 1959, using a digital computer. The accuracies of the new epicentres and times of origin are about $\pm 0{\cdot}1$ degrees and 1·0 seconds respectively. P_n arrivals at epicentral distances less than 16° were used, together with an iteration method which used the local velocity of P_n waves. The standard deviation in both latitude and longitude for the new epicentres is less than $\pm 0{\cdot}1$ degree. The same authors have also investigated the mechanism of some seventy earthquakes which occurred in or near Turkey between the years 1936 and 1965.

Help with Computers

THE Ministry of Technology is continuing to support the development in Britain of new computer techniques. It has just announced that, under its Advanced Computer Techniques Project, twenty-one new contracts worth more than £1 million, and supported jointly by the Government and industry, have been placed in the past year. The Government has also allocated an additional £600,000 for new contracts which are expected to be placed by April 1967.

The project was started under the Department of Scientific and Industrial Research in 1963, when the then government considered there was not enough research and development to maintain a viable competitive position in the world computer market. Seven contracts costing about £325,000 have been completed so far. The ministry's aim is to support projects which are too uncertain or too long term to commend themselves to industry, but which lie in areas that must be developed if the industry is to grow.

The prevailing view is that research and development are chiefly required in the development of fast storage devices, the design of systems and in the working out of a cheap and reliable technology of peripheral equipment and software.

Fresh Fish to Eat

THE report for 1965 of the Director of the Torry Research Station (H.M.S.O., 5*s*. 6*d*.) lays greatest stress on the need for technical innovation in the British fishing industry. The traditional fishing grounds are yielding fewer fish of the types most appreciated by the British public—cod, haddock, plaice and herring—so that the station's development of methods which minimize wastage of fish between catching and consumption is of particular importance. Superchilling of fish in liquid air, pre-packaging wet fish and dip coating of fish in liquids which produce a flexible surface film are methods which have been tried. Another possibility is to fillet the fish before freezing at sea in order to eliminate thawing and refreezing of fish for filleting to be carried out.

Even if spoilage of fish is minimized, however, it seems unlikely that all needs can be satisfied without the introduction of hitherto unexploited species such as redfish, dogfish, saithe and mackerel. These fish could be marketed in traditional ways or as components of made-up products such as fish sausages or fish cakes. A further possibility is to extend the fishing grounds to the South Atlantic, and work has been done on the preservation of fish at higher ambient temperatures in preparation for such a development.

Since these problems are essentially of a practical nature, a shift in the emphasis of the station's work away from basic research has occurred, and will be encouraged. Basic research work, however, still makes up a considerable proportion of the station's effort, and bacteriological and biochemical investigations have continued to be carried out.

Microbiologists in Moscow

AT the end of July over five thousand microbiologists from nearly fifty countries assembled at Moscow State University for their ninth quatrennial congress. More than 1,500 papers were presented or made open for discussion and they covered most aspects of microbiology. Statistics such as these immediately raise the obvious question of the usefulness of this kind of meeting (writes one of the participants). Indeed, the poor standard of many of the communications was such that one would have wished for a more thorough selection in the preparation of the programme. Be that as it may, a great deal of interest emerged during the course of the congress which afforded—and which, through the agency of its published proceedings, will continue to afford—an ideal opportunity for microbiologists to survey their rapidly expanding subject.

One of the undoubted consequences of this huge meeting was the chance of informal discussion with colleagues, and a recurrent theme in these exchanges,

particularly as expressed by Soviet and other scientists from the Soviet Union and from Eastern Europe, was the applicability of microbiological discoveries to industry and agriculture. Several sessions were concerned with applied aspects; and discussions of the automatic control of microbiological processes, and of the engineering equipment required, attracted much attention. Developments in this area of microbiology in the next few years will be rapid and far-reaching. The fuller exploitation of micro-organisms in the production of vaccines, antibiotics and a vast array of organic materials, as well as the problem of producing more food, are of the greatest importance. It was clear from the views expressed in Moscow that the increasing contributions of industries based on microbiology hold promise for the future.

Important information on the complex interactions between microbes and their hosts is coming from what is called gnotobiology — investigation with organisms free from germs or specifically inoculated. These are uniquely useful in the study of physiological processes and diseases in the absence of a microbial vector. With the advent of commercially available animals free from germs, and the isolator chambers for keeping them like that, gnotobiology is advancing rapidly. Included in the programme at Moscow was the use of germ-free techniques in the care of patients, which can be critical for those with impaired immunity mechanisms, as well as in containing contagious diseases in hospitals.

The gnotobiotic implications of space travel were also discussed. During long space flights there may be a high probability of a lethal simplification of the gut flora and the cross-contamination of pathogens. This less publicized problem of space exploration is of great practical significance to the technologist.

Apart from presenting a comprehensive report of the contemporary scene, an international meeting of this nature must also look to the future. To a gratifying extent the organizers of the Moscow congress were successful in establishing this balance which, considering the great diversity of subject matter embraced by microbiology, was no small achievement.

Population Excursions

THE birth rate is still falling steadily in Britain, according to the provisional figures for the June quarter recently issued by the Registrar General; it is estimated that there were 215,000 live births during the quarter, giving a rate of 17·9 per thousand population compared with rates of 18·5 and 19·2 for the corresponding quarters of 1964 and 1965. At the same time, the infant mortality rate has remained low—18·2 per thousand live births is estimated for the second quarter, versus the 1965 average of 19 per thousand. Only Sweden and the Netherlands have appreciably lower rates, while the United States averaged 25 deaths per thousand live births in 1965.

The birth rate may be falling but it is still well above the mortality rate; the Registrar General's Quarterly Return for the March quarter of 1966 reports that there were 862,290 live births in 1965 and only 549,379 deaths, of which 24,254 were from violent causes. The most prolific killer is still coronary and arteriosclerotic heart disease; it accounted for more than a fifth of all deaths in 1965 and at 113,451 is the highest number recorded. In the past 10 years, deaths from this cause have increased by 50 per cent. However, the number of deaths from myocardial degeneration has been steadily declining and reached a record low last year of 38,267. The other three main causes of death were pneumonia, bronchitis and stroke—the first two accounted for 60,595 deaths, and the latter for 78,149. Compared with these figures, the number of violent deaths seems quite small and is the lowest figure in 3 years; accidents involving motor vehicles caused 7,515 deaths, which is an increase in the past 2 years. The most publicized causes of all—murder and war—were responsible for only 317 deaths.

For the future, the Registrar General estimates that the population of the United Kingdom will have increased by 7·0 million by 1981, and that nearly all this increase will have occurred in England. The largest growth is expected to be in East Anglia, the Midlands and south-west England, with percentage increases of 30, 20 and 17 respectively; south-east England, at present containing 35·5 per cent of the English population, is expected to grow at only 12 per cent over the period, or less quickly than the national average.

Respiration Physiology

DR. PHILIP HUGE-JONES writes: The scientific publication explosion is becoming a threat to the assimilation of knowledge almost as alarming as the population explosion to the assimilation of food. So it is now no longer possible to welcome a new journal, as it was a few decades ago, without first assessing the need for its existence. The first number of *Respiration Physiology* has just been published this year with an eminent international editorial board and two distinguished physiologists as editors—Pierre Dejours of Paris as the day-to-day editor and Wallace Fenn of Rochester New York as honorary editor. Is there need for such a journal?

It is interesting to reflect on where articles about respiratory physiology, published in English, have hitherto appeared. In Britain the obvious place has been the *Journal of Physiology*, but with the outstanding success and interest in neuro-muscular physiology in Britain during the past decades, the proportion of articles in that journal about the physiology of respiration has declined. Thus a rough analysis of the contents of the *Journal of Physiology* for 1925 compared with 1965 showed the following percentages: in 1925, neuro-muscular articles 22 per cent, cardio-respiratory 28 per cent, endocrine 28 per cent, other 22 per cent, compared with 1965, neuro-muscular 53 per cent, cardio-respiratory 13 per cent, endocrine 15 per cent, other 19 per cent. During the period the total size of the journal increased from 46 articles to 201; the number of neuro-muscular articles increased from 10 to 106 while those on cardio-respiratory subjects increased only from 13 to 26.

It might seem that in Britain interest has shifted away from human and mammalian respiratory physiology (so ably pioneered here by Haldane, Barcroft, Hill and others) towards neuro-physiology after the success of Sherington, Adrian, and others who have followed the Oxford and Cambridge Schools. In fact, with the decline in interest in respiration among British physiologists there has been a marked increase

in interest among clinical research workers. Some of this applied and clinical respiratory physiology has been published in journals such as *Clinical Science*, but much has been published in the United States. There the *Journal of Applied Physiology* has increased, not only in absolute size but in the proportion of its articles on respiratory physiology, so that the number in March 1966 was a mammoth volume weighing 2·75 lb. and having no less than forty-six articles in it on respiratory physiology out of a total of about seventy. Obviously, there is a demand for space to publish articles about applied respiratory physiology.

What are the credentials of the new journal *Respiration Physiology*? First, it is an international journal in English. Secondly, it seems to aim to bring together the different fields of respiratory physiology from cellular respiration, through comparative respiratory physiology in animals, to applied clinical physiology of man. Unlike the *Journal of Applied Physiology* it is a speciality journal for respiration physiology—as its name implies.

In the first number of *Respiration Physiology* there are nine papers of original research including articles on a theoretical analysis of aquatic gas exchange, experimental work on respiration in an elasmobranch fish, chemical control of breathing in rats, on the pressure flow characteristics of isolated dog's lungs, on temperature and respiration on sleep, and articles on human breathing including gaseous diffusion on the airways, the effects of posture on compliance, on chemoreceptor block and on the determination of carbon monoxide transfer—a wide variety within the speciality of respiration by authors from the United States, Britain, Germany and New Zealand. If this journal can maintain the high standard of contents and layout of its first number it should surely succeed.

Information Exchange

SOVIET scientific and technical information specialists have just completed a three-week tour of Britain, visiting institutions and organizations concerned with storage, dissemination and retrieval of scientific and technical information. A similar visit to the Soviet Union was made by a team of British information specialists in May 1963. The tour was organized by the International Scientific Relations Division of the Department of Education and Science in Britain. The nine delegates, led by Mr. N. B. Arutynov, head of the Directorate of Scientific and Technical Information, State Committee for Science and Technology, Moscow, visited twenty government and industrial libraries and research establishments.

Appointments

DR. J. B. ADAMS, director of the Culham Laboratory, has been appointed a full-time member of the United Kingdom Atomic Energy Authority for 5 years as from October 1.

MR. G. J. BELL has been appointed director of the Royal Observatory, Hong Kong, in succession to Dr. I. E. M. Watts, who has been appointed to the headquarters of the World Meteorological Organization in Geneva.

PROF. D. J. FINNEY, professor of statistics in the University of Edinburgh, and Mr. J. K. Steward, head of the Mathematics and Computer Department of Imperial Chemical Industries, Ltd., have been appointed to the Computer Board. These appointments complete the membership of the board, the establishment of which was announced in the House of Commons on August 3.

PROF. I. S. ZHELUDEV has been appointed deputy director general of the International Atomic Energy Agency for the Department of Technical Operations, in succession to Prof. G. A. Yagodin.

Announcements

THE Culham Laboratory of the U.K. Atomic Energy Authority is to be awarded a contract worth roughly £3 million by the European Space Research Organization (ESRO). The authority will be the principal contractor in the development and construction of the first of the three satellites now included in the ESRO programme. This will be largely concerned with astronomical work. The intention is to launch a 30-in. Cassegrain telescope working in conjunction with a spectroscope working in the ultra-violet between 900 Å and 3300 Å. It is hoped that the alignment of the telescope will be accurate to within one-tenth of a second of arc. The entire payload will weigh 500 lb. and the entire package will be 10 ft. long and 40 in. wide. The present plan is that the satellite will be launched in 1971 using the ELDO launcher for getting off the ground.

FOUR new coastal surveying ships, designed to operate in any part of the world independently for long periods, have been ordered by the British Royal Navy. The new ships, being built by Brooke Marine, Ltd., of Lowestoft, will replace the four converted *Bay* class frigates which have been in service since 1950. When they are completed, the Royal Navy's surveying fleet will consist of four ocean survey ships, four coastal survey ships and seven smaller boats.

THE Canadian Society for Chemical Engineering has been formed within and as a part of the Chemical Institute of Canada. The new society will represent the institute in the field of chemical engineering, both nationally and internationally. The society's first officers are those of the institute's former Chemical Engineering Division, now replaced by the new society, and are: *President*, Dr. D. B. Robinson, chairman of the Department of Chemical and Petroleum Engineering in the University of Alberta; *Vice-President*, Dr. W. H. Gauvin, director of the Noranda Research Centre, Pointe Claire, Quebec; *Secretary-Treasurer*, Dr. N. E. Cooke, Engineering Department, CIL, Montreal. The new society's first annual meeting will take place during the sixteenth Canadian Chemical Engineering Conference which it is sponsoring in Windsor, Ontario, during October 17–19.

THE Harkness Fellowships of the Commonwealth Fund of New York are offered each year for study and travel in the United States. Candidates must be citizens of the United Kingdom or a present British Colony, and must be between the ages of 21 and 32. Further information can be obtained from the Warden, Harkness House, 38 Upper Brook Street, London, W.1.

A JOINT meeting of the Biological Engineering Society and Stichting voor Biofysica will be held at Utrecht during September 22–24. Further information can be obtained from W. J. Perkins, The National Institute for Medical Research, Mill Hill, London, N.W.7.

A CONFERENCE on "Advances in Electron Microscopy", arranged by the Electron Microscopy and Analysis Group of the Institute of Physics and the Physical Society and the Electron Microscopy Section of the Royal Microscopical Society, is being held at the Institution of Electrical Engineers during September 26–28. Further information can be obtained from the Meetings Officer, the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

THE third field meeting of the North of England Soils Discussion Group is being held at Merlewood Research Station, Grange-over-Sands, Lancashire, and in the Morecambe Bay area, during September 26–28. Further information can be obtained from H. T. Davies, National Agricultural Advisory Service, Yorks and Lancs Region, Government Buildings, Lawnswood, Leeds, 16.

THE ninth International Cancer Congress will be held, under the auspices of the International Union against Cancer, in Tokyo during October 23–29. Further information can be obtained from the National Organizing Committee, Ninth International Cancer Congress, c/o Cancer Institute, Nishisugamo, Toshima-ku, Tokyo.

A CONFERENCE on "Electron Nuclear Hyperfine Interactions in Spectroscopy" will be held in Wellington during October 17–21. Further information can be obtained from Dr. R. M. Golding, Conference Secretary, Royal Society of New Zealand, c/o Chemistry Division, D.S.I.R., Private Bag, Petone, Wellington.

THE sixteenth Canadian Chemical Engineering Conference of the Chemical Institute of Canada will be held in Windsor, Ontario, during October 17–19. Further information can be obtained from Dr. P. M. Reilly, Polymer Corporation, Ltd., Sarnia, Ontario.

THE sectional meeting of the World Power Conference, the theme of which is "Problems of Future Years in Energy-Utilization", will be held in Tokyo during October 16–20. Further information can be obtained from the Japanese National Committee, World Power Conference, c/o Japan Power Association, Daido Building, 1–46 Shiba Minamisakuma-cho, Minato-ku, Tokyo.

A SYMPOSIUM on the "Optimization of Chemical Processes", organized by the Royal Institute of Chemistry and the Society of Chemical Industry, will be held at the University of Manchester on October 12. Further information can be obtained from Mr. L. Winestone, Geigy (U.K.), Ltd., Tenax Road, Trafford Park, Manchester 17.

A CONFERENCE on "Engineering of the Future in the Rubber Industry" will be held at Royal Leamington Spa during October 6–7. Further information can be obtained from Mr. A. L. Smith, Controller of Development Compounding, Tyre Compounds Laboratory, Dunlop Rubber Co., Ltd., Fort Dunlop, Birmingham 24.

THE sixth International Congress of Clinical Pathology will be held in Rome during October 3–8. Further information can be obtained from Segreteria del VI Congresso Internazionale di Patologia Clinica, Via dei Penitenzieri 13, Rome.

CORRESPONDENCE

We Wuz Robbed

SIR,—If the object of the World Cup series were to determine the identity of the best playing team, your editorial comment would be justified. But in fact its object, albeit inexplicit, is something much more sophisticated—the generation, abreaction and (one must hope) the control of aggressions in a ritualized form. Because we badly need an emotional technology of this kind I for one would be sorry to see it reshaped, unless it were in the direction of making the therapeutic acting-out more effective. For the operation to succeed, there must be a climatic final contest, there must be built-in uncertainties and unfairnesses (otherwise nobody would have the opportunity of being robbed in a context which does not generate military spirit), and there must be a referee to respect or blame: possibly the ritual would be more effective if, symbolically at least, he were eventually killed and eaten, but the present system is an insightful compromise.

The selection of the "best" team is accordingly a pretext for an important anthropological manoeuvre. That this manoeuvre has grown intuitively rather than as the result of planning adds to its effectiveness, and is typical of a rite. It is an inexpensive project compared with, say, the literal enactment of the shamanic enterprise of Moon travel, and with the restraint imposed by sportsmanship, however variously interpreted, I think it is beneficial. We have too few safe outlets for aggression, rivalry and boasting. We should not let scientism disturb those which we have.

I am, Sir, etc.,

ALEX COMFORT

Department of Zoology,
University College,
London.

Drop More Names

SIR,—I am a regular user of *Science Citation Index* (S.C.I.) and wish to comment on your recent remarks about it (*Nature*, August 6, 1966) because I suspect that you have not used the *Index* for its proper purpose, which is to search the literature for references pertaining to a current scientific problem.

Your description of the principle on which the Index is compiled is correct, but your statement of the fundamental idea behind S.C.I. and your implication that the use of S.C.I. is limited to the pursuit of this idea in any particular case appear to me superficial. In my view S.C.I. enables one to answer the questions: "Has it been done? Has it been thought of? Has it been criticized, proved, or disproved?" S.C.I. permits the questioner to exploit fully his own intelligence and experience in his analysis of the literature without having to rely on the arbitrary choice and use of key-words by abstracters. An abstracter might neglect minor points which later become of major importance to someone else and might even misunderstand a paper.

The following example illustrates the way in which citations found in S.C.I. act as the "labels" by which "ideas" in the literature can be traced. A researcher planning an experiment to distinguish between certain hypotheses considers the range of possible results and their bearing on his hypotheses. Even at this stage he can make a list of the key papers to which he thinks he would have to refer when finally writing his own paper on this research, choosing this list so that it characterizes as many as possible of the aspects of his research. He now finds the citations of his listed papers in S.C.I. Publications which cite two or more papers from his list are likely to be useful. Many of those which cite only one can quickly be dismissed as irrelevant on the basis of their authorship, or the journals in which they appear, or their titles (which are found in the *source index*). If there are no relevant citations, the research worker's idea is probably new.

My experience suggests that literature problems can be solved with S.C.I., within an hour or two of raising them, more completely and quickly than with conventional abstracts. Your alternative suggestion of search by computer is open to the objection that those with the most to learn, undergraduates and graduate students, might not have rapid access to the computer service. Furthermore, although computers are quick, a citation service by computer would suffer from delays in booking time and in supplying in the correct form the information required for a search. The questioner would probably want to alter this information on seeing the results of the search, whereas the individual user of S.C.I. can modify his search procedure as he goes, according to his findings.

Yours faithfully,

MARTIN DAVIES

Harvard Medical School,
Boston, Massachusetts.

FOUR YEARS OF INFORMATION EXCHANGE

THE National Institutes of Health in the United States have for four years been running a rapidly growing system for the exchange of preprints between scientists working in biological fields. The first information exchange group, started in April 1961 on an informal basis, has now been joined by six other groups. The speed with which these networks, which have sometimes been called "invisible societies", are growing can be told from the fact that information group No. 7, barely a year old, had 1,100 members in April of this year and is expected to reach 2,000 by the end of 1966. The organizers of the scheme are now reaching the point at which they must apply for permanent financial support for a version of the experiment which has been running, and an appropriate request has apparently been made through the administration of the National Institutes of Health. At the same time, what is called *A Report to the Members* has been circulated by Dr. Errett C. Albritton, the director of the information exchange programme. This is valuable insight into the working of the information exchange and its problems.

Faster Publication

According to Dr. Albritton: "The overall objective [was] to accelerate the advance of medical science by subsidizing quick scientist-to-scientist communication on an experimental basis within a group of scientists (and later six more groups) in which there existed a high degree of identity of research interests". He argues that the informal exchange of information between scientists working close to each other in laboratories, or sharing the same coffee room, is a model for the kind of exchange of information that information exchange groups should promote. "Since taxpayer's dollars would support this experiment, the IEG had to be clearly differentiated from media of communication already serving science, and IEG's service had to be convincingly related to actual acceleration of the advance of science. The distinction between the service to science as proposed, and that supplied by journals, involved dimensional differences on two separate scales, one of speed of communication of research findings, the other of excellence of what was communicated. . . . Journals sacrifice speed to achieve excellence; IEG would sacrifice excellence—if it did not already exist in a communication—to achieve speed. If it did not already exist [sic], for many or even most of the communications in this subsidized professional correspondence might be of such high excellence that the journal would require no alterations whatever. But that would be an individual matter—the IEG would have no screening or review mechanism for assuring excellence."

Dr. Albritton compares the exchange of information within the exchange groups to the exchange of information at symposia and also cites the practice of the *Proceedings of the U.S. National Academy of Sciences*, in which papers submitted by members of the Academy are published without being refereed. He adds that the justification for spending taxpayers' money on such a scheme cannot simply be speed and that there must be "convincing reason to believe that the experimental operation proposed would actually accelerate scientific discovery and thus lie within the mission of the NIH". He quotes Dr. Philip Handler, one of the original sponsors of the information exchange groups, as saying that the chief object is to "accelerate the progress of scientific discovery" by updating "the frame of reference in which the scientist makes his day to day research decisions". There are said to have been two chairmen of information exchange groups who have supplied instances in which members were saved from embarking on projects because they learned, through the information exchange group to which they belonged, that the work had already been done elsewhere.

"Polls of membership by two IEG chairmen have supplied actual instances in which members were saved embarking on a project that another member had just completed, and instances of radical revision of plans, triggered by incoming information. Dr. Handler did not expect these spectacular instances to be frequent in occurrence; IEG's main impact would lie in the more informed decisions it made possible, throughout the research area, in *every* research laboratory.

"Sufficient documentation does not exist as yet for an appraisal of the impact IEG is having to accelerate the advance of scientific discovery. Statistical data on the average and range of the time-span between dispatch of scientific memos from the NIH print shop and the appearance of the same research findings in print in a journal are not as yet available. But the longer that time, the more near to 'actually current' is each recipient-scientist's frame of reference for his day-to-day research decisions."

On the cost of the system, the *Report to Members* has very little specific detail to provide. The cost for 1966 is said to be "by no means small" and equivalent to twenty average research grants. Dr. Albritton has calculated that if all the 18,000 recipients of National Institutes of Health grants were members of information exchange groups, the overall cost would be $5 million. This, of course, is probably a figure which takes account of the financial economies that would arise if there were even more members—in other words the present cost per member is likely to be greater than it would be when all National Institutes of Health grant holders were enrolled.

The *Report to Members* points out that the seven information exchange groups are formally autonomous. "The IEG Office does the observing, but otherwise serves only as a mail drop for scientists who wish to communicate their research findings speedily to their colleagues around the world. The experiment is costly and thus must be limited. These seven groups are regarded as a sufficient pilot run out of the 250 to 300 possible scientific groups the NIH might come to support."

Dr. Albritton's memorandum continues:

"*Priority.* Reference to priority in a recent memo from the writer needs elaboration. The IEG clearly has some of the attributes of a scientific society, formed, however, solely to speed communication. Basic in such a 'society'

is the observance of the ethics of priority. Without confidence in such observance, no scientist would care to accept membership. When the successive IEGs were organized, each, through its chairman and/or co-chairman, adopted as one of the conditions of membership the commitment to observe priority.

"One of the gratifying findings in the experiment has been the almost complete absence of any failure to observe priority, traced in its rare occurrence to a hasty reading of a memo received. The finding has been communicated to the membership for the benefit of any of the more recent members who may have less than complete security in quick communication of their own research findings.

"*Quoting Material in Memos.* The consensus among the IEG chairmen regarding bibliographic references to scientific memos may be repeated here for the benefit of the newer members. It is that inasmuch as a memo is not a 'publication' (it falls in the category of private professional correspondence), it may not be referred to in a bibliographic reference except as a 'personal communication' from the author of the memo. (It should, of course, be cited, if the writer of the published paper is a member of the IEG or has had access to an IEG memo through the courtesy of a member.) Prudence suggests that the writer of the paper [should] consult with the author of the memo to see if he still stands by the statement to be quoted. (Some IEG members make it a practice to dispatch at once to the author of a particularly interesting memo a request for a reprint of the article when it finally appears in a journal.)

"*Membership Policy.* Again for the sake of the newer members: Every research finding in an IEG's research area is potentially needed, quickly, by some member. If the finder is not a member, his findings are missing from the information stream. And just this much is missing from the stream's impact to accelerate research progress. Scientists *not* doing research in an IEG's research area belong in some other IEG, maybe yet to be formed; but scientists who *are* doing research in the IEG's limited area should be *in*, if they are capable of independent research.

"*The Recent Delay in Dispatch of Memos.* In the past two months the time between receipt of memos and their dispatch has been rapidly extended in the NIH Print Shop, until, in some instances, a memo has stayed in the shop as long as two months. This has been explained to us as temporary, due to a heavy load of research grants printing falling on the Print Shop at the same time as they were suffering from an unforeseen shortage of operating staff. The emergency has not ended at the time of this writing, but we are assured that they will try to move back to a week or ten days as their normal production time.

"*What is the Future for IEG?* The IEG remains an experimental programme. The chairmen and co-chairmen and other close observers believe IEG is here to stay. If it is, the programme will be taken out of the experimental category and will be able to extend its coverage to other research areas. However, still under consideration by higher administrators is the question whether the amount of acceleration IEG gives to scientific progress is worth the cost. An increase in speed seems a possibility; cutting costs is a possibility. Either should help toward an affirmative answer."

Dr. Albritton says that there has been an increase in the number of memoranda contributed by each member of the group as the years have gone by. He estimates that 80 per cent of the memoranda circulated at the beginning of the experiment eventually appeared in print in ordinary journals, and suggests that the present ratio is nearer 90 per cent.

It would, of course, be wrong to think that the volume of literature flowing into the National Institutes of Health can be processed without trouble. In an earlier note headed "Memos Must Be Camera-Ready!" Dr. Albritton advised members that "the IEG Office is receiving so many manuscripts (typescripts) each week that have to have additional work before they can be printed, that we are having to make a drastic change in procedure. Hereafter, memos that are camera-ready will be processed first and rushed on to the NIH print shop, but those requiring more work will be set aside in a waiting file—to be attended to when we can get to them. It is worth your investigating whether your secretary has the erroneous idea that a typescript that is 'journal-ready' is automatically also 'camera-ready'. What we send to the NIH print shop must be *ready for the camera*".

This note goes on to explain that pages must be numbered, glossy prints must be mounted, and that pages that have to be turned sideways must be legible when the top is turned to the left. By the end of the year (1966) Dr. Albritton expects that the delay in processing manuscripts before sending them to the "print shop" would be a month. He follows with practical advice "Addressed to Secretaries" which includes the helpful paragraph: "Now for a suggestion that will give more 'class' to the memo, if it does not seem too complicated to you. If all tables and all figures are segregated to the back of the memo, it gives more class to the printed job if FIGURES can head a right-hand page; and the same for TABLES, and the same for BIBLIOGRAPHY. Right-hand pages are odd, you remember. If one of these headings is about to be on an even-numbered page you can prevent it by inserting a blank page, giving it the even number, and proceeding on from there".

Why Not Get it to Us NOW?

Dr. Albritton writes:

Information Exchange Group No. 1, now entering its sixth year with over 700 members, has demonstrated conclusively that research findings can be disclosed through the IEG months before journal publication, *without jeopardizing priority*. In fact, it is now clear, the IEG has brought into existence *new and potent safeguards* to priority. The protective factors seen to exist within the IEG, that do not exist outside, may be pointed out:

1. Assume that a memo by scientist "*A*" contains a research finding that must be mentioned in a memo or published paper by scientist "*B*", else he fails to give *A* due priority credit. Every scientist is motivated to respect priority, but scientist *B* has added motivation: he has committed himself in one of the conditions of IEG membership. *B* honours his commitment in relation to *A* all the more in view of the fact that all his peers in the group are witness to *A*'s priority.

2. If *B*'s memo should actually fail to give *A* the priority credit due him, *A* has the opportunity to call it to *B*'s attention, privately, before the paper comes out in a journal. (If *B*'s paper is already in print, *A* still has recourse to the next procedure.)

3. If *A* does not get satisfaction from *B* in private correspondence, *A* can lay the matter before the IEG chairman. The chairman will, of course, satisfy himself that *A* has a valid complaint, but then can privately bring irresistible pressure on *B*. *A* also has the option of sending a memo through the Exchange.

There are now 2,500 members in the seven IEGs, and 1,200 memos have been transmitted, all *priority protected*. It is obvious, of course, that a paper sent through the IEG is better protected than one published without prior circulation through the IEG. Even more obvious: the earlier a paper is sent to the IEG Office, the surer the author can be that he actually will have priority. Surest of all will be the author who sends the paper through IEG as soon as the last page is pulled out of the typewriter. Waiting until it is accepted by a journal is an unnecessary precaution—the delay, even detrimental.

LIQUID STATE PHYSICS

By PROF. H. N. V. TEMPERLEY

Department of Applied Mathematics, University College, Swansea

LIQUID state physics has had a very curious history of leaps forward with periods of stagnation. The present advances were triggered by some extensive machine calculations published in 1957 (refs. 1 and 2). Because of the importance of the liquid state to almost every human activity, empirical knowledge abounds. Ever since the atomic hypothesis was taken seriously there have been attempts to understand the properties of liquids at a more fundamental level and it has probably always been realized that atoms and molecules show weak attractions at long distances and strong repulsions at short distances, and that the most characteristic properties of a liquid (a finite bulk modulus yet no resistance to shear) are somehow accounted for by such interactions. There are still many gaps in our knowledge; for example, we still do not know the most stable solid structure associated with any reasonable law of interaction. It is highly probable (but has not yet been formally proved) that the most space-saving way of packing spheres is in a cubic or hexagonal close-packed arrangement, but recent work by Bernal, Frank and others makes it plausible that various disordered arrangements become possible at densities only slightly less than close-packed. Intuitively it can be expected that a disordered structure can be distorted with little expenditure of energy if the average density is kept constant, so that the absence of permanent resistance to shearing forces can be understood.

So matters stood when van der Waals published his famous equation in 1873. This showed that the critical temperature of a gas, below which condensation becomes possible, could be understood as a balance between energy and entropy. Put in another way, above this temperature the effect of thermal agitation always overcomes the effect of the attractive forces between molecules and the substance fills the vessel uniformly; below this temperature, for sufficiently high densities, the attractive forces prevail and most of the assembly condenses. Very recently, rigorous work by Uhlenbeck and others has shown that a van der Waals's type of equation is the rigorous consequence of giving the attractive forces a range which is long compared with the molecular diameter. Forces between real molecules fall off fairly rapidly with distance, and it is now known that the corresponding equation of state would be more complicated analytically. Nevertheless, van der Waals's theory remains a very valuable first approximation to the description of condensation and the liquid state.

No real advances in our knowledge took place until the thirties and forties except the pioneer efforts of Lindemann (later Lord Cherwell), Braunbek, Born and others to understand how a solid crystal could become unstable at a sufficiently high temperature. These gave some very important insights, but the concept that was to be the analogue for a liquid of the lattice structure of a solid only emerged very gradually. This key concept is that of the distribution function, discovered experimentally in the twenties by X-ray studies of liquids. Imagine an observer standing on a particular molecule. Because of its finite size, neighbouring molecules can only approach to a certain distance. A typical molecule will be surrounded, on the average, by a "shell" of nearest neighbours, these in turn by a second "shell" and so on. The hypothetical observer would then see an average density of matter, oscillating with the distance from him with a period comparable with the molecular diameter, merging eventually into a constant density at very large distances from the typical molecule. This distribution function can be inferred from X-ray or neutron scattering experiments. In 1935 Kirkwood showed that it was in principle possible to calculate this function $g(r)$, the local density at a distance r from a typical molecule, theoretically, given the function $V(r)$, describing the interaction energy of two typical molecules at a distance r. At very low densities, the probability of finding two typical molecules at a distance r apart is proportional simply to the Boltzmann factor $\exp(-V(r)/kT)$; however, as the density increases, this has to be modified to allow for the fact that our typical pair of molecules is surrounded by others, the problem resembling the "self-consistent" calculations of quantum mechanics. By successive approximation we obtain a series expansion of $g(r)$ in ascending powers of the density. Various approximate summations of this expansion lead to integral equations for $g(r)$, which can be solved numerically if $V(r)$ is assumed known. The early studies of Kirkwood[3], and Born and Green[4], turned out to be rather unreliable at liquid densities, but we now have a considerably better understanding of the mathematics and physics of the problem of calculating $g(r)$, and thus of the equation of state, of a "simple" liquid like argon, for which the molecules can be safely considered to be spheres which interact with forces that are central and non-directional. (A molecule like water or alcohol has none of these desirable properties, and the theoretical study of such liquids is still at an early stage.) For simple liquids, methods now available enable the equation of state to be predicted to within a few per cent; the main uncertainty is now in our knowledge of $V(r)$ for real molecules.

It can be said that the direct study of the distribution function $g(r)$, which can be measured experimentally apart from its connexion with the equation of state, has practically superseded most other models of the liquid state, though these are still valuable physically. Among them we may mention the following:

(*a*) The "hole" model. A liquid was thought of as being like a crystal lattice from which some of the molecules are missing. If we suppose that the lattice becomes unstable when a certain percentage of places are empty we obtain a rough theory of melting.

(*b*) The "cell" model. A typical molecule is supposed to be confined to a small region of the liquid by the repulsive fields of its neighbours. Occasionally it can squeeze between its neighbours and migrate through the liquid, this process being associated with viscous flow.

(*c*) The "significant structure" or "cybotactic" model. A typical molecule is supposed to spend a proportion of its time in surroundings resembling a crystal and the remainder in a gas-like environment. This statement is to be understood statistically; the model does not actually assert that crystal fragments of various sizes are physically present in the liquid.

The experience with these tentative models has been very similar to that with the older theory of van der Waals, namely, that by suitable choices of constants some liquid properties can be accounted for numerically, while others are reproduced in order of magnitude. In the light of recent work it seems that such models stand or fall to the extent that they approximate to the surroundings of a molecule in a real liquid. These will certainly be something between those of a crystal and a gas, but the "significant structure" approximation is probably rather crude. The "cell" model takes account of just the first

"shell" of neighbours surrounding a typical molecule and smooths out the effect of the more distant shells, which are allowed for in theories involving the distribution function explicitly. The approximation behind the "hole" model is also rather rough.

Within the past five years there has been a considerable revival of interest in theories of the liquid state. This interest has been mainly confined to classical liquids. The theory of the two forms of liquid helium has followed the general lines laid down by Landau and Feynman with considerable success, but further progress probably awaits a better understanding of the fundamental theory of classical assemblies, with which this article is concerned.

In 1957 the theorist received a serious shock. Reliable machine calculations[1,2] then became available for the equation of state of the simplest assembly that has any claim to resemble a real liquid, the assembly of rigid spheres without attractive forces. At densities that can reasonably be considered to correspond to the liquid state (about 60–70 per cent of the close-packed density) it was found that none of the then available theories was satisfactory. The integral equation approaches due to Kirkwood[3], and to Born and Green[4], predicted pressures that, at such densities, were too low by factors of the order of three. The "cell" or "free volume" model fared almost equally badly, and it has since been found that it is a more appropriate description of the solid rather than of the liquid state. Finally, a comparison with an equation of state based on the first four virial coefficients confirmed what had long been suspected, that expansions in ascending powers of the density become poor at liquid densities, an impression that was confirmed by calculations of the fifth and sixth virial coefficients for this model.

Fortunately, theoreticians were not deterred by this challenge. Examination of the Kirkwood and Born–Green approaches showed that, by a piece of bad luck, the fundamental "superposition approximation" that they made becomes unreliable at unexpectedly low densities. It does correctly describe the initial departure of any gas from perfection and, in fact, also correctly reproduces the third virial coefficient, but will not bear the long extrapolations from densities at which departures of a vapour from perfection first become noticeable to the density of the liquid (which may be several hundred times greater). In 1958 Percus and Yevick[5] introduced a new form of integral equation, effectively based on the Debye–von Kármán picture of a solid (in which the normal modes are elastic waves, and the displacements of atoms from their equilibrium positions in the lattice are assumed to be small). They had to introduce a number of approximations of rather obscure significance in discussing how the spectrum of elementary excitations might vary with density, and how this spectrum of a liquid is related to that of a solid.

The proof of the pudding is in the eating; there can be no doubt that the Percus–Yevick equation fares better than most of its rivals in describing the consequences, not only of the "rigid sphere" interaction, but also of other interactions, such as the "Lennard–Jones 6–12" interaction, that are reasonably good representations of the attractions and repulsions of real molecules[6]. Direct comparison with experiment is inconclusive, even with a simple liquid such as argon—the interaction function is imperfectly known and interactions involving three or more atoms are perhaps significant. It has become usual, therefore, to compare the predictions of integral equations with Monte Carlo calculations of the equation of state for the same interaction, rather than directly with experiment, when trying to test their accuracy.

Why does the Percus–Yevick equation fare so very much better than the Kirkwood or Born–Green approach when it also involves approximations the effect of which is hard to assess? This question is under active investigation; so far only a few hints have emerged. Passing from solid to liquid densities involves a factor of only about 1·1–1·3 compared with factors of the order of hundreds when passing from vapour to liquid. A solid-like model thus has a better chance *a priori*. It has also been shown that some of the terms omitted by the Percus–Yevick equation tend to balance out[7]. These are only hints that make us ask for more!

The study of transport effects, initiated by Kirkwood in 1946, has also continued intensively, notably by Prigogine and his school in Brussels[8], and by S. A. Rice and his collaborators in Chicago[9]. Gradually we seem to be progressing from "order of magnitude" to reasonably reliable calculations of transport coefficients. As with equilibrium properties, some approximations are always necessary, and the penalties exacted for sloppy derivations or injudicious approximations can be even more severe than in equilibrium theory.

It is pertinent to ask what we can expect in the way of "technological fall-out". The conferences on the liquid state held in 1963, sponsored by the Institute of Physics and by General Motors, and the Washington Conference on Critical Phenomena held in 1965 all concentrated mainly on fundamentals. The same is likely to be true of a Faraday Society Discussion planned for April 1967. It is very satisfying that we seem to be approaching a situation in which we shall be able to relate equilibrium and transport properties of a liquid to the intermolecular interaction function in the sorts of ways that are already possible for a solid or gas, but can we expect spectacular developments in liquid state physics comparable with the transistor, the laser, the superconducting magnet, the special magnetic materials and the magnetic storage devices that solid state physics has given us?

It can justly be said that studies of equations of state are matters for experts only, but this is far from being the case with the closely related subject of phase transitions of liquids and solutions. A particularly striking feature of the 1965 Washington Conference on Critical Phenomena was its emphasis on the fundamental similarity of many different types of phase transition. In some cases the nature of the assembly and the precise details of the interaction seem to matter little, in others they can be crucial.

The technical importance of being able to predict in what circumstances a moving liquid will boil, emit gas, deposit a solid precipitate or freeze scarcely needs emphasis, yet the controlling factors are nearly always poorly understood. The emission of gas from a liquid can have very serious results, as in the formation of bubbles in the blood or in cavitation in pipes and near propellers, or in the formation of voids in castings. (In other cases, as in the design of pumps and impellers, it is possible to exploit the occurrence of cavitation. The lifting effect of gas bubbles in a liquid can help its circulation in certain types of process.) In other situations it may be very important to prevent the formation of bubbles. In certain types of bearing the lubricant is in a state of tension and it may be important to know in what circumstances one may expect to avoid cavitation. In botany various situations are known in which sap is under tension. The rising of sap appears to result, at least partly, from tension developed in the leaves by evaporation through the stomata[10]. The ejection of spores by the common fern arises[11] from the gradual drying out of certain cells, the liquid contents of which finally break, enabling the cell to act like a catapult when the tension is suddenly released.

It can also be important to know in what circumstances a liquid may be expected to freeze or to deposit a solid. As with gaseous solutions, a mere knowledge of equilibrium thermodynamics or statistical mechanics is not enough. It is well known that a solution of a gas or solid in a liquid can often be preserved indefinitely at considerable supersaturations if suitable precautions are taken, but why can some solutions supersaturate so much more than others? The equilibrium theory of solutions can be

said to be reasonably well understood and it has many similarities with theories of phase transitions, but non-equilibrium phenomena show many more idiosyncrasies than do equilibrium ones. Why, for example, can many liquids be obtained in a glassy state but others, such as water, can never be supercooled below a certain limiting temperature? When ice is formed from supercooled vapour, why can its crystal habit be changed quite a number of times within quite a small range of temperature? These are a few of the fascinating questions which are poorly understood, yet are likely to be of great technical significance.

Mention of the phase transitions of water reminds one of their meteorological importance. Condensation of water vapour plays a dominant part in cloud formation and is one of the main sources of energy of tornadoes and hurricanes. Many problems of cloud physics, such as the icing of aircraft, are closely related to the problems of the supercooling of water and of nucleation effects. So far, the progress of experiments in "rain making", and in modifying the course of hurricanes, by the artificial seeding of clouds has proved rather disappointing, but this is not a reason for saying that theoretical and experimental study of these matters is useless. The mechanism of electrification of thunderclouds is, according to some theories anyway, intimately associated with the freezing transition. The suggestion that an artificial thundercloud could be produced in the laboratory and then used as a cheap high voltage generator probably belongs to science fiction, but there is a strong case for careful study of effects associated with the freezing and evaporation of polar liquids (on which very little is known theoretically). One question of very considerable technical importance is a study of the factors that determine the rate of evaporation of a water surface. Can it be significantly retarded by any feasible surface treatment? If so, this would be of importance in irrigation and the management of reservoirs. Or can it be hastened? This would be of importance in the distillation of sea water and the design of boilers and related equipment.

Until very recently, it has been almost taken for granted that the interactions between ions in a liquid metal were of "many body" type. However, recent work by March[12] and his colleagues suggests that there is an effective interaction between ions not differing greatly from the "screened coulomb" interaction between ions in an electrolyte. Such work must lead, in due course, to better understanding of the crystallization and melting of metals. If the interaction can indeed be treated as effectively between pairs of ions only, the theoretical treatment becomes very much simpler than if it is of the many body type. Thus, we may expect this work to have considerable impact on metallurgy.

These are some of the likely direct benefits to various sciences of a study of the liquid state. Among indirect benefits mention can be made of the study of fluidized powders and slurries. A powder can be brought into a state bearing striking resemblances to a liquid if a gas is blown through it from below. The effect is that each particle is no longer completely imprisoned by its neighbours, but migrates over a certain "free volume". This relationship is very like that between neighbouring molecules in a liquid. Indeed, a fluidized powder can have a "free surface" and a "vapour phase" and can "find its own level" and have other properties very like those of a liquid. So far the study of fluidized powders has been mainly on empirical lines, but comparison with the "rigid sphere" model of a liquid is likely to be extremely fruitful, and should throw light on the transport of solid particles through pipes by liquids or by gases—a subject of considerable technological importance.

We indeed see a strange history of fundamental advances such as the discovery of the van der Waals equation, Kirkwood's pioneer work on the liquid distribution function and on transport coefficients and modern machine calculations on the equations of state of simple models having been separated by long gaps. At the 1963 conference in London a large number of experimental studies were reported, using techniques ranging from neutron scattering to spectroscopic study of melts, while theoretical work ranged from studies of "lattice" and other idealized models to the thermodynamics of mixtures. Although the subject is still mainly one for specialists it calls for relatively small investments of money and technical effort. Because it is hard to think of a science that is not in some way concerned with the properties of liquids and solutions, any advances in our knowledge seem certain to find applications somewhere. If one were asked to name a field in which a given expenditure of money and manpower were likely to produce a good return in the form of technological fall-out, this would be a likely choice.

This subject is also one in which cross-fertilization between industrial and university research is likely to benefit both. It may be quite feasible to carry out a continuous industrial process with a supersaturated solution which "ought", on equilibrium theory, to deposit a solid or to emit a gas, but a small alteration in the conditions may take one "over the hump" and thus bring the process to rest. At present, much effort is going into the study of the statistical mechanics of steady state and other non-equilibrium situations. This is just one example of cross-fertilization possibilities. Because virtually every science is concerned in some way with liquids and solutions other possibilities must abound.

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So many of the properties of matter, especially when in the gaseous form, can be deduced from the hypothesis that their minute parts are in rapid motion, the velocity increasing with the temperature, that the precise nature of this motion becomes a subject of rational curiosity. Daniel Bernouilli, Herapath, Joule, Krönig, Clausius, etc., have shewn that the relations between pressure, temperature, and density in a perfect gas can be explained by supposing the particles to move with uniform velocity in straight lines, striking against the sides of the containing vessel and thus producing pressure. It is not necessary to suppose each particle to travel to any great distance in the same straight line; for the effect in producing pressure will be the same if the particles strike against each other; so that the straight line described may be very short. M. Clausius has determined the mean length of path in terms of the average distance of the particles, and the distance between the centres of two particles when collision takes place. We have at present no means of ascertaining either of these distances; but certain phenomena, such as the internal friction of gases, the conduction of heat through a gas, and the diffusion of one gas through another, seem to indicate the possibility of determining accurately the mean length of path which a particle describes between two successive collisions. In order to lay the foundation of such investigations on strict mechanical principles, I shall demonstrate the laws of motion of an indefinite number of small, hard, and perfectly elastic spheres acting on one another only during impact.

J. C. MAXWELL

Phil. Mag., January, 1860.

BOOK REVIEWS

PARANORMAL EVOLUTION

The Living Stream

A Restatement of Evolution Theory and its Relation to the Spirit of Man. By Alister Hardy. Pp. 292. (London: William Collins, Sons and Co., Ltd., 1965.) 30*s*. net.

THE Gifford Lectures, delivered annually at the University of Aberdeen, were intended by their founder, Lord Gifford, for "promoting, advancing, teaching and diffusing the study of Natural Theology, in the widest sense of the term". This has provided considerable scope for lecturers in past years; the list of past lecturers is an impressive one, including, among others, philosophers, physicists, astronomers, theologians and a distinguished physiologist. The series of lectures during the session 1963–64 were given by a distinguished zoologist, Sir Alister Hardy, and the first of the series has now been published under the title *The Living Stream*.

Hardy approaches the task assigned to him primarily as a student of the evolutionary adaptation of animals to their environment, and, in agreeing with the definition of natural theology as a theism which is derived empirically from the study of nature, man and human history, he is concerned to emphasize that current ideas of evolution are not fully explanatory of this process. "I venture to suggest", he writes, "that there is something more about the process of evolution than is generally conceded by most biologists today; and this 'something more' does, I believe, link natural theology to the biological scheme". It is not easy in a short review to summarize, without doing injustice to his various lines of reasoning, all the arguments on which the author relies for substantiating his main thesis, particularly as this book may be assumed to be a general biological survey intended to preface the second series of lectures that now await publication. Hardy makes it clear in his introduction that, while rational theology has usually maintained that God's existence could be proved by reason alone, "to this I do not subscribe". He also makes a distinction between natural history as "the qualitative description of nature" and science "the quantitative and experimental analysis of its interactions". Whether such a distinction is accepted as valid or not, some may find it difficult to accord these preliminary statements with the exhortation in the last sentence of the book that we need to "show the way to a re-establishment of the idea of God as both a philosophical and scientific reality". But the inconsistency of thought implied in these references is perhaps more apparent than real when considered in relation to the detailed discussions generally. The author gives a remarkably lucid and concise account of the development of modern evolutionary ideas and the nature of the gene, and he agrees that selection acting on small random changes in the inherited nuclear material is the main physical mechanism of evolutionary change. He lays particular emphasis on the consciously directed behaviour of animals in seeking out new environments, or in attempting to adapt themselves to changing environments, as one of the most important factors for evolutionary progress by providing the opportunity for the selection of appropriate mutations as they arise. Further, he envisages a "group mind" or a "species mind" representing a psychic side of animal life, and determining certain configurations of form and behaviour unfolding automatically according to the DNA code of the particular species concerned. He suggests, however, that the psychic side of an animal may be independent of the DNA code, though it may also interact with the physical system in the evolutionary process through organic selection. Finally, he invokes the phenomenon of telepathy (in his view, as well as in the view of other students of psychical phenomena, well established by experimental tests such as card guessing) for an explanation of the rapid and widespread development of new behaviour patterns among different geographical groups of the same species.

No doubt there are those who will feel disinclined to follow Hardy so far in such a speculation, but it is well to note that he himself remarks that "these fancies, without more facts and experiment, lead to folly". Indeed, he goes on to say "I do not expect that my speculation is right, but by being shown to be wrong or impossible it may give rise to better thoughts which may help towards a solution of some of our conundrums". It will be at once admitted that to disprove a hypothesis of communication by telepathic means between individuals of a species would certainly be very difficult indeed. But the question will inevitably be asked whether it is in fact necessary, or useful, with our present knowledge of the potentialities of known evolutionary mechanisms, to postulate a conception such as telepathy as an additional factor determining biological adaptations. Opinions will no doubt vary widely on this matter, but all readers of Hardy's book will share the opinion that it is eminently readable as well as being pleasingly provocative.

W. E. LE GROS CLARK

MUSCLE CONTRACTION

Molecular Biology of Muscular Contraction

Edited by S. Ebashi, F. Oosawa, T. Sekine and Y. Tonomura. (BBA Library, Vol. 9.) Pp. xii + 206. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 110*s*.

RECENT research has simplified our ideas on the biochemistry of skeletal muscle and there is now widespread acceptance of the grosser aspects of contraction: contraction occurs as the result of rigid filaments sliding past one another; energy is provided by the hydrolysis of ATP and calcium released from the sarcoplasmic reticulum provides the trigger for contraction. This has led to a division of interest among research workers. On the one hand how does the action potential cause the release of calcium, and on the other hand how is this calcium able to elicit contraction of the myofibril? In either case the interest lies in the explanation of these events at the molecular level.

Molecular Biology of Muscular Contraction is a tribute to Prof. Kumagai, who has done much to foster the vigorous groups in Japan working on muscular contraction. It was his discovery of the granular nature of the relaxing factor that paved the way to its identification as fragmented sarcoplasmic reticulum. Nineteen contributions from the United States and Japan cover many aspects of the field. However, considering the title of this book, it is unfortunate that no mention is made of the molecular architecture of the filaments. The absence of a paper on tropomyosin is also surprising as Japanese workers have been largely responsible for elucidating the role of this protein in the myofibril.

The papers are divided into six sections on ion binding, myosin, actin, actomyosin, comparative aspects of muscle

and contraction–excitation coupling. Singling out a few papers for mention, Tonomura and co-workers summarize and interpret their data on the kinetics of the interaction of ATP analogues with myosin systems. Kielley reviews his evidence for the existence of three polypeptide chains in myosin and for the arrangement of the meromyosin moieties within the molecule.

The role of the bound nucleotide of actin remains a challenging issue, and Oosawa and his colleagues conclude that, while not indispensable, it does exert a large effect on actin polymerization. They also contribute new evidence that conformational changes in the actin polymer occur during contraction.

A salutary warning to biochemists, who perforce are often obliged to use mixtures of red and white muscle, is given by Gergely and his co-workers. They find very large differences in the properties of fragmented sarcoplasmic reticulum, of the myofibrils and even of the purified myosin obtained from red and white fibres. They make the interesting suggestion that red muscle myosin may be related to cardiac myosin. Finally, Ebashi discusses how the action potential arriving at the central tubule of a triad could elicit the release of calcium at the lateral vesicles.

My main criticism of this book is that the contributions are specialist in nature and are not general reviews. Thus the book cannot be recommended to students and others unfamiliar with the field, whereas there certainly is a need for a modern text-book on the subject.

G. W. OFFER

VETERINARY CODEX

British Veterinary Codex 1965

Pp. xxxv+843. (London: The Pharmaceutical Press, 1965.) 105*s*.

THE long-awaited new edition of this book follows closely the layout and content of its predecessor. As an authoritative reference book for those concerned with the manufacture, supply and use of drugs in veterinary practice, it will no doubt continue to be of great value. It is unfortunate, however, that an interval of twelve years should elapse between editions even when an interim supplement is published.

The *Codex* consists of three main parts; monographs on drugs and chemicals, on antisera and vaccines, and a formulary. This is followed by seventeen appendixes and a therapeutic index.

Part I has been extensively revised by the addition of 56 new monographs, while 106 have now been deleted. Several drugs in current use are not included. I was struck in particular by the absence of griseofulvin, while acepromazine, bendrofluazide, framycetin, heparin, phencyclidine, tripelennamine and tylosine are also missing. Conversely, is it still necessary to include camphor, chenopodium and rhubarb? The monographs are generally useful and informative although the description of the administration of halothane (p. 180) did not impress. The potential toxic hazards of stilboestrol implantation in lambs are not mentioned, although similar effects in cattle and poultry are noted.

Part II (biological products) has been revised and contains several additions. Four new appendixes have been added, including a useful section on pharmaceutical adjuvants. The appendixes on feed supplements and gas–liquid chromatography are, however, too short to be of any great value. Brief descriptions of complex subjects are rarely satisfactory and adequate enlargement could scarcely be justified in a veterinary codex. The therapeutic index is also of limited value, although it could be useful for reference or possibly in an emergency.

This is an essential publication which would benefit from more frequent revision, so that changes in the field of veterinary therapeutics can be more rapidly assimilated.

J. SANFORD

FROM WOAD TO WEED

Weeds of the World

Biology and Control. By Lawrence J. King. (Plant Science Monographs.) Pp. xxxii+526+23 plates. (London: Leonard Hill; New York: Interscience Publishers, Inc., 1966.) 105*s* net.

THIS book is another volume in the Plant Science Monograph series, which is designed primarily to bring together the scattered literature of important specialities in the plant sciences, with the ultimate and indirect object of helping to feed and clothe the increasing world population and to improve productivity and hence living standards. There can be no doubt of the suitability of weeds as a subject in this series, nor of the need of a review of research in this subject. The present book, announced at least five years before publication, aroused high expectations, so that it is a pity to have to register a measure of disappointment.

The first eleven chapters are devoted to weed biology in the widest possible sense of both words. One of the objects of these monographs is to cover all aspects of the subject concerned, and Dr. King has certainly attempted to do this, even to the extent of listing some of the more extraordinary uses to which man has put weeds, from using a *Lithospermum* for contraception to an *Angelica* root as a talisman for gamblers. The chapters are a series of reviews, each with its relevant bibliography, and the author has made much use of original publications, especially for their graphical and pictorial material. In the first chapter there is a lengthy consideration of the words used for weeds in several languages, from the possible origin of "weed" from "woad" (*Isatis tinctoria*) in English, to the interpretation of Chinese and Japanese ideographs. Various definitions of weeds follow and these too are considered in detail.

Having started in such detail and diversity, it is perhaps not surprising to find a certain imbalance in the volume. Matters that might have furthered the aims of this series, such as the significance and increasing importance of rhizomatous weeds in certain systems and areas of agriculture, are mentioned only in scattered references, whereas the importance of weed seeds is well considered, presumably in view of the author's association with the Boyce Thompson Institute.

The four chapters on weed control could well have been omitted: they add nothing to existing works and are noticeably dated. For example, in a book published in 1966, where (apart from one list in an appendix) are the bipyridyls? In addition, the naming of herbicides is inconsistent and the primary authors are not always quoted. This is the case with the grouping of triazines on structural affinity.

Despite these criticisms this is a valuable book, which will undoubtedly be read to great profit by all those interested in weeds, for it refers frequently to earlier biological or more purely botanical publications that are not always appreciated or even discovered by those involved in more practical aspects. R. J. CHANCELLOR

PLANT LATIN

Botanical Latin

History, Grammar, Syntax, Terminology and Vocabulary. By William T. Stearn. Pp. xiv+566. (Edinburgh: Thomas Nelson and Sons, Ltd., 1966.) 105*s*.

SYSTEMATIC botany is probably the only discipline which uses Latin as a regular, indeed compulsory, means of

communication. Under the International Code of Botanical Nomenclature no new taxon can be validly published without at least a diagnosis in Latin, so some knowledge of botanical Latin is essential to every working taxonomist.

It is therefore remarkable that no guide to the subject in English, other than more or less complete glossaries, has been published for more than a century. Dr. Stearn's book supplies what is, for once, really a long-felt want, and does it with his usual accuracy, wide knowledge and eye to practical needs.

After a brief introduction to the method of using the book the reader is entertained with a history of the development of botanical Latin terminology, which is valuable not only for an understanding of modern Latin but also for the interpretation of earlier works.

In the second section of the book some 70 pages are devoted to grammar, and here hard work for the reader begins. The young systematist with no previous knowledge of Latin will have to make a determined effort, if he is really going to master this without assistance. It may in fact prove better to pass on to the third section, syntax and other matters, and try to model a diagnosis or description on one of the many good examples given in this part and then to get it corrected, and so learn by trial and error. The examples are drawn from all groups of plants, except bacteria and fossils; therefore a wide range of terminology is available. In addition there is an extensive vocabulary at the end so that by selecting appropriate words and substituting them in the chosen example the beginner can get quite a long way.

If an adverb is being sought, the list on pages 105–107 should be consulted, as well as the vocabulary, as there are differences between them both in content and, to a lesser extent, in the English equivalent given. A few examples, taken at random, will illustrate this: the vocabulary lists *abundanter* and *copiose*, which are absent from the list, and gives *disgustingly*, *badly* as the meaning of *putide*; the list has *infauste*, missing from the vocabulary, and gives *badly*, *absurdly* as the meaning of *putide*.

Other valuable special lists are those dealing with geographical names, colour terms etc., while lichenologists and phycologists will find the list of chemical names particularly useful.

Dr. Stearn claims that botanical Latin should be regarded as a language distinct from classical Latin and most botanists would support this claim at least as far as it justifies the simplification of grammar, the use of a much bigger range of colour terms and other new words or words used in special senses, and the retention of certain non-classical spellings such as *bacca* and *annulus*. To argue the case further is a matter for the language expert. A professor of classics once remarked that botanical Latin was the kind of Latin the Romans would have used if they sent telegrams, that is clear, simple and concise.

Altogether this is a most useful and interesting book from which beginners and experts alike can profit. It has already been used in an unexpected way by a botanist with a knowledge of Latin but little acquaintance with English to produce English descriptions of plants.

T. G. TUTIN

"PHYSICS OF THE OCEANS"

Elements of Physical Oceanography

By Hugh J. McLellan. Pp. ix+150. (London and New York: Pergamon Press, Ltd., 1965.) 63*s*. net.

THE increasing interest in oceanography in recent years has given rise to a number of popular books on the subject, while a succession of review volumes at the research level has appeared, summarizing advances in special fields. At the same time, the student coming to the subject with no systematic knowledge of it and requiring a sound treatment of its principles has found a very meagre selection of texts. This book by Prof. McLellan is a valuable contribution to filling this gap, so far as physical oceanography is concerned. It is based on a course of lectures given at Texas A and M University and it bears the mark of having been written by a teacher who knows from experience what questions students will ask and which points they will find difficult. It is intended to provide a broad look at the subject, for biologists, chemists and geologists, as well as for physical oceanographers. The treatment is necessarily selective, but is sufficiently representative of the whole field, and each topic is treated rigorously within the limits which the author sets himself.

The three parts into which the book is divided deal respectively with descriptive oceanography, oceanic movements and selected topics. Starting with a view of the oceans in perspective, Part I goes on to describe the chemical nature of the ocean, the measurement and distribution of temperature and salinity and the fields of density and pressure. This leads to a discussion of water masses and a description of the general oceanic circulation. The basic dynamical concepts are well explained in Part II, including a good discussion of geopotential surfaces and stability. Geostrophic currents, wind drift, turbulence, inertial motion and waves are among the subjects treated, and this part ends with a brief description of tides. Two of the chapters in Part III deal with light in the sea and the heat budget of the ocean, essential topics in a study of physical oceanography, and these are followed by a brief chapter on thermohaline circulation. The other two selected topics are sound propagation and estuaries.

There is only one point about which I feel inclined to quibble and that is the indiscriminate use of the terms "force" and "acceleration" in dealing with the effects of the earth's rotation. It is disconcerting to read of "centrifugal acceleration" away from the axis of rotation and "Corilis acceleration" acting to the right of a moving element in the northern hemisphere. This confusion in nomenclature is not a feature peculiar to this particular book, however.

The book is well illustrated throughout by appropriate diagrams. Those taken from other sources are wisely selected and clearly reproduced and the original ones are also simple and clear. There are useful lists of references and sources of additional information at the end of each chapter.

K. F. BOWDEN

ELECTROMAGNETISM IN GEOPHYSICS

Electromagnetism and the Earth's Interior

By Tsuneji Rikitake. (Developments in Solid Earth Geophysics, Vol. 2. Pp. xi+308. (Amsterdam, London and New York: Elsevier Publishing Company, 1966.) 130*s*.

MANY advances have been made in the study of the Earth's magnetic field since 1940, when Chapman and Bartels first published their treatise *Geomagnetism*. In that comprehensive work the absence of an adequate theory of the source of the field was notable, and in this direction particularly significant developments have taken place. Prof. Rikitake's book is an up-to-date account of theoretical geomagnetism and it reflects how much more satisfactory our understanding of the fundamental processes has become in recent years.

The book falls into two main parts. The first deals with the main geomagnetic field, giving a wide-ranging review of the theories of its production and its long period variations. Here the author (rather cautiously) advocates the self-exciting dynamo mechanism and he shows how this accounts successfully for most of the observations.

The second part of the book is concerned with electromagnetic induction in the mantle and the oceans, produced by magnetic fluctuations. Highly detailed mathematical theories are developed to cover all the various cases, including the thin-sheet approximation to deal with ocean screening and the spherically symmetric model for the Earth as a whole.

The author's approach has been to survey the original papers, many of them his own, choosing certain of them for detailed exposition. Here I think Prof. Rikitake is open to criticism. The book would have benefited greatly from a more unified approach instead of the mixture of styles and notations resulting from adhering closely to the original sources. An important gain from this would have been a new emphasis on the fundamental principles. For example, the choice of particular papers given detailed analysis in the first section does not seem to me to be very sensible. Many pages are devoted to the rather unsatisfactory Inglis model for field production, while the basic papers of Bullard and Gellman and of Elsasser are given much less space. I also feel that if he had considered the overall logic of the book more carefully the author might then have included more discussions of the physics involved, rather than being carried away by the mathematics as sometimes happens in the present volume.

As the book stands it represents a fairly complete summary of the important work in this field. The list of references is excellent so that the reader may easily follow up any line which is not fully dealt with in the text. The diagrams are clear and the mathematics well produced. There are a few printing mistakes and in places the English is idiosyncratic, but this does not seriously detract from the general readability of the work. Prof. Rikitake has performed a useful service in gathering all this material together in one volume, but I feel the work would have been more valuable if he had developed the subject logically from the physical background in his own way.

R. L. PARKER

FLUID FLOW

Flow Measurement by Square-edged Orifice Plate Using Corner Tappings

By W. J. Clark. Pp. xiii + 226. (London and New York: Pergamon Press, Ltd., 1965.) 200*s*. net.

THE square-edged orifice plate meter is probably the simplest device available for metering the flow of fluid in a duct, and, for that reason, it is the most commonly used instrument. Large quantities of often valuable fluids can flow through such a meter and it is necessary to ensure that the orifice plate is correctly installed if the flow readings are to be accurate. This monograph is the result of Dr. Clark's experience with vast numbers of such instruments in a large chemical works, and methods of designing such metering installations are described using charts and a special slide-rule to calculate the orifice diameter and flow characteristic. A number of specimen calculations and installations are included and the importance of regular maintenance is emphasized in what is essentially a practical approach to the subject.

It is inevitable that many of the data presented in the monograph are basically the same as those contained in the British Standard on flow measurement (*B.S.* 1042: 1964). The use in the text of non-standard Reynolds number correction factors could give rise to a little confusion, although this does perhaps illustrate the point that metering perfection cannot be expected even under ideal laboratory conditions.

The monograph contains a fuller examination than is usual of the influence of flow pulsations on metering errors, although it is a little disappointing that the author has not included references to the more recent work in this field. While Dr. Clark emphasizes that, in the majority of cases, errors due to this effect are negligible, the difficulty lies in detecting the presence of such errors. The cases brought to my notice have usually arisen where a user has observed a plant thermal efficiency greater than 100 per cent, or where the indicated flow is in the opposite direction to the known mean flow. Less extreme cases often go unnoticed, as a steady meter reading is so easily confused with an accurate one. Dr. Clark devotes his attention mainly to the pulsation errors associated with the flow through the orifice or primary element, but the errors often spring from the manometer not indicating the true time mean of the pressure differential. Greater attention could have been given to the possible sources of these manometer errors and to the conditions necessary for their elimination. For example, the advice that damping should be confined to the manometer liquid rather than to the leads could be challenged, as linear damping devices in the leads near the orifice connexions can prove very successful in eliminating manometer errors. Symmetry in the manometer lines is no guarantee of accuracy since equal volumes in both lines can produce errors of the order of 100 per cent.

T. J. WILLIAMS

ORGANOMETALLIC COMPOUNDS

Advances in Organometallic Chemistry

Vol. 2. Edited by F. G. A. Stone and Robert West. Pp. ix + 440. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1964.) 107*s*. 6*d*.

THE intensified study of organometallic compounds, as well as notable extensions in the field they embrace, makes the appearance of volumes such as this a dire necessity for chemists. Surely it is here, if anywhere, that all the main branches of chemistry meet and overlap. But perhaps for that very reason there is a subconscious tendency to regard organometallic chemistry as "nobody's baby", and nowhere has this been more apparent hitherto than among the compilers of reviews.

The present volume, under its able editorship, maintains the aims and standards of the first. The titles of the chapters are well chosen and avoid overlap with the first volume, but that is not to suggest that they fill in more than a very few of the gaps it left, and chemists will be hoping that this matter will in time be rectified by the due appearance of further volumes in fairly rapid succession. The one now presented is comprised of seven more or less extensive reviews written by specialists in the respective subjects, each review being subdivided into sections.

The alkali metals, nickel and boron, are particularly well represented. Indeed, well over half the book is devoted to some aspect of their chemistry, so that workers interested in these elements will find this volume especially important. It should be emphasized that the review by R. Köster is not the same as that entitled "Organoboron heterocycles" which he published almost simultaneously in *Prog. Boron Chem.*, **1**, Chapter 7, but is quite different in presentation and to a large extent also in the material incorporated. The subject is in any case too vast to be conveniently covered by a single review, so that Koster's two reviews can be regarded as complementary.

The various chapters are not all equally exhaustive in the treatment of their subjects, but in this respect those by H. A. Skinner, R. B. King and J. Birmingham on the strengths of metal-carbon bonds, the reactions of alkali metal derivatives of metal carbonyls and related compounds, and on the synthesis of *cyclo*pentadienyl metal compounds, respectively, may be singled out as coming to full comprehensiveness. It was almost inevitable that *cyclo*pentadienyl and allyl complexes of nickel each come up for treatment in two different chapters, but otherwise there is no overlap. The chapters are all well written, and one is scarcely ever aware that English is not the mother tongue of several of the authors. Indeed,

the only serious criticism one can make against this volume is that, in the absence of a formula index, the six-page subject index is much too brief and no reliable guide as to what may be found in the book. There is a twenty-page author index. L. H. LONG

GASEOUS OXIDATION PROCESSES

Oxidation and Combustion Reviews

Vol. 1. Edited by C. F. H. Tipper. Pp. 344. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 80*s*.

THE series *Oxidation and Combustion Reviews* is intended to deal with both pure and applied aspects of oxidation and combustion—a wide field in which chemistry, physics and engineering overlap. The first volume concentrates on chemical aspects of oxidation by molecular oxygen. Nevertheless, the field covered is wide; oxidation both in the gas phase and at the surfaces of solids is dealt with in six chapters by different authors.

The first chapter, "Application of the Theory of Branched Chain Reactions in Low Temperature Combustion", by R. Ben-Aim and M. Lucquin, gives a brief but useful summary of the theories of self ignition and describes some of the methods of studying it. It then discusses the application of these to real oxidation systems, dealing particularly with the cool flames of hydrocarbons and of their oxygenated derivatives. The morphology of cool flames and associated phenomena is well reviewed, but important recent developments in our knowledge of the chemical mechanism of oxidation under these conditions are given too brief a treatment.

"Oxidation Reactions Induced by Ionising Radiation", by G. Hughes, provides a useful catalogue of the reactions that occur when high energy radiation interacts with substrates containing oxygen. The author purposely excludes the irradiation of aqueous solutions, concentrating on the less-well-documented reactions of pure inorganic and organic substrates.

"Gas Phase Photo-oxidation", by G. R. McMillan and J. G. Calvert, compares photo-oxidation with thermal oxidation as a method of providing reliable information on elementary combustion reactions. Many examples are given (and critically discussed) of the derivation of rate constants and other mechanistic information from the results of photo-oxidation experiments. The elementary processes discussed include reactions of alkyl, acyl, alkylperoxy, peracyl and alkoxy radicals. The chapter is well written and logically presented.

The fourth chapter, "Oxidation Reactions involving Nitrogen Dioxide", by J. H. Thomas, is a good, up-to-date, descriptive review. It describes in detail recent kinetic work on the use of nitrogen dioxide as an oxidant, as a sensitizer and as an inhibitor in gas phase oxidation reactions. Slow combustion, flames and explosive reactions are all reviewed and particular attention is paid to the controversial nature of the initiation step in reactions involving nitrogen dioxide. Nitration reactions are, however, excluded.

In the fifth chapter, "Oxidative Degradation of High Polymers", W. L. Hawkins describes the deleterious effects on polymers of thermal and light-induced oxidative degradation and discusses methods of retarding these reactions. The "hydroperoxide chain theory", which describes the autoxidation of volatile hydrocarbons, is applied to the oxidation of hydrocarbon polymers, and ways are suggested in which this mechanism must be modified to apply to non-hydrocarbon polymers. No mathematical treatment of polymer degradation is included, but the qualitative treatment of the possible reactions involved is good.

The book ends with "The Heterogeneous Selective Oxidation of Hydrocarbons", by R. J. Sampson and D. Shooter, which describes the effects of the physical and chemical properties of solid catalysts in determining the selectivity of oxidation processes at their surfaces. These processes are exemplified by the oxidations of ethylene to ethylene oxide, propylene to acrolein, C_4 hydrocarbons to maleic anhydride and to butadiene, and aromatic hydrocarbons to anhydrides. As well as considering the basic mechanism of these processes, the authors present results of practical interest, listing 122 references to the patent literature. The chapter would benefit, however, by the inclusion of a summary of factors affecting selectivity (so far as they are known) at the expense of detailed description of results.

The volume as a whole is a mine of useful information on gaseous and gas–solid oxidation processes; it is recommended for workers in these fields. A. FISH

Anthology of Orthopaedics

By Mercer Rang. Pp. xi + 243. (Edinburgh and London: E. and S. Livingstone, Ltd., 1966.) 42*s*. net.

SOME medical authorities dislike eponyms: others, particularly medical historians, commend them as giving honour to the discoverer of a disease or injury or its first exponent. In this well-written book Mr. Rang, senior lecturer in surgery at the University of the West Indies, studies his specialty historically, and gives us not only a brief biography of each surgeon or eponymous author, but also an extract from the book or journal in which the condition was first described. Portrait sketches are included as well as anatomical drawings, X-ray photographs and descriptions of apparatus and splints.

Mr. Rang states in his preface that the book is not comprehensive and a number of omissions of orthopaedic eponyms will be noted by experts; but, on the whole, the more important ones are given, in addition to others less well known.

Orthopaedics is a comparatively modern specialty. Formerly, it was practised by the general surgeon who took a special interest in the subject. Physicians and neurologists also have contributed to orthopaedic eponyms. Hippocrates wrote a book on fractures and another on articulations. Paul of Aegina (625–690) described ganglion in a tendon and its treatment. W. J. Little (1810–94) introduced tenotomy into Britain after studying Stromeyer's results in Hanover. Intending to be a surgeon, he founded the Royal Orthopaedic Hospital which is now the Royal National Orthopaedic Hospital in London. He turned from surgery to medicine, became physician to the London Hospital, and wrote on tenotomy, club foot and scoliosis. He described the spastic state caused by birth injury to the brain, which is known as Little's disease. A pioneer in specialized orthopaedics was the general practitioner, Hugh Owen Thomas (1834–91), who invented the Thomas splint and advocated prolonged rest for tuberculous joints. His nephew, Sir Robert Jones (1857–1933), specialized entirely in orthopaedic surgery from 1905 and, with Dame Agnes Hunt, founded the Robert Jones and Agnes Hunt Orthopaedic Hospital at Oswestry. Another famous pioneer was Sir Henry Gauvain (1878–1945), who obtained successful results through the open-air treatment of bone and joint tuberculosis. He described spasm of the muscles around the affected hip joint as a diagnostic sign of tuberculosis. Jones and Gauvain by their work made orthopaedics a specialty. ARTHUR MACNALTY

The Determination of Toxic Substances in Air

A Manual of I.C.I. Practice. Revised edition. Edited by N. W. Hanson, D. A. Reilly and H. E. Stagg. Pp. xii + 200. (Cambridge: W. Heffer and Sons, Ltd., 1965.) 50*s*.

THIS book, which was first issued in 1956, has been revised by a sub-committee working under the general

direction of the Analytical Methods Committee of Imperial Chemical Industries Limited. The revised edition, like its predecessor, is a manual of Imperial Chemical Industries practice, consisting mainly of the description of methods of analysis for the determination of low concentrations of a range of toxic substances found in factory atmospheres. New or improved analytical methods up to the end of 1963 have been examined for some sixty substances or groups of substances, and details are given, for each selected method, of the apparatus and reagents required, the sampling and test procedures and the calculation of results.

For many substances several test procedures, which fall into three categories, are described: rapid field tests based almost entirely on the series of booklets *Methods for the Detection of Toxic Substances in Air* published by H.M. Factory Inspectorate, Ministry of Labour; more precise methods including gas chromatographic techniques which require the facilities of a laboratory; and indicator tube tests for selected substances with the commendable advice to all users to follow Imperial Chemical Industries practice and calibrate each batch of tubes before use by means of atmospheres of known composition.

Brief descriptions are also given of the apparatus required for sampling industrial atmospheres (18 pp.), chemical methods of measuring toxic substances in air (8 pp.) and, in a chapter new to this edition, static and dynamic methods of preparing atmospheres of known composition (7 pp.).

Although there is a growing awareness in industry of the need to protect workers from the hazards of repeated exposure to low concentrations of air-borne contaminants, few books describing suitable methods of air analysis are available. This compilation of methods based on the wide experience of a large industrial organization is a welcome contribution to the subject. The book is well arranged, clearly and concisely written and contains useful diagrams, an ample index and adequate references. Without doubt it will be consulted frequently by those interested in the field of industrial hygiene. A. A. CHRISTIE

Calculus of Variations and Partial Differential Equations of the First Order

By C. Carathéodory. Part 1: Partial Differential Equations of the First Order. (Holden-Day Series in Mathematical Physics.) Pp. xvi+171. (San Francisco, London and Amsterdam: Holden-Day, Inc., 1965.) $8.50.

CARATHÉODORY'S *Variationsrechnung* first appeared in 1935 and was reviewed in *Nature* in the November 23 issue of that year. Part I of the book, entitled "Partial Differential Equations of the First Order", is described in that review as "an account remarkable . . . for lucidity and completeness"; and the review of the whole work concludes with the sentence "In sum, this book is indispensable".

In the 30 years since the publication of Carathéodory's book many advances have been made in the mathematical theory of partial differential equations, but most of these occurred in branches of the theory outside the area covered by Carathéodory, and the latter's book remains a classic. In view of continuing interest, Hölder edited a (German) reprint of Part I. The present translation is based on this reprint, which appeared in 1956 and in which a few minor errors, both typographical and mathematical, were corrected, some short comments were added in the text or in footnotes, and a brief supplement was provided.

Carathéodory's presentation of partial differential equations of the first order combines geometrical insight with analytical rigour, and the availability in English of this presentation will be welcome to many. The English translation is by J. J. Brandstatter of the Stanford Research Institute and is always accurate, and mostly readable and idiomatic. A. ERDÉLYI

MEETINGS

INTERNATIONAL NUTRITION

THE members of an international congress of biochemists concern themselves with biochemistry, and pure and applied chemists in the main discuss chemical science. Even physicists when they meet restrict themselves to matters of physics, leaving discussion of nuclear power to engineers, military strategists and politicians. At the seventh International Congress of Nutrition held in Hamburg during August 3–10, however, three kinds of discussions took place. The first of these comprised strictly scientific reports of experimental studies and clinical investigations, and the second were surveys of food intake in relation to nutritional requirement and population density; both clearly relevant to the professional competence of the participants of the congress. But who is professionally qualified to say how one nation, once poor, develops and becomes rich, why another falls into anarchy, poverty and malnutrition, while a third, desperately embracing technological innovation, loans, gifts and advice nevertheless fails to improve the lot of its population and while categorized as a "Developing Nation" finds itself unable to develop?

Perhaps nutritionists are unwise to argue as hotly as they do that nutrition is a science. Biochemistry budded off as a part of physiology, and nutrition—as a scientific speciality—was in its turn an offshoot of biochemistry. But nutritionists, having established themselves by such studies as the chemical analysis of foodstuffs, elucidation of the crystalline structure of thiamine (vitamin B_1) and of its biochemical mechanisms, have extended their territory to include "Food Habits in Pre-industrial Societies in Mezoamerica", "Rituals and Taboos" and "Poverty Dissected: An Anatomy Lesson in Rural Paraguay"—to cite just three of the papers read at Hamburg. It is true that the traditional customs in Central America and taboos and rituals in primitive and sophisticated countries alike exert an important influence on the foods the people eat and on their consequent state of nutrition. But a scientist whose authority depends on his training in biochemistry and physiology is an amateur when he attempts to draw deductions about matters on which the judgment of an ethnologist is needed. Understanding of the habits and customs of a nation or tribe will be as baffling to a dietitian educated in Boston who is mistakenly attempting to introduce the bottle feeding of infants into Tanzania as will the oddity to a visitor from Central Africa of the fact that the British are happy to receive pooled blood plasma by injection although during the Second World War they refused with disgust to allow the Ministry of Food to utilize the separated corpuscles for the manufacture of black pudding to be taken by mouth.

Because the study of nutrition throws light on the human condition, it is inevitable that nutritionists feel moved to allow their hearts and consciences to be influenced by what the evidence of their intellects brings to light. In Peru, the advent of industrialization and mining attracts peasants from the highlands of the interior to the slums of Lima where, as Dr. G. G. Graham of Baltimore observed, and showed to the Congress in a series of horrifying photographs, their children sicken and die of marasmus. Yet at the same time a developing fishing industry provides a flourishing export of fish flour. The adults who work in the copper mines and in the fish-flour factory share in the modest material prosperity, yet the young children suffer. Where, then, shall the nutritionist draw the line: at the scientific deduction that marasmus is a syndrome in infants resulting from lack of calories and protein? that the disease is aggravated by the export of fish rather than its distribution among the shanty dwellers of Lima? or that a more vigorous export

drive combined with an increase in industrialization would further enrich the nation and enable more peasants to work for better wages in the towns rather than suffer the hardships of a primitive agrarian existence?

The proceedings of the International Congress showed quite clearly that nutritionists now recognize that nutrition is something more than a scientific speciality. Dr. Walter Kraak of Bonn chose as the title of his paper, "Co-ordination of Research, Economics, Technology and Public Sanitation as a Basis for Proper Nutrition". Prof. K. Breirem of Vollebekk, Norway, in introducing a session to discuss problems of world nutrition in the future, quoted the aphorism that "nutrition is not a science but is a meeting place for scientists". But is this enough? By their recognition that economics, sociology, ethnology, anthropology—no one had the courage to mention the very Prince of Denmark himself, namely, politics—are germane to their studies, the nutritionists at Hamburg showed a welcome awareness of the implications of the problems with which they were trying to deal. But even if in the future they invite other kinds of scientists than those expert in human and animal nutrition to contribute to their congresses, will they succeed then in talking sense? Should such meetings be restricted only to scientists?

On the final day at Hamburg, Prof. H. Wilbrandt of Gottingen came to the heart of the matter. Scientific studies in underdeveloped countries have brought to light the causes of nutritional diseases and thus shown the means to cure and prevent them, selfless workers from international organizations have striven with devotion to aid, succour and teach the unfortunate. Nevertheless it is hard to deny that in too many instances the end result has been worse than the beginning. The introduction of many Western ideas—white bread, carbonated beverages, even canned milk—has done harm rather than good. Food gifts from rich nations, said Prof. Wilbrandt, are no real assistance to those who receive them. They keep the poor countries impoverished. The present policies are erroneous and lead to perdition. It is in those countries where people leave the land, abandon a policy of peasant agrarianism and embrace a philosophy of industrialization that agriculture becomes efficient and productive.

Nutritional scientists have no title to discuss economics, industrialization or such political and philosophical matters as whether in equity a country that indulges in the sentimental luxury of supporting an enormous population of unproductive cows and an even larger population of even less productive monkeys can rationally expect to receive large gifts of "surplus" wheat. But the fact that some at least of the nutritionists at Hamburg realized that this was so, that they recognized as windy platitudes the inevitable speeches about their goal being to banish all hunger from all the populations of the globe (even before breakfast), that they can point out the need for rational and informed discussion of the problem —these things justified the meeting at Hamburg and are an augury for better congresses in the future.

MAGNUS PYKE

REPORTS

ZOOPLANKTON SAMPLING

AT the symposium on zooplankton production organized by the International Council for the Exploration of the Sea (ICES) in Copenhagen in 1961 (ref. 1) a sub-committee was set up to look into the possibilities of standardization of zooplankton sampling. Similar aims were expressed by the Scientific Committee on Oceanic Research of the International Council of Scientific Unions (SCOR) and by the U.S. National Academy of Sciences Committee on Oceanography (NASCO), and it was obviously desirable that a single committee be asked to look into this question on an international basis. A joint committee was set up by ICES, SCOR and Unesco as three international bodies which would give other national committees, as well as NASCO, the opportunity to pass on the benefits of their experience through their membership of SCOR.

The first meeting of this committee was held in Paris in January 1964 under the chairmanship of Dr. J. H. Fraser, with Dr. G. Humphrey (the past president of SCOR), Dr. T. Parsons (then of the Office of Oceanography, Unesco) and Professor J. Frey (then chairman of the ICES Plankton Committee). Because zooplankton includes such a variety of organisms, it was obviously impossible to recommend a single apparatus and it was decided to set up four working parties to deal with organisms in four arbitrarily chosen size ranges.

Working party 1 was asked to deal with the microzooplankton, at present sampled by water-bottle, very fine meshed nets, or filtered from pumped supplies. Working party 2 was given the next size range, at present sampled by a great range of techniques including the almost traditional use of a No. 3 mesh. Working party 3 was asked to consider the plankton often sampled by stramin and other coarse meshed nets, while working party 4 was asked to deal with the fast moving macroplankton, including euphausids and small fish.

The working parties were given two years to report and could not themselves undertake research. Working parties 1 and 4 completed their work by correspondence only; working party 2 met in Villefranche in September 1965 immediately before the ICES and IBP meetings in Rome. Working party 3 met in Sydney in February 1966.

Working party 1 assumed an arbitrary upper limit of 200μ for the organisms dealt with, as this had been selected by working party 2 as its lower limit. In its report, emphasis was placed on the need for more knowledge of the microzooplankton and its importance in energy transfer. Existing methods were reviewed and a majority of the group preferred the use of a 10-l. water bottle to give a significant sample of forms up to about 150μ; the disadvantage of having to take many samples to cover different depth ranges was also emphasized. Organisms 75–200μ, especially the more sparsely distributed species, might best be sampled by a fine-meshed net.

Methods of estimating biomass call for standardization, but the basic difficulties of separating dead from living matter and phytoplankton from zooplankton seem as yet to forestall any completely satisfactory method. Uniformity of approach to the problem of production must also be sought and active investigation into these fields is recommended.

Working party 2 decided that their most hopeful approach towards standardization would be to design a new but simple net aimed at giving an assessment of the biomass of the plankton from 200μ upwards to about 10 mm. A prototype design was agreed for testing during the symposium at Sydney, and as a result of these tests and further experimental trials by Dr. Paul Smith of the Fishery Oceanography Centre of the U.S. Fish and Wildlife Service some modifications were made. The net being considered has a mouth opening 57 cm in diameter (0·25 m^2) and a filtration area of 6 : 1 with nylon 'Nytal *P*7' basket weave, which has a mesh aperture of 200μ and a 45 per cent porosity (ratio of mesh aperture to total area). It has a cylindrical section of 95 cm followed by a conical section with a side length of 166 cm. The turbulence of the water flow which results from the ring creates an undulating movement of the cylindrical part which tends to clean it and gives it a superior sustained filtering efficiency. The nylon is attached to the ring by a canvas band of 10 cm width and there is also a 10 cm canvas band 57 cm below the upper canvas band where a throttling line can be fitted for closing the net. The ring is 57 cm

internal diameter, 1·5 cm diameter thickness with three attached eyelets for the bridles and rope lead attachments. The three bridles are each 57 cm in length attached to a swivel, and the nylon rope leads are to take a 25 kg weight under normal usage, though a 40 kg weight is desirable under conditions when the wire angle would exceed 25°. It is recommended that a flowmeter (*TSK* or equivalent) should be fitted from three points on the ring and accurately centred 14·25 cm from the rim. The codend should be a light-weight bucket (7·5 cm diameter) of polyvinyl chloride or brass, fitted with windows of the same mesh as the net, and with a volume of 150–200 c.c. (when plankton is required alive a volume of 500 c.c. is preferable), but for use in very rich waters a bag of the same mesh as the net is more convenient. The filtration efficiency of this net is 94 per cent. It should be used vertically, lowered at 60 m/min and hauled 45 m/min which should give a towing duration of 4·5 min for 200 m water column during which time clogging should not normally reduce the efficiency of the net to less than 85 per cent. Where possible a second flowmeter should be attached outside the rim to give an indication of efficiency, and if this is less than 85 per cent the haul should not be considered as quantitatively representative.

Working party 2 also recommends taking two simultaneous samples, or splitting the sample into two halves, one for biomass determination by the Lovegrove method[2,3] and one for counting and taxonomic analysis. It also recommends more research into net design, water flow, plankton behaviour and patchiness, and on net material. The development of telemetering depth-flowmeters, and electrical methods of opening and closing nets, are desirable as well as research into the effect of shock waves arising from the ship and noise on plankton sampling.

Working party 3 considered that an encased high-speed sampler would be the best sampler for their size range of organisms, but that none of current design was near enough to the ideal. It recommended further research to produce a suitable design and suggest the features to be considered as ideals, although it must be realized that some compromises may be necessary to make it a workable and practical design. In the meantime it recommends a net with a mouth opening of 1 m^2, the net made of monofilament nylon of 1 mm mesh aperture having a cylindrical forward part 57 cm and a conical after part 200 cm in length and strengthened with six longitudinal tapes not more than 2 cm wide. It should have a throat of sailcloth not more than 12 cm wide wrapped around the ring, which should be of galvanized tubing of about 2 cm outside diameter and with three bridles equal in length to the mouth diameter. A flowmeter should be fitted in the mouth area 25 cm from the ring and the collecting bucket should be light in weight. This net should be towed at 2–3 knots using a sinker of 40 kg or an equally efficient depressor. The organisms sampled by this net would overlap those taken by the net designed by working party 2 mainly for biomass determination. Use of the 1 mm mesh advocated by working party 3 has the advantage that it is possible to filter far greater quantities of water and so sample the larger species which are usually less abundant. These will be retained relatively free from contamination by those great quantities of small organisms which interfere with sorting and which by adhering during fixation make taxonomic separation difficult. The report from this working party also recommends further research to find the best means of telemetering information from the sampler to the surface and telemetering control signals to the sampler.

Working party 4 considered that the organisms in its size range (about 2–20 cm) would be best termed micronekton rather than macroplankton as this would reduce confusion with the range considered by working party 3. Gelatinous organisms and other animals greater than 10 cm will occur in the catches and these must be considered separately from the main sample. The working party considered that of the methods available only one sampler has widespread use—the Isaacs–Kidd Midwater Trawl—though it notes that as there is no method of measuring the volume of water filtered it is only semi-quantitative. The net should be large and the 6 ft. or 10 ft. net (that is, depressor spread) should be used according to the available shipboard facilities. The mesh should be 0·5 in. (12·5 mm) stretched nylon supported within an outer net of about 2·5 in. (6·5 cm) knot to knot. The use of mixed or graded meshes is deprecated even though the catch is maintained in better condition if the codend is preceded by a short length of 3 mm mesh (any resulting small organisms in the catch could be disregarded).

The speed of the ship should be maintained at 3 knots, wire should be paid out at 40 metres/min, hauled at 40 metres/min for oblique tows or 60 for horizontal tows. Oblique hauls should sample 200–0 m depth and for thorough depth sampling by horizontal tows sampling should be at 50 m intervals to 1,000 mm and 250 mm intervals beyond. The use of a depth-time recorder or depth telemeter is a prerequisite of midwater sampling because accurate knowledge of the depth of fishing is essential to a proper interpretation of the data. A flowmeter should be used to measure the relative length of tow.

The reports of all four working parties are to be collated into one which will be published in due course as a Unesco monograph.

J. H. FRASER

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OBITUARIES

Patrick B. Kennedy

PATRICK BRENDAN KENNEDY, first professor of mathematics at the University of York, died suddenly on June 8, 1966, aged only 37. He was born at Cork and educated there at North Monastery and later University College. After obtaining an M.A. at Cork he went to the then University College of the South-west at Exeter to study the theory of functions of a complex variable, and this subject remained one of his main interests. His Ph.D. thesis was stimulated by a conjecture of M. H. Heins, which he disproved, and contained a discussion of the minimum growth of an integral function with k symptotic values together with counter-examples to show that all his theorems were the best possible. To construct these counter-examples he provided a method for approximating certain subharmonic functions by logarithms of integral functions which proved then and is now a most valuable tool. The distinction of this work was recognized when after a short period as assistant lecturer at Aberystwyth and lecturer at Cork he was appointed professor of mathematics at Cork in 1956. He was appointed to the chair at York in 1963.

Kennedy had a delightful Irish humour, which always made him charming company, but he was nevertheless an extremely serious person and drove himself hard. He took a strong personal interest in all his students. He found it hard to delegate work to his junior colleagues, feeling that they should not be overburdened with chores but should have the opportunity for their own researches. He was also keenly interested in all matters of education and had been a member of the teaching committee of the Mathematical Association. At the time of his death he was writing two books, conducting the first degree examinations at York and also acting as external examiner at Cork. He is survived by his wife, whom he married in 1954, and three children.

W. K. HAYMAN

IDENTIFICATION OF RADIO GALAXIES AND QUASI-STELLAR OBJECTS

By J. G. BOLTON
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ABOUT 2,000 radio sources between declinations +20° and −90° have been catalogued during the past four years with the 210 ft. telescope at Parkes. The finding survey for the catalogue was made at a frequency of 408 Mc/s and additional observations were made of most sources at 1,410 and 2,650 Mc/s. The nominal lower limit of flux density for most of the survey was 2·5 flux units (10^{-26} W m^{-2} $(c/s)^{-1}$) and the accuracy of the source positions is estimated to be ±1 minute of arc. More precise positions (±12 seconds of arc) have been determined for 640 sources with flux densities greater than 1·6 flux units at 1,410 Mc/s. A search of the *Palomar Sky Survey* prints in the area between declinations 20° and −33° which is common to both the *Sky Survey* and the *Parkes Catalogue* has yielded about 400 identifications. The various zones of the *Catalogue*[1], the precise positions[2] and details of the identifications[3] are at present being published in the *Australian Journal of Physics*.

So far as identifications are concerned, the statistically most complete sample is that of 383 sources between declinations 20° and −30° for which accurate positions are available. Their distribution in celestial co-ordinates and the identification status of each source is shown in Fig. 1. All the sources shown on this diagram are believed to be extragalactic. In the area inside the dotted line, the sources are partly of galactic origin and the fraction of sources which can be identified is reduced by obscuration within the Galaxy. 143, or 38 per cent, of the 383 sources can be identified with galaxies and 100, or 26 per cent, with quasi-stellar objects. About 60 per cent of the quasi-stellar objects have been confirmed by optical investigations. Somewhat similar results have been obtained by Wyndham[4] from an investigation of 300 sources from the revised *3C* catalogue with flux densities greater than 9 flux units at 178 Mc/s. Of the 242 identifications in the present sample, sixty are either previously well known identifications, or have been independently identified by Wyndham. Wyndham's ratio of quasi-stellar objects to galaxies is somewhat lower than that of the present results; this probably arises from the difference in the survey frequencies used in the *3C* and *Parkes Catalogues* and the difference in radio spectra for quasi-stellar objects and galaxies.

This difference between the radio spectra of quasi-stellar objects and galaxies was first noted by A. J. Shimmins of this laboratory (private communication). It is illustrated in Fig. 2, which shows the distribution of the quasi-stellar objects, the galaxies and the unidentified sources in terms of the ratio of their flux densities at 1,410 and 2,650 Mc/s. Most of the galaxies lie well to the right of the line at a ratio of 1·6, whereas the peak of the distribution of quasi-stellar objects is to the left. Thus, the quasi-stellar objects can be distinguished from galaxies by their high-frequency spectra and, on this basis, Fig. 2*c*

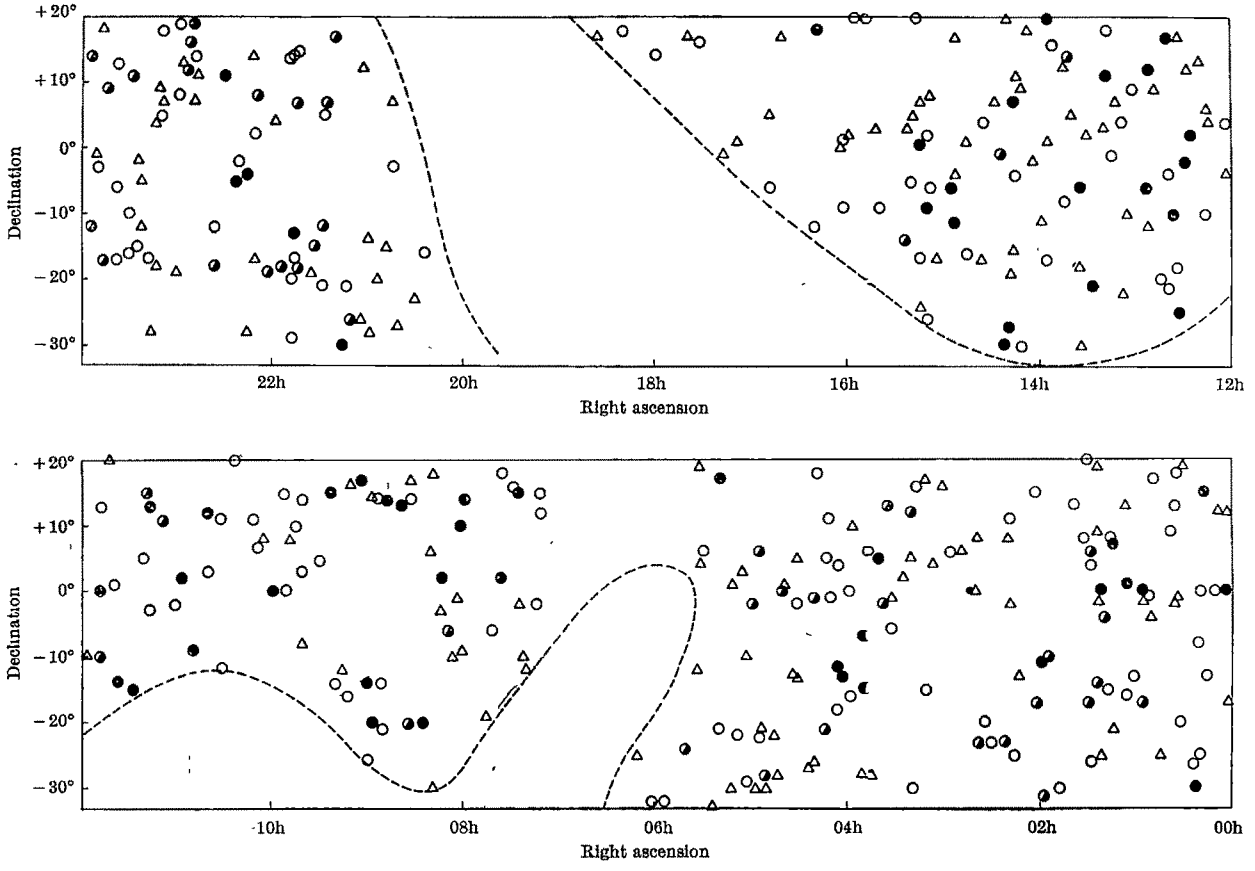


Fig. 1. Distribution of 383 radio sources between declinations 20° and −30° for which precise positions are available. ○, Unidentified sources; △, galaxies; ●, confirmed quasi-stellar objects; ◑, possible quasi-stellar objects. Dashed line encloses area containing a fraction of galactic sources or regions of obscuration.

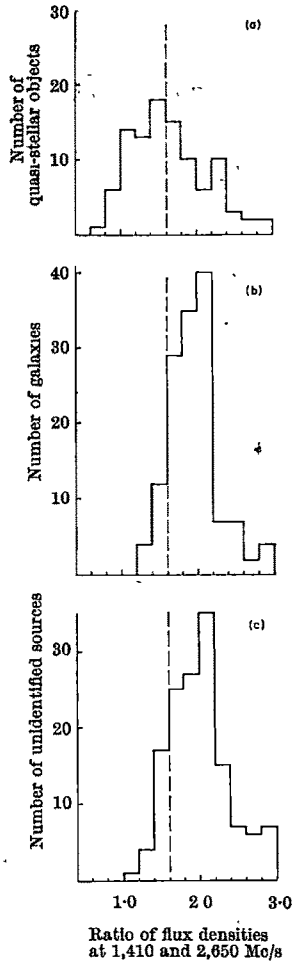


Fig. 2. Histograms of the number of sources with a given ratio of flux density at 1,410 and 2,650 Mc/s for: (*a*) quasi-stellar objects, (*b*) galaxies; (*c*) unidentified sources.

strongly suggests that the majority of unidentified sources are galaxies which are presumably fainter than the plate limit of the *Sky Survey*. Two further arguments may be advanced to support this result.

The first depends on the distribution of radio and optical magnitudes for the identified sources shown in Fig. 3, in which the radio magnitude scale at 1,410 Mc/s has been adopted such that 1 flux unit is equivalent to a radio magnitude of 10·5. Most of the optical magnitudes were estimated by comparison of their images on the *Sky Survey* plates with those of objects for which photometric magnitudes are available. For faint objects these estimated magnitudes probably have quite large errors but not of sufficient extent radically to alter Fig. 3. Fig. 3*d* for the galaxies shows the well-known large range in optical magnitudes corresponding to a small range in radio magnitudes. The distribution is markedly skew and strongly reflects the effect of the plate limit of the *Sky Survey*. The distributions for quasi-stellar objects show a much closer relationship between optical and radio magnitudes: there is no suggestion of a cut-off owing to plate limit, which would probably be at least one magnitude fainter for stellar objects than for galaxies. The sharp cut-off in the magnitude distribution for the galaxies suggests that the unidentified sources may well be galaxies beyond the *Sky Survey* plate limit. The magnitude distribution for quasi-stellar objects suggests that the identifications with quasi-stellar objects are essentially complete for the present sample, and that quasi-stellar objects between the nineteenth magnitude and the plate limit would be found among radio sources which are fainter than those of the present sample.

A second argument is provided by observations for interplanetary scintillations of about 200 of this sample of sources (due to R. D. Ekers of this laboratory). Such scintillation is an indication of the small angular size of a source (2 sec of arc or less). Ekers found that more than 50 per cent of the suggested quasi-stellar objects scintillate, whereas only 20 per cent of the galaxies fainter than the seventeenth magnitude do so. He found that only 25 per cent of the unidentified sources scintillate and, on the assumption that they are, in general, more distant than identified sources of the same class, his results strongly suggest that they are galaxies rather than quasi-stellar objects.

Source Counts. The anomaly of "too many faint sources" in the counts of radio sources with flux density is now well established. The slope of the graph of the logarithm of the number of sources (N) with a flux density greater than S against this value of log S is approximately $-1{\cdot}8$, compared with an expected slope of $-1{\cdot}5$ for a uniform distribution of sources in a Euclidean universe. This result has been interpreted either as indicating an evolving universe in which radio sources were more numerous or intrinsically brighter in its earlier history, or in terms of a local deficiency in a fraction of the population, which is presumed to be of galactic origin. Véron[5] and Longair[6] have independently pointed out that the slope of the log N–log S curve is significantly different for different classes of sources. They find the slope for galaxies is of the order of $-1{\cdot}5$, for quasi-stellar objects $-2{\cdot}2$, and even steeper for unidentified sources. The obvious inferences have been drawn—that the quasi-stellar objects and unidentified objects are responsible for the anomaly on the evolving universe model—or, for example, in a model by Sciama and Saslaw[7], that the unidentified sources and possibly some of the quasi-stellar objects for which red-shifts are not yet available, represent a galactic component.

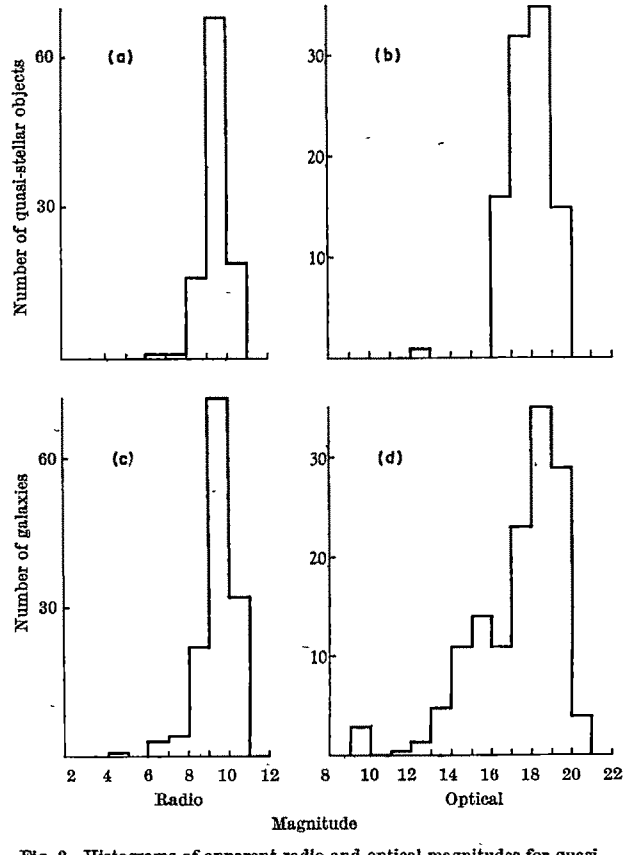


Fig. 3. Histograms of apparent radio and optical magnitudes for quasi-stellar objects and galaxies.

The present observations substantiate the results of Véron and Longair. The counts for the three classes at 1,410 and 2,650 Mc/s are shown in Fig. 4*a*, *b* and *c*. The differences between the slopes are not as extreme as those of Véron and Longair, possibly because of the larger number of samples involved, but they are nevertheless quite pronounced. The log N–log S relationship for galaxies does not, in fact, have a constant slope but is markedly flatter towards the faint end. Such an effect would result from the cut-off imposed on identifications of galaxies by the plate limit of the *Sky Survey*, for the fraction of identifications possible will decrease with the flux density. If the previous deduction that the unidentified sources are predominantly galaxies is correct, then these sources should be included with the galaxies in the count. The log N–log S relationship for galaxies and unidentified sources is shown in Fig. 3*d*, where its slope can be seen to be indistinguishable from that of the quasi-stellar objects. It will be seen that the log N–log S slope for the quasi-stellar objects is slightly flatter at 2,650 Mc/s than at 1,410 Mc/s. No significance should be attached to this result; it arises from the high fraction of flat spectrum sources among the quasi-stellar objects, and the selection criteria for the source sample. The galaxy counts do not show this effect, as the fraction of flat-spectrum sources among them is negligible.

The result that the modified log N–log S slope for galaxies is the same as that for quasi-stellar objects is perhaps not surprising for, if the red-shifts of the quasi-stellar objects are cosmological, the quasi-stellar objects and galaxies in the present sample span much the same volume of space. Most of the sources under consideration have

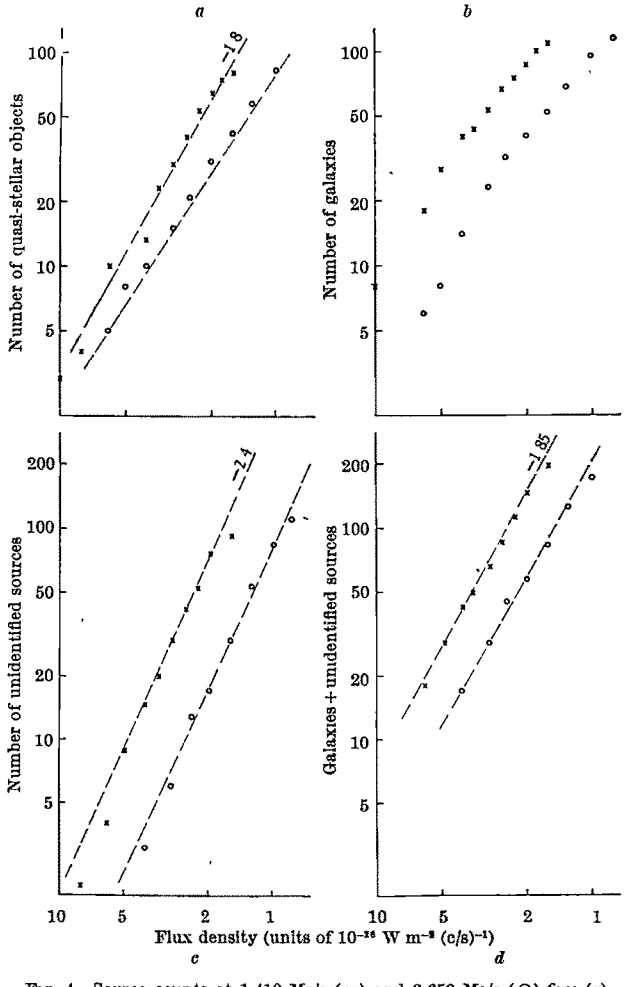


Fig. 4. Source counts at 1,410 Mc/s (×) and 2,650 Mc/s (○) for: (*a*) quasi-stellar objects; (*b*) galaxies, (*c*) unidentified sources, and (*d*) composite of galaxies and unidentified sources.

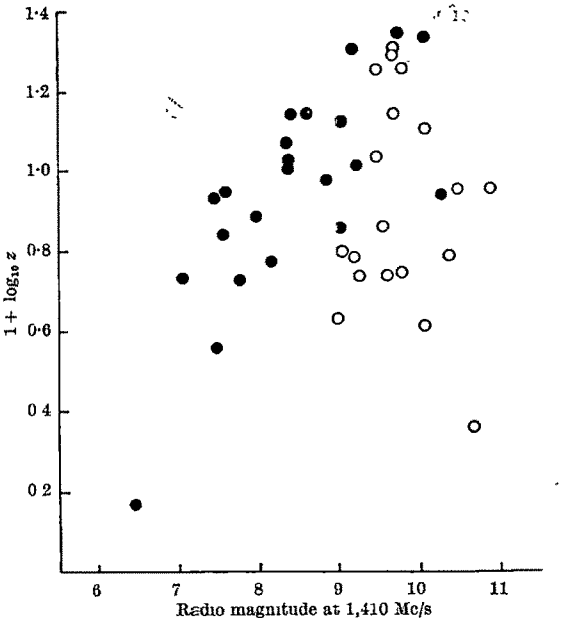


Fig. 5. Radio magnitude–red-shift diagram for quasi-stellar objects. ●, Objects with flat or relatively flat spectra; ○, objects with steep radio spectra.

apparent radio magnitudes between 9 and 10. In this range seventy-eight sources can be identified with galaxies and eighty are unidentified. A few of the latter may be faint or unrecognized quasi-stellar objects and some are probably N- type or compact galaxies above plate limit—which are difficult to distinguish from stars on the *Sky Survey* prints. However, at least fifty are probably galaxies beyond plate limit. These will have absolute radio magnitudes equal to or brighter than $M_r = -32$. The exact $M_r = -32$ cannot be determined, but it would certainly extend to form of the luminosity distribution beyond $M_r = -34$, because objects of this luminosity are known to exist (for example, Cygnus-*A* and 3*C* 295). Galaxies in this range will have distance moduli between 41·5 and 43·5, and the red-shifts of the most distant will be well in excess of unity. The similarity of the log N–log S for the galaxies and quasi-stellar objects within the same range of red-shifts may indicate a parallel evolutionary development.

Red-shift—Magnitude Relation for Quasi-stellar Objects. So far in this article it has been assumed that the red-shifts of the quasi-stellar objects are cosmological in origin. A number of alternative suggestions have been made to overcome difficulties associated with their energy supply and incompatibilities between their angular diameters and short period light variations. However, no really insurmountable objections to a cosmological origin have been raised, and the alternative hypotheses appear to provoke greater difficulties than they aim to overcome.

Hoyle and Burbidge[8] have recently questioned the cosmological red-shift on new grounds. They point out that there is no relationship between the red-shift and radio magnitude at low frequencies (178 Mc/s), and that even the relationship between red-shift and optical magnitude appears to be simply a scatter diagram. However, such a result does not necessarily vitiate a cosmological red-shift; it may merely be an indication of a large dispersion in the relevant absolute luminosities. To obtain a reasonable relationship demands an intrinsic property which is comparatively constant, as is the absolute optical magnitude for the radio galaxies. The large variations in the light output of some of the quasi-stellar objects would suggest that the optical magnitude is not such a property. About half the objects considered by Hoyle and Burbidge have fairly steep spectra, and

quite a few of this class of quasi-stellar objects are known to have components of considerable angular and presumably linear size. Such components may be the results of singular events, and similar to the large physical components of radio galaxies—for which there is certainly a very large range in intrinsic luminosity and a correspondingly large scatter in their radio magnitude–red-shift diagram. It is possible that the radio emission from quasi-stellar objects with flat radio spectra, which might result from a continuous process in a small physical volume, could be a better indicator of intrinsic luminosity. In Fig. 5 the relationship between the radio magnitude at 1,410 Mc/s and the red-shift is shown, with different symbols for the objects with relatively flat and steep spectra. While there is no very distinct relationship, the scatter for the flat-spectrum sources is considerably lower than that in either of the diagrams given by Hoyle and Burbidge. There is a suggestion that radio spectra might be useful in assigning a luminosity classification, and that a search for objects with very large red-shifts could be most successful among faint radio sources with flat spectra.

I thank many of my colleagues at the Radiophysics Laboratory who provided the basic data used in this investigation, particularly Dr. Margaret Clarke and Mrs. Jennifer Ekers for their work on identifications. I also thank Dr. T. D. Kinman of the Lick Observatory for providing optical confirmation and accurate optical positions of many of the southern quasi-stellar objects, and Dr. Margaret Burbidge for providing red-shift data in advance of publication.

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RESOLUTION OF X-RAY SOURCES AT LOW GALACTIC LONGITUDE

By Dr. PHILIP C. FISHER, Dr. WILLARD C. JORDAN, ARTHUR J. MEYEROTT, LOREN W. ACTON and DOUGLAS T. ROETHIG

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The second of two rockets instrumented primarily for locating celestial X-ray sources at low galactic latitude ($|b^{II}| < 20°$) was flown on September 30, 1965. During 1964, three different groups[1–3] examined most of the longitude interval $330° < l^{II} < 25°$ and a number of X-ray sources were located. In addition to some discrete sources, a significant residue of counts was found above background[1] which did not appear to be associated with any of the discrete sources. Part of the 1965 flight was arranged to determine if these residual counts represented an extended source or a number of individual sources. It was also hoped that a better determination of the galactic latitude of the sources already observed might provide some indication of their relative position within the Galaxy.

An *Aerobee* rocket fitted with a roll-stabilized attitude control system (ACS) was used and flown from the White Sands Missile Range. The ACS was programmed such that two differently oriented X-ray detectors could make two short scans approximately parallel to the galactic equator. Owing to a failure in the ACS, a third scan normal to the galactic equator was not carried out. Gas proportional counters with berylium windows were used to measure the energy of each X-ray detected, while photometers sensitive to visible light were used to obtain information concerning stellar aspect. Pulse heights of individual events in the X-ray detectors, detector scan rates, and stellar signals from the aspect photometers were all telemetered to the ground. The intermittent behaviour of the signals from the optical photometers (believed to be related to a visible glow observed around the rocket[4]) has meant that information is of little use for the results presented here. It should be noted that a small part of the sky near the galactic centre was not observed on the 1965 flight owing to the presence of the Moon, which lay at $\alpha = 17h\ 22m$, $\delta = -24{\cdot}6°$ and passed through the field of view of detector *A* near 150·5 sec after the launching.

One major difference between the 1964 and 1965 flights was that the fields of view of the 1965 detectors were reduced to a half and a fifth that of the field of detector *C* used in 1964. Table 1 indicates the characteristics of the three detectors. Counter *A* had a detection efficiency similar to that of the counter used in obtaining the 1964 data, while counter *B* was more sensitive above 5 keV because of a higher filling pressure and because xenon as well as argon and methane were used for the filling gas.

Fig. 1 presents the observed counting rate as a function of time for segments of the two flights; the 1965 data have been corrected for the dead time of the counter's electronics—this correction ranges from about 15 per cent when no discrete source was within the field of view to about 50 per cent for the peak rates seen at 138 sec and 152 sec after launch. No correction of the 1964 data was necessary. The rockets were scanning at rates of 2·03, 1·08, and 0·58 degrees/sec while the data of detectors *C*, *A* and *B* respectively were being acquired. Therefore the time scales of the data have been adjusted for these different scan rates in order to show the relative galactic longitude of the various sources that were observed. The discussion of how the high count regions were correlated with position on the celestial sphere, and so with the galactic longitude scale given in Fig. 1, will be presented later. The data for detector *A* given in Fig. 1 were acquired at about the same galactic latitude as the data for detector *C*. While six high count rate regions were suggested by the 1964 data, on at least seven occasions during the first 1965 scan the signal for counter *A* indicates the presence of an X-ray source. Another significant difference in the Fig. 1 data occurs near $l^{II} = 0°$, corresponding to the

Table 1. X-Ray Detector Characteristics for Data presented in Fig. 1

Detector	Flight date	Effective window area (cm²)	Field of view* (degrees)	Approximate energy interval (keV)	Counter filling gas
C	Oct. 1, 1964	263	2·9 × 26·5	4–8	90% argon, 10% methane
A	Sept. 30, 1965	195	1·8, × 16·7	2–9	90% argon, 10% methane
B	Sept. 30, 1965	235	1·7 × 7·4	3–11	69·5% argon, 20% xenon, 10% methane, 0·5% helium

* Full width at half maximum sensitivity.

time interval of detector A of 148 sec to 152 sec after the launching. It may well be that the major part of this difference is the result of the quite different sizes and slightly different orientations of the field of view of the two detectors. For example, one of the 1964 sources[1] may lie outside the field of view of detector A, or several of the 1964 sources[1-3] may have been observed simultaneously (despite the improved angular resolution of the detectors used) on the more recent flight. A decrease in the absolute flux of one of the 1964 sources cannot be ruled out. In addition to these differences, new X-ray sources were observed by detector A near 160 sec and 129 sec after the launching. The latter source was so close to the horizon of the 1964 flight that it may not have been observed at that time. Although the long dimension of field of view of counter B was only half that of the field of detector A, on this first scan detector B observed all but the weakest source seen by detector A. Except for this source, the first scan data from detector B show count rate variations similar in time and amplitude to those of detector A. There is a possibility that detector B saw simultaneously the first two sources which came into view of detector A at 130·5 sec after the launching.

The data given in Fig. 1 for detector B were acquired during the second scan of the 1965 flight which was carried out at about a 5° lower latitude than that used in obtaining the detector A and C data shown. Considering the scan rate and the counter background, the highest detection sensitivity of the experiment (about 0·1 photons cm^{-2} sec^{-1}) was attained while the data given in Fig. 1 for detector B were being obtained. Only two sources were observed by detector B on this scan. Although no unambiguous location of the X-ray source near 218 sec has been obtained, the other source at 207·4 sec after the launching seems to be the same as that observed by detector A near 152 sec.

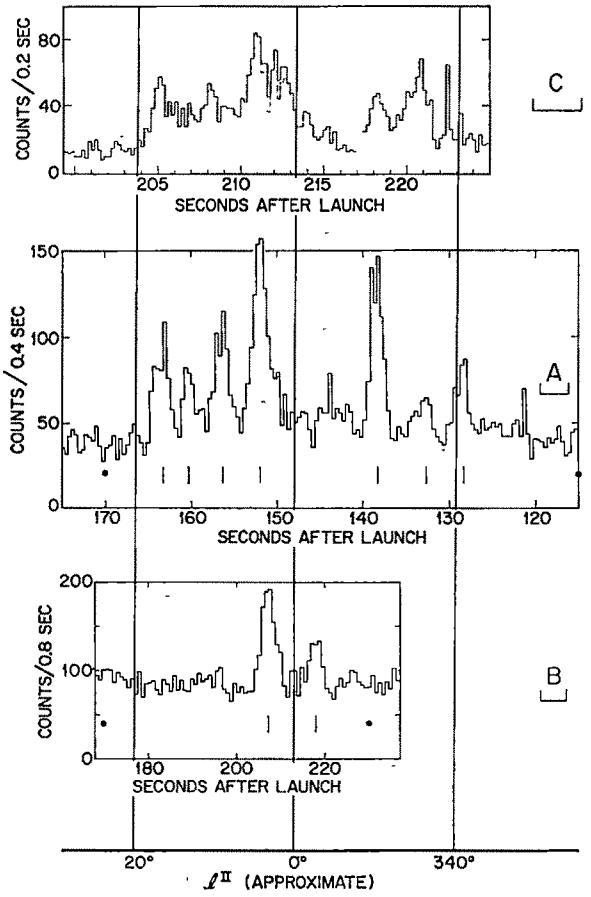


Fig. 1. Counts observed during two rocket flights, while scanning in longitude near the galactic equator. Letters at the right of the figure denote which detector the data were derived from, and the line segments under these letters indicate the time required for a point source to pass completely through the narrow dimension of the field of view of the detector. The time intervals between the black dots shown with the data of detectors A and B indicate the duration of the two 1965 scans. Dotted lines in the detector C data near 212 sec show the count rate anticipated after subtraction of effects from the brightest source in Scorpius which lay near the edge of the field of view of detector C at this time.

The regions of higher count rate observed by detectors A and B have been correlated on the basis of the collimator orientations on the rocket and on the assumption that no important spectral differences existed for a given (postulated) source. Although the statistics are poor, this assumption seems valid as no striking spectral differences appear in the data of the two counters for any of the seven sources. The correlations lead to a determination of: (1) the distance and direction of each of the given sources from the scan path; (2) the distances between sources measured along the scan path; (3) the strength in counts cm^{-2} sec^{-1} of each source observed. Relative position along the scan path can be computed from the elapsed time between observation of X-ray sources. For the 148–165-sec time interval of the data for detector A, no action of the ACS occurred which would have affected the position of the detector along the scan path. Consequently, the various points on the 1965 grid of relative positions for this interval are known to about $\pm 0{\cdot}6°$ along the scan path, while an error of about $\pm 1°$ is appropriate for the position of a source off the scan path.

The grid of relative positions from the 1965 experiment can be oriented on the celestial sphere by assuming the scan paths of the detectors are those prescribed by the roll-stabilized ACS. This assumption makes it possible to make some detailed correlations between the high count regions of the two flights. The data of detector C from the 1964 flight were supplemented by information on the galactic latitude of *SCO XR*-3[2] and *SCO X*-2[3]. Fig. 2 presents the result of this correlation. The heavy diagonal line indicates the galactic equator while the lighter long diagonal lines indicate the scan path of the centre of the field of view of the two 1965 detectors. Although the scan path of detector C is not shown, it lay slightly below the galactic equator. The longitudes of the sources observed[1] in 1964 are indicated by the shaded swaths nearly perpendicular to the galactic equator. The inter-comparisons of X-ray data indicate a lateral shift of about 1°, and a skew of about 1°, of the detector's actual scan path as compared with the path originally prescribed for the ACS. This shifted path has been used in Fig. 2. Such a shift is within the nominal errors established for operation of the roll-stabilized ACS. The position of the scan path shown in Fig. 2 is believed to be known to within about $\pm 1°$, and this uncertainty provides the chief contribution to the error in source location indicated by the areas outlined. It is hoped that reduction of the optical aspect data will lead to an improvement in the determination of the location of the X-ray sources.

The positions and fluxes of the X-ray sources shown in Fig. 2 have been summarized in Table 2. In computing the counts cm^{-2} sec^{-1} of the sources observed by detector A, a background rate was used that was the average of the counts observed in the 121–126 and 166–171 sec time intervals. Although this rate indicates a significant residue of counts above background for the interval $350° < l^{II} < 0°$, the data seem inadequate to specify further the significance (or lack thereof) of these residual counts.

In only a few instances is the position information now available for the brighter X-ray sources adequate to attempt correlations with known features of the Galaxy. It does seem significant that source numbers 4 and 7 in Table 2 fall within $\pm 1{\cdot}5°$ of the nearby thermal radio sources M 8 and M 16. As noted earlier, the error in relative galactic longitude of these sources as seen by detector A is probably no more than $\pm 0{\cdot}6°$. The angles between count rate peaks which can be interpreted as the M 16 and M 8 signals on the first detector B scan (M 16 was too near the edge of the field of view to be detected on the second scan), and the angle between the M 8 and

Table 2. POSITION AND COUNT RATE INFORMATION FOR X-RAY SOURCES OBSERVED

Source No.	Detector *A* data of 1965: Time from the launching (sec)	Counts cm^{-2} sec^{-1} Measured	Corrected*	Approximate position (1950 epoch) α	δ	Detector *C* data of 1964: Time from the launching (sec)	Corrected* counts (cm^{-2} sec^{-1})	1965 to 1964 count rate ratios: 1964 Lockheed data	1964 NRL data	Remarks†
1	128 4	0·6	—	—	—	—	—	—	—	(i)
2	132·8	0 4	0 6	17h 23m	−44·2°	220 8	1·1	0·5	0 5	(ii)
3	138·3	1·5	1·5	17 08	−36 4	218·3	0 8	1 9	1·4	(iii)
—	—	—	—	—	—	212 6	0 9	—	—	(iv)
4	152·5	1·8	2·0	18 02	−24·9	211 0	1 4	1·4	1 5	(v)
5	156·3	1 0	1·0	18 03	−20 7	208 2	0·8	1·2	—	
6	160·3	0·6	0 6	18 11	−17 2	—	—	—	1·7	(vi)
7	163·3	0 9	0 9	18 14	−14·3	205 1	0 9	1·0	—	

* The measured count rates were corrected to that value which would have been observed if the source lay on the collimater's centreline.

† Remarks: (i) Source was below horizon in 1964 experiment (ref. 1) and lies within the large rectangular region of the 1965 experiment closest to the horizon in Fig. 2. Agreement in the count rates seen by detectors *A* and *B* suggests the X-ray source lies near $\alpha = 16$h 52m, $\delta = -46{\cdot}6°$. (ii) Air absorption has not yet been allowed for in the 1965 experiment, so this source's flux may or may not have decreased with time. The NRL position of *SCO XR*-3[2] has been given for this source and the comparisons of count rates were made assuming the X-ray source lies at this position. (iii) The NRL position of *SCO XR*-2[2] has been given for this source and the comparisons of count rates assuming the X-ray source lies at this position. (iv) The existence and/or location of this source is now in doubt. (v) As noted in the text, the observed X-rays may actually come from several sources. The most likely division of the observed flux seems to yield a bright source at the position given, and a fourfold weaker source near $\alpha = 17$h 37m, $\delta = -22\ 9°$. (vi) The *SGR XR*-2 source of NRL[2] has now been resolved into at least three different X-ray sources.

M 16 signals on the first and second scan of detector *A*, are all equal to within ±0·6° of values appropriate to their known differences[5] in position. Although *M* 8 and *M* 16 are not point sources in the radio or optical portions of the observable spectrum, Fig. 1 gives little or no indication that the (possibly) related X-ray sources are other than point sources. While the arguments concerning identification of *M* 8 and *M* 16 with X-ray sources seem sound, the only other bright thermal radio source in this range of longitudes, *M* 17, was apparently not observed.

To our knowledge, no theoretical estimates have been made for the X-ray flux associated with H II regions such as *M* 8, *M* 16 and *M* 17. However, if either their radio[6,7] or optical[7,8] fluxes are fitted to the spectral distribution of the Crab Nebula[9], X-ray fluxes are predicted which are comparable with or larger than those actually observed. Allen's short lists[10] of bright radio sources and diffuse nebulae suggest several other similar objects which might be examined for X-ray emission in order to provide greater confidence in the tentative identification of *M* 8 and *M* 16. For example, consider the X-ray sources in Cygnus observed by the Naval Research Laboratory (NRL) group[11]. Although *NGC* 7000 was omitted, both the Cygnus Loop and Cygnus *X* were noted in some of the data and on the most recent X-ray sky map of the NRL group[11]. The close proximity of the brightest radio portion of Cygnus *X* to the locations of Cygnus *A* and Cygnus *XR*-1 makes it quite difficult to correlate X-ray and radio sources in this region. However, *NGC* 7000 and Cygnus *X*, and the brightest radio emission region of the Cygnus Loop, all appear to lie quite close to high count locations noted by the NRL workers[11]. Such an association is at least consistent with the tentative *M* 8 and *M* 16 identifications. A further test and understanding of the hypothesis that bright and nearby radio sources may also be observable X-ray sources could be provided by examination of the Orion Nebula, *M* 42. Although the absence of dark globules in this object is an indication that it is fundamentally different[12] from *M* 8 and *M* 16, a similar naive fitting of radio and optical source strength with a Crab-Nebula-like spectrum suggests a measurable X-ray flux.

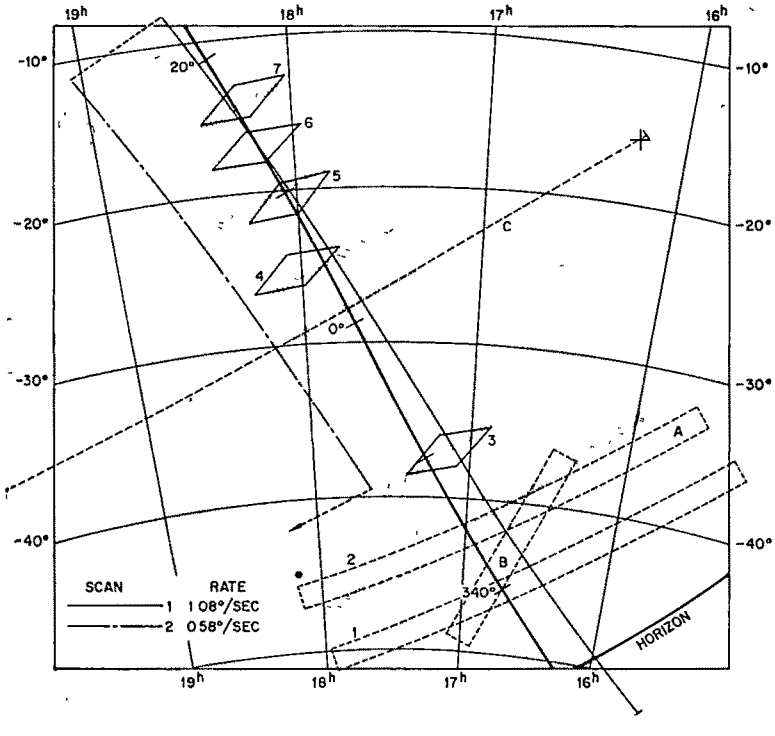


Fig. 2. Approximate positions of the brighter low-longitude sources observed on September 30, 1965. Co-ordinates of right ascension and declination are indicated as well as the galactic longitude. The outlined regions in which the sources are believed to lie are numbered as in Table 2. The large dotted rectangles near the lower right corner show the maximum latitude coverage and half the longitude coverage of the fields of view of detectors *A* and *B* when observing source Nos. 1 and 2 of Table 2. The orientation of these detectors relative to detector *C* of the 1964 experiment is apparent from the dotted line near the centre of the figure which shows the maximum galactic latitude coverage of detector *C*. The narrow shaded swaths indicate the central part of the regions where the 1964 measurements showed different sources must lie, the + at the upper right representing the position found in 1964 for the brightest X-ray source in Scorpius.

It appears to be significant that a fairly large fraction of the few radio sources which are nearby, intense, and have a relatively flat spectrum can be found in the vicinity of presently observed X-ray sources. These particular X-ray sources may well lie no more than several kiloparsecs away, those at low galactic longitude being no more distant than the Sagittarius arm of the Galaxy. While several new X-ray sources have been discovered and tentative associations with *M* 8 and *M* 16 have been made, an essential point of these data is the relatively large number of X-ray sources in a small area of sky. Although still more information (and possibly additional source positions) may yet be derived from the 1965

flight data, a significant further improvement of both angular resolution and detection sensitivity seems necessary before a more meaningful identification can be made of all the low longitude counts at present observed.

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RATIOS OF OXYGEN-18 : OXYGEN-16 IN PHOSPHATE AND CARBONATE FROM LIVING AND FOSSIL MARINE ORGANISMS

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Earlier I reported[1] the results obtained by measuring the oxygen isotopic composition of phosphate from the shells of some living marine organisms. A summary of the technique used for the purification of the phosphate, the removal of the oxygen from the final product (bismuth *ortho*phosphate) by fluorination with bromine trifluoride, and its conversion to carbon dioxide for spectrometric measurement was also given.

This article reports the results of further measurements of oxygen isotopic composition carried out on shells of both living and fossil marine organisms together with some of the previous results. The main purpose of these measurements was to determine the relationship between growth temperature of shells and the abundance of oxygen-18 in their calcium phosphate. Such a relationship would help in measuring palaeotemperatures and in estimating the oxygen isotopic composition of ocean water during the past from the equation provided by Epstein *et al.*[2] for carbonates and a similar equation for phosphates. In both equations the unknowns are the growth temperature and the oxygen isotopic composition of the environmental water. In previous calculations of palaeotemperatures the oxygen isotopic composition of ocean water was assumed to have been equal to that of modern mean ocean water.

One problem in the establishment of a temperature scale is to obtain samples grown under controlled temperature conditions. This is very difficult in the case of phosphates owing to the small percentage of phosphate generally contained in the shells of marine organisms and to the difficulty of growing inarticulate brachiopods, which contain fairly large amounts of phosphate, in an aquarium. Crustaceans, the phosphate content of which is also high, do not seem to precipitate their phosphate in isotopic equilibrium[3]. The average growth temperature of the shells was calculated from the oxygen isotopic composition of the calcium carbonate of each shell, by means of Epstein *et al.*'s equation. It was assumed that both calcium phosphate and calcium carbonate were deposited contemporaneously. The results obtained were corrected for the isotopic effect of the environmental water. Eighteen specimens of fourteen different species of Lamellibranchiata, Gastropoda, Brachiopoda, Cirripedia, Cephalopoda and Echinodermata were measured.

The analytical results are given in the δ terminology, where δ is the deviation of the ratio of oxygen-18 : oxygen-16 in the sample from that of a working mass spectrometer standard :

$$R = (1 + \delta) R_{std}$$

The δ values are expressed in parts per thousand (mil) and reported against carbon dioxide obtained from *PDB*-1 standard by reaction with 100 per cent phosphoric acid and standard mean ocean water (SMOW) as defined by Craig[4].

The amount of phosphate (III) ion present in the shells ranged from traces to about 0·3 per cent. No investigations have as yet been made on the crystal chemistry of the phosphate. McConnell[5] showed that phosphate in *Lingula* shells is present as francolite (carbonate fluorapatite). In shells containing very small amounts of phosphate, however, it is possible that a significant percentage of the phosphate is in the organic matter of the shell itself (proteins). Some investigations are now being made on this subject.

The species measured, their locations and the results obtained are listed in Table 1. In Fig. 1 the oxygen

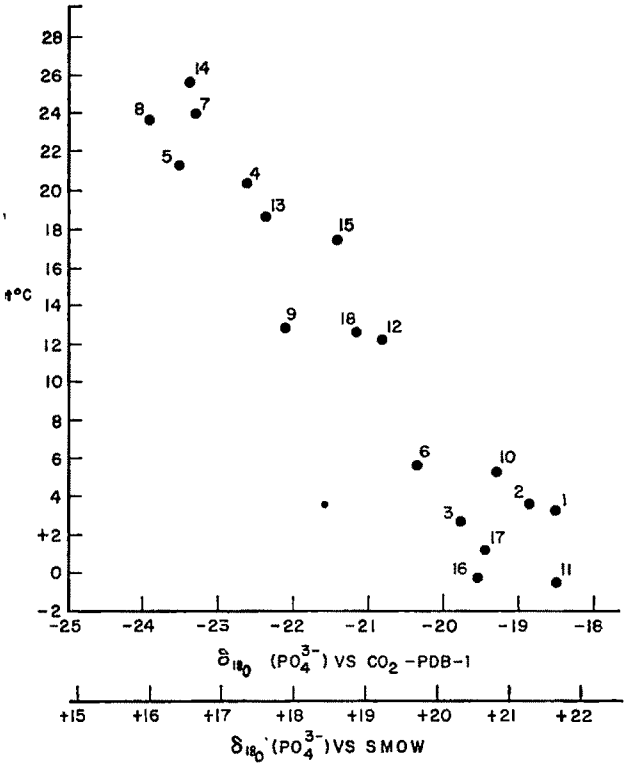


Fig. 1. Oxygen isotopic composition of phosphate from shells of living marine organisms versus their average growth temperature as calculated from carbonate δ's. The δ's are corrected for the effect of the isotopic composition of the environmental water. Numbers refer to Table 1.

isotopic composition of the phosphate is given against the average growth temperature of the shells as calculated from the oxygen isotopic composition of the carbonate. The dispersion of the points can be explained as arising from the error in the determination of carbonate δ's (standard deviations σ = ±0·1 per mil), the error in the evaluation of the correction for the H_2O isotopic effect (presumably between ±0·1 and ±0·2 per mil) and the error in the determination of the phosphate δ's (σ = ±0·18 per mil). The σ for phosphate δ's has been calculated from the results obtained on about 150 measurements.

Least-squares treatment of the data in Fig. 1 gave the following equation for the straight-line relationship between δ_{18O} (PO_4^{3-}) and t °C

$$t = -90{\cdot}0 - 4{\cdot}8\,(\delta_p - \delta_w)$$

where t is the temperature in °C, δ_p refers to the ratio of oxygen-18 : oxygen-16 in the phosphate (III) ion measured by the process described earlier[1], δ_w is the δ value of carbon dioxide equilibrated isotopically at 25·2° C with the environmental water, measured against the same mass spectrometer standard gas as used for the carbon dioxide obtained by conversion of the oxygen extracted from calcium phosphate. The calculated σ for this equation is ±2·70° C. A direct comparison of the coefficients in this equation with those in the carbon equation is difficult owing to the fact that the two equations are of different order; however, the slopes of these two equations are rather close. Plotting δ_{18O} (PO_4^{3-}) against δ_{18O} (CO_3^{2-}) (Fig. 2), the slope is found to be about 1·4. From the similarity in the slopes and from the precision one can obtain in measuring ($\delta_p - \delta_c$) (about ±0·3 per mil) the uncertainty with which the temperature t and the oxygen isotopic composition of the environmental water (δ_w) could be calculated for fossils containing both carbonate and phosphate is

$$t \pm 4{\cdot}5°\text{ C},\ \delta_w \pm 0{\cdot}8 \text{ per mil}$$

This temperature uncertainty is quite large for measuring the palaeotemperatures with reasonable accuracy; however, it is possible that the slope of the final equation will differ somewhat more from that of the carbonates. The uncertainty in the calculation of δ_w is small enough to make it possible to calculate whether or not there have been large variations of the oxygen isotopic composition of ocean water through geological time.

The oxygen isotopic composition of teeth of two fish was also measured in order to obtain information on the possibility of using fossil teeth for measurements of palaeotemperature. The following results were obtained:

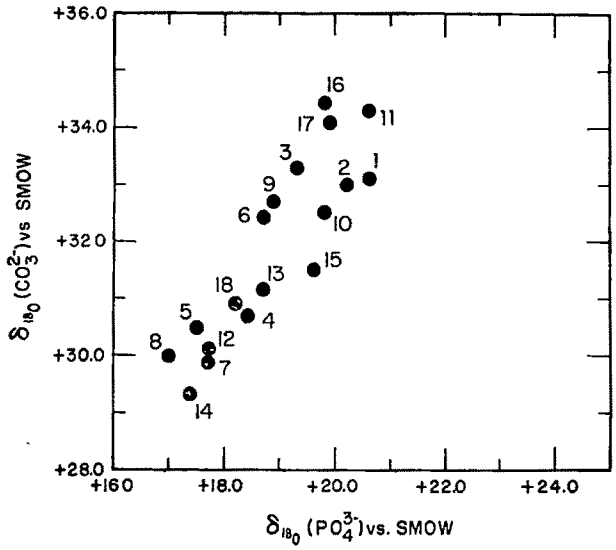


Fig. 2. Oxygen isotopic composition of co-existing carbonate and phosphate from shells of living marine organisms. Numbers refer to Table 1.

Oxyrina spallanzani (Adriatic Sea). Presumed temperature range 12°–23° C; average temperature recorded by the phosphate in the tooth 14·8° C.

Lagenorinchus albirostris (Northern Sea). Presumed temperature range 5°–17° C; average temperature recorded by the phosphate in the tooth 15·5° C.

Both the results are within the expected range of temperature even though they are near the limits.

On the basis of the results obtained it is possible to draw the following conclusions. (1) A general relationship exists between the temperature of deposition and the oxygen isotopic composition of phosphate in many marine invertebrates. From the good agreement shown by a considerable variety of organisms it can be assumed that in these cases the precipitation of phosphate occurs under isotopic equilibrium conditions. (2) The slopes of the phosphate and carbonate equations are rather close. On the basis of the results obtained so far it seems that isotopic palaeotemperatures cannot be measured with

Table 1

No. of sample	Species	Location	Depth (m)	$\delta_{18O}(PO_4^{3-})$ vs. CO_2-*PDB*-1	$\delta_{18O}(PO_4^{3-})$ vs. SMOW	$\delta_{18O}(CO_3^{2-})$ vs. *PDB*-1	t (° C) from $\delta_{18O}(CO_3^{2-})$	$\delta_{18O}(H_2O)$ vs. SMOW
1	*Spisula solidissima*	44°11′ N. 49°25′ W.	50	−19·50	+20·6	+2·45	+ 3 3	−1·00
2	*Chlamys islandica*	45°02′ N. 51°33′ W.	80	−19·86	+20 2	+2 34	+ 3 7	−1·00
3	*Balanus* sp.	45°02′ N. 51°33′ W.	80	−20 72	+19 3	+2·65	+ 2·7	−1 00
4	*Mytilus galloprovincialis*	43°38′ N. 10°17′ E.	5	−21 61	+18·4	+0 12	+20 4	+1·00
5	*Sepia officinalis*	Tyrrhenian Sea	Surface	−22 50	+17·5	−0·10	+21·4	+1·00
6	*Clinocardium ciliatum*	45°02′ N. 51°33′ W.	80	−21 37	+18·7	+1·78	+ 5·6	−1·00
7	*Ostrea edulis*	40°28′ N. 17°14′ E.	1–2	−22·27	+17·7	−0·66	+24·0	+1·00
8	*Ostrea edulis*	40°28′ N. 17°14′ E.	1–2	−22·91	+17 0	−0·61	+23·8	+1·00
9	*Terebratula vitrea*	42°37′ N 8°41′ E	150	−21 09	+18 9	+1·90	+12·7	+1 00
10	*Spisula solidissima*	44°11′ N. 49°25′ W.	50	−20·30	+19 8	+1·85	+ 5 3	−1·00
11	*Balanus* sp.	52°10′ N. 54°33′ W.	250	−19 51	+20 6	+3 65	− 0 5	−1 00
12	*Mytilus edulis*	Zeeland coast (Netherlands)	1–2	−22 31	+17·7	−0·47	+12 2	−1·50
13	*Sepia officinalis*	Tyrrhenian Sea	Surface	−21 34	+18·7	+0 52	+18·6	+1·00
14	*Echinometra lucunter*	S. Florida	1–2	−22 60	+17 4	−1·25	+25·0	+0·60
15	*Ostrea lamellosa*	43°37′ N. 10°17′ E.	5	−20 42	+19·6	+0·79	+17·4	+1·00
16	*Fusus* sp.	54°37′ N. 54°56′ W.	220	−20·29	+19·8	+3·83	− 0 3	−0·75
17	*Fusus* sp.	54°37′ N. 54°56′ W.	220	−20 17	+19 9	+3·43	+ 1·0	−0 75
18	*Gryphaea* sp.	Char. Maritime (France)	2	−21 80	+18 2	+0 28	+12 8	−0 60

reasonable accuracy by a carbonate–phosphate temperature scale. It should be possible, however, to obtain a good set of data on the possible existence of major variations of the oxygen isotopic composition of ocean water during geological time.

Measurements on Fossils

The oxygen isotopic composition of both carbonate and phosphate from some fossil marine organisms was measured. The fossils come mainly from Silurian, Carboniferous, Jurassic and Cretaceous formations in Belgium, Denmark, England, France, Germany, Greenland, Italy, The Netherlands, Scotland, Somaliland, the United States and the Soviet Union. Samples were mainly belemnites and pelecypods, together with a few brachiopods, rudists, crustaceans and fish teeth. The content of the phosphate (III) ion in these fossils ranges from about 0·05 up to about 34·0 per cent by weight. In species with very low phosphate contents two or three specimens from the same layer were processed together. The species measured, their locations, their ages, and the oxygen isotopic compositions of the phosphate (III) and carbonate (II) ions are given in Table 2. In Fig. 3 these phosphate δ's are plotted against the geological age attributed to each fossil.

A wide range of values was obtained. The most negative are those of Silurian and Carboniferous fossils. Among the Palaeozoic specimens there are freshwater and seawater fossils and a tooth of an unknown fish. All of them were very badly preserved, shells of *Obolus* had undergone recrystallization and the original material of the tooth was more than 50 per cent replaced by charcoal. The δ values of their phosphate are very close to one another and very negative. For *Obolus apollinis* the carbonate δ's (−8·2 versus *PDB*-1) are those of a carbonate which has almost completely re-equilibrated with ground (rain) water.

Among Jurassic fossils, belemnites have very large positive phosphate δ's which, on the tentative scale, correspond approximately to a temperature of about −10° C. Three pelecypods and one brachiopod show more negative δ values.

As regards Cretaceous fossils, a Lower Cretaceous belemnite is still as positive as Jurassic ones. A group of Middle Cretaceous fossils (chiefly pelecypods) gives δ values about midway between the most positive and the most negative. Several specimens from Upper Cretaceous formations (belemnites, a tooth of a fish and

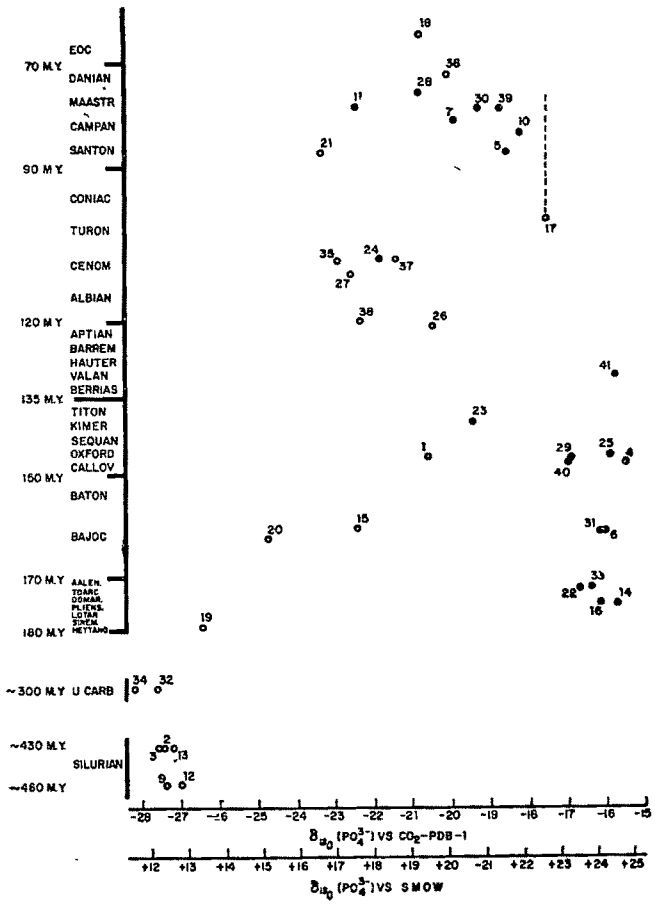


Fig. 3. Oxygen isotopic composition of phosphate plotted against geological age for fossil shells of marine organisms. Numbers refer to Table 2. ●, Belemnites; ○, other fossils.

Table 2

No. of sample	Species	Age and location	$\delta_{18O}(PO_4^{3-})$ vs. CO_2-*PDB*-1	$\delta_{18O}(PO_4^{3-})$ vs. SMOW	$\delta_{18O}(CO_3^{2-})$ vs.*PDB*-1	$\delta_{18O}(CO_3^{2-})$ vs. SMOW
1	*Gryphaea cfr. arcuata*	Callovian-Oxfordian—France	−20·60	+19·4	−0·80	+29·8
2	*Lingula lewisii*	Silurian–England	−27 48	+12·3	–	–
3	*Lingula lewisii*	Silurian—England	−27·63	+12·1	–	–
4	*Cylindroteuthis puzosiana*	Callovian—England	−15·45	+24·8	+0 75	+31·4
5	*Belemnitella* sp.	Santonian—Belgium	−18·55	+21·6	−0·15	+30·4
6	*Megateuthis giganteus*	Bajocian—Germany	−16·04	+24·2	−0 60	+30·0
7	*Belemnitella mucronata*	Campanian—Belgium	−19 84	+20 2	+0 31	+30·9
9	*Obolus apollinis*	Ordovician—Soviet Union	−26·46	+13·4	−8·19	+22·2
10	*Belemnitella quadrata*	Campanian—Belgium	−18·20	+22·0	−0·76	+29·8
11	*Belemnitella* sp.	Maastrichtian—The Netherlands	−22 46	+17·5	+0·15	+30·7
12	*Obolus apollinis*	Ordovician—Soviet Union	−26 98	+12 8	−8 32	+22·0
13	*Lingula sysmondii*	Silurian—England	−27 16	+12 6	–	–
14	*Passaloteuthis* sp.	Pliensbachian—Germany	−15·69	+24·6	−0 81	+29 8
15	*Lopha cristagalli*	Bajocian—Germany	−22·44	+17·5	−3·08	+27·4
16	*Passaloteuthis* sp.	Pliensbachian—Germany	−16·10	+24·1	−0·15	+30·4
17	*Pseudoranenella* n. sp.	Turonian-Maastrichtian—Greenland	−17·54	+22·6	–	–
18	Unknown decapod	Eocene—France	−20·81	+19·2	−1 80	+28·7
19	*Gryphaea arcuata*	Hettangian—Germany	−26·44	+13 4	−2·80	+27·7
20	*Terebratula digona*	Bajocian—France	−24·72	+15 2	−3·26	+27·2
21	*Jopha* sp.	Santonian—Italy	−23·35	+16·6	−2·96	+27·5
22	*Acrocoelites* sp.	Toarcian—Germany	−16·67	+23·5	−1·72	+28·8
23	*Cylindroteuthis* sp.	Kimeridgian—Scotland	−19·42	+20·7	−1·73	+28·8
24	*Belemnites* sp.	Cenomanian—Belgium	−21·82	+18·2	−0·52	+30·1
25	*Cylindroteuthis puzosiana*	Oxfordian—England	−15 91	+24·3	−0·49	+30·1
26	*Exogyra latissima*	Aptian—Somaliland	−20·45	+19 6	−2·61	+27·9
27	*Exogyra flabellata*	Albian-Cenomanian—Somaliland	−22·58	+17·4	−4·08	+26·4
28	*Belemnitella mucronata*	Maastrichtian—The Netherlands	−20·86	+19 2	+0·59	+31·2
29	*Pachyteuthis excentralis*	Callovian-Oxfordian—England	−16·90	+23·3	−0 50	+30·1
30	*Belemnitella americana*	U. Campanian—(New Jersey) U.S.A.	−19·27	+20·8	+0·23	+30·8
31	*Megateuthis giganteus*	Bajocian—Germany	−16·14	+24·1	−0 05	+30·5
32	*Antraconauta phillipsii*	U. Carboniferous—England	−27·63	+12·1	–	–
33	*Salpingoteuthis* sp.	Toarcian—Germany	−16·38	+23·8	−2·54	+28·0
34	Tooth of unknown fish	U. Carboniferous—England	−28·19	+11·6	–	–
35	*Alectryonia scyphax*	Cenomanian—Italy	−22·92	+17·0	−3 29	+27·2
36	*Lamna appendiculata* (tooth)	Danian—Denmark	−20·09	+19·9	–	–
37	*Alectryonia scyphax*	Cenomanian—Italy	−21·46	+18·6	−2·93	+27·6
38	*Exogyra latissima*	Aptian—Somaliland	−22 33	+17·7	−3 48	+27·0
39	*Belemnitella americana*	U. Campanian—(New Jersey) U.S.A.	−18·75	+21·4	−0 23	+30·3
40	*Cylindroteuthis puzosiana*	L. Oxfordian—England	−17 02	+23·2	−0·10	+30·5
41	*Belemnites pistilliformis*	Neocomian—Germany	−15·76	+24·5	−0 16	+30 4

Pseudoranenella) show rather scattered δ values which are again rather positive. The average δ value of this group corresponds approximately to a temperature of about +2° C. There also seems to be a tendency towards more negative δ values in younger specimens. In the case of fossils other than belemnites one is led to the conclusion that there is a general relationship between the ages of these fossils and the oxygen isotopic composition of their phosphate, their δ values becoming more and more negative with time. If this is correct, this can be caused by an isotopic exchange between the oxygen of the phosphate and the oxygen of ground waters. Despite the high bond energy between oxygen and phosphorus atoms, this exchange seems to reach a very high rate in Palaeozoic fossils. In fact, the freshwater fossil *Antraconauta phillipsii* (No. 32) shows the same oxygen isotopic composition as the sea-water fossils *Obolus* and *Lingula*.

If this is a correct interpretation of the values obtained from fossils other than belemnites, it can be concluded that for carbonates, because the bond energy of oxygen to carbon atoms is much lower than that of oxygen to phosphorus atoms, an isotopic exchange between carbonate and ground water took place in Mesozoic fossils.

The most difficult problem is to interpret the results obtained from belemnites. So far as I know, there are no natural fractionation processes which fossils normally undergo during the diagenesis of sediments which tend to enrich a fossil in oxygen-18. The fact that belemnites generally show very positive δ values could result from four different causes: (*a*) a biological fractionation effect; (*b*) the effect of some solid-state exchange (for example, between phosphate and carbonate); (*c*) the presence of secondary phosphate in the fossils; (*d*) a lesser exchange with ground water during diagenetic processes than observed in pelecypods and brachiopods.

The first hypothesis looks to be rather improbable as a biological fractionation effect would not adequately explain the difference between the δ values obtained from Jurassic and Upper Cretaceous belemnites. Moreover, modern Cephalopods, such as *Sepia officinalis*, seem to precipitate both their calcium carbonate and their calcium phosphate under isotopic equilibrium conditions. Finally, it must be remembered that a tooth from a fish (*Lamna appendiculata*) and a *Pseudoranenella* n. sp. from Upper Cretaceous show δ values very close to those of belemnites of the same age.

The possibility of some solid-state exchange should be rejected as it seems very difficult to explain how such a process could produce opposite fractionation effects in belemnites from those in pelecypods.

As regards the third hypothesis, it seems quite strange to obtain such positive δ's from phosphate of secondary origin. The natural exchange processes which fossils normally undergo during the diagenesis of sediments generally tend to deplete a fossil in oxygen-18. The same process can be supposed for phosphate on the basis of the results obtained from Palaeozoic specimens. However, the distribution of phosphorus in Jurassic and Cretaceous belemnites is now being studied by means of a microprobe. If the phosphate is of secondary origin, the phosphorus atoms should be concentrated along the apical canal and along the external surface, these areas being those in which an exchange or a replacement process can occur most easily. A study will also be made on the crystal chemistry of the phosphate in these belemnites.

The fourth hypothesis becomes acceptable on the basis of the following assumption. Considering the exchange surface, S (cm²), of a fossil and its mass, M (grams), the ratio S/M gives an idea of the probability of the presence of a significant rate of exchange between ground water and the fossil. For belemnites this ratio is about 1, while for pelecypods and brachiopods it reaches values of 10, 15 and even more. This means that the probability of an isotopic exchange of the oxygen between phosphate (or carbonate) and ground water is relatively low for belemnites and very high for brachiopods and pelecypods. This has been generally proved by measurements of palaeotemperature carried out by several authors on the carbonate of fossils. Pelecypods and brachiopods normally show more negative δ values than belemnites of the same age and from the same area. This is even observed in Upper Cretaceous fossils. A similar behaviour can be expected for phosphate.

Let us suppose that the very positive δ's are the closest to the original values and that the deposition of the phosphate always occurred under conditions of isotopic equilibrium. Assuming that the temperature range of the Jurassic ocean was not very different from that of the modern oceans, say 20 ± 10° C, one should conclude that the oxygen isotopic composition of ocean water was much more positive at that time than it is now. The presumable difference is of the order of magnitude of 5–9 per mil. Since this difference is extremely large, it is rather difficult to accept these values, without even questioning their validity. The possibility of a variation of the oxygen isotopic composition of the ocean water with time was discussed by Silverman[6], who considered that the continuous deposition (either organic or inorganic) of carbonates, phosphates, silicates, sulphates, etc., would progressively deplete sea water of oxygen-18 because of the fractionation effect produced during the deposition of these chemical compounds. The most recent arguments put forward in this field were by Craig[7], who pointed out the difficulty of making any kind of detailed balance for the oxygen isotopic composition of sea water during the past. The principal processes involved in a possible evolution of the oxygen isotopic composition of sea water[7,8] are weathering, erosional and sedimentation processes and the possible addition of juvenile water. No evaluation can be made of the contribution of juvenile water and, consequently, no conclusions can be drawn even if it seems likely that there has been a progressive depletion in oxygen-18 in sea water.

In general, the results obtained from Upper Cretaceous fossils seem to agree with such a hypothesis. Following the same considerations as for Jurassic samples, the difference in the oxygen isotopic composition between Upper Cretaceous water and modern water is reduced to about 1–5 per mil. However, a variation of several per mil in less than 100 million years seems to be quite large. In order to make a first approximation to the possible effect on the isotopic composition of ocean water of only the sedimentation processes, let us assume the total mass of ocean water to be $1{\cdot}37 \times 10^{24}$ g and the value of 0·2 cm/1,000 years to be the most probable one for the rate of accumulation of *Globigerina* ooze sediments. Assuming this as an average rate of sedimentation for the whole area covered by oceans, such a sedimentation, acting over a 100 million year period, would produce a variation in the average oxygen isotopic composition of sea water of about +2·5 per mil. This assumes an average value of 1·03 for the fractionation factor of oxygen (α) for the precipitation (either chemical or biological) of the different chemical compounds in the sediments and does not take into account the effect of the other variables which can greatly increase or reduce the isotopic effect.

If one tries to calculate t and δ_w from equation (1) and the Epstein equation it can easily be seen that the system cannot be solved with the values obtained from the measurements of fossils, either belemnites or other organisms.

Plotting δ_{18O} (PO_4^{3-}) against δ_{18O} (CO_3^{2-}), independently of the oxygen isotopic composition of the ocean water, a distribution similar to that in Fig. 2 should be obtained. In Fig. 4 it can be seen that this does not happen. In the case of samples 9 and 12 (the only two Palaeozoic fossils containing measurable amounts of both carbonate and phosphate) the values, which are very different from all the other results obtained, again lead to the conclusion that an extensive isotopic exchange occurred for both

carbonate and phosphate. It is extremely difficult to draw any definite conclusions in the case of the other fossils. It can be said, generally speaking, that belemnites show more positive δ values, both for carbonate and for phosphate. Further measurements will be required to decide whether this is the result of better preservation of the original oxygen isotopic composition or of other causes; it must be pointed out, however, that spot measurements of the oxygen isotopic composition of carbonate carried out on some Jurassic belemnites between the apical canal and the external surface showed that, in the specimens measured, some isotopic exchange occurred between carbonate and ground water. The results obtained from sample No. 23 are given in Fig. 5; similar results were obtained from other belemnites. The external portion of the rostrum and the portion near the apical canal show δ values which evidently arise from an isotopic exchange with ground water. Clayton and Stevens[9] obtained results of the same type from New Zealand belemnites.

Further measurements on phosphatic fossils of different ages are now in progress.

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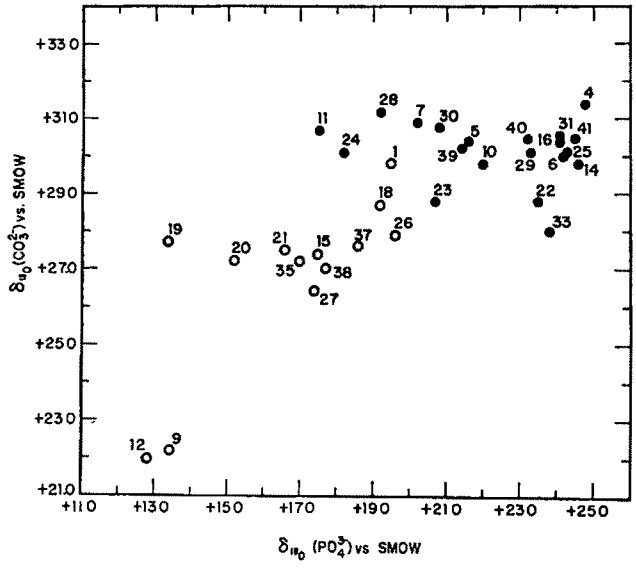


Fig. 4. Oxygen isotopic composition of co-existing carbonate and phosphate from marine fossils. Numbers refer to Table 2.

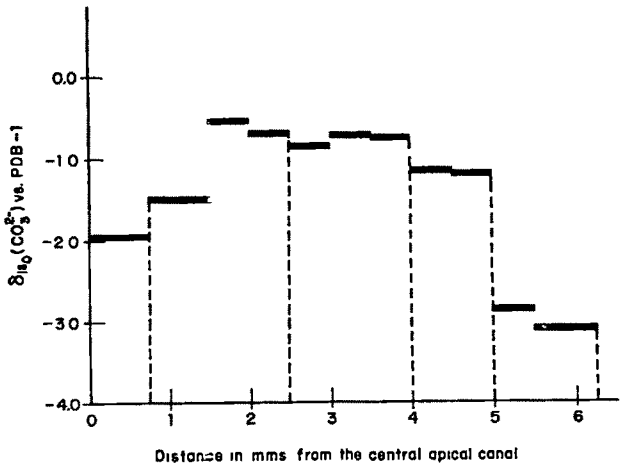


Fig. 5. Incremental carbonate δ's versus distance from the central apical canal in a Kimeridgian belemnite (sample No. 23). Dashed vertical lines indicate boundaries between growth rings.

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DETERMINATION OF THE ABUNDANCE OF CARBON AND NITROGEN ISOTOPES IN DUTCH COALS AND NATURAL GAS

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It has been pointed out by Patijn[1] that the presence of large deposits of natural gas in the north-east of The Netherlands may be the result of a recoalification process in the subsurface coal seams of great parts of The Netherlands. Although this theory would seem to give a reasonable explanation of the formation of methane, it does not account for the occurrence of varying and relatively large amounts of nitrogen in Dutch natural gas. Consequently, it has been assumed[2] that the nitrogen in this gas is of atmospheric origin.

In this connexion, it seemed of interest to determine the abundance of the carbon and nitrogen isotopes in coal samples of different seams as well as in samples of natural gas and mine gas.

The determination of the isotope ratios was carried out using an '*MS3*' mass spectrometer, manufactured by Associated Electrical Industries, Ltd., of Manchester, incorporating the modifications outlined by Shackleton[3]. This modified instrument is capable of measuring differences as small as 0·001 per cent in the isotope abundance of carbon-13 and nitrogen-15 in carbon dioxide and nitrogen samples, respectively.

For the measurement of the carbon isotope ratios, a primary and a secondary standard were used.

Diphenantryl ($C_{28}H_{18}$) was taken as the primary standard. The abundance of carbon-13 was determined by measuring the ratio of the peak heights at *m/e* 354 and 355. (This measurement was carried out on an A.E.I. type *MS* 9 mass spectrometer with high resolving power.) The resulting value, which was corrected for a small contribution of the dihydro- and tetrahydro-compounds, amounted to 1·079 ± 0·001 atom per cent. After combustion to carbon dioxide no difference could be detected between the values for the carbon dioxide contents of the primary and secondary standards; the latter standard was used for comparison with the carbon dioxide obtained by combustion of the coal and gas samples.

Atmospheric nitrogen was applied as a standard for the measurement of the nitrogen isotope abundance. This standard was based on a sample of pure nitrogen from an air separation plant and a sample of air which had been freed from oxygen by absorption in an ammoniacal solution of cuprous chloride. The two samples showed the same abundance of nitrogen-15 isotope.

The combustion of the coal samples, which had been dried at 100° C, was carried out in a stream of pure dry oxygen in an electrically heated tube. The resulting carbon dioxide was trapped on silica at −78° C. After the oxygen had been pumped off at −196° C, the carbon dioxide was desorbed in the sample bottle to be analysed in the mass spectrometer. In addition to this procedure, coal samples were burnt in a combustion calorimeter at an oxygen pressure of 20 atm. In this case also the oxygen was removed from the carbon dioxide by pumping at −196° C.

A definite amount of natural gas (300 ml.) was burnt by passing it in a closed circuit over copper oxide at a temperature of 850° C. After removal of the carbon dioxide the sample could also be used for the determination of the nitrogen isotope ratio.

Finally, the organic nitrogen of coal was converted to elementary nitrogen by Kjeldahl digestion and subsequent oxidation of ammonium chloride with sodium hypobromite.

The characteristic properties of the coal samples and the composition of the gas samples used in this investigation are summarized in Tables 1 and 2. Table 3 lists the isotope ratios for the different coal samples as well as for the gas samples.

From the results it appears that the variations in the isotopic ratio for coal samples with a wide stratigraphical range and a great difference in degree of coalification are only slight. In view of these variations, the mine gas samples should be considered as strongly depleted in carbon-13. The natural gas from the Slochteren bore, however, seems to be only slightly depleted in carbon-13.

It is well known[4] that in irreversible decomposition reactions the ^{12}C—^{12}C bond is slightly more liable to rupture than the corresponding ^{12}C—^{13}C bond. This isotope effect is dependent on temperature, in the sense that high temperature processes show little, if any, isotope discrimination.

The carbon isotope effect, which has been established for a number of decarboxylation reactions[5], may also be expected to occur in the demethanation process. Since the reaction with the heavy isotope lags behind that with the lighter isotope, and the amount of methane evolved is small compared with the total amount of carbon available (the amount of methane liberated in coalification from 34–31·5 per cent volatile matter is 12 m³ per ton of coal[6]), it may be expected that the mine gas methane is depleted in carbon-13. This is confirmed by our results.

The dependence of the isotope fractionation on temperature is demonstrated by the slight depletion of the methane obtained in the carbonization experiment with a sample of the Emma coal seam, which was heated at a rate of 3° C/min. The gas sample, predominantly consisting of hydrogen and methane, was taken in the temperature range 400°–600° C.

The depletion found for the Slochteren gas supports the view that the Dutch natural gas originates from a recoalification of coal seams located at depths between 3,000 and 5,000 m below the sea level[1]. During the recoalification at these depths the temperature may have been higher than in the coalification of the Limburg coal seams, and this may explain, at least qualitatively, the different degrees of depletion. (The estimated temperature gradient during the Carboniferous era is 12–15 m/°C (ref. 1). The present temperature gradient in The Netherlands in formations overlying the Palaeozoic is 25–30 m/°C.)

As regards the abundance of nitrogen-15 in the nitrogen from coal, compared with that in the nitrogen present in natural gas and mine gas, it is interesting to note that the nitrogen in the gases is enriched in nitrogen-15 (Table 4). On the other hand, ammonia from coke-oven gas, which

Table 1. ORIGIN OF COAL SAMPLES USED IN THIS INVESTIGATION

Origin	Depth (m)	Volatile matter (per cent)
Central Limburg, State Mine Beatrix, Bore LXX, seam *GB*9 (137 m below Wasserfall horizon)	1196·6–1197·3	7·4
Central Limburg, Peel region, Bore LXXVI, seam *GB*4a (160 m above Sarnsbank horizon)	831·9– 832·9	11·1
Central Limburg, State Mine Beatrix, Bore LXX, seam *GB*36/37 (46 m above Catharina horizon)	524·0– 525·2	15·8
South Limburg, State Mine Maurits, seam IX, *GB*29 (55 m below Catharina horizon)	660	21·5
South Limburg, State Mine Emma, seam *C*¹, *GB*45 (70 m below Domina horizon)	700	28

Table 2. COMPOSITION OF GAS SAMPLES

	CH_4	C_2H_6	C_3H_8	C_4H_{10}	C_5H_{12}	CO_2	H_2	N_2	Ar*	He*
Mine gas, State Mine Emma, seam *K*1, *GB*53*a* (V.M. 33 per cent)	98 5	0 05	< 0 01	< 0·01	< 0·01	0·3	0·01	1·0	0·01	< 0·01
Mine gas, State Mine Emma, seam *C*¹, *GB*45	98·4	0·05	< 0·01	< 0·01	< 0·01	0·4	< 0 01	1 0	–	–
Natural gas, Slochtern bore	81·6	2·2	0·3	0·1	0·1	0 9	–	14·8	0·011*	0·037*

* Calculated from the mass spectrometric analysis after combustion of the hydrocarbons and subsequent absorption of carbon dioxide.

Table 3. CARBON ISOTOPE ABUNDANCES FOR COAL AND GAS SAMPLES

Sample	Carbon-13* (atom per cent)	δ $^{13}C/^{12}C$ (per mil)†
State Mine Beatrix, bore LXX, seam *GB*9 (V.M. 7·4 per cent)	1·079[5]	0
Peel Region, bore LXXVI, seam *GB*4*a* (V.M. 11·8 per cent)	1·078[4]	− 1
State Mine Beatrix, bore LXX, seam *GB*36/37 (V.M. 15·8 per cent)	1·078[6]	0
State Mine Maurits, bore IX, seam *GB*29 (V.M. 21·5 per cent)	1·074[3]	− 4
State Mine Emma, bore *C*¹, seam *GB*45 (V.M. 28 per cent)	1·073[3]	− 5
Mine gas, State Mine Emma, seam *K*1, *GB*53*a*	1·037[7]	−39
Mine gas, State Mine Emma, seam *C*¹, *GB*45	1·063[3]	−14
Natural gas, bore Slochteren	1·071[3]	− 7
Methane from carbonization of a sample from State Mine Emma, seam *C*¹, *GB*45 (3 per cent by weight in relation to the origin coal)	1·073[3]	− 5

* Carbon-13 atom per cent = $\frac{^{13}CO_2}{^{12}CO_2 + ^{13}CO_2} \times 100$

† $\delta\ ^{13}C/^{12}C = \frac{^{13}CO_2/^{12}CO_2\ (\text{sample}) - ^{13}CO_2/^{12}CO_2\ (\text{standard})}{^{13}CO_2/^{12}CO_2\ (\text{standard})} \times 1{,}000.$

Table 4. NITROGEN ISOTOPE ABUNDANCE FOR COAL AND GAS SAMPLES

Sample	Nitrogen-15* (atom per cent)	δ $^{15}N/^{14}N$† (per mil)
Natural gas, Slochteren bore	0·367[3]	+18
Mine gas, State Mine Emma, seam *C*¹, *GB*45	0·364[3]	+12
Coal, State Mine Maurits, seam IX, *GB*29	0·362[3]	+ 2
Pyridine from coke-oven gas	0·361[6]	0
Ammonia from coke-oven gas	0·358[3]	− 9

* Nitrogen-15 atom per cent = $\frac{100}{2R + 1}$ with $R = \frac{^{14}N\ ^{14}N}{^{14}N\ ^{15}N}$

† $\delta\ ^{15}N/^{14}N = \frac{^{15}N/^{14}N\ (\text{sample}) - ^{15}N/^{14}N\ (\text{standard})}{^{15}N/^{14}N\ (\text{standard})} \times 1{,}000.$

in contrast with pyridine must have been formed by the rupture of C—N bonds, appeared to be depleted in nitrogen-15.

As it is difficult to conceive of chemical processes for the conversion of the organic nitrogen to elementary nitrogen and as, moreover, such processes would discriminate in favour of the lighter isotope, it must be concluded that the nitrogen in the natural gas was probably not originated in the coal.

The relation with the atmospheric nitrogen must, however, be left for further consideration. The enrichment in nitrogen-15 could be conceived to result from bacteria fractionation (for example, nitrogen fixation processes) which has already been reported for sulphur[7] and carbon isotopes[8], but so far no evidence in support of this view has been found.

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A HOMINID TOE BONE FROM BED I, OLDUVAI GORGE, TANZANIA

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During the excavation of site *FLK* North (*FLK N1*) at Olduvai Gorge, a hominid toe bone (Fig. 1, *a–e*) was recovered on November 16, 1961, by Mr. Joseph Mutaba, an assistant of Dr. L. S. B. Leakey.

The bone was found at Level 5, one of four superimposed occupation levels 3–3·5 ft. below the marker bed in the upper part of Bed I, and was associated with chopper tools and other implements typical of the Oldowan culture. In addition numerous broken mammalian bones were found. Despite extensive excavation no further hominid bones were found. From the site and stratigraphy, it seems that the toe bone has no direct connexion with the other hominid remains already known from the *FLK* area; the *Australopithecus* (*Zinjanthropus*) *boisei* skull and the *Homo habilis* cranial and post-cranial material from Bed I (including the foot) were recovered from much lower levels. On a purely stratigraphic basis, Hominid 16 from *FLK* II (Maiko Gully) is probably the nearest, as this skull seems to have eroded out of the clay 4–5 ft. above the marker bed; however, Maiko Gully is several hundred yards distant from *FLK* North.

The bone is the right hallucial terminal phalanx of an adult hominid, and is complete and undistorted. The reason for ascribing this bone to the right side is given below. In shape the bone is short, broad, and flattened from above downwards. It presents an oval base, which bears a divided facet for articulation with the head of the proximal phalanx, and lateral tubercles for the attachment of the collateral ligaments of the interphalangeal joint. The base is marked underneath with an arcuate tubercle for the attachment of the flexor hallucis longus, while the head bears a prominent tuberositas unguicularis. From above, the bone displays a broad head and a broad base separated by lateral asymmetrically arranged mid-shaft indentations. These are so arranged that the head of the bone is tilted to the right. Finally, and perhaps, from a functional point of view, of most significance, when viewed from the front, the bone is twisted in its length, a condition known as axial torsion (Fig. 1*e*).

The toe bone has been compared with samples drawn from populations of modern man (including Bushman), gorilla and chimpanzee; comparison has also been made with casts of the Neanderthal hallucial terminal phalanges from Kiik-Koba and Skuhl. Anatomically, the Olduvai terminal phalanx shows greatest resemblance to that of modern man, but its morphology is not precisely matched by any of the hominoid bones or casts which we have examined.

Metrically the most striking feature of the bone is its exceptionally high length/breadth index (Fig. 2); it is broader than it is long, a condition not found in modern man but shared with the Kiik-Koba bone alone.

It has been shown that valgus deviation of the terminal phalanx of the great toe is invariably present in modern human adults, as well as in children and stillborn foetuses drawn from several races[1,2]. This deviation, present even in the unshod, has been linked with man's unique gait[2]. Measurements made in a series of modern human and pongid bones confirm the previous findings and suggest that this deviation is more often absent or has a varus inclination in pronograde quadrupedal primates (Fig. 3).

The Olduvai toe bone has a deviation of 2° to the right (determined by a triangulation method and confirmed by the tilt of the head and the asymmetry of the mid-shaft waisting); this figure is outside the modern human range in our series and also in that of Barnett[2]. In order to decide for an unknown bone whether the direction of this deviation is varus or valgus, it is necessary to establish to which side the bone belongs. Wilkinson[3] states that in modern man the tubercle for the insertion of flexor hallucis longus extends more distally on the fibular side than on the tibial side of the terminal phalanx, and may even reach the unguicular tuberosity. On these grounds the Olduvai toe bone can be referred to the right side and the deviation thus confirmed as being valgus in direction.

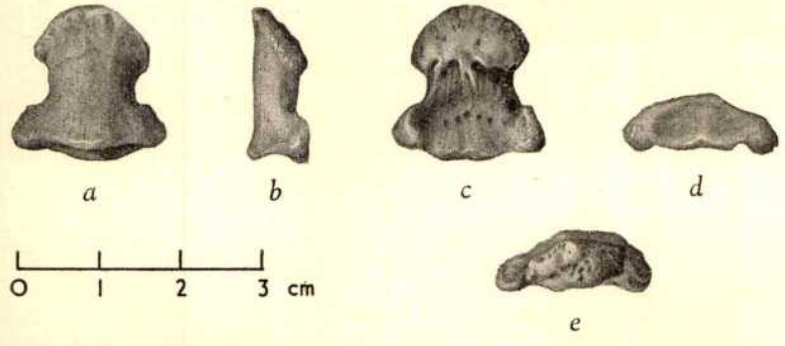


Fig. 1. The terminal phalanx from Olduvai Gorge (Hominid 10), drawn by Mrs. A. Besterman.

A further feature of the Olduvai ungual phalanx to which particular attention must be directed is its degree of axial torsion. Modern human terminal phalanges possess a range of lateral torsion which does not seem to have been described previously. An assessment of this characteristic and its possible phylogenetic and functional significance will be published elsewhere by one of us (M. H. D.); meanwhile the normal ranges of variation for this feature are given for modern man (including Bushman), gorilla, chimpanzee, and for two single examples of Neanderthal man (Fig. 4). The Olduvai and Neanderthal toe bones lie squarely within the modern human range for this feature, while the modern pongids

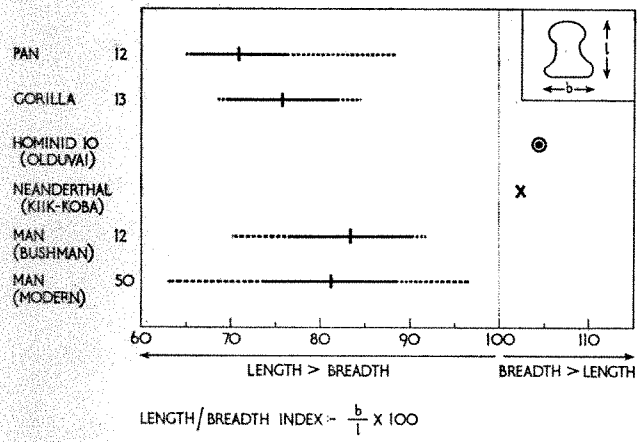


Fig. 2. The length/breadth index of the Olduvai terminal phalanx compared with that of modern man, Neanderthal man, modern gorilla and modern chimpanzee (mean, total and *S.D.* ranges).

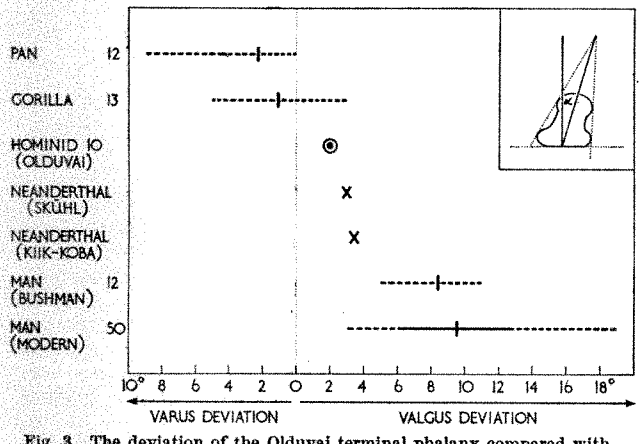


Fig. 3. The deviation of the Olduvai terminal phalanx compared with that of modern man, Neanderthal man, modern chimpanzee and modern gorilla (mean, total and *S.D.* ranges).

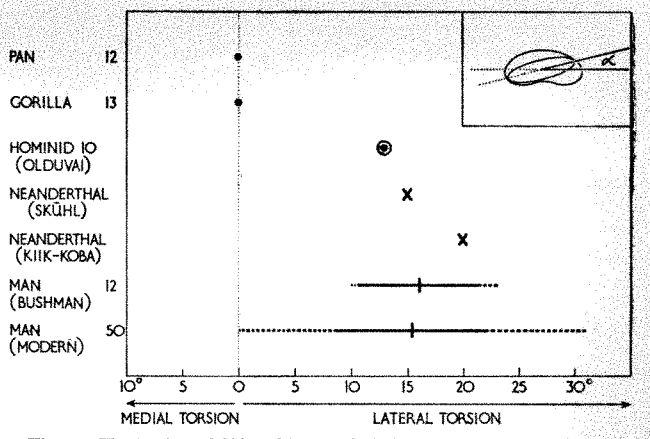


Fig. 4. The torsion of Olduvai terminal phalanx compared with that of modern man, Neanderthal man, modern chimpanzee and modern gorilla (mean, total and *S.D.* ranges).

examined show no lateral torsion at all. With regard to the magnitude of axial torsion of the terminal phalanx of the great toe there is, therefore, a clear distinction between those forms which have a pronograde quadrupedal gait and those with a plantigrade bipedal gait.

The implications of the combined presence of valgus deviation and lateral torsion are of the greatest functional significance. With respect to valgus deviation of the great toe it has been said: "Unfortunately the distal phalanx of the hallux is seldom recovered in fossil form, but the discovery of a valgus deviation in the bone of an extinct creature would provide evidence for a human type of gait"[2]. The discovery of lateral torsion, another specific feature associated with a modern type of gait, allied with valgus deviation in the same fossil bone strongly suggests, therefore, that the Olduvai toe bone belonged to an upright bipedal hominid possessing a plantigrade propulsive gait.

On the basis of an isolated toe bone it is clearly not possible to allocate the fossil to a known specific taxon; no comparable bone of any other form is available from Lower or Middle Pleistocene deposits. Nevertheless it must be borne in mind that a comparable type of gait has already been ascribed to the *Homo habilis* foot recovered from a lower level in the same deposits at Olduvai Gorge[3].

This terminal phalanx from Bed I (Hominid 10) provides further evidence for an advanced form of bipedal gait in the early hominid forms which were evolving in East Africa during the Upper Villafranchian.

We thank Dr. L. S. B. Leakey for the opportunity to study and report this new material.

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COMPUTER RAY TRACING OF THE OPTICAL SYSTEM OF THE SCHEMATIC EYE

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The theory of the schematic eye, as originally developed by Listing and Helmholtz and improved by Gullstrand and others[1-3], has played an important part in characterizing the image-formation capabilities of the normal, the myopic, and the hyperopic eye. A large part of present knowledge concerning the human eye arises from these remarkable investigations, even though Gaussian geometric optics, in which the aberrations are entirely ignored, were used.

Within the past decade, however, the method of ray tracing by means of electronic digital computers[4-7] has revolutionized the analysis of optical systems, since it

Table 1. PARAMETERS OF THE SCHEMATIC EYES (BASED ON GULLSTRAND'S MODEL)

Parameters	Relaxed[8]	Accommodated (at 300 mm)[11]	Relaxed[8]	Accommodated (at 100 mm)[8]
Radius of curvature (mm)	*A*	*B*	*C*	*D*
Cornea anterior surface	7·7	7·7	7·7	7·7
Cornea posterior surface	6·8	6·8	6·8	6·8
Lens anterior surface	10·0	7·89	10·0	5·35
Core-lens anterior surface	–	–	7·911	+2·655
Core-lens posterior surface	–	–	−5·76	−2·655
Lens posterior surface	−6·0	−5·564	−6·00	−5·33
Position of vertex of refracting surfaces (mm)				
Cornea anterior surface	15·6 (0·0)	15·6 (0·0)	0·0	0·0
Cornea posterior surface	16·1 (0·5)	16·1 (0·5)	0·5	0·5
Lens anterior surface	19·2 (3·6)	18·8653 (3·2653)	3·6	3·2
Core-lens anterior surface	–	–	4·146	3·8725
Core-lens posterior surface	–	–	6·565	6·5275
Lens posterior surface	22·8 (7·2)	22·9717 (7·3717)	7·2	7·2
Index of refraction				
Cornea	1·376	1·376	1·376	1·376
Aqueous humour	1·336	1·336	1·336	1·336
Vitreous body	1·336	1·336	1·336	1·336
Outer portion of lens	1·410	1·410	1·386	1·386
Core-lens	–	–	1·406	1·406

automatically encompasses all the aberrations that occur. Even though this technique has been extensively applied to the design of photographic lens systems, to our knowledge it has never until now been applied to the investigation of the optical system of the human eye. The purpose of this article is to describe our first results with such an approach. In particular, we present here the effects on image formation of variations in pupil size, refractive indices, and curvatures of the cornea and lens, utilizing schematic-eye values based on Gullstrand's model. (See Table 1.)

Computer Programme. A ray-tracing computer programme called *ARIS* (Automatic Retinal Image Study)[8] was formulated. The programme traces a large number of rays from an object point on the optical axis and computes for each the refraction as it passes through the surfaces of the cornea and the lens, including core surfaces, in a multi-layered lens. In addition, the programme can automatically adjust the object distance for retinal image formation, and can vary the curvatures and indices of refraction of the optical elements of the eye for iterative ray-tracing computations. Because *ARIS* does not involve the limitations of a Gaussian approximation, those parameters most commonly considered in optometry such as refractive power were not used, and a more suitable set of parameters was chosen. Fig. 1 shows the parameters actually used in *ARIS*.

Aperture Size Variation. Fig. 2*a* shows the effects of variation in aperture diameter (which is proportional to pupil size) on the location of the least-confusion disk with

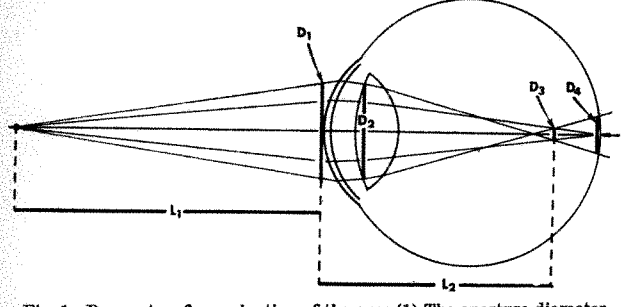


Fig. 1. Parameters for evaluation of the eye: (1) The aperture diameter at the anterior pole (D_1). (2) The pupil diameter (D_4). (Note that the pupil diameter is about 89 per cent of the aperture diameter at the anterior pole on a range from 1 mm to 8 mm.) (3) The least-confusion disk diameter (D_2). (Note, for the case of myopia, that the least-confusion disk is in front of the retina; and for hyperopia, at the back of the retina.) (4) The retinal image diameter (D_4). (5) The source distance (L_1) is that between the source point and the anterior pole of the eye. (Note that if the least-confusion disk corresponding to this source point is formed on the retina, the source distance is called the focusing distance.) (6) The least-confusion disk distance (L_2) is that between the least confusion disk and the anterior pole.

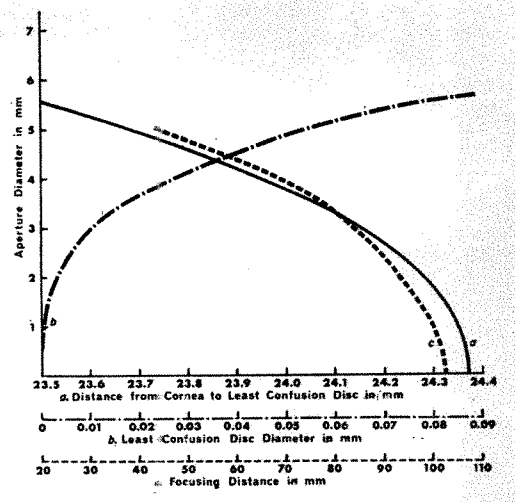


Fig. 2. Effect of pupil aperture diameter on (*a*) distance from cornea to least-confusion disk, (*b*) diameter of least-confusion disk, and (*c*) focusing distance. Curves (*a*) and (*b*) are based on the schematic eye parameters of column *C* of Table 1; curve (*c*) is based on column *D* of Table 1.

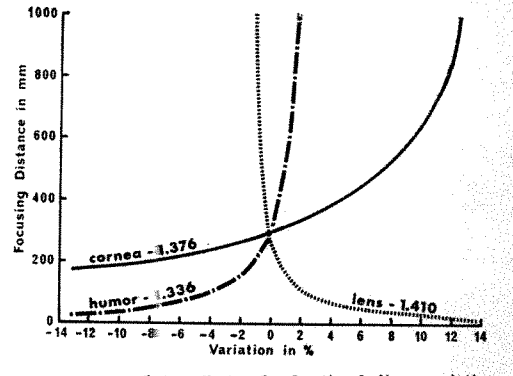


Fig. 3. Comparison of the effects of refraction-indices variation. The curves are based on schematic eye parameters of column *B* in Table 1.

respect to the cornea. The curves indicate that increasing the pupil size moves the least-confusion disk towards the cornea (that is, away from the retina in myopic eyes); this result is consistent with the phenomenon of "night myopia" for emmetropes and myopes, and indicates the expected improved image quality for hyperopes. Fig. 2*b* shows the results of varying aperture size on the diameter of the least-confusion disk. The figure demonstrates quantitatively how the image quality deteriorates with increasing pupil size. Fig. 2*c* shows the effect of aperture size on focusing distance. Here the focusing distance is measured from the front of the cornea to the point object which has its least-confusion disk on the surface of the retina. This figure illustrates that improving the ambient light, and thus reducing the pupil size, will improve vision at greater object distances. For example, well-lighted traffic intersections will enable direction signs to be read more easily from farther away for the same illumination on the sign.

Refractive Index Variation. Fig. 3 shows the effect of variation of the indices of refraction on the focusing distance. In this figure many results are combined in a single diagram. The point of intersection of all the curves represents the nominal indices and optical-element curvatures given in Table 1. Each curve shows the effect on the focusing distance of varying only the index associated with it, all other indices and curvatures remaining at the values given in Table 1. Note that as the index of both the vitreous and aqueous humour increases and approaches that of the lens, the curve rises asymptotically to a value beyond which a real focus in space will not occur. Reducing the index of the lens toward the value of the humour has a similar effect. These results are consistent with the

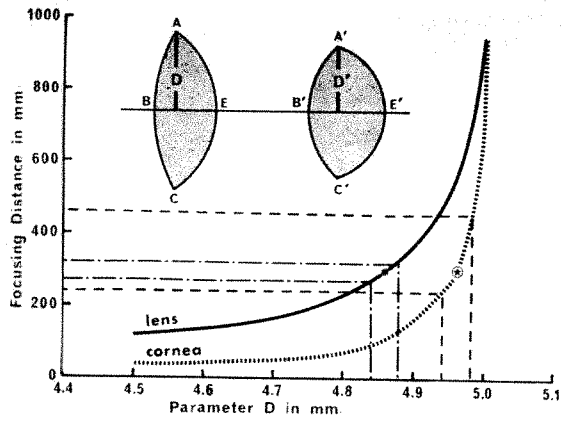


Fig. 4. Comparison of effect of curvature variation. Parameter D is the distance from centre to circumference of the lens or corneal section varied. The insert shows the manner in which D affects the curvature of the lens surfaces. The arc lengths ABC and AEC are held constant (at 10·47 mm and 8·83 mm respectively) as the distance D is varied. (For example, as D is reduced from 5·00 mm to 4·51 mm, the radius of curvature ABC is reduced from 10·00 mm to 5·62 mm and that of AEC is reduced from −6·00 mm to −4·77 mm.) Similarly, the curvatures (both positive) of the two surfaces of the cornea are varied by changing the centre-to-circumference distance. The solid curve shows the effect on the focusing distance of varying D for the lens with the curvatures for the corneal surfaces held at their original nominal values; the dotted curve shows the effect on the focusing distance of varying D for the cornea while the curvatures of the lens surfaces are held constant at their original nominal values (represented by the dark circle on the solid curve). Note how much greater is the effect of the same change in D applied to the cornea than applied to the lens. This is because the principal refraction takes place at the cornea-to-air interface. The curves are based on the schematic eye parameters of column B of Table 1.

claim that an increase in the index of refraction of the lens in diabetics can result in transient myopia[9].

Curvature Variation. Fig. 4 gives the effect of changes in the curvatures of the lens and cornea on the focusing distance. The circle on the lens curve and the star on the cornea curve are the "normal" values given in Table 1. The changes of curvatures are represented by varying a chord length D while the arc lengths subtended by the chord are held constant for the lens and for the cornea (see insert in Fig. 4)—although of course the lens and cornea do not change curvature in this manner. The curve for the lens gives the effect on the focusing distance of the change of curvature of the lens surfaces for the nominal corneal curvatures. The curve for the cornea gives the effect on the focusing distance of the change in curvature of the cornea surfaces for the nominal lens curvatures. Notice that the same change ΔD in chord length will have about four times the effect for the cornea as for the lens on the focusing distance.

Limitations and Conclusions. The limitations of our initial investigations using the programme *ARIS* (but *not* the limitations of the *ARIS* programme itself) are in the approximation (inherent in the Gullstrand schematic eye) of a centred system, spherical optical surfaces, and too few layers of the lens. In future investigations, however, it is intended to take these effects and many more into account, and also to include consideration of entire image formations and physical-optics interactions in the image formation[10]. The computer ray-tracing method presents a powerful new tool for investigating in detail the many effects that must be taken into account if a thorough understanding is to be obtained of the image-forming capabilities of the eye.

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SEQUENCE OF LINKAGE BETWEEN THE PROSTHETIC GROUPS AND THE POLYPEPTIDE CHAINS OF HAEMOGLOBIN

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ALTHOUGH the biosynthesis of tetrapyrroles and the synthetic pathways of polypeptides have been extensively investigated, little is known about the site and sequence of combination between haem and the polypeptide chains of haemoglobin. Haemoglobin A has a molecular weight of 64,000 and consists of four polypeptide chains, namely two alpha and two beta chains, each of which carries a haem molecule. In a recent publication, it has been shown that globin has a molecular weight of 32,000 and the composition alpha-beta[1]. Addition of more than 4 moles of haemin in a solution containing cyanide to two moles of globin resulted in a protein that was indistinguishable by a variety of criteria from cyanmethaemoglobin A and that had a sedimentation coefficient of 4·1 and 4·4, depending on protein concentration, thus indicating the formation of a haemoglobin molecule containing four polypeptide chains[1]. It was therefore concluded that the combination with haem favoured the association between the polypeptide subunits.

The overall reaction is given by formula 1*.

$$\underset{\text{Globin}}{2\,\alpha\beta} \xrightarrow{4\ \text{haem}} \underset{\text{Haemoglobin A}}{\alpha^h\alpha^h\beta^h\beta^h} \qquad (1)$$

On theoretical grounds, it could, therefore, be postulated that the combination between haemin and globin involves the following steps (formula 2)*

$$\underset{\text{Globin}}{2\,\alpha\beta} \xrightarrow{+1\,h} \begin{matrix}\alpha\beta^h\\ \alpha^h\alpha\beta\beta\\ \alpha\alpha\beta^h\beta\end{matrix} \xrightarrow{+1\,h} \begin{matrix}\alpha^h\alpha\beta\,\beta\\ \alpha^h\alpha\beta^h\beta\\ \alpha\alpha\beta^h\beta^h\end{matrix} \xrightarrow{+1\,h} \begin{matrix}\alpha^h\alpha^h\beta\beta\\ \alpha^h\alpha\beta^h\beta^h\\ \alpha^h\alpha\beta^h\beta^h\end{matrix} \xrightarrow{+1\,h} \underset{\text{Haemoglobin A}}{\alpha^h\alpha^h\beta^h\beta^h} \qquad (2)$$

Haemoglobin in solution is in rapid association–dissociation equilibrium according to the formula*

$$\alpha^h\alpha^h\beta^h\beta^h \underset{1}{\rightleftharpoons} 2\,\alpha^h\beta^h \underset{1}{\rightleftharpoons} 2\,\alpha^h + 2\,\beta^h \qquad (3)$$

The formula $\alpha^h\beta^h$ on addition of $2h$ to $2\alpha\beta$, therefore, appears unlikely, as this would immediately lead to the association, resulting in the formation of haemoglobin. This, in turn, would exclude the formation of compounds having three haems per four polypeptide chains. On the basis of formula 2, three classes of intermediary compounds in the combination between globin and haemin were to be expected, having the ratios haemin : polypeptides 1 : 4, 2 : 4 and 3 : 4, respectively. All possible formulae of these

* The symbol h denotes one molecule of haemin.

three intermediary compounds are listed in formula 2. A search for these postulated compounds was, therefore, started. The results reported here demonstrate the existence of such compounds and give some of their characteristics.

All chemicals used in the experiments were reagent grade. Globin was prepared as previously described[1]. Haemin was prepared according to the method of Labbe and Nishida[2]. Haemin solutions were obtained by dissolving 2–3 mg of haemin in 0·2 ml. of 0·1 N sodium hydroxide and subsequent dilution to the desired concentration, with 0·01 M sodium hydrogen phosphate containing 100 mg potassium cyanide/l., after the *p*H of this buffer had been brought to 7·5 with concentrated phosphoric acid.

Intermediary compounds were obtained by mixing 2 moles of globin with less than four times equimolar amounts of haemin. For the isolation of intermediary compound II (I.C. II) 2·0 moles of haemin were added per 64 mg of globin.

The intermediary compound II was isolated on a column 50 cm long and 9 mm in diameter, packed with '*CM* Sephadex *C* 50' (Pharmacia). The column was equilibrated with 0·01 M sodium hydrogen phosphate which contained 100 mg potassium cyanide/l. The *p*H of this buffer had been brought to 6·8 by addition of concentrated phosphoric acid. Haemin–globin mixtures containing about 100 mg protein in about 5 ml. were dialysed overnight against the same buffer and then applied to the column. Elution was carried out by a non-linear gradient, obtained by the addition of 0·02 M sodium hydrogen phosphate containing 100 mg potassium cyanide/l. to a 250 ml. constant-volume mixing chamber containing the buffer of *p*H 6·8.

Estimation of molecular weight was carried out on a column 150 cm long and 11 mm in diameter, packed with 'Sephadex *G* 100' bead form and equilibrated with 0·1 m *tris* hydrochloric acid buffer *p*H 7·2 containing 100 mg potassium cyanide/l. Bovine plasma albumin grade *A*, bovine pancreas ribonuclease grade *A* (both from Calbiochem), ovalbumin five times crystallized (Serva Entwicklungslabor Heidelberg, Germany), blue dextran (Pharmacia, Uppsala), HPO_4^{2-} ions and glycine served as markers. For the determination of the elution volume the first point of inflexion of the optical density curve at 280 m was taken. This closely approximated half the maximum height of the curve. The protein samples were applied in 0·2 ml. of the buffer used for equilibration. After the sample had sunk into the 'Sephadex' gel, the walls of the glass column above were rinsed three times with 0·2 ml. of buffer and the sample afterwards eluted with the same buffer. Starch gel electrophoresis was performed in a cold room at 4° C with a discontinuous buffer system at *p*H 8·6 using 0·076 M *tris* (2-amino-2-hydroxymethylpropane-1 : 3 diol) and 0·005 M citric acid for the gel and 0·30 M boric acid – 0·06 M sodium hydroxide for the buffer vessels[3]. Spectra were obtained with a model *DK* 2 Beckman automatic recording spectrophotometer.

Globin A prepared by our methods always shows on starch gel electrophoresis a main zone comprising more than 95 per cent of the protein and a minor zone with slightly greater anodic mobility. In contrast to the main band, this zone gives a slight blue colour reaction with benzidine stain, thus indicating the presence of haem.

Addition of 4 μmoles haemin to 2 μmoles globin (mol. wt. 32,000) results in the formation of a protein which behaves electrophoretically like haemoglobin A (Fig. 1). Addition of less than 4 μmoles of haemin to 2 μmoles of globin results in the formation of three electrophoretically distinct bands which, at *p*H 8·6, show an anodic mobility between globin A and haemoglobin A (Fig. 1). The addition of small amounts of haemin (approximately 1 μmole per 2 μmole globin) leads to the intensification of the slightly more acidic band (Fig. 1, iii) (intermediary compound I). Addition of 2 μmoles of haemin leads to the formation of another still more acidic protein in much larger quantities (intermediary compound II), with a corresponding decrease in density of the globin and intermediary compound I bands. At this point, a third weak band with still greater anodic mobility (intermediary compound III) frequently appears and often a band with the mobility of haemoglobin A as well. On further addition of haemin, the globin band, and often also the band of intermediary compound I, disappear. The increase in density of the haemoglobin A band is accompanied by the successive disappearance of the bands of intermediary compounds II and III, until finally, by adding more haem, all the protein has been converted into haemoglobin A (Fig. 1). Intermediary compound II appeared as the quantitatively most important band and its isolation was attempted by chromatography on '*CM* Sephadex' (Fig. 2). Fig. 3 shows the starch gel analysis of pooled fractions which had been concentrated afterwards with concurrent dialysis*. The proteins in each of the sections obtained by pooling a number of chromatographic fractions could be converted into haemoglobin A by sufficient addition of haemin (Fig. 3).

The section containing only intermediary compound II was further investigated. Spectral analysis revealed that the compound had an ultra-violet absorption spectrum which closely resembled that of cyanmethaemoglobin[1] (Fig. 4). The spectrum in the range of visible light showed a marked quantitative difference although its shape was very similar to cyanmethaemoglobin (Fig. 4), and the positions of the absorption maxima and minima are identical (Fig. 4). Thus absorbance ratios of the protein maximum at 274 mμ and the maxima and minima in the range of visible light showed a marked difference.

The results of gel filtration on 'Sephadex *G*100' are summarized in Fig. 4. In the range of protein concentra-

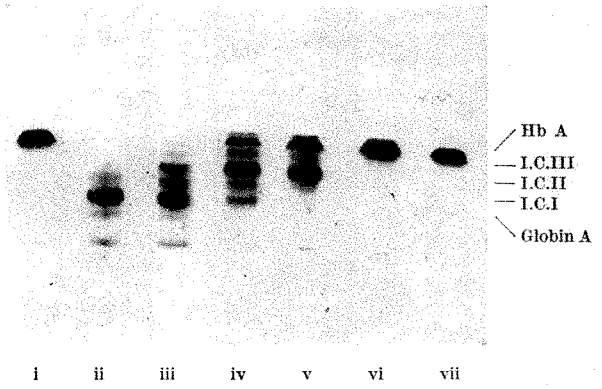


Fig. 1. Starch-gel electrophoresis. Discontinuous buffer system *p*H 8·6 (ref. 3). i, Haemoglobin marker; ii, globin A; iii, globin A + 1 mol haemin/2 moles globin; iv, globin A + 2 moles haemin/2 moles globin; v, globin A + 3 moles haemin/2 moles globin; vi, globin A + 4 moles haemin/2 moles globin; vii, haemoglobin marker.

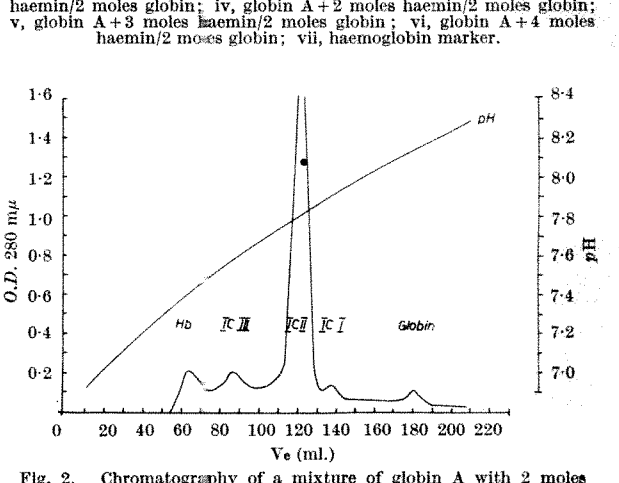


Fig. 2. Chromatography of a mixture of globin A with 2 moles haemin/2 moles globin. Conditions are given in the text.

tions used there was only very little concentration dependence of the elution volume for haemoglobin and none for globin. By comparison with the elution volumes of the marker proteins, a molecular weight of haemoglobin of 46,000 and one of 28,000 for globin has been determined. The elution volume of I.C.II and that for haemoglobin were identical. While the haemoglobin peak was almost symmetric the I.C.II peak showed some trailing.

By adding increasing but small amounts of haemin to a globin solution, the existence of three intermediary compounds—postulated on theoretical grounds—has been demonstrated. Quantitatively, the formation of I.C.II and haemoglobin is favoured. This is in agreement with the results from equilibrium studies between methaemoglobin and another haem-binding apoprotein (apomyoglobin). From their data[5] the authors concluded that the fixation of the first haem enhanced the affinity for the second. The fourth and last haem was captured more readily than any of the other three. The change of quantitative distribution between the intermediary compounds on addition of small amounts of haem further supports that concept.

From the results of gel filtration experiments it can be concluded that the I.C.II molecule must consist of four polypeptide chains, and that in size and shape it must closely resemble haemoglobin. Since I.C.II can be completely converted into haemoglobin by mere addition of haem, it must contain two alpha and two beta chains. The molecular weight of 46,000 obtained for haemoglobin —due to its subunit structure in rapid association–dissociation equilibrium—is in agreement with the results obtained by others[6]. The increased trailing observed in the elution pattern of I.C.II, however, is in keeping with the idea that dissociation into two subunits occurs more

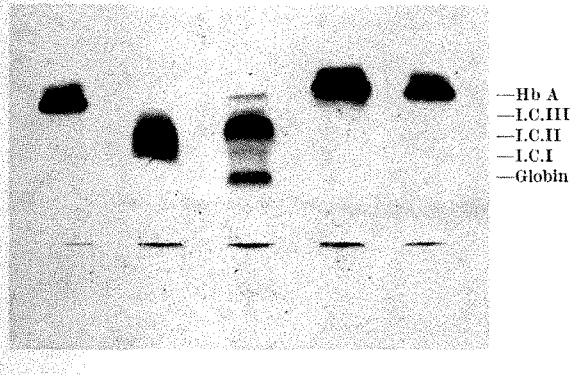


Fig. 3. Starch-gel electrophoresis conditions as in Fig. 1. i, Hb A marker; ii, isolated I.C.II; iii, globin A + 2 moles haemin/2 moles globin; iv, I.C.II + excess haemin; v, Hb A marker.

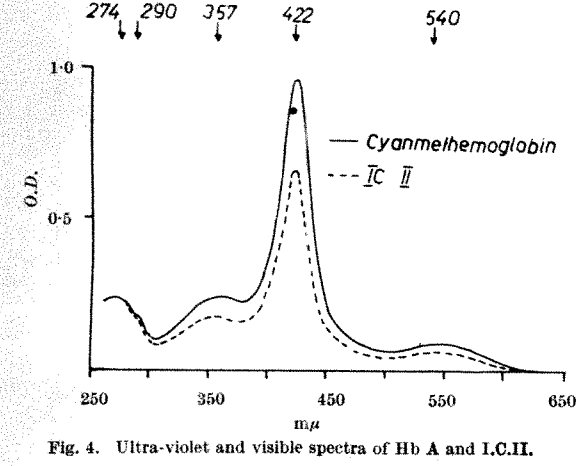


Fig. 4. Ultra-violet and visible spectra of Hb A and I.C.II.

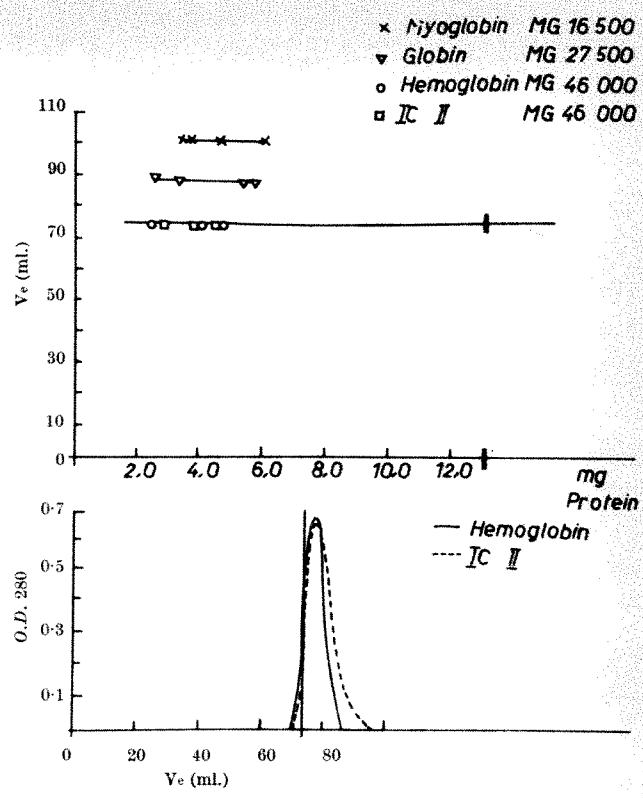


Fig. 5. Gel filtration on 'Sephadex G100'. Dependence of the elution volume on total amount protein added in 0·2 ml. (top). Elution volume and shape of eluted peak for Hb A (Cyanmet) and I.C.II (bottom).

readily in I.C.II than in haemoglobin. The concept that I.C.II must have a molecular arrangement similar to that of haemoglobin is further supported by the observation that the maxima and minima of optical density have the same position, and the differences are merely quantitative ones.

The electrophoretic mobility of the band designated I.C.II is in keeping with the idea that it contains half the prosthetic group found in haemoglobin. The fact that addition of half the amount of haem needed for primary saturation of all specific binding sites leads to the formation of I.C.II (Fig. 2) supports the same idea. From the data presented in this paper we cannot decide, however, which of the three formulae postulated for I.C.II applies (formula 2). Experiments to solve this question are in progress.

Data presented in a recent publication[7] led to the conclusion that the amino-acid sequence alone was sufficient to determine the conformation of apomyoglobin in solution. Addition of haem led to a specific conformational change (significant gain of helix). From the overall formula $2\,\alpha\beta + 4\,h \longrightarrow \alpha^h\alpha^h\beta^h\beta^h$ it is obvious that addition of haem to apohaemoglobin leads to a considerable configurational change. The observation that I.C.II—in contrast to globin—consists of four polypeptide chains with an arrangement closely resembling that in haemoglobin implies that an important part of this rearrangement had taken place when the second haem is fixed.

I thank Prof. F. Leuthardt, who has kindly permitted this work to be carried out in his laboratory, and Mrs. V. Pünter and Miss R. Hess for technical assistance.

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T SYSTEM CONNEXIONS WITH THE SARCOLEMMA AND SARCOPLASMIC RETICULUM

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THE interfibrillar spaces of muscle fibres contain two membranous systems, the *T* system and the sarcoplasmic reticulum. Practically all the *T* system is oriented transversely between the fibrils. The sarcoplasmic reticulum can be looked on as longitudinally oriented between the *T* system. The terminal segments of the sarcoplasmic reticulum are, however, in close apposition to the *T* system. The space between the sarcoplasmic reticulum and the *T* system at the sites of apposition is remarkably constant and this space contains electron-opaque material which has been variously described. Recent investigations[1–3] have shown that the membranes of the *T* system are continuous with the sarcolemma at the level of the *Z* band in fish and frog skeletal muscle fibres. Similar continuity of the *T* system membranes and sarcolemma in muscle fibres of other vertebrates might also exist[1–3]. Nevertheless, it seems desirable to make observations within the group of vertebrates (reptiles, birds, and mammals) with the *T* system located at the junctions of the *A* and *I* bands.

In the interior of vertebrate muscle fibres the *T* system (*T*) is closely associated with the terminal segments of the sarcoplasmic reticulum (*R*) on either side of it. The central *T* element and the lateral *R* elements have been called a triad[4]. Numerous investigations have called attention to the constancy of the space between *T* and *R*. Lines of density alternating with less dense areas in the space between *T* and *R* have been reported[5] for bat cricothyroid muscle fibres. It has been suggested[6] that structures crossing the space between *T* and *R* are membrane-like in rat gastrocnemius muscle fibres. On the other hand, it has been suggested[7] that the apposed membranes of *T* and *R* might be fused to form tight junctions like those formed by the apposed plasma membranes of various cells[8–10]. However, it was pointed out[7] that fusion of apposed membranes of *T* and *R* has not been observed clearly. These differences in electron-microscope observations[6,7] emphasize the need for further investigations of structures at the site of the apposed membranes of *T* and *R*. The need for these investigations is enhanced by the efforts at present in progress to determine the relationship of *T* and *R* to transmission of excitation from the sarcolemma to the interior of the fibre. In the work reported here an attempt is made to show triple-layered apposed membranes of *T* and *R* and to describe the connexions between these membranes.

For investigations concerned primarily with connexions between the sarcolemma and *T* we used glutaraldehyde fixation which has been shown to be highly effective for such investigations[1–3] on fish and frog muscle fibres. Small bundles of rat gastrocnemius muscle fibres, removed from animals anaesthetized with 'Nembutal', were tied to 'Plexiglas' stays and fixed in glutaraldehyde by the method of Sabatini *et al.*[11] slightly modified. The fibres were post-fixed with osmic acid by the Palade method as modified by Caulfield[12]. The tissues were dehydrated for 10 min in 50 per cent alcohol and then placed overnight in 70 parts absolute alcohol and 30 parts saturated aqueous solution of uranyl acetate. After completion of dehydration with 95 and 100 per cent alcohol, the fibres were embedded in 'Maraglas'. Sections of the tissues were made with a Porter-Blum microtome and stained with lead hydroxide solution according to the method of Lever[13]. Sections exhibiting grey interference colours and near 500 Å in thickness were examined with a Siemens 'Elmiskop I' electron microscope.

For some of the observations on apposed membranes of *T* and *R*, fibre bundles were fixed with osmic acid, embedded in methacrylate and otherwise prepared as described. However, in these cases the sections were cut somewhat thicker and showed silver interference colours.

The characteristic apposition of membranes of *T* and *R* was found in triads at the subsarcolemmal level. This structural feature of *T* and *R* membranes made identification reliable even in sections that showed only one of the two regions of apposed membranes. In rat gastrocnemius muscle fibres the tubules of the *T* system, viewed in cross-section, are usually elliptical in shape. The long axis of the ellipse is 800–1200 Å and the short axis is 200–500 Å. Assuming that the size and shape of the opening of the *T* system in the sarcolemma are similar to interfibrillar size and shape of *T*, as seen in cross-section, it should be possible to make sections 500 Å thick whose thickness would fall within the long axis of the opening and exclusive of the boundary walls on either side of the opening. Sections thus oriented would show distinct continuity of *T* with the sarcolemma.

The connexions between *T* and the sarcolemma are found near the junctions of the *A* and *I* bands in moderately stretched fibres. The connexions occur at points where the sarcolemma shows an invagination. The connexions thus formed show a continuity between the extracellular fluid and the fluid contents of *T* tubules. The electron micrograph in Fig. 1 shows the location of the *T* system (*T*) in relation to the *A*–*I* junction and the *Z* band. The invagination of the sarcolemma and its connexion with *T* are located below the star in Fig. 1. The plane of the section is about 15° oblique to the longitudinal axis of the fibre and the terminal segment of sarcoplasmic reticulum (*R*) is shown on the left side only of *T*. In this

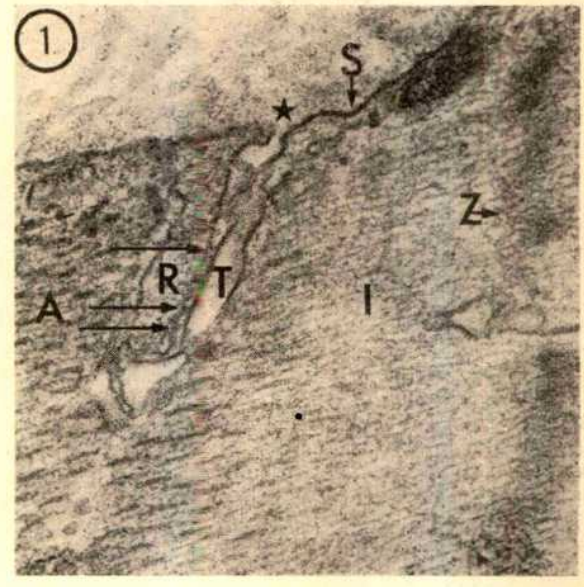


Fig. 1. Electron micrograph of rat gastrocnemius muscle fibre with the plane of the section passing about 15° oblique to the longitudinal axis of the fibre. The star indicates a point where the sarcolemma (*S*) is invaginated and connected with membranes of the transverse system (*T*). The arrows point toward dense zones across the space between apposed membranes of *T* and a terminal segment of sarcoplasmic reticulum (*R*). *A*, *A* band; *I*, *I* band; *Z*, *Z* band. Glutaraldehyde fixation. 'Maraglas' embedding. (× *c.* 90,000.)

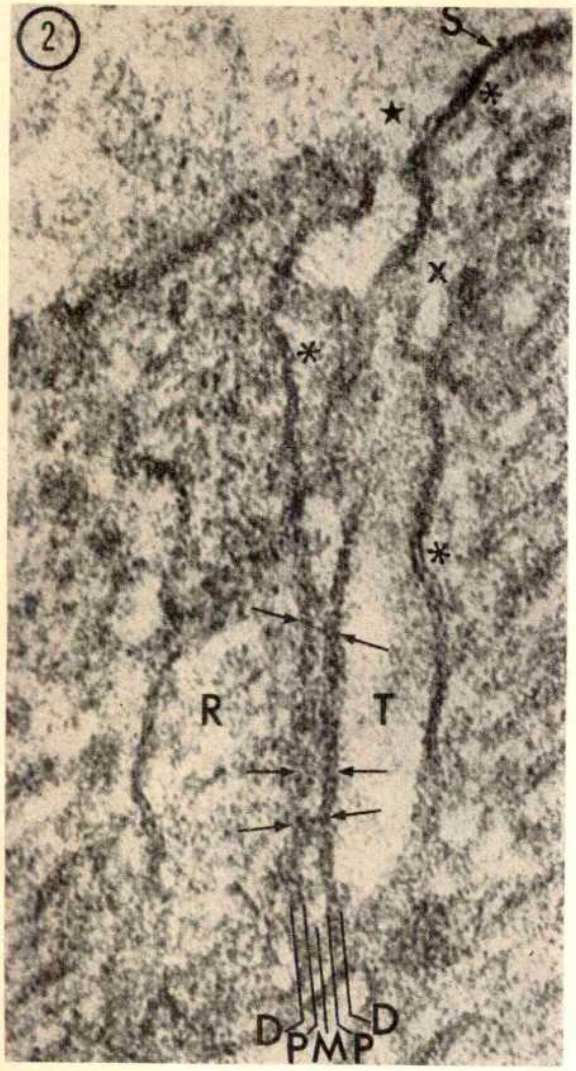


Fig. 2. A high magnification of a part of Fig. 1 showing details of the connexions of the T system with S and R. The asterisks indicate points where the triple layers of S, T, and R membranes are visible. The X marks a point where the section apparently includes a tangential view of the convoluted tubular wall of T. The points of the arrows are placed at the boundaries of distal outer dense layers of triple-layered apposed membranes of T and R. M, Medial space between membranes of T and R; P, proximal outer dense layers of triple-layered membranes; D, distal outer dense layers. (× c. 260,000.)

section the width of the space between apposed membranes of T and R is not uniform throughout. In more favourable sections showing all three elements of the triad, like the one shown in Fig. 4, the space width between T and R is more constant. At a few points in Fig. 1 dense zones across the space between apposed membranes of T and R are indicated by arrows.

A part of the section in Fig. 1 is shown at higher magnification in Fig. 2. The continuity of the extracellular fluid with the fluid content of the T tubule is clearly illustrated (star). The point indicated by X apparently shows a convolution of the T tubule that causes a tangential view of the tubular wall to be included in the section. It is possible to observe the similarity of membrane structure in sarcolemma, T, and R at points of favourable section (asterisks). The membranes show two outer dense layers separated by a less dense middle layer. The total thickness of the three layered membrane of T is about 65 Å. Similar thickness is shown for the R membrane apposed with T. The sarcolemma is slightly thicker and more heavily stained. The triple layers in T and R membranes and the space between these membranes can be seen at the same level of apposition in some cases (arrows). For purposes of presentation in this investigation the space between apposed membranes of T and R and the structures within this space will be regarded as medial. The nearest and farthest outer dense layers of the apposed three layered membranes will be designated as proximal and distal, respectively. The arrows in Fig. 2 mark the boundaries of distal outer dense layers of T and R membranes at points where the triple layers can be seen in each of the apposed membranes. Now, if fusion of the type described for plasma membranes of adjoining cells[8–10] were present, the proximal outer dense layers of apposed membranes should be fused to form the intermediate layer of a five-layered structure. This would mean that the total thickness of the two membranes at the points of fusion would be somewhat less than twice 65 Å, say 120 Å. Examination of the distances between arrows in Fig. 2 shows an average distance slightly greater than 200 Å. If the location and measurements are correct, fusion of the proximal outer dense layers of the apposed membranes of T and R is impossible at the points indicated by arrows. Actually the dense zones across the space between T and R suggest intermittent connexions of the apposed membranes.

In preparations fixed initially with osmic acid, openings in the sarcolemma continuing as T tubules were never found. On the other hand, extension of T to the subsarcolemmal region of the fibre was seen several times. An example of T and R at the subsarcolemmal level, as they appear in preparations fixed with osmic acid, is shown in Fig. 3. The triple layers of the T membrane are rather well depicted in the wall of the tubule on the left side. The triple layers of the apposed membrane of R are also visible at some points on the left side of T. Here again, as in the case of Fig. 2, the apposed membranes of T and R occupy a distance (space between points of arrows) that is incompatible with the concept of fusion of these membranes. The thickness of the apposed membranes of T and R is about 75 Å in Fig. 3. This means that the combined thickness of the two apposed membranes is about 150 Å. The distance between the points of the arrows is about 225 Å. There appear to

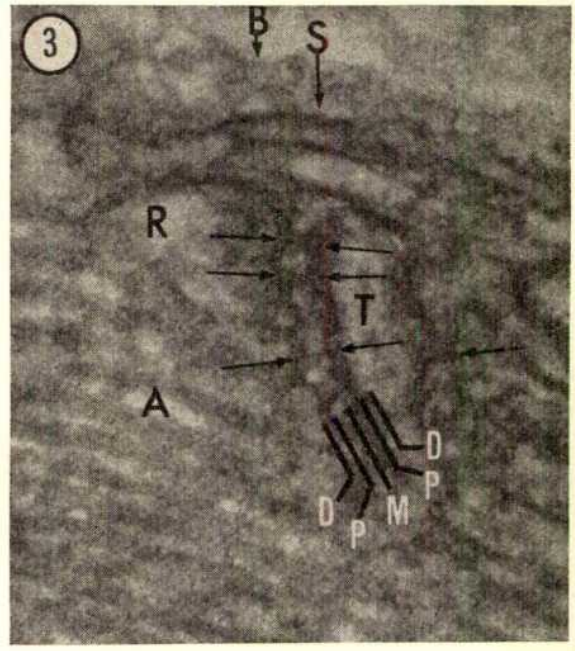


Fig. 3. Approximate longitudinal section of a fibre showing T and R near the sarcolemma (S). The points of the arrows mark the outer boundaries of apposed membranes of T and R and the connexions across the space between T and R at these points suggest the presence of triple layers typical of membrane density pattern. A, A band; B, basement membrane. Labels M, P, and D, as in Fig. 2. Osmic acid fixation. Methacrylate embedding. (× c. 280,000.)

be four rather clearly defined connexions across the space between the apposed membranes of T and R on the left side of T. At the points indicated by arrows, a membrane-like appearance of the connexions is suggested by parallel lines of density separated by a less dense space. At one point on the right side of T a membrane-like connexion between T and R is indicated by an arrow.

Fig. 4 is shown as an example of a section passing through the three elements of the triad. The section includes a relatively long longitudinal view of T. At points where the triple layers of the apposed membranes of T and R are visible at the same level there is evidence that the membranes are not fused. The arrows which mark the boundaries of distal outer dense layers of triple-layered apposed membranes are separated by about 220 Å, more or less. The thickness of the membranes is about 75 Å. This means that the distance between the proximal outer dense layers of the apposed membranes should be about 70 Å. Measurements show the average of this distance to be approximately 70 Å. Connexions across the space between T and R can be seen at four points on the left side of T, and at seven points on the right side of T. Membrane-like structures, which are comparable to those previously reported[14], are visible within R. Connexions of these membrane-like structures with the limiting membrane of R facing T (asterisks) apparently contribute to the scalloped appearance of this limiting membrane.

Contact of the T system with the sarcolemma has been shown by Revel[5] with a unique method of preparation. Fibres of bat cricothyroid muscle fixed with osmic acid in the presence of 0·01 M calcium chloride and with no subsequent staining showed rather marked electron density in the T system and the sarcolemma, in sharp contrast with other structures of the fibres which showed virtually no staining at all. Since the terminal segments of the sarcoplasmic reticulum did not show electron density, it was possible for Revel[5] to conclude that the

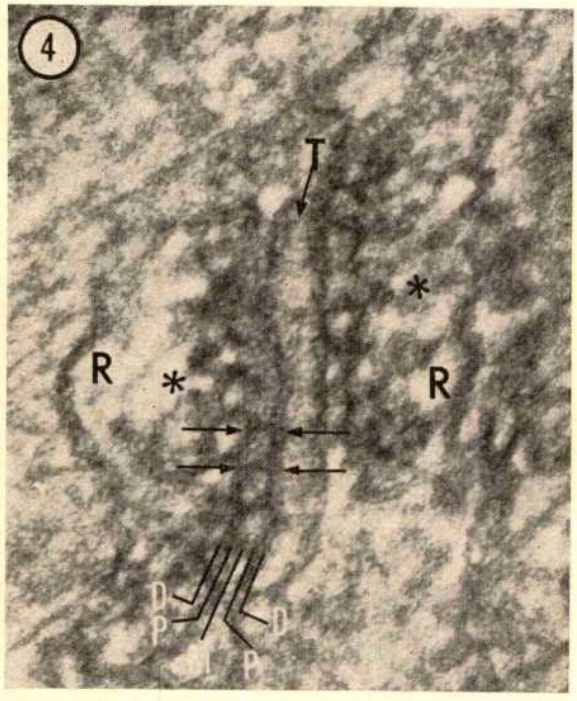


Fig. 4. Longitudinal section of a fibre showing a longitudinal section of T with R on either side of T. Four connexions across the space between T and R can be seen on the left side of T and seven on the right side. The points of the arrows mark the outer boundaries of apposed membranes of T and R. The asterisks are placed near connexions of membrane-like structures within R with the limiting membrane of R facing T. Labels M, P, and D as in Fig. 2. Osmic acid fixation. Methacrylate embedding. (× *c.* 275,000.)

structure making contact with the sarcolemma at the level of the A–I junction is indeed the T system. H. E. Huxley[15] demonstrated the presence of ferritin molecules only in the T system of frog muscle fibres soaked in Ringer's solution containing ferritin, before fixation with osmic acid. The failure to observe openings in the sarcolemma at the level of the T system led Huxley[15] to the suggestion that osmic acid fixation brings about the closure of openings of the sarcolemma into the T system. The presence of the ferritin molecules in the T system was taken as evidence that the openings are present in the living state of the fibre. At approximately the same time, Franzini-Armstrong and Porter[1] were able to demonstrate invaginations of the sarcolemma to form connexions with membranes of the T system in fish muscle fibres when they used glutaraldehyde as the fixative. The observations recorded here illustrate the superiority of glutaraldehyde over osmic acid as a fixative for demonstrating connexions between the sarcolemma and the T system.

The dense zones that traverse the spaces between apposed membranes of T and R have been designated as connexions although they have not been unequivocally established as connexions in this investigation. However, there is substantial support of the view that the dense zones are indeed connexions. In the first place, the space between the apposed membranes of T and R narrows at the level of the connexions. Secondly, the connexions appear to be membrane-like at favourable points of observation. The average distance between apposed membranes of T and R at the level of connexions (paired arrows in Figs. 3 and 4) is 73 Å. The average distance at the level of the less dense areas, interspersed between these connexions, is 92 Å. The fact that the distance at the level of connexions is shorter suggests that a holding force is exerted on the apposed membranes at this level. Such a holding force would require the dense zones to be connexions, rather than studs, between the apposed membranes of T and R. In attempts to demonstrate the presence and nature of connexions between the apposed membranes of T and R, methacrylate has a slight advantage over 'Maraglas' as an embedding material. The connexions across the space between apposed membranes are more clearly defined (Figs. 3 and 4) and a membrane-like appearance is indicated in some of these connexions. The exact nature of the connexions is not yet determined, but the results recorded here do support the view that these connexions are membrane-like. Furthermore, the results (Figs. 2, 3 and 4) show definitely that fusion of proximal outer dense layers of three-layered apposed membranes of T and R is not found with either of the two methods of preparation used in this investigation.

It is true that an anatomical tight junction, such as the one described for fused plasma membranes of adjoining cells of various types[8–10], would provide the structural requirements for the electrotonic spread of current from T to R if it were present in the apposed membranes of T and R. However, as we have suggested in the proposal of a structural model for transmission of excitation from the sarcolemma to T and R of muscle fibres[16], there is another possible structural design at the site of the apposed membranes of T and R that might form a "physiological tight junction" compatible with the electrotonic spread of current from T to R. This model is based on the observations that connexions between T and R are seen in cross-sections of the triad like that shown in Fig. 2 of a previous report[14] and in longitudinal sections as described here. These observations support the hypothesis that the less dense areas between connexions across T and R are completely surrounded by the connexions and the apposed membranes of T and R. The less dense areas may be thought of as completely sealed off from the surroundings like a sealed compartment of a honeycomb. Under these conditions, the less dense areas between T and R would be completely separated from the surrounding sarcoplasm. This structural design, if

present, might permit the effective spread of electrotonic current from T to R.

As already suggested[17], the continuity of extracellular fluid with the fluid in the T tubules indicates that the sodium concentration in these fluids is relatively high when compared with intracellular fluid. This means that a sodium concentration gradient might exist across the membranes of the T system. In the presence of an excitatory impulse the sodium gradient could play a part in current flow through the membranes of the T system.

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TWO COMPONENTS OF THE CARDIAC ACTION POTENTIAL

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THE notion of a cardiac action potential composed of fast and slow components (Fig. 1*A*) has often appeared in recent literature. Attempts to interpret the findings in terms of ionic movements across the cell membrane[1–6] contain evidence which suggests that (*a*) the initial fast component is related to an increase in sodium (chord) conductance and (*b*) a decrease in potassium conductance underlies the slow component. The data presented in this article add support to this concept and bring out the point that the slow component can exist and propagate even when the fast component seems to be absent.

Inspection of the transmembrane action potentials recorded from various regions of the heart suggests that, on the basis of the voltage-time relationship, they can be classified in three main groups which differ in terms of the amplitude and the maximum depolarization rate of the initial fast component of depolarization. In the rabbit atrium the three groups are represented by (*a*) cells of the pectinate muscles and crista terminalis; (*b*) perinodal cells; and (*c*) slowly conducting cells of the sinoatrial or atrioventricular nodes. Transmembrane action potentials recorded from ordinary atrial muscle fibres of the first group exhibit an initial fast phase of depolarization which extends to the peak of the action potential (Fig. 1*B*). In records obtained from perinodal cells, the initial fast depolarization finishes well before the peak of the action potential (arrow, Fig. 1*C*). The remainder of the depolarization is effected at a much slower rate. In records obtained from "true" nodal cells (sinoatrial nodal pacemaker and N region of atrioventricular node[15]) no initial fast depolarization can be detected. The transmembrane action potential arises with a very low velocity and the contour of the upstroke is smooth (Fig. 1*D*).

The cells which constitute these three groups reveal a number of differences in their pattern of response to various agents. The local application (by hydraulic ejection from a micropipette) of perfusion medium containing acetylcholine (ACh) causes the changes shown in Fig. 2. It can be seen that the initial fast component of the action potential, when present, is not depressed by ACh (Fig. 2*A* and *B*). Records of the first derivative of the transmembrane action potential show that the maximum rate of depolarization is actually increased because of the hyperpolarization caused by ACh (ref. 7.) On the other hand, the slow component of these action potentials is markedly depressed both in amplitude and duration (Fig. 2*A* and *B*). In "true" nodal cells, where a significant fast component is not present, ACh causes partial or complete abolition of electrical activity by depression of the isolated slow component (Fig. 2*C*). This action results in a failure of propagation in both the sinoatrial and atrioventricular nodes.

Local depolarization of atrial muscle fibres by the hydraulic application of potassium-rich perfusion medium from a micropipette decreases both the maximum rate of rise and the peak potential of the initial fast component of the transmembrane action potential, as would be expected[1]. The decrease in peak potential of the fast component exposes the rising phase of the slow component, which seems to be relatively insensitive to experimental modification (Fig. 3*A*). In the case of nodal cells, where

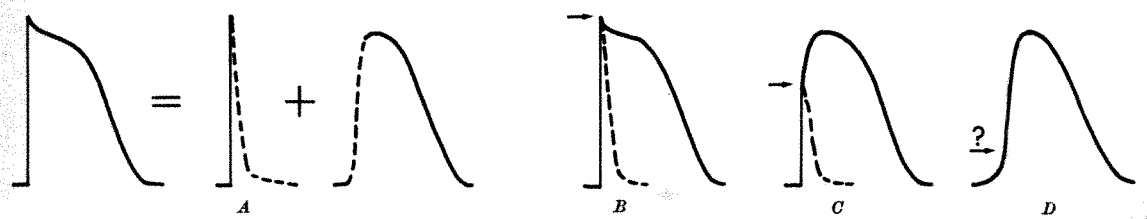


Fig. 1. *A*, Hypothetical representation of the summation of the fast and slow components of depolarization to produce the transmembrane action potential recorded from a common cardiac fibre (first group). *B*, *C*, and *D*, Schematic representation of typical transmembrane action potentials recorded from each of the three cell types described. The solid lines depict the actual voltage-time course of the record; the interrupted lines depict the assumed voltage-time course of the potential due to the isolated fast component; arrows show the peak potential attained during the fast component.

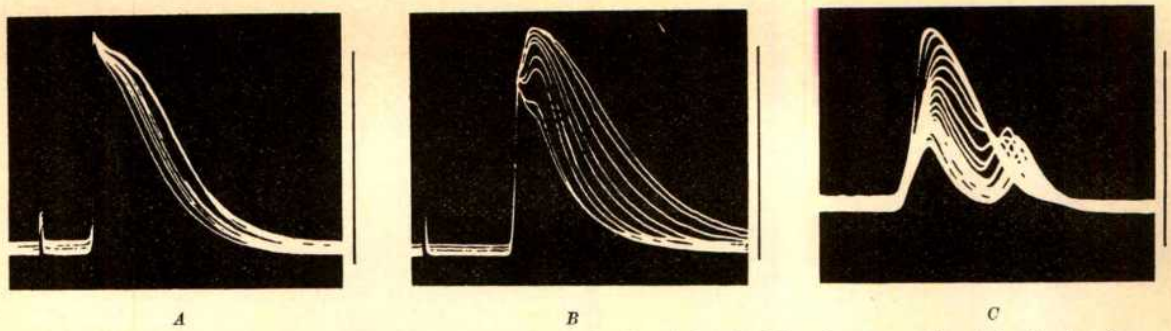


Fig. 2. Transmembrane action potentials recorded from electrically driven isolated rabbit atrium preparations during local application of ACh-containing medium. (*A*) Atrial muscle fibre, (*B*) perinodal fibre and (*C*) fibre in the *N* region of the atrioventricular node. Each record shows a number of superimposed traces recorded just before and during the local application of ACh. Note that in *A*, although there is a decrease in duration of the action potential, the upstroke and peak potential are essentially unchanged. In *B*, although the initial fast component of the upstroke is unchanged, loss of the slow component causes a decrease both in peak potential and duration. In *C*, application of ACh causes a progressive decrease in the rate of rise of the entire upstroke and also a decrease in peak potential. Also shown in *C* are potential changes by re-entrant excitation; these are not pertinent to the present discussion. Vertical lines at the right side of each block represent 100 mV.

an initial fast component is not apparent, partial depolarization by excess potassium does not significantly change the maximum rate of rise of peak potential caused by the isolated slow component (Fig. 4). Using an atropinized preparation, local depletion of sodium (by hydraulic ejection from a micropipette of perfusion medium in which 95 per cent of the sodium is replaced by choline chloride) causes the expected marked decrease in the rate of rise

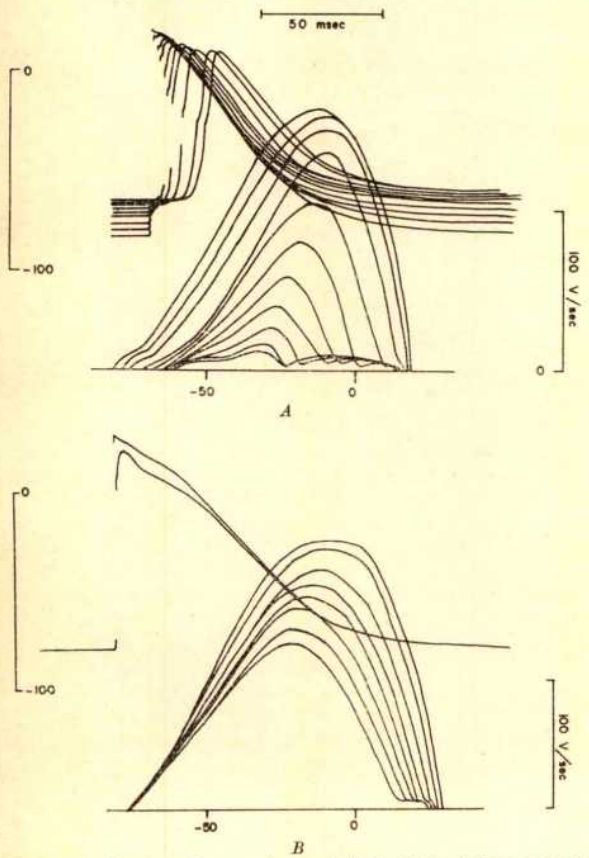


Fig. 3. *A*, Transmembrane action potentials obtained from a single atrial fibre of an electrically driven isolated rabbit heart preparation. Tracings of records showing the effects of local application of potassium-rich solution. Above are shown changes in transmembrane potential as a function of time and, below, the rate of change of potential as a function of voltage during the upstroke. The voltage calibration applies to the upper traces and represents 100 mV; the abscissa for the lower traces shows potential in mV. Note that, although there is a marked decrease in the maximum rate and magnitude of depolarization due to the initial fast component, the time course and magnitude of depolarization due to the slow component remain essentially unchanged. *B*, Tracings of records, as in *A*, showing the effects of local ejection of a sodium-depleted (95 per cent) medium. Effective sodium concentration at recording site is higher than in the ejected fluid due to its dilution in the surrounding medium. Note the small changes in peak potential and duration. See text for description.

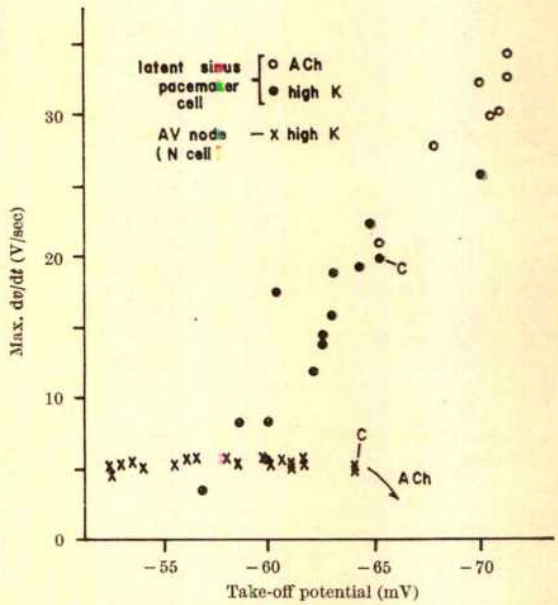


Fig. 4. Plot of maximum rate of rise (max. dv/dt) in V/sec, against resting potential before excitation (take-off potential), in mV. Circles depict data obtained from a latent sinus pacemaker cell which exhibited an initial fast component similar to that shown in Fig. 1*C*. Crosses relate to data obtained from an atrioventricular nodal *N* cell, like the one shown in Fig. 1*D*. Note that depolarization leaves practically unaltered the rate of rise of the slow component upstroke of the nodal cell. C, Control.

and in the amplitude of the depolarization due to the initial fast component. Sodium depletion, however, has a much smaller effect on the slow component as is shown by the minimal change in its peak potential (Fig. 3*B*).

The findings which we have summarized suggest to us that two distinct and different mechanisms may be responsible for depolarization of cardiac muscle or for the development of the cardiac action potential. The first mechanism is probably the voltage dependent and regenerative increase in sodium conductance which has been shown to be the cause of the action potential upstroke in the squid giant axon[8] and other excitable tissue. In atrial, ventricular and Purkinje fibres this mechanism is the cause of the rapid phase of the upstroke of the action potential, and its transient nature accounts for the voltage-time variation of the fast component. It shows the expected dependence on transmembrane potential and extracellular sodium concentration, but is not directly influenced by ACh. The second mechanism is sensitive to ACh and is relatively uninfluenced by changes in membrane potential; it is less sensitive to a decrease in extracellular sodium concentration. This mechanism appears to be the sole cause of the slowly rising action potentials

recorded from true nodal fibres. It also contributes the plateaux of the action potentials of Purkinje, atrial and ventricular fibres and is responsible for the final slow portion of the upstroke of perinodal fibres. We have no direct evidence concerning the nature of the mechanism underlying the slow components; however, its sensitivity to ACh suggests that it may be a decrease in potassium conductance. This hypothesis is supported to some extent by the fact that the apparent resistance to weak current pulses applied through microelectrodes is markedly reduced only during the fast component. This holds true even when the fast component constitutes a minor part of the upstroke of the action potential; in such cases, the polarization resistance is high for the remainder of depolarization[9].

Whatever mechanism is responsible for the slow component, it is apparent from the peculiar behaviour of sinoatrial and atrioventricular nodes[10] that its appearance in isolation imposes electrophysiological characteristics on these tissues which may differ markedly from those observed in the presence of the fast component.

This work was supported by grants from the U.S. Public Health Service.

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REFLEX INNERVATION OF THE TEMPORO-MANDIBULAR JOINT

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THE neurology of the temporo-mandibular joint has never been adequately investigated, despite its obvious importance to dental physiologists and surgeons. It is not generally appreciated, for example, that many of the functional hypotheses of orthodontic surgery relating to this joint[1] are merely inferential, and are not founded on any systematic experimental knowledge of its innervation. Appropriate clinical investigations, however, over the past 10 years suggest that its nerve supply probably contributes to the reflex regulation of the masticatory musculature[2–5] and to mandibular kinaesthetic perception[6]. In order to remedy this situation, we have examined the innervation of the temporo-mandibular joints in twenty cats with macroscopic, microscopic and neurophysiological techniques similar to those used previously in this laboratory for the investigation of the knee[7], ankle[8] and laryngeal[9,10] joints.

Extrinsic articular innervation. Both macrodissection and microdissection studies show that the innervation of the temporo-mandibular joint in the cat is provided[11] from articular branches of the auriculo-temporal, masseteric and deep temporal nerves, in a pattern similar to that in man[6,12]. The joint is not innervated from the facial nerve.

The auriculo-temporal nerve supplies the posterior and postero-medial aspects of the joint capsule and the related articular fat pad as it curves behind the joint. Further articular branches are provided to the lateral capsule and ligament of the joint as the more distal part of the nerve crosses the ramus of the mandible below the joint, ascending (deep to the masseter muscle) in the periosteum covering the lateral surface of the ramus. The masseteric nerve gives off numerous articular branches which form a plexus on and within the anterior capsule of the joint, as the nerve passes through the sigmoid notch of the mandible to innervate the masseter muscle. The deep temporal nerves, which innervate the temporalis muscle, give off variable articular branches within the substance of the muscle that pass from its deep surface to the anterior and anteromedial aspects of the joint capsule.

Articular nerve endings. Neurohistological examination (in gold chloride, frozen and paraffin silver and methylene blue preparations of fresh tissue) of the terminations of the articular nerves in the capsular, ligamentous, synovial, meniscal and adipose tissues of the temporo-mandibular joint reveals that the nerve endings are similar in type to those in the limb joints[7,8,11]. In terms of the relative numerical proportions of the different types of ending (*q.v.*), the temporo-mandibular joint is more like the ankle[8] than the knee[7] joint.

Myelinated afferent fibres of medium (6–12μ) diameter in the articular nerves terminate in clusters of encapsulated Type I and Type II corpuscles[5,7,13] embedded in the fibrous capsule on all aspects of the temporo-mandibular joint. The population density of these end organs is greatest posteriorly and postero-laterally, and least in the medial part of the joint capsule. As in the limb joints[13], the fibres innervating the Type II corpuscles are larger than those innervating the Type I corpuscles (8–12μ compared with 6–9μ). Type II corpuscles are also present in large numbers in the fat pad situated posterior to the temporo-mandibular joint, as is the case with articular fat pads elsewhere in the body[7,8,13]. Larger (up to 17μ in diameter) myelinated afferent fibres, located mainly in the articular branches of the auriculo-temporal nerve, innervate Type III corpuscles located on the superficial surface of the lateral capsular ligament: these end organs are identical with those located in joint ligaments elsewhere[5,7,8,13].

The many unmyelinated and small (less than 5μ) diameter myelinated fibres present in all the articular nerves terminate in plexuses and free nerve endings that are distributed throughout the fibrous capsule and the posterior fat pad related to the joint, and in the walls of the articular blood vessels. These unorganized terminals constitute the Type IV variety of articular nerve ending[5,13] and are identical with those in other joints[7–10].

No nerve endings are present in the synovial tissue of the temporo-mandibular joint, as is the case with all other joints[7–10,13]. Likewise, there are no nerve endings of any type in the joint meniscus[4,11,14], apart from some Type IV terminals distributed in the most peripheral fibrous layers of the disk at the points where it is attached to the joint capsule—as is the case with the menisci in the knee joint[7,13].

Neurophysiological investigations have shown[5,8,10,13,15] that in all joints the corpuscular end organs are mechanoreceptors with differing behavioural characteristics. Discharges from Type I corpuscles are slowly adapting and from Type II corpuscles rapidly adapting, in response to changing mechanical stresses in the tissues in which they lie; both types of corpuscle have relatively low thresholds. The Type III corpuscles in the joint ligaments are high threshold, slowly adapting mechanoreceptors that are activated only at the extremes of joint movement. The non-corpuscular Type IV terminations provide the articular pain receptor system[4,5,7–9,13].

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Articular mechanoreceptor reflexes. The reflex influence of discharges from the Type I and Type II mechanoreceptors in the temporo-mandibular joint on the masticatory muscles was examined in multichannel electromyographic investigations of motor unit activity in these muscles during passive movements of the isolated temporo-mandibular joint, using cats under light monitored pentobarbitone anaesthesia. The experimental procedure was similar to that used in this laboratory for the demonstration of articular reflexes in the larynx[10] and at the ankle[8].

After inducing anaesthesia, all the supra- and infra-mandibular muscles were detached at their insertions from one half of the mandible—care being taken to preserve the articular and muscular branches of the related nerve trunks. Pairs of fine, stainless steel electrodes (insulated except at their tips) were then inserted into the temporalis, masseter and pterygoid muscles on each side of the head, from which simultaneous bipolar electromyograms were recorded on a Grass polygraph. An insulated brass rod was wired to the left horizontal ramus of the mandible; this rod was manipulated between stop bars to produce measured passive movements of the mandible in opening, closing, protrusive, retrusive and side-to-side movements (the latter with the mandible in the open and in the closed, but not dentally occluded, position), after immobilization of the animal's skull and body in the prone position by attachment to a brass scaffold bolted to the operating table. The degrees of jaw movement were measured with a transparent protractor on enlarged photographs of the movement, made with an electronic flash camera. In some experiments, the mandible was left intact so that both sides were moved simultaneously, the muscular activity on the intact side being used for synchronous comparison with that on the operated side; in others, the symphysis menti was divided and each half of the mandible was moved independently—the other side being immobilized by fixation to the scaffold. In various experiments, simultaneous records were made of the animal's electrocardiogram, electroencephalogram, respiratory movements, pulmonary ventilation and limb muscle activity for monitoring purposes. The animals were kept warm on a thermostatically controlled, heated operating table.

Preliminary investigations showed that the basic patterns of muscular activity during each of the passive jaw movements listed, performed with the muscles intact, are similar in the lightly anaesthetized cat to those associated with comparable voluntary jaw movements in man[2,3]. After the muscles had been detached on one side, reciprocally related facilitation and inhibition of motor unit activity could still be provoked (although at lower amplitude) in the detached muscles by movements of the ipsilateral temporo-mandibular joint, even though occlusion of the teeth was avoided during such movements. In these circumstances the muscular responses to passive movement cannot arise from myotatic or dental receptors, and for this reason they must be evoked by discharges from the mechanoreceptors in the capsule of the moving temporo-mandibular joint. Furthermore, analysis of the electromyograms shows that the motor unit responses thus provoked fall into two categories—rapidly adapting (within 1·5 sec or less) effects that occur only at the onset of movement, and slowly adapting effects that develop during movement and persist at lower intensity throughout maintenance of the new jaw position. The characteristics of these responses are totally different from the very slowly adapting myotatic reflex responses produced in response to direct stretch of the detached muscles, but are identical with the rapidly and slowly adapting articular reflex responses previously demonstrated in the muscles of the limbs[8] and larynx[10].

Confirmation of the articular origin of the residual motor unit responses to mandibular movement in the detached jaw muscles is provided by the following additional observations that were made in these experiments.

(1) The responses to all movements were reversibly abolished, in the detached muscles only, for periods of 50–60 min by infiltration of the capsule of the ipsilateral temporo-mandibular joint with a local anaesthetic solution (1 per cent lignocaine hydrochloride)—as is the case with other articular reflexes[8,10,15]. In this respect, it may be noted that local anaesthesia of the temporo-mandibular joint also produces impaired mandibular positioning in man[6].

(2) The responses to all movements were irreversibly abolished, in the detached muscles only, by electrocoagulation of the capsule of the ipsilateral temporo-mandibular joint—as is again the case with other articular reflexes[8,10,15].

(3) The responses to all movements were irreversibly abolished, in the detached muscles only, by ipsilateral condylectomy or disarticulation of the ipsilateral temporo-mandibular joint.

(4) Similar responses could be provoked in the detached muscles (in the absence of jaw movement) by direct traction on the residual capsule of the ipsilateral temporo-mandibular joint after removal of the head of the mandible from the joint cavity.

(5) As is the case in the limbs[8], the articular reflex responses in the jaw muscles are abolished selectively by a slight increase in the degree of general anaesthesia, at a stage when the direct myotatic reflexes in the same muscles remain unimpaired. They return when anaesthesia is allowed to diminish again, but are totally absent as long as anaesthesia is maintained at the stages customarily used for surgical procedures.

The last of these observations (with others) suggests that the articular mechanoreceptor reflex system related to the temporo-mandibular joint is polysynaptic, in contrast to the monosynaptic nature of the masticatory myotatic reflex arc[16]. Articular reflex systems related to other joints are polysynaptic[8,10,15]; and preliminary microelectrode recordings of the potentials evoked in the lower brain stem nuclei by passive movements of the jaw in cats[17] add further support to the view that they are likewise polysynaptic in relation to the temporo-mandibular joint.

These investigations demonstrate that the articular tissues of the temporo-mandibular joint are liberally provided with rapidly and slowly adapting corpuscular mechanoreceptors, and with an elaborate non-corpuscular pain receptor system. They further show that discharges from the articular mechanoreceptors make significant contributions (both facilitatory and inhibitory) to the reflex co-ordination of masticatory muscle activity during jaw movements in all directions. Clearly, then, changes in temporo-mandibular articular reflex activity must now be considered as contributing to the production of various clinical types of abnormal jaw relationships and to the variety of masticatory responses to different types of orthodontic treatment.

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IMMUNOLOGICAL DETERMINATION OF HUMAN FOLLICLE STIMULATING HORMONE

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ALTHOUGH immunoassays for human chorionic gonadotrophin (HCG) and human pituitary luteinizing hormone (LH) have been described[1], similar assays for human follicle stimulating hormone (FSH) have not yet been reported. The reason for this is probably the lack of a specific antiserum. Recently there have been a number of reports on haemagglutination-inhibition experiments using antisera raised to human pituitary FSH preparations[2,3], but correlations of biologically and immunologically determined FSH were not demonstrated.

Extensive investigations in this laboratory[4] have shown that antisera to human pituitary FSH fractions neutralized the biological activity of FSH but contained immunological contaminants, notably to albumin and to an antigen common to most preparations of HCG. The interference from these contaminants in haemagglutination-inhibition tests could be largely removed by absorption with albumin and HCG, but the estimations of FSH activity remained unreliable and there were possibly still other immunological contaminants. New methods of removing these contaminants were recently reported[5]. This article describes haemagglutination-inhibition tests carried out for FSH with two antisera raised against the purified preparations.

Human pituitary FSH was prepared as previously described[6], and was purified by partitioning in the system urea: ammonium sulphate : ethyl 'Cellosolve' and electrophoresis on starch gel. The solutions used for immunization contained about 400 mg IRP-HMG (international reference preparation of human postmenopausal gonadotrophin), that is, 57 I.U. FSH/ml., and showed no detectable LH activity by the ovarian ascorbic acid depletion method, or albumin by the haemagglutination-inhibition test. Other human pituitary FSH preparations were fractions obtained at various stages of the extraction procedure. Human pituitary LH fractions were kindly supplied by Dr. A. S. Hartree, Cambridge[7]. Samples of urinary FSH materials were mainly stock preparations of this laboratory, obtained either from the urine of postmenopausal women (HMG) or from the urine of normal menstruating women by means of kaolin absorption and reprecipitation from ammonium acetate solutions with alcohol. Other urinary extracts (Fig. 3) were prepared from daily collections of early morning specimens of urine, when 20 ml. of urine were precipitated by benzoic and tungstic acids[8], washed in alcohol and ether and finally dissolved in 0·5 ml. of saline to make a fortyfold concentrate of the original urine. In all immunological determinations, HMG 613 (N.V. Organon–Oss, Holland) was used as standard preparation.

Inoculations were carried out by combining intramuscular injections of antigen suspended in Ramon's adjuvants with intravenous injections of antigen adsorbed on to bentonite. The total amount of antigen given to one animal was approximately equivalent to 6 g IRP-HMG.

Two antisera obtained from two rabbits showed similar although not identical properties. After absorption with normal human serum they completely inhibited the action of the equivalent of 3 mg IRP-HMG when given to immature mice in doses down to 0·0012 ml. In agar diffusion and immunoelectrophoretic experiments, the two antisera formed two precipitin lines with most human pituitary and urinary FSH preparations. One of the lines —that which occurred in the albumin position—was not observed when albumin-free HMG 613 was used as antigen. LH did not form a precipitin line with one of the antisera but, when added in increasing amounts to the antiserum as an absorbent, one of the two lines formed with FSH was progressively removed. Precipitin lines formed with HCG were weak with either antiserum.

The haemagglutination-inhibition method was used for quantitative immunological estimations. Sheep red cells were tanned and later coated with a 'Cellosolve' partition fraction of human pituitary FSH because the most purified fractions of starch gel did not appear to become sufficiently firmly attached to the cells. The coating was performed by incubating 1 ml. of a 10 per cent suspension of tanned cells with 100 μg antigen for 1 h at 37°–40° C.

Comparisons were made between the biological activity of several preparations as tested by the ovarian augmentation assay and immunological activity using a human pituitary FSH haemagglutination system. All results were expressed in terms of IRP-HMG. Fig. 1 shows FSH determinations of ten consecutive collections of urines over a period of 72 h from a female patient. The pattern of the biological activity of the extracts is reflected fairly well in the pattern of the immunological results although the reflexion is not quantitatively precise.

Fig. 2 shows the degree of correlation between the biological and immunological estimations of FSH activity of twelve HMG extracts, one extract from specimens of urine from a menstruating woman and six human pituitary extracts. In order to calculate the mean immunological values with upper and lower limits, the method developed by Butt *et al.*[1] was applied. Except for those few urinary extracts with low biological activity which showed no immunological activity up to 5 mg/ml., only crude acetone dried pituitary powder gave a largely misleading immunological result. The majority of biological and immunological figures were of the same order within each pair (Table 1). Because crude pituitary powder contains LH as well as FSH, purified fractions of LH were next studied. It was found that they reacted strongly in this system—the results did not correlate with either the LH activities or the FSH activities of the fractions (Table 2).

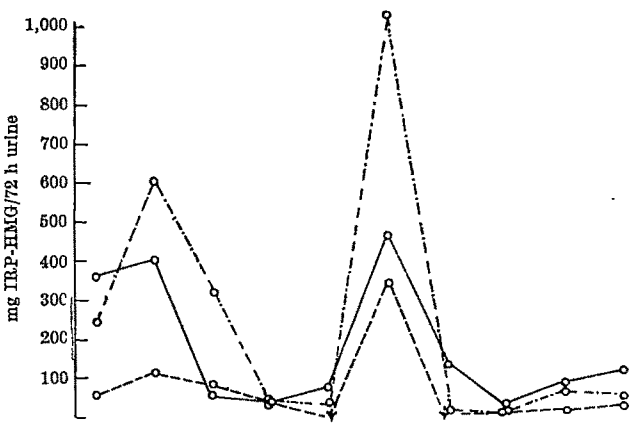


Fig. 1. Extracts of ten consecutive collections of urine (each taken over a period of 72 h) from a female patient assayed by the ovarian augmentation test (———), and by two antisera independently in the haemagglutination test (– – – –, and ——·——).

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Table 1. BIOLOGICAL AND IMMUNOLOGICAL ESTIMATIONS OF FSH ACTIVITIES

	Immunological activity in terms of mg IRP-HMG/mg or /ml. or /ampoule	Biological activity in terms of mg IRP-HMG /mg or /ml. or /ampoule
Human pituitary FSH fractions		
Crude extract from acetone-dried pituitary powder, freeze dried	3100 (2900–12000)	240
CM-cellulose fraction (ref. 6)	547 (450–1800)	1000
CP 1 fraction (ref. 6)	2200 (1800–7000)	3000
Partition fraction (ref. 5)	1100 (700–3000)	3000
Starch gel fraction 3 (ref. 5)	360 (300–1200)	200
Starch gel fraction 4 (ref. 5)	47 (30–150)	< 30
Human urinary FSH extracts		
Crude HMG (lab. standard 1)	2·7 (1·8–7·5)	1·17
Crude HMG (23 + 30)	2·2 (1·8–7·5)	1·56
„ HMG (29/3)	1·0 (0·8–3·0)	0 04
„ HMG (104)	1·14 (0·9–3·5)	2·34
„ HMG (1)	4·4 (3·6–14·4)	4·4
„ HMG (2)	2·0 (1·8–7·2)	8 5
„ HMG (Y 3)	72·0 (54–216)	56·0
„ HMG (lab. standard 2)	20·0 (18–75)	22·7
Purified HMG A (Paines and Byrne, Ltd.)	30·9 (22–94)	86·0
Purified HMG B (Paines and Byrne, Ltd.)	39·5 (28–112)	176
Purified HMG (Pergonal)	29·0 (22·5–90 0)	30
Purified HMG, organic phase (Pergonal)	10·0 (7·5–3·0)	15
Crude urinary FSH (menstruating woman)	0 9 (0·7–3·0)	0 39
HMG 613, used as standard	183	183

Table 2. BIOLOGICAL FSH AND LH ACTIVITIES AND ENDPOINTS IN AN FSH HAEMAGGLUTINATION SYSTEM

Substance	LH activity in ovarian ascorbic acid depletion assay in terms of mg IRP-HMG/mg	FSH activity in ovarian augmentation assay in terms of mg IRP-HMG/mg	Endpoint in haemagglutination-inhibition test in terms of biological units/mg
HMG 613		183/ampoule	1·12 mg IRP-HMG
Human pituitary FSH, fraction *CP* 1 (ref. 6)	550	3000	1·2 mg IRP-HMG
Human pituitary LH, fraction DEAE I	6000	3·8	0·46 I.U. HCG*
Human pituitary LH, fraction DEAE	1200	50·0	0·02 I.U. HCG
Human pituitary LH, Sephadex-fraction	7000	< 3·8	0·31 I.U. HCG
HCG (Leo-Pharmaceutical Products)	1250 I.U. HCG/mg		125 0 I.U. HCG
Human serum albumin	–	–	> 500 μg/ml.

* The conversion factor from mg IRP-HMG to I.U. HCG was 0 31 (obtained from Dr. Butt).

In Fig. 3, only immunological FSH estimations are shown. Daily specimens of morning urine for three consecutive menstrual cycles were pooled into ten specimens per cycle. The urines were collected by a patient for a control month and then for 2 months while she was taking contraceptive pills. There was not enough urine to carry out bioassays; however, the pattern of the control month fits well into the patterns obtained by bioassays on FSH changes during the menstrual cycle[9]. During the 2 months the patient was on contraceptive pills the release of FSH was largely suppressed.

In contrast to antisera which had been raised to human pituitary FSH fractions on previous occasions and had not

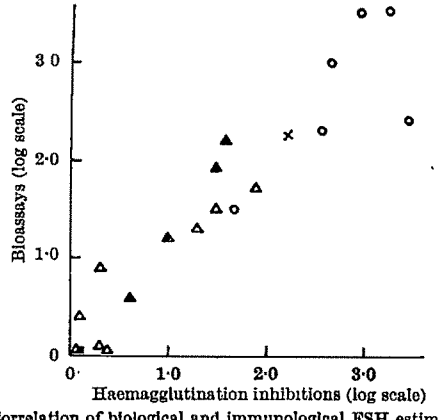


Fig. 2. Correlation of biological and immunological FSH estimations of human pituitary FSH preparations (○), crude HMG preparations (△), purified HMG preparations (▲) and a urinary extract of a menstruating woman (■). HMG 613 standard (×).

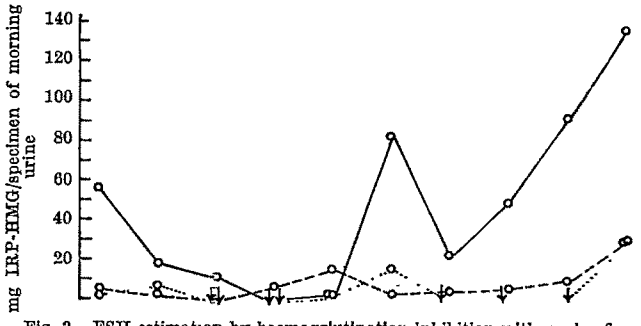


Fig. 3. FSH estimation by haemagglutination inhibition with pools of urines of three consecutive menstrual cycles. Control month (———), first month on contraceptive pills (– – –), second month on pills (·····).

been treated with organic solvents and urea, the antisera reported here readily reacted with urinary FSH preparations in the haemagglutination technique. The correlation between the figures obtained for the biological and immunological activities of pituitary and urinary FSH is fairly good, although the reason for the strong interference of LH remains obscure. It was found that, in terms of I.U., HCG had a much less pronounced inhibitory effect than LH, and that LH preparations did not react according to their biological activities. The results of the experiments suggest the presence of an unspecific immunogenic component originating from the pituitary and which is common to both LH and FSH preparations. This suggestion is supported by two further observations. First, although the FSH fractions used for inoculation contained no LH activity which was biologically detectable, it was impossible to absorb the antisera by LH without losing a large proportion of the haemagglutination titres. Secondly, it was observed that a crude extract from acetone dried pituitary powder and an LH fraction which showed comparatively little biological activity exhibited a particularly strong inhibitory effect in haemagglutination tests. It is therefore clear that in certain circumstances misleading results may occur; however, in view of the correlation shown for both biological and immunological determinations of FSH, it is unlikely that the unspecific component interferes in a misleading way when immunological investigations are carried out on purified preparations of FSH. This is also true when the investigations are carried out on extracts of body fluids which have been submitted to an identical extraction technique. It is possible that the component is present at a consistent ratio in such extracts.

It can be concluded that the precision of the haemagglutination assay for FSH is limited. However, this appears to apply to similar assays of other protein hormones as well as for HCG[10]. Nevertheless, the method may be helpful for overall checks in clinical work.

I thank Dr. A. C. Crooke for encouraging this work and Dr. W. R. Butt for the numerous fractions of FSH of known potency, especially prepared for this investigation. I also thank Dr. F. Ingrassia, Florence, for some of the urinary extracts, and Organon for their gift of HMG 613. This work was carried out under a grant from the Medical Research Council.

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LEUCOANTHOCYANINS FROM THE LEAVES OF EUROPEAN LARCH (*Larix decidua*, MILL)

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WHILE investigating the mobilization of iron by aqueous extracts of leaves which had fallen during the autumn from various species of trees, I noted that a dilute aqueous extract of larch leaves, kept at 0° C for a week, deposited a precipitate with properties typical of a leucodelphinidin. The slight mobility of the product on paper chromatograms, in both aqueous and organic solvents, indicates that it is not a monomeric leucoanthocyanin. The structure of the leucodelphinidin has not been investigated in detail, but its unusual nature, and that of the unknown leucoanthocyanidin described here, seem to warrant a preliminary report of their properties.

There are few published accounts of the chemistry of the leaves of *Larix decidua*. That of Brown *et al.*[1], who described the formation of delphinidin and cyanidin when leaves are heated with concentrated hydrochloric acid, is relevant to the present work. They found that a streak of small mobility in aqueous chromatographic solvents exhibited the properties of a leucoanthocyanin.

Larch Leaf Leucodelphinidin

Larch leaf leucodelphinidin, accompanied by a water insoluble organic calcium compound, separates at 0° C from aqueous extracts containing about 20 per cent dry matter, or from more concentrated extracts at room temperature. Larger quantities can be obtained by further concentrating the filtrate. The total yield of leucodelphinidin and the calcium compound varies with samples gathered in different years; it is usually 2–3 per cent of the dry weight of milled leaves, and it remains unaffected whether the leaves are dried by freezing or heating in an oven after collection. Approximately equal amounts of the leucodelphinidin and the calcium compound are present in the alcohol-washed and dried precipitate. The leucodelphinidin is difficult to purify, but a sample which can be shown to be homogeneous by chromatography can be prepared by stirring the crude precipitate with ten times its weight of water, filtering, evaporating the filtrate to dryness *in vacuo* and washing the residue with methanol. The final product is amorphous, and has a melting point $<350°$ C. The calcium compound is insoluble in water and in methanol. It has no reducing properties but reacts strongly with Morris's anthrone reagent[2].

On two-way paper chromatograms the leucodelphinidin moves as a streak with a rate of flow (R_F) of 0–0·5 in 6 per cent acetic acid, and also as a streak with an R_F of 0–0·12 in Forestal solvent (acetic acid/water/concentrated hydrochloric acid, 30 : 10 : 3 v/v) (ref. 3), but is immobile in organic chromatographic solvents. The streak reacts with the ferricyanide reagent[4] and becomes pink when heated after spraying with either 2 N aqueous hydrochloric acid or 3 per cent alcoholic toluene *p*-sulphonic acid[5]. An aqueous solution of the leucodelphinidin becomes brown on adding 3 per cent ferric chloride.

Delphinidin is formed when the leucoanthocyanin is heated at 100° C with *n*-butanol/concentrated hydrochloric acid (20 : 1 v/v)—this can be confirmed by spectrophotometry and paper chromatography with Forestal solvent, 90 per cent formic acid/hydrochloric acid 3 N (1 : 1 v/v) (ref. 6) and *n*-butanol/acetic acid/water (6 : 1 : 2 v/v). After extracting the delphinidin, glucose and arabinose can be detected in the aqueous hydrolysate by paper chromatography with water-saturated phenol and *n*-butanol/acetic acid/water (6 · 1 : 2) (ref. 7).

Visual comparison of the chromatograms with standards and spectrophotometric determination[8] of the sugars extracted from the excised spots indicate a molecular ratio of 1 : 1 for the two sugars. The presence of 16 per cent glucose, determined directly on various samples of the leucodelphinidin with anthrone[2], with which arabinose does not react, indicates a molecular weight of about 1,200, assuming that the compound contains one molecule each of glucose and arabinose.

The ultra-violet absorption spectrum of the leucodelphinidin in water shows little absorption throughout the range 200–400 mμ, with a broad maximum between 260 mμ and 280 mμ, $E^{1\%}_{1\,cm}$ (275 mμ) = 19. The spectrum in 0·1 M aqueous sodium hydroxide shows no maximum, only a flattening of the curve between 275 mμ and 285 mμ. It seems that the leucoanthocyanin is probably a polymeric leucodelphinidin in which one or more of the vicinal hydroxyl groups in the pyrogallol ring is masked, possibly by the sugar residues, leading to a loss of reactivity to ferric chloride.

The existence of true leucoanthocyanins, that is glycosides of leucoanthocyanidins, has seldom been reported. Shantz and Steward[9] claim to have isolated such a monoglucoside from the liquid endosperm of *Aesculus woerlitzensis*. Swain and Bate-Smith[10], and Swain[11], suggest that, in general, some polysaccharide may be entrained in the course of extracting a complex leucoanthocyanin, or that the sugar is in the form of a polymer on which the phenolic substance is adsorbed. This seems not to be so with the larch leucodelphinidin, which is isolated as a coprecipitate with the unknown calcium compound from a dilute aqueous extract in which polysaccharide would be expected to remain in solution. In these circumstances, the high glucose content (16 per cent), plus the arabinose, makes the possibility of entrainment or adsorption unlikely.

Anthocyanidins from Larch Leaves

Larch leaves do not contain free anthocyanins and anthocyanidins, but a treatment whereby the leaves are boiled for 30 min with alcoholic hydrochloric acid extracts delphinidin, cyanidin and an unknown anthocyanidin. The last-mentioned compound has very different chemical properties from any natural anthocyanidin so far reported and would seem to be derived from a new type of leucoanthocyanin or leucoanthocyanidin.

Extracts prepared by repeatedly treating larch leaves with cold water give successively smaller amounts of these anthocyanidins when heated with alcoholic hydrochloric acid. The amount increases again when the partially extracted leaves are extracted with hot water. Although it is not known whether the delphinidin prepared from these extracts is derived from a common leucoanthocyanin, the solubility of the isolated leucodelphinidin (above) differs from that of the leucocyanidin and the unknown leucoanthocyanin, neither of which separates in an insoluble form from a cold-water extract of larch leaves. All these leuco-compounds are contained in the streak (R_F, 0–0·5) in 6 per cent acetic acid, which is always present on chromatograms of fresh aqueous extracts of larch leaves.

The relative proportions of the three anthocyanidins were estimated by heating the dry milled leaves with

Table 1. FORMATION OF ANTHOCYANIDINS FROM PICKED (GREEN) AND FALLEN *L. decidua* LEAVES

Sample	Wt. of leaves (g)	E_{max}* (550 mμ)	E_{max}/100 mg oven-dry leaf	Anthocyanidin (per cent)
Fallen leaves (1963)	0·521	0 845	0·162	1·70
Fallen leaves (1963) after cold-water extraction	0 419	0·413	0 099	1·05†
Fallen leaves (1964)	0 588	0 955	0 163	1·80
Picked leaves (1964)	0·516	0·437‡	0 085	0 89

* Calculated as cyanidin, E = 0·477 for a 0·1 per cent solution in *n*-butanol/concentrated hydrochloric acid (20 : 1 v/v). Test solutions made up to 50 ml. and diluted 1 . 10.

† Represents 62 per cent anthocyanidin unextracted by cold water.

‡ Corrected for absorption of chlorophyll at 550 mμ.

n-butanol/concentrated hydrochloric acid (20 : 1 v/v) in a closed tube for 30–35 min[12], and separating the anthocyanidins as bands on Whatman No. 3 paper before spectrophotometric examination. Absorbances (in *n*-butanol/concentrated hydrochloric acid 20 : 1 v/v) are: delphinidin, E = 0·662 (558–560 mμ); cyanidin, E = 0·239 (550 mμ), measured at the wavelengths of maximum absorption on a Unicam *SP* 500 spectrophotometer, giving a ratio of delphinidin : unknown anthocyanidin : cyanidin of 2 : 1 : 0·36. The total amount of anthocyanidin formed was also estimated by measuring E_{max} of a suitable dilution of the original hydrolysate, basing the measurement on the absorbance of pure cyanidin of known concentration[12] (Table 1).

The unknown anthocyanidin can be prepared on a larger scale by distilling off the solvent from the hydrolysate (50 ml.) from 5 g leaves and extracting the residue with 2 N hydrochloric acid. This extract is chromatographed on a column of 'Solka Floc' powdered cellulose with Forestal solvent as eluent, and purified by banding the crude unknown anthocyanidin on thick paper and chromatographing with 90 per cent formic acid/3 N hydrochloric acid (1 : 1 v/v).

The unknown anthocyanidin also differs in its chromatographic behaviour from any anthocyanidin so far reported; in Forestal solvent, the R_F is 0·75 (R_{Cy} = 1·42); in 90 per cent formic acid/3 N hydrochloric acid (1 : 1 v/v), the R_F is 0·29 (R_{Cy} = 1·29); in *n*-butanol/acetic acid/water (6 : 1 : 2 v/v), the streak R_F is 0·63–0·90. When exposed to ammonia vapour its spot, originally similar in colour to cyanidin, changes to green, in contrast with the blue of the common anthocyanidins; the green colour persists for at least 1 h for papers run in Forestal solvent. When the anthocyanidin is taken to dryness from a methanol/hydrochloric acid solution it does not re-dissolve in methanol alone; a blue-purple suspension forms and a drop of concentrated hydrochloric acid is needed for complete solution and return of the usual red colour.

In Robinson and Robinson's qualitative tests[13]: (*a*) the colour of the anthocyanidin in *iso*-amyl alcohol is partly destroyed by oxidation and becomes orange yellow; (*b*) an *iso*-amyl alcohol solution becomes blue on adding sodium acetate, and is unchanged on adding ferric chloride; (*c*) at least 15 volumes of benzene are required to transfer the colour of an *iso*-amyl alcohol solution (1 vol.) to 0·5 per cent aqueous hydrochloric acid (3 vol.) and when the benzene is added, the upper layer of mixture changes colour to blue. The aqueous hydrochloric acid layer becomes pink, however, and the anthocyanidin can be re-extracted unchanged by *iso*-amyl alcohol. When dissolved in lower alcohols containing hydrochloric acid, the colour of the anthocyanidin resembles that of cyanidin.

In the visible region the absorption spectrum of the compound is characteristic of an anthocyanidin having at least two adjacent —OH groups in the *B* ring, that is λ_{max} (0·01 per cent methanolic hydrochloric acid)[14] is at 540–542 mμ, shifting +28 mμ on adding ethanolic aluminium chloride[15]. In *n*-butanol/concentrated hydrochloric acid (1 : 1 v/v) λ_{max} is at 456 mμ. The ultraviolet absorption spectrum between 200 mμ and 400 mμ shows a series of shoulders at 250–255 mμ, 298–308 mμ and 345–354 mμ.

The leucoanthocyanins of larch leaves must be regarded as major constituents of the aqueous extract; the leucodelphinidin, isolated as a precipitate, with the unknown calcium compound together make up 10–15 per cent of the dry matter of the extract.

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NON-ASSOCIATIVE BEHAVIOURAL MODIFICATIONS IN NEREID POLYCHAETES

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THERE is no doubt that nereid polychaetes are capable of learning in at least some situations. Habituation is usually regarded as the simplest type of learning, and in the three nereids so far investigated—*Nereis diversicolor*, *N. pelagica*, and *Platynereis dumerilii*—the withdrawal response habituates to repeated stimulation[1–3]. Habituation of this response is, however, probably a complex central process. It has been shown that both the sensory giant axon synaptic junctions and motor giant axon junctions are capable of accommodating to repeated tactile and electrical stimulations in *N. virens*[4] and to sudden changes in illumination in *N. diversicolor*, *N. virens* and *P. dumerilii*[5]. Habituation cannot, however, be attributed solely to motor fatigue, or to accommodation of synapses on the motor side of the reflex, because worms which have habituated to one stimulus are still capable of responding if the nature of the stimulus is changed[2]. Similarly, sensory accommodation cannot account for the habituation process because one response is often replaced by a different one; withdrawal responses, for example, which always occur in response to initial anterior stimulations in *N. diversicolor* and *P. dumerilii*, are sometimes replaced by a response in which the worms attack the seeker used to stimulate them[6].

Four types of associative learning have been investigated in nereids, but the only evidence that these animals can learn associations comes from their behaviour in "T" mazes. If worms which make a "correct" choice in the maze are allowed to remain in a darkened compartment for at least five minutes, they are able to learn to

avoid the punishment in the "incorrect" arm to a criterion of ten consecutive "correct" choices. *N. virens* did this in an average of 68·2 trials, *N. diversicolor* in 85·5 trials, and *Perinereis cultrifera* in 85·0 trials[7]. In a subsequent investigation *N. diversicolor* reached the same criterion in an average of 84·4 trials[8].

The reason for regarding maze learning as a manifestation of associative learning is that higher animals undoubtedly learn to select one arm of a maze by associating some feature of it with a reward or avoidance of a punishment. Nereids in the mazes were not learning in this manner, however, because no clues were provided. It is possible that they are able to learn the maze because the anterior sense organs are stimulated differently when left and right choices are made and, in fact, removal of the anterior sense organs does disrupt a previously learned habit[8]. But proprioceptive sensory information may be used in some circumstances, because almost completely deafferentiated worms can still learn to avoid the punishment[8].

Investigations of the earthworm have indicated that unilateral stimulation given outside the maze can induce a turning preference when earthworms are introduced into a maze[9]. Sessions of unilateral tactile stimulation and stimulation from a bright light induce turning away from the side stimulated and weak illumination induces turning towards the side stimulated. It was suggested that the establishment of the habit of turning in a given direction may be considered as a type of pseudo-conditioning in earthworms—the unconditioned stimulus eliciting a given direction of turning, which is made apparent when the animals are forced to make a turning choice in a maze[9]. But the punishment is not given unilaterally to either earthworms or nereids in the maze-learning situation, and in any case it is well known that nereids do not react in this way to unilateral tactile stimulation at least[6]. The effect of unilateral stimulation cannot therefore be used to explain the learned behaviour of nereids in the maze. Nevertheless, it does raise the possibility that associative learning may not be the only means by which an animal's behaviour can become modified so that it selects the "correct" arm in a maze.

In three other experimental situations which were designed to investigate the ability of nereids to learn by association, modifications in behaviour were observed, which were the result of non-associative processes, but could be mistaken for associative learning. One instrumental-learning situation and two conditioning procedures have been investigated. In the instrumental-learning situation the worms were given a slight electric shock whenever they reached the exit of a 'Perspex' channel. The intention was to test the ability of the worms to avoid the punishment by refusing to crawl along the channel[10,11]. In one series of conditioning experiments, a sudden change in illumination (conditioned stimulus) was paired with food presentation in order to investigate conditioning of the feeding response[12]. In another series of experiments, designed to investigate conditioning of the withdrawal response, an electric shock was paired with the presentation of food[12]. Normally, associative learning is only possible when there is close temporal contiguity between the conditioned and unconditioned stimuli. It was shown in these experiments, however, that nereids learn just as effectively if the unconditioned stimulus is presented between trials as when it is paired with the conditioned stimulus; furthermore, apparent conditioning can sometimes be induced by a single presentation of the unconditioned stimulus. There is little doubt that, in these instances, the behaviour of the worm was modified non-associatively by the sensitization effects of the unconditioned stimulus; that is to say, the behaviour of the worms was modified by the punishment they received with the result that the animals now reversed or remained stationary in the 'Perspex' channel—either course of action enabled them to avoid the shock given at the exit (Fig. 1).

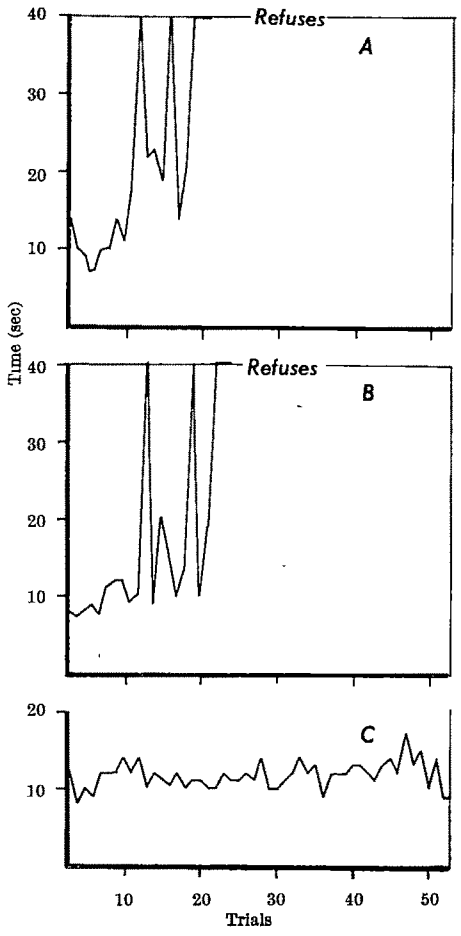


Fig. 1. Time taken for individual *Nereis virens* to crawl to the end of a 'Perspex' channel *A*, Punishment between trials (when the worm is not in the apparatus); *B*, punishment at the exit of channel; *C*, no punishment. (In part after Evans (ref. 10).)

In the conditioning experiments it was found that feeding modified the behaviour of the worms so that they crawled to the ends of the glass tubes containing them in response to sudden changes of illumination and electric shocks. It was noted that tactile stimuli induced greater reactivity of the withdrawal response than did sudden changes in illumination. In these situations the behaviour of the worm can be regarded as learning in the sense that it was modified with experience, but clearly the new habits cannot be considered to have been a manifestation of associative learning.

The chief difference between associative and non-associative learning is the manner in which learning is acquired. Normally, animals are only capable of forming associations under very restricted conditions; for example, conditioned responses can be established by forward conditioning procedures but only with difficulty, if at all, by backward, trace and delayed procedures. Non-associative learning, on the other hand, can be achieved by all these procedures. There are, however, many similarities between associative and non-associative learning and, indeed, it is only with difficulty that the two processes are distinguished. In both processes, the animal's reaction to a stimulus or stimulus situation (conditioned stimulus) becomes modified as a result of the effects of a reward or punishment (unconditioned stimulus) and the modifications can be retained after the new habit has been acquired. Retention of non-associative learning in nereids appears, however, to be short-lived; for example, retention of the habit learned in a 'Perspex'

channel is less than 24 h (ref. 10). The similarities that exist suggest that non-associative learning may be achieved by mechanisms which are not unlike those involved in associative learning; indeed, mechanisms may exist in nereids resembling those which Young[13-15] believes to be involved in associative learning in *Octopus*.

Young suggests that in *Octopus* exploratory responses to unfamiliar objects are elicited by a change in the visual situation. At first the response is weak, but if it is reinforced by a reward the animal responds more rapidly. There is, therefore, a mechanism for producing graduated commands to attack so that the octopus responds quickly to a cue-stimulus which has proved favourable. However, if the cue-stimulus is accompanied by an unfavourable situation—one in which the animal is punished—the tendency to explore or attack the object is reduced. Overall there is a balancing of signals indicating reward or punishment; the tendency to explore is increased by reward and reduced by punishment. Young believes that visual learning in *Octopus* is controlled by the vertical and median superior frontal lobes of the brain. He suggested that the vertical lobe is a centre that tends to reduce the tendency to attack if the animal is punished, and that the median superior frontal lobe tends to promote it if the animal is rewarded. This view is supported by experimental evidence; in some cases, at least, it is found that removal of the vertical lobe in *Octopus* increases the tendency to attack (removes the inhibition) and removal of the median superior frontal lobe decreases the tendency to attack[14].

Behaviourally, nereids resemble *Octopus* in the sense that a stimulus is capable of eliciting either a positive or a negative response, depending, at least partially, on the reinforcement the animal has recently experienced. In the laboratory, a nereid reacts to stimuli, such as various chemical stimuli[16], sudden changes in illumination[12], mechanical stimuli[1], and anterior tactile stimuli[17] in one of two ways. It may either react positively, by crawling along the tube which it is inhabiting, often exploring the area adjacent to the mouth of the tube, or negatively, by withdrawing, by reversing in the tube, or by ignoring the stimulus altogether. Sometimes both a withdrawal (negative) response and a positive response may occur to the same stimulus; in this event, withdrawal is always followed by the worm crawling to the end of its tube. Punishment tends to promote negative (avoidance) responses. In the 'Perspex' channel it inhibits movement and facilitates reversal; this enables worms to avoid a punishment at the end of the channel. Punishment given to worms in glass tubes causes the withdrawal response to become more reactive to sudden stimulation. Conversely, reward promotes positive responses; food presentation facilitates exploratory responses to sudden changes in illumination. Thus it would seem that, like *Octopus*, nereids may have mechanisms producing graduated commands for exploratory and avoidance behaviour, which are influenced by the nature of the reinforcement that is received by the animal. If paired centres do exist in nereids, and possess functions similar to those suggested for the vertical lobes and median superior frontal lobes in *Octopus*, it is possible that they are housed in the supra-oesophageal ganglion and sub-oesophageal ganglion. In fact, Prosser[18] has already suggested that in annelids, the supra-oesophageal ganglion may be an inhibitory centre and the sub-oesophageal ganglion an excitatory one.

The functions of the sub-oesophageal ganglion in annelids are difficult to evaluate from extirpation experiments because removal of this ganglion necessarily disconnects the supra-oesophageal ganglion and anterior sense organs from the rest of the central nervous system. However, extirpation is generally followed by inactivity, which might be predicted if this is an excitatory centre.

Fortunately, removal of the supra-oesophageal ganglion has less drastic effects. Its extirpation does not prevent earthworms from crawling, burrowing, withdrawing, and copulating[19], and similarly decerebrate nereids can withdraw, swim, crawl, irrigate, and burrow[20]. However, both earthworms[21] and nereids[20], deprived of the ganglion, become hyperactive, and decerebrate nereids irrigate their tubes incessantly and more energetically than before[21]. It would appear, therefore, that in the intact animal the supra-oesophageal ganglion is inhibitory at least as regards the performance of certain activities. If the supra-oesophageal ganglion of the annelid acts as a centre that inhibits positive responses and facilitates avoidance responses—which is the suggested function of the vertical lobes in *Octopus*—removal of it should tend to increase positive responses at the expense of negative ones (in *Octopus* removal of the vertical lobes increases the tendency to attack objects[14]). Hess[24] found that after removal of the supra-oesophageal ganglion, the normal negative response of worms in turning away from bright light was reversed. Before extirpation, only nine responses in a total of 144 were turns towards the source of illumination, but after extirpation of the ganglion this increased to 132 out of 144 responses. More recently Ratner and Stein[25] also found that decerebrate earthworms responded positively to sudden increases in illumination more frequently than did intact worms.

Similar functions for the vertical lobes in *Octopus* and for the supra-oesophageal ganglion in annelids are also suggested by the behavioural effects resulting from extirpation of these centres in problems involving avoidance learning. Thus Boycott and Young[26] found that an *Octopus* lacking the vertical lobes could only learn not to attack an isolated crab if there was an interval of only 2 min between trials. Similarly, Wells and Wells[27] found that removal of the vertical lobes made it difficult or impossible for blinded octopuses to learn a tactile discrimination when the intervals between trials was 1 h, although they could do so when this time interval was only 5 min. Furthermore, it has been established that decerebrate nereids are only able to learn to avoid a punishment at the end of a 'Perspex' channel if a massed training technique is used (Fig. 2). In this event the learned response never involves a reversal in the channel; instead the decerebrate worms remain stationary[10]. Similarly, it appears that the withdrawal response of decerebrate earthworms can only be conditioned by massed training[28].

Young[15] suggests that a permanent memory is not actually stored in the vertical lobes in *Octopus*, but that these are only temporarily involved in setting up a memory trace. Nereids are similar in this respect, because although the supra-oesophageal ganglion is not the site of retention (worms trained in an avoidance-learning situation retain the learned response even if they are later decerebrated[10]),

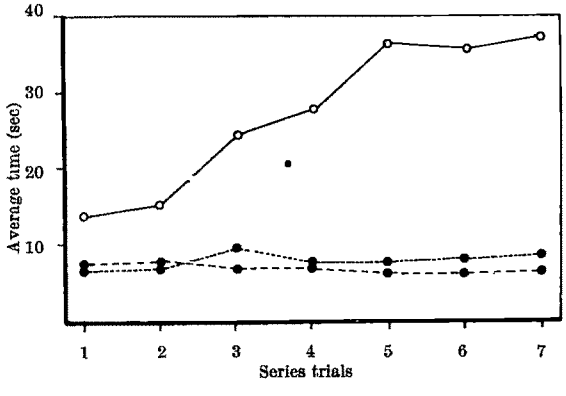


Fig. 2. Learning in intact and decerebrate *Nereis virens* with spaced trials. Average results plotted. ○———○, Intact worms trained in series of ten trials at 30 min intervals. ● – – – ●, Decerebrate worms trained in series of ten trials at 15 min intervals. ●·····●, Decerebrate worms trained in series of ten trials at 30 min intervals. (After Evans[10].)

it is clearly important in the establishment of memory traces. Naive, decerebrate worms are able to learn to avoid th shock at the end of the 'Perspex' channel in about the same number of trials as intact worms, but they are unable to retain the learned behaviour for even 1 h, whereas intact worms can retain it for 6 h (ref. 10).

Although the brain of *Octopus* has been mapped far more thoroughly than the ganglia of any annelid, a certain number of anatomical similarities do exist between the *Octopus* brain and the supra-oesophageal ganglion of annelids. The features of particular interest in the "brains" of many annelids are the corpora pedunculata. These consist of numerous small, globuli cells which resemble the small cells characteristic of the learning centres of cephalopods and vertebrates. Although the functions of the corpora pedunculata of annelids have never been explored, it is quite possible that these are centres concerned with behavioural modifications—particularly in view of the fact that a large proportion of the rest of the supra-oesophageal ganglion is neurosecretory in function.

The major difference between learning in *Octopus* and *Nereis* is that, as Young[13] points out, in *Octopus* the response does not remain "unconditional" and the signals received from either the punishing or rewarding event (the reinforcing signals) condition the distance receptor mechanisms that have been active. In nereids, in at least some learning situations, the mechanism necessary for this conditioning to occur does not exist. The reinforcing signals probably activate all receptor systems non-associatively and association between the reinforcing signals and the receptor system activated is extremely unlikely because a single unconditioned stimulus can modify the behaviour of the worm. For example, one tactile stimulus can induce a withdrawal response to a sudden decrease in illumination in a previously habituated worm, and similarly a single presentation of food can sensitize the feeding response to a sudden increase in illumination[12]. It is a most unlikely explanation of these results to suggest that one-trial learning is occurring, as there is no temporal contiguity between the conditioned and unconditioned stimuli involved.

Associative learning mechanisms of the kind described in *Octopus* may have evolved from non-associative mechanisms such as those in nereids. In both *Octopus* and nereids a reinforcing event has a similar effect on the subsequent responses the animal makes to stimuli, but conditioning mechanisms in *Octopus* have been evolved which enable learned responses to be linked with a particular stimulus situation. Conditioning mechanisms exist in a variety of vertebrates and higher invertebrates and, although they may have evolved independently on more than one occasion, Young[13] has pointed out that there are apparent similarities in the associative learning mechanisms of animals which are as widely separated phylogenetically as mammals and cephalopods. In mammals the situation is undoubtedly extremely complex, but the hypothalamus does contain pairs of centres producing opposite effects and, furthermore, it is linked by pathways to the septum which may contain similar centres. The hippocampus and the amygdaloid nuclei are necessary for the establishment of memory but are not the site of retention. This suggests that these regions of the mammalian brain may have a function similar to that of the vertical lobes in *Octopus*, and it has been found that in mammals bilateral removal of the hippocampus may grossly disturb memory for recent events.

In many animals conditioning mechanisms operate under very restricted conditions and conditioning can only be established if the conditioned (cue) stimulus is closely associated with the reinforcing event (unconditioned stimulus). In many mammals the optimum conditions occur if the unconditioned stimulus occurs 0·5 sec after the onset of the conditioned stimulus[29]. But this is not always so; for pigs the optimum is believed to be 4 or 8 sec[30], and for the rhesus monkey 2 sec[31]. For fish it is also 2 sec[32]. It may be physiologically difficult to associate the conditioned stimulus with the signals of the reinforcing stimulus unless they occur concurrently and in the correct sequence. As Young[13] has pointed out, there is a delay between the receipt of the one stimulus by the nervous system and the reinforcement signals which follow. In order to produce conditioning, the address of the appropriate elements in the memory system must be held during the delay. The delays in holding the necessary information during trace and delayed conditioning procedures may be too great to enable conditioning to occur. In backward conditioning it would be necessary to hold information of the reinforcing event until the receipt of the cue-stimulus. Such a mechanism may well not exist after all, for learning to associate a conditioned stimulus with an event that has already occurred is most probably of no biological value whatsoever.

Conditioning mechanisms have probably evolved with the evolution of sense organs that are capable of making detailed sensory discriminations. Animals such as *Octopus* have well developed sense organs and are capable of accurate sensory discriminations. Consequently they are able to associate one particular, say visual, stimulus with a particular reinforcing event and another visual stimulus with another reinforcing event. Indeed, it appears that they have well developed abilities to do so[33]. Presumably they are also able to learn to recognize both prey and predators and respond accordingly. Animals such as nereids, on the other hand, are almost certainly unable to distinguish between visual stimuli caused by predators and those by food because their eyes are incapable of forming images. A sudden change in illumination, such as a shadow, can be detected by nereids but could be caused by a predator, by food, such as floating seaweed and, innocuously, by a passing cloud. Even if the necessary mechanisms existed, conditioning would be difficult to establish in this situation and would be of doubtful value to the animal. This is because in nature conditioned stimuli probably occur at random with different kinds of reinforcing events.

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LETTERS TO THE EDITOR

ASTRONOMY

Evidence on the Evolutionary Character of the Universe derived from Recent Red-shift Measurements

Hoyle and Burbidge[1] have recently examined the red-shifts of a number of quasi-stellar radio sources[2,3] and have plotted their radio flux densities (S) against red-shift (z); they conclude that the results are inconsistent with a cosmological interpretation of these red-shifts.

This communication shows that this conclusion is not valid and that indeed the observed relationship between S and z provides valuable new information on the evolutionary nature of the Universe.

There is now considerable evidence in favour of evolutionary cosmologies, the most important observations being (*a*) the counts of radio sources[4,5], (*b*) the detection of cosmic radiation with a black-body spectrum[6-9], and (*c*) the present abundance of cosmic helium[10,11]. The possibility that quasi-stellar sources are of relatively local origin is now difficult to accept in view of (*a*) the isotropy of the counts of faint radio sources over the northern sky[12,13], (*b*) the absence of quasi-stellar sources showing blue-shifted spectral lines[14] and (*c*) the marked similarity between the radio properties of a number of quasi-stellar source counts has been considered by Davidson and *MSH* 14-1-21 (ref. 16) and 3*C* 245 (ref. 17) all appear optically to be quasi-stellar sources and yet have a radio structure very similar to radio galaxies such as Cygnus *A* and 3*C* 33.

The nature of the evolutionary changes in the properties of radio galaxies which are necessary to explain the radio source counts has been considered by Davidson and Davies[18] and more recently by myself[19]. In the second article, it was shown that changes with epoch of either the mean spatial density (density evolution) or the mean luminosity (luminosity evolution) of powerful extra-galactic radio sources could explain the excess of faint sources in the log N − log S relationship. It has been suggested that quasi-stellar sources may account for a large part of this excess since it has been shown that the slope of the source counts for such sources is steeper than that of the total source counts[19,20].

In cosmologies involving density evolution, the sources at all distances have the same distribution of radio luminosity, P, and the plot of S against z will follow the usual relationship:

$$S = \frac{P}{r^2(1+z)^{1+\alpha}}$$

where α is the spectral index ($S \propto \nu^{-\alpha}$) and r the distance of the radio source which is given by

$$r = \frac{2c}{H}(1-(1+z)^{-\frac{1}{2}})$$

in the Einstein-de Sitter world model. Because more than 80 per cent of the local quasi-stellar radio sources ($z < 0{\cdot}5$) have luminosities in the range $4 \times 10^{25} < P_{178} < 6 \times 10^{26}$ W(c/s)$^{-1}$ ster.$^{-1}$, it is possible to draw on the $S-z$ diagram the limits within which most of the quasi-stellar radio sources should lie.

If, on the other hand, the excess of faint sources is attributed to luminosity evolution, the $S-z$ relationship must be modified to take account of the increased luminosity of sources at earlier epochs. As Longair[19] has shown, the luminosity evolution required may be defined by $\bar{P} \propto t^{-x}$ where $x \sim 2$. ($\bar{P}$ is the mean luminosity at epoch t.) Lines on the $S-z$ diagram corresponding to the limiting values of P_{178} at the present epoch may again be drawn. The results of these calculations for the two types of evolution are shown in Fig. 1 superimposed on the distribution of points presented by Hoyle and Burbidge restricted to sources for which $S_{178} \geqslant 8 \times 10^{-26}$ W m^{-2}(c/s)$^{-1}$. (Only at values greater than this flux density have complete searches for quasi-stellar sources been carried out and of those found, roughly 50 per cent have measured red-shifts.)

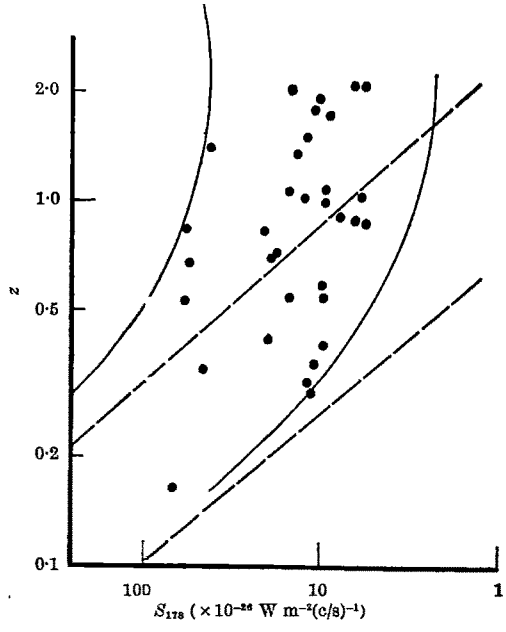


Fig. 1. The red-shift flux density plots expected in evolutionary cosmologies in which density evolution (dashed lines) and luminosity evolution (full lines) are included. The distribution of observational points is that presented by Hoyle and Burbidge restricted to $S_{178} \geqslant 8 \times 10^{-26}$ W m^{-2}(c/s)$^{-1}$.

It can be seen from Fig. 1 that cosmologies involving luminosity evolution can explain entirely the wide dispersion of points in the $S-z$ plot whereas, in those involving density evolution, only 45 per cent of the quasi-stellar sources lie within the expected limits. In the latter type of evolutionary cosmological model, it is difficult to account for those sources lying appreciably above the upper limit.

These results suggest that the observed log $N-\log S$ relationship arises from the increased luminosity of powerful radio sources at early epochs. Such a variation of luminosity with epoch implies that sources at a given flux density should include a wide range of red-shifts and this provides a natural explanation for the presence of distant quasi-stellar sources even at high flux densities

(for example, $3C298 : z = 1{\cdot}436$, $S_{178} = 44 \times 10^{-26}$ W $m^{-2}(c/s)^{-1}$).

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An Apparent Increase in Galactic Radio Noise during a Total Solar Eclipse

DURING the total eclipse of 1965, May 30, observations were made on the island of Manuae (lat., 19° 15′ 43″ S.; long., 158° 57′ 43″ W.) of changes in the *D* layer absorption of radio signals, and also of fluctuations in the galactic noise level at 18·2 Mc/s. The latter observations in particular produced an unexpected effect which is reported here.

The apparatus used in measuring the noise consisted of two half-wave dipoles in phase, with reflectors, the main lobe being directed at R.A. 1h 20m, Dec. 16° 21′ S., that is 8° from the zenith in a direction W. 20° S. This aerial fed a low noise receiver of 10 kc/s bandwidth, and the audio-output was amplified, rectified and used to drive a pen recorder. The system was calibrated with a 5722 noise diode.

Observations were made between 18h 45m and 21h 00m U.T. on every day between May 27 and June 2. Time markers were received from station *WWVH* and placed manually on the chart.

The unexpected feature consisted of two large bursts of noise, each of 13 sec duration, the leading edges of which coincided to 3 sec with the times of optical second and third contacts. The mean power level in each burst was about 2·5 times the mean noise power during the eclipse. No such similar bursts occurred at any other time while observations were being made. Because all expeditions on the Island had collaborated in the detection, identification and elimination of any sources of local radio interference, we are satisfied that the noise originated from a source outside the island.

Reduction of the data on this and other observations is not yet complete.

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PLANETARY SCIENCE

A Method for Translunar Radio Communication

THE increase of human activity on the lunar surface that can be expected to follow the first manned landings will present a special problem in translunar communications. The lack of an ionosphere about the Moon, the lack of any appreciable atmosphere on the lunar surface, and the nearness of the lunar horizon, require solutions to this problem that may be somewhat different from those most easily available on the Earth's surface. Extended radio coverage about the lithosphere, such as occurs on Earth because of the atmospheric density gradient and temperature inversions, will not be possible because of the vanishingly low density of any gaseous envelope that may exist around the Moon.

Consideration of the special properties which are believed to be likely for the lunar lithosphere[1,2] indicates a possible solution: that the sub-lunar surface materials can be used as a propagation medium for translunar communication.

The immediate surface layers have an extremely low thermal conductivity and a very low content of liquid water, as shown by microwave thermal observations. Thus there is some evidence that the outer layers of the Moon contain considerable quantities of material similar to earthly pumice, in that this material has been expanded by gas bubbles during some stage of formation, and/or was formed from meteoric splash dust and thermal cycle spallation products, re-welded on succeeding surfaces by direct solar action.

The depth to which these conditions exist is of course at the moment a matter of pure speculation. However, if they persist for an appreciable depth, then a considerable volume of the outer part of the Moon is likely to be transparent to radio waves in the range from the lowest frequencies that can be radiated by practical antennae up to frequencies in the tens of megacycles per second. The depth of this transparency is probably a function of radio frequency, because microwave thermal observations[3] show some transparency even for microwaves in a thin layer of the outer surface.

Antennae placed on the surface of the Moon should give coupling to sub-surface waves with which astronauts can communicate beyond the lunar horizon. Trailing wire antennae can be simple and inexpensive as compared with arrays of microwave dish antennae, which have the additional disadvantage of being confined to line-of-sight use.

The advantages of sub-lunar low frequency communications seem obvious. Very compact receivers of low mass and transmitters of great reliability are available from simple designs using solid-state active elements and microcircuitry type construction. The principal problem is the primary power. Storage cells and solar cells in combination have already shown their adaptability in the lunar surface environment, in connexion with the *Surveyor* soft landing and picture transmission.

The remaining problem is that of determining the true nature and extent of the outer portion of the Moon in respect to its radio transmission properties. If the outer layers are sufficiently transparent to a suitable radio frequency, the internal reflexion from the lunar surface and the ray curvature resulting from increased density as a function of depth should ensure a strong wave component along and just below the surface, with sufficient evanescent wave penetration through the surface to ensure antenna coupling.

The foregoing possibilities make it very desirable to obtain measurements of lunar sub-surface properties in relation to radio transparency and the depth of the transparent layers, before the arrival of manned landing parties. This information should be obtainable by means

of a suitable circumlunar transmitting or receiving satellite. Which of these approaches is most economical depends on a detailed assessment of ionosphere penetration of signals at the Earth terminal and of the effect of Earth atmospherics.

Because this evaluation will be long and involved, the results that can be expected from a basic satellite transmitter system will be outlined here as an example from which we can judge the information that is possible from this type of experiment. Reflected wave-lengths of metre size and greater indicate a smooth figure for the lunar surface as a whole[4]. The portion of the Moon which is transparent to radio waves should act as a spherical lens and therefore as a gigantic focusing antenna. If the index of refraction of the transparent lunar material for radio waves lies between 1·25 and 2, as is true for light terrestrial rocks, then a satellite orbiting at a distance of between 300 miles and 50 miles above the lunar surface will produce a strongly focused beam from the Moon, which acts as a lens antenna. As the transmitting satellite passes behind the Moon from the Earth, this beam should pass across the surface of the Earth.

The strength of the central lobe of this beam will depend on the transmitter power and radiator gain, and on the degree of transparency and area of this lunar material. The angular size will depend on the relation between the satellite position and the ideal focal point of the Moon as a lens, as well as the relation between the lunar diameter and the wave-length. The relative angular size and phase of the side-lobe pattern will depend on the radial depth of the transparent layer and the transmitted frequency. Several passes, each transmitting a different frequency, or a time-sharing system between pulses of various frequencies to obtain data from several frequencies at a single pass, should produce data equivalent to an imagined X-raying of the Moon. Thus a small unmanned lunar satellite could be used as a vehicle to determine the feasibility of a miniature and simple system of translunar voice communication, and at the same time could considerably increase our knowledge of the internal structure of the Moon. The cost of this effort would be quite small compared with the cost of a manned landing, and could contribute considerably to the safety and comfort of future lunar explorers.

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Occurrence of Hydrogen Peroxide in Sea Water

TRACES of dissolved substances, of the order of 10^{-7} M or less, can or may have far-reaching effects on the type or growth rate of organisms in the sea[1]. We now wish to report the occurrence of a possible ectocrine, hydrogen peroxide, in surface sea water samples taken near Port Aransas. We have no direct evidence suggesting that hydrogen peroxide in sea water has any biological or chemical significance; however, from continuing experiments on the growth of single cells of coccoid blue-green algae on agar plates[2,3], we are led to propose that it could be a significant ecological variable.

Hydrogen peroxide was measured in sea water samples using the sensitive scopoletin-peroxidase fluorescence technique of Andreae[4] and Perschke and Broda[5]. For the values reported here a stoichiometry of one part scopoletin to one part hydrogen peroxide was used. Changes in fluorescence were detected with a barrier cell (*IRC A-10M*) and measured on a Keithley model 150*A* microvolt–ammeter. (An American Instrument Co. photomultiplier microphotometer was used in later work.)

Table 1. HYDROGEN PEROXIDE IN SURFACE SEA WATER SAMPLES

Location	μg hydrogen peroxide/l.
Boat basin adjacent to Institute	1 6, 1·6, 2·8
Tide pool near beach	0 5, 6 7, 2 6
Beach near South Jetty	5 8, 1 0, 4·0
Open Gulf	5·5, 3·3

For the first three samples: Date and weather conditions shown in order left to right: April 21, mostly cloudy, air temp. 27° C; June 3, bright sun, air temp. 31° C, south-easterly wind at 15–20 m.p.h.; July 8, bright sun, air temp 31° C, south-easterly wind at 20–22 m.p.h. Open Gulf samples: first sample June 11, 10 miles offshore, blue water, clear sky, south-easterly wind at 5–10 m.p.h.; second sample September 14, 5 miles offshore, turbid water, clear sky, south-easterly wind at 12–15 m.p.h.

The sea water samples were collected in 'Pyrex' or plastic containers. They were assayed as soon as possible, usually within 30 min. A typical assay consisted of the following: 6 ml. of sea water (unfiltered); 0·5 ml. (1 μg) of scopoletin (Mann Laboratories); and 0·5 ml. of 0·08 M *tris* buffer, *p*H 8·0. The initial fluorescence baseline was measured, then 0·05 ml. (200 μg) peroxidase, Sigma Chemical Co., Type II, was added. The addition of catalase to samples of sea water which gave strongly positive assays of hydrogen peroxide resulted in complete loss of activity in the scopoletin-peroxidase system.

Table 1 shows some of the results found. Our experience so far would suggest that the presence of traces of hydrogen peroxide is a constant feature of sea water taken from the Port Aransas area.

We make no specification as to the source of the hydrogen peroxide. It could be formed photochemically in the atmosphere, photochemically in sea water itself through catalysis by dissolved pigments[6] or possibly even through the metabolic activity of micro-organisms. The data so far do not allow a choice to be made from these possibilities. This work was supported by the National Science Foundation.

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Surface Tension of Slick Patches near Kelp Beds

DURING a voyage off southern California near Santa Catalina Island some surface tension measurements were made on the open sea and over and around the slick areas associated with kelp beds. The usual methods of measuring surface tension involve laboratory equipment which is not feasible for use aboard a small sailing ship. For this reason, these tests were made with drops of a series of ten oil solutions, calibrated so that each solution would just spread against a different specific surface pressure, or lowering of surface tension. This calibrated series made it possible to measure, by inspection, surface tensions in the range 76–30 dynes/cm.

Based on N. K. Adam's[1] technique, a series of solutions of a hydrophilic compound, *n*-dodecyl alcohol, and non-spreading oils was standardized so that a drop of each oil solution just spread against a measured surface tension. Solutions of known surface tensions, determined by a Du Nuoy tensiometer calibrated with distilled water, were

made with potassium chloride, a surface-active agent known as 'Aerosol *OT*', and distilled water, as listed in Table 1. 'Aerosol *OT*' is a waxy solid form of sodium dioctyl sulphosuccinate produced by the American Cyanamid Co. The quantities of 'Aerosol *OT*' used varied from 0 to 0·1 per cent by weight, and the oils used were Sargent's light mineral oil (80°–90° Saybolt, 35 dynes/cm surface tension) and *n*-decane (Eastman Organic Chemicals *P* 2405). The amounts of *n*-dodecyl alcohol (Eastman 873) in the spreading oils varied from a trace in the 76 dynes/cm solution to 0·18 per cent by volume in the 30 dynes/cm solution. The small amounts of *n*-dodecyl alcohol were added in the form of a 10 per cent solution of the alcohol in *n*-decane.

Table 1. COMPOSITION OF CALIBRATED SURFACE TENSION OILS

Surface tension (dynes/cm)	Standardizing solutions	Spreading oil base
76	Potassium chloride (14 wt. per cent)	Mineral
74	Potassium chloride (6 8 wt. per cent)	Mineral
72	Distilled water	Mineral
70	'Aerosol *OT*'	Mineral
68	'Aerosol *OT*'	Mineral
61	'Aerosol *OT*'	Mineral
58	'Aerosol *OT*'	Mineral
50	'Aerosol *OT*'	20 per cent decane in mineral
40	'Aerosol *OT*'	*n*-decane
30	'Aerosol *OT*'	*n*-decane

A sample of open sea surface water taken from near the mid-point of the Outer Santa Barbara Channel was stored in an overflowing plastic container. Its surface tension was measured 6 days later with a Du Nuoy tensiometer. We found a value of 74 dynes/cm, which agrees with that expected for open sea water. This value can be used as a standard of comparison for the surface tension values measured on the slick patches. All other measurements were made by direct application of the oil drops to the sea surface.

Sieburth and Conover[2] reported decreases of 1–3 dynes/cm in the surface tension of natural slicks in the Sargasso Sea, compared with non-slick areas nearby. They considered that the slick areas were formed by the ripple damping action of monomolecular layers of organic matter produced by blue-green algae *Trichodesmium erythraeum*. The present report deals with the slick patches associated with the kelp beds near the shore of Santa Catalina Island. The predominant kelp of these beds is *Macrocystis pyrifera*[3], commonly called "Devil's Apron".

The areas of testing were two coves, Howland's and Moonstone Beach, on the north-east side of Santa Catalina, each of which had a fairly sheltered harbour and slick kelp patches near the shore. When measurements were made, our boat was the only one in the harbour and the oil drops were applied from a small skiff drifting over the areas.

In both coves the surface tension of the open rippled water measured consistently between 70 and 72 dynes/cm. Landward, the surface tensions varied from 68 to 70 dynes/cm at the outer edge of the slick areas to 58 and 61 dynes/cm directly over the kelp plants.

At Moonstone Beach it was possible to make surface tension measurements along a line extending from the clear water in the harbour, across a large kelp bed slick, to the shore, with a strong 8–10 m per sec on-shore wind blowing. The values of surface tension in the clear water upwind of the slick were 70 dynes/cm; on the very edge of the slick, 61 dynes/cm; over the kelp, 58; downwind towards shore, 50; and still closer to shore, less than 50 but more than 40 dynes/cm. In the backwash from a small tidal cave the surface tension measured 58 dynes/cm.

These values suggest that the metabolic processes of the kelp produce organic material which lowers the surface tension of sea water. The organic material from the kelp evidently stays concentrated near the surface, for in the backwash of a tidal cave the decrease in surface tension was less than in the surrounding area, owing to the admixture of under-surface water.

We thank Dr. Alexander Goetz for making possible the trip on his boat.

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Occurrence of Permafrost at an Arctic Lake

A FIELD investigation was carried out in April 1961 to determine the distribution of permafrost below a lake in the Mackenzie River delta[1–3]. This study was of special interest inasmuch as the delta lake is situated in an old river bed and the occurrence of permafrost is considerably affected by the large number of lakes and channels in the immediate vicinity. A similar but more detailed investigation was undertaken by the Division of Building Research of the National Research Council of Canada during April and May 1964 at a small lake about 5 miles north-east of Inuvik, Northwest Territories. This lake was selected because it was completely isolated from other bodies of water which might influence the ground thermal regime.

The lake is located on the edge of the rolling upland which forms the eastern boundary of the Mackenzie delta. It is about 1·25 miles from the delta at an elevation about 340 ft. above the delta, and is nearly rectangular in shape (about 800 ft. long by 400 ft. wide) with a maximum depth of 19 ft. No streams flow into the lake.

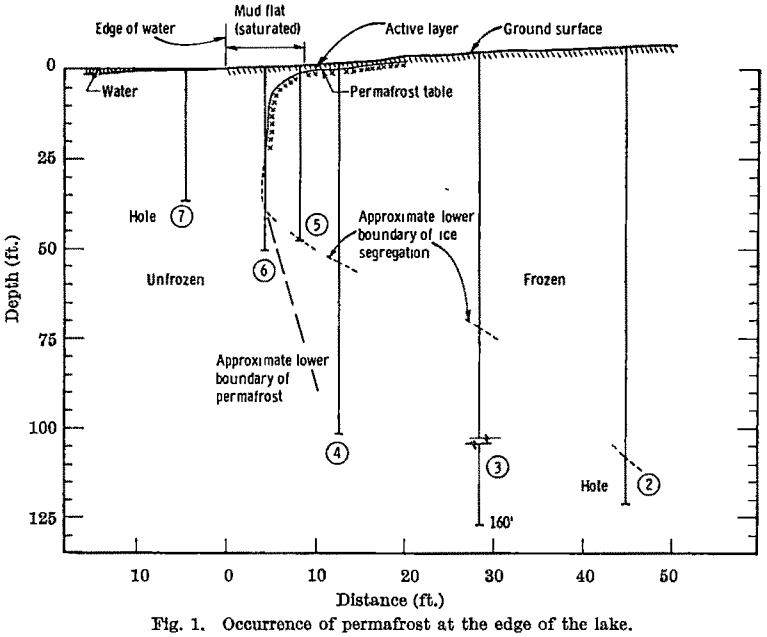


Fig. 1. Occurrence of permafrost at the edge of the lake.

It is replenished by snow melt water from its very small drainage basin. A small stream issues from the south-west end and drains to the delta. The lake is situated in the transition zone between the tundra to the east and the forested delta to the west. Although stunted white spruce are scattered over the area, the predominant vegetation consists of willow and alder and low brush. *Sphagnum* and other mosses with patches of lichen form the ground cover. The entire area surrounding the lake is covered with large hummocks up to 2 ft. high and 7 ft. in diameter. The mean annual air temperature at Inuvik is about 14° F. Permafrost is continuous below the ground surface and is hundreds of feet thick.

The main intention of the field programme was to determine the distribution of permafrost near the edge of the lake and to provide instrumentation which would enable an analysis to be made of the ground thermal regime below and adjacent to the lake. The investigation consisted of drilling and sampling nine holes at or adjacent to the east edge of the lake. Good cores were recovered of most of the frozen materials encountered.

Hole No. 1 (312 ft. deep) was situated on the top of a ridge about 850 ft. from and 50 ft. higher than the lake. Hole No. 9 was drilled to a depth of 320 ft. below the lake (in 12 ft. of water) about 60 ft. from shore. The remaining seven holes were drilled to depths of 40–160 ft.; five of them were on the shore and two in the lake (Fig. 1). A number of hand probings were also made between these holes to determine the maximum seasonal frost penetration (May) and depth of thaw (September). Thermocouple cables were installed to depths of 50–300 ft. to measure ground temperatures in five of the holes. In addition, three specially designed and fabricated temperature recorders (which will run unattended for at least one year) were installed. Surveys were also made to map lake bottom and ground surface contours.

From 2–35 ft. of organic silt overlies 100–150 ft. of dense stony till. The till is underlain by a thinly stratified shale. The depth to which ice segregation was noted increased with distance from the edge of the lake. The lowest depths at which ice segregation was observed in the holes at the edge of the lake are indicated in Fig. 1. No frozen ground was encountered in the three holes below the lake. At hole No. 6 (5 ft. from the edge of the lake), permafrost occurred from about 24 ft. to 40 ft. Initial ground temperature observations indicate a thickness of permafrost of about 250 ft. in hole No. 3, which was about 28 ft. from the edge. Frozen ground exists for the full depth of hole No. 1 and a thickness of more than 400 ft. is indicated by the ground temperatures. Ice segregation was observed to a depth of about 290 ft. in this hole. The drilling operation considerably disturbed the ground thermal regime in the boreholes and thermal equilibrium has not yet been restored. Measurements are at present being made to determine the mean lake bottom temperature, the mean ground surface temperature and the geothermal gradient.

It is possible to draw some general conclusions from the initial field observations. It is probable that the size and shape of the lake have remained unchanged for a long period of time. Little variation in water level and a very slow accumulation of bottom sediment would be expected because the lake is replenished only by local snow melt each year, and no sign of large-scale erosion or slumping has been observed on the perimeter of the lake. The small stream draining the lake would provide a natural control on the water level.

The effect of this very small water body on the occurrence of permafrost is quite pronounced. The existence of a deep thawed zone below the lake is a direct result. It is probable that permafrost is completely absent below the lake. Although the thawing effect of the water is confined to the ground lying beneath the lake, as shown by the presence of permafrost at the shoreline, the initial ground temperature measurements indicate that the thermal effect of the lake extends for some distance beyond its perimeter.

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Some Seismological Features of the North Macquarie Ridge

THE recent demonstration of the existence of a ridge connexion between Macquarie Island and New Zealand, by Brodie and Dawson[1], has clarified several aspects of the regional seismology. Fig. 1 relates epicentres determined during the past 5 years by the United States Coast and Geodetic Survey in the area, to the bathymetry as presented by Brodie and Dawson. Seismicity is seen to be definitely associated with the ridge structure. There is one case (about 55° S., 163° E.) notably distant from the

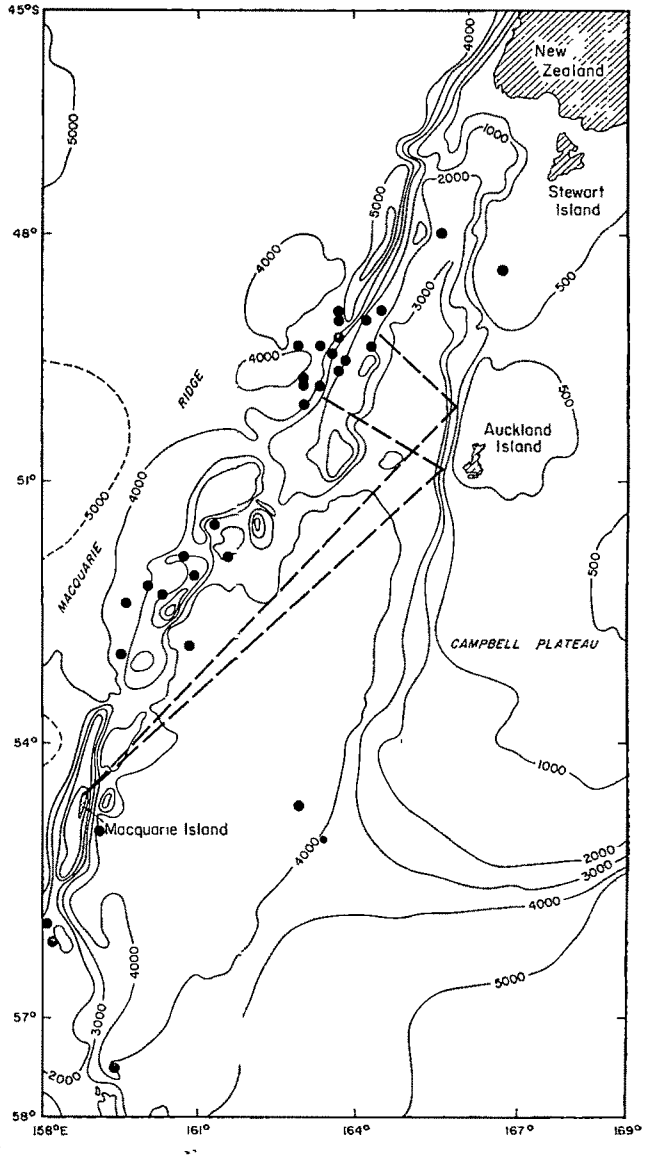


Fig. 1. Depths in metres.

ridge; from consideration of P phase arrivals at Australian stations, this is believed to be a mislocated ridge shock. Thus the Campbell Plateau appears to be aseismic, at least during this period. The marked offset in the ridge at about 51° S., noted by Brodie and Dawson, could suggest a fracture zone similar to those reported along the equatorial part of the mid-Atlantic ridge by Heezen *et al.*[2]. However, no epicentres have been located near this feature during the period considered.

The Bureau of Mineral Resources has operated a seismological station at Macquarie Island since 1951. A puzzling feature of Macquarie Island records of shocks in the group north-west of Auckland Islands has been a frequent anomalously late T phase arrival. These arrivals had been provisionally interpreted as T waves reflected at the edge of the Campbell Plateau. The new bathymetric map makes this interpretation entirely feasible. Typical paths for such reflected T phases are shown in Fig. 1.

Material on the T phase as recorded at Macquarie Island is being prepared for publication. This will include more detail concerning these reflexions.

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P Amplitudes from *Longshot* at Australian Stations

THE P phase from the nuclear explosion *Longshot*, detonated beneath Amchitka Island at a depth of 2,300 ft. on October 29, 1965, was well recorded at most Australian seismograph stations. The amplitudes of ground displacement at these stations have been plotted against distance in Fig. 1. These amplitudes were calculated from the peak-to-peak amplitude of the first cycle of P motion on each record. U.S. Coastal and Geodetic Survey station abbreviations have been used in the figure, with the addition of the recently established Warramunga array at Tennant Creek (TCA), and the South Australian station at Cleve (CLV). Details of the stations have been given by Doyle and Underwood[1].

Despite the scatter common to all amplitude studies, the data clearly show a sharp decrease in amplitude between 92° and 93°. It is unlikely that this marks the shadow boundary of the core, especially as studies of PcP from nuclear explosions[3-4] indicate that the depth of the core-mantle boundary is not far from that calculated by Jeffreys[5]. It is more probable that the effect is due to the influence of the D'' layer[6]. A recent study of travel times by Cleary and Hales[7] suggests that $dT/d\Delta$ is approximately constant from about 93°. A condition

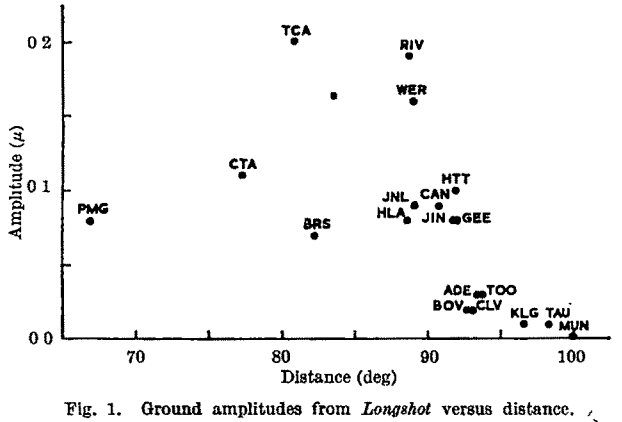


Fig. 1. Ground amplitudes from *Longshot* versus distance.

approaching this would account for the decrease in amplitude.

At Cleve and Adelaide, the small P amplitudes disclose a larger impulsive arrival about 1 sec later. This is too late to be pP, and any arrival which had travelled along the path of the initial P would be similarly diminished in amplitude. The phase is therefore most readily interpreted as PcP, providing further evidence that the bottom of the P ray path is above the core-mantle boundary for this distance.

The period of P decreases from 1·1 sec to 0·8 sec as the distance increases within the range, but the decrease appears to be fairly uniform and unrelated to the amplitude change. This phenomenon, together with travel time observations, will be discussed in detail elsewhere.

I thank the directors of the Bureau of Mineral Resources, the Geophysics Departments of the Universities of Brisbane, Adelaide and Hobart, and Riverview Observatory, for making records available. R. Underwood supplied seismograph calibrations for all stations operated by the Australian National University.

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A Model for the Occurrence of Large Earthquakes

THIS communication outlines a probability model which seems to provide an adequate basis for making predictions concerning the occurrence of largest earthquake magnitudes over time: (*a*) The number of earthquakes in a year is a Poisson random variable with mean α; (*b*) X, the earthquake magnitude, is a random variable distributed with cumulative distribution function:

$$F(x) = Pr\,(X \leqslant x) = 1 - e^{-\beta x},\ x \geqslant 0 \qquad (1)$$

From these assumptions it follows that Y, the largest annual earthquake magnitude, is distributed with cumulative distribution function $G(y)$, where

$$G(y) = Pr\,(Y \leqslant y) = \sum_{k=0}^{\infty} \frac{e^{-\alpha}\,\alpha^k}{k!} \left[F(y)\right]^k$$

$$= \exp\left\{-\alpha\left[1-F(y)\right]\right\} = \exp\left(-\alpha e^{-\beta y}\right),\ y \geqslant 0 \qquad (2)$$

The cumulative distribution function (2) is called the "type 1" distribution of largest values in the terminology of Gumbel[1].

In order to estimate the parameters α and β from $y_1, y_2, \ldots, y_n$, the largest annual earthquake magnitudes in n years, one arranges these values in order of increasing size $y_{(1)} \leqslant y_{(2)} \leqslant \ldots \leqslant y_{(n)}$, where $y_{(j)}$ is the jth smallest annual maximum magnitude. To each $y_{(j)}$, one associates the value $G(y_{(j)}) = j/(n+1)$. The parameters α and β are found from the least-square fit to the equation

$$\log\left[-\log G(y)\right] = \log \alpha - \beta y \qquad (3)$$

derived from equation (2).

It should be noted that $\alpha e^{-\beta y}$ is the expected number of earthquakes, N_y, in a given year which have magnitude exceeding y. It follows that

$$\log N_y = \log \alpha - \beta y \qquad (4)$$

which is of the same form as the widely used empirical formula

$$\log_{10} N_y = a - by \tag{5}$$

(see, for example, ref. 2). The parameters a and b are related to α and β as follows:

$$a = \log \alpha / \log 10 \qquad b = \beta / \log 10 \tag{6}$$

The parameter β also has a physical meaning, since $1/\beta$ is the mean magnitude of all earthquakes of magnitude $x > 0$. If X_0 is the magnitude threshold of the observations, then $X_0 + 1/\beta$ is the mean magnitude over the data range used.

In addition to relation 4, several other useful ones can be obtained from the model. It is, for example, an immediate consequence of the model that T_y, the mean return period (that is, the mean interval in years) between earthquakes having magnitude greater than y is given by

$$T_y = 1/N_y = \exp(\beta y)/\alpha \tag{7}$$

Another quantity of interest is $\tilde{y}$, the modal (most probable or most frequently observed) annual maximum magnitude. Since $\tilde{y}$ is the value for which $g(y) = G'(y)$ becomes maximum, it can be shown that

$$\tilde{y} = \frac{\log \alpha}{\beta} \tag{8}$$

It is readily verified that $N\tilde{y} = 1$, that is, $\tilde{y}$, is the magnitude such that we can expect one earthquake having magnitude $\tilde{y}$ or more in a given year (another way of saying this is that the mean return period of $\tilde{y}$ is $T_{\tilde{y}} = 1$). Also, $G(\tilde{y}) = e^{-1}$; that is, $\tilde{y}$ is that maximum annual magnitude which is exceeded in the long run 63 per cent of the time.

The modal earthquake magnitude in a T year period is

$$\tilde{y}_T = \log(\alpha T)/\beta = \tilde{y} + \frac{\log T}{\beta} \tag{9}$$

The value y_p of the maximum annual earthquake magnitude which is exceeded with probability p is found by solving the equation

$$\exp(-\alpha e^{-\beta y_p}) = 1 - p \tag{10}$$

which yields

$$y_p = \tilde{y} - \frac{\log[-\log(1-p)]}{\beta} \tag{11}$$

More generally, $y_p(D)$, the value of the maximum earthquake magnitude which is exceeded with probability p in a D year period, is given by

$$y_p(D) = y_p + \frac{\log D}{\beta} \tag{12}$$

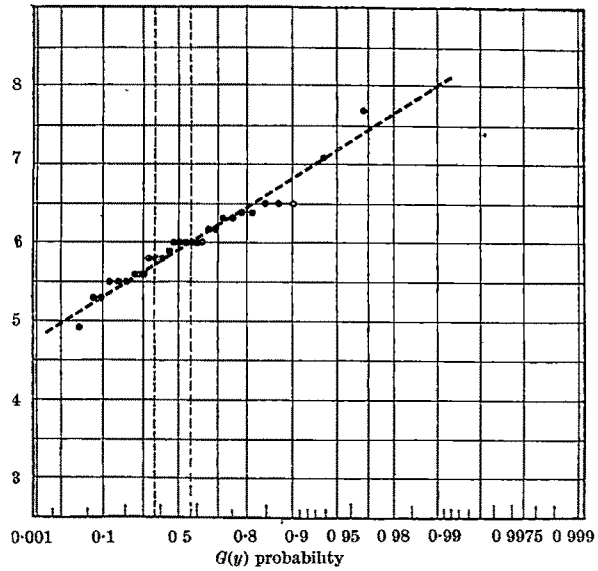


Fig. 1.

Finally, the earthquake risk $R_D(y)$ (ref. 3), that is, the probability of occurrence of an earthquake of magnitude y or more in a D year period, can be written as

$$R_D(y) = 1 - \exp(-\alpha D\, e^{-\beta y}) \tag{13}$$

Calculations based on this model have been carried out for earthquakes in California for the period 1932–62. Table 1 lists the magnitude maxima by increasing size.

Table 1. CALIFORNIA YEARLY EARTHQUAKE MAXIMA (1932–62) BY ORDER OF INCREASING SIZE

j	$y(j)$	$G[y(j)]$
1	4·9	0·03125
2	5 3	0 06250
3	5 3	0 09375
4	5·5	0 12500
5	5·5	0 15625
6	5 5	0 18750
7	5·5	0 21875
8	5·6	0 25000
9	5 6	0 28125
10	5 6	0·31250
11	5 8	0 34375
12	5·8	0 37500
13	5·8	0·40625
14	5·9	0 43750
15	6·0	0·46875
16	6·0	0 50000
17	6·0	0 53125
18	6·0	0·56250
19	6 0	0·59375
20	6 0	0 62500
21	6·2	0·65625
22	6 2	0 68750
23	6 3	0 71875
24	6 3	0 75000
25	6 4	0 78125
26	6·4	0·81250
27	6·5	0 84375
28	6·5	0 87500
29	6 5	0 90625
30	7 1	0·93750
31	7·7	0·96875

The values given in Table 1, plotted on extremal probability paper (ref. 1), yield an acceptable straight line fit, as shown in Fig. 1. From this graph it is possible to determine approximate values for the parameters α and β of the distribution $G(y)$ of annual extreme earthquakes in California. More accurately, the data given in Table 1 can be fitted to equation (3) by a least-square regression procedure such as *REGRESS*, a computer routine available within the *STATPAK* statistical system at the University of California[4]. Application of this procedure to the data in Table 1 yields 11·43 and 2·00 as estimates, respectively, of $\log \alpha$ and β. The standard deviation of β was estimated as 0·08 by the same programme.

Gutenberg and Richter[5] give $0{\cdot}88 \pm 0{\cdot}03$ as an estimate of their parameter b for Southern California, which yields (see equation 6)

$$\beta = 2{\cdot}03 \pm 0{\cdot}07$$

which gives reasonable agreement with our estimate derived from only thirty-one annual maxima. Our predicted modal annual maximum (equation 8) is:

$$\tilde{y} = 11{\cdot}43/2{\cdot}00 = 5{\cdot}715$$

In view of the fact that the modal value $\tilde{y}$ is exceeded 63 per cent of all years we have $G(\tilde{y}) = 0{\cdot}37$, which corresponds to a magnitude $\tilde{y} = 5{\cdot}8$ in Table 1. Again, the agreement is excellent.

Let us now compute N_y, the expected number of earthquakes per year, and T_y, the mean return period, for shocks of magnitude greater than or equal to y in California. Using equations (4) and (7) with the values of α and β already obtained we find the estimates given in Table 2.

Table 2. PREDICTED YEARLY NUMBERS AND RETURN PERIODS FOR CALIFORNIA EARTHQUAKES

Magnitude y	Yearly expected number N_y	Mean return period T_y
3	228	1·6 days
4	20 7	17·6 days
5	4 18	87·3 days
6	0·57	1·8 years
7	0 076	13·2 years
8	0 010	100·0 years
9	0 0014	720 years

The model presented in this note should be considered as a first approximation to the real situation. In an area as large and complex as California there is considerable geographical variation both as to frequency and magnitude of occurrence of earthquakes. A more refined model would be obtained by dividing the area into two or more regions and fitting the seismicity in each of these regions by the model. However, despite the known lack of homogeneity of earthquake occurrence in California the present simplified approach provides reasonable estimates for the occurrence of large earthquakes within the uncertainty due to the shortness of the available historical record.

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PHYSICS

The Temperature Scale

THE temperature scale recently proposed by Georgian[1] has stimulated us to spend some time considering both the temperature scale and the dimensions to be associated with temperature. We shall begin by outlining details of various temperature scales which have been suggested during the past hundred years:

Rucker, 1889[2]. Let one degree be the temperature increase required to add one erg of energy to 1 c.c. of air at 0° C and one atmosphere. Rücker made his suggestion *exempli gratia*, saying that ". . . our knowledge of the molecular construction and dynamics of solids and liquids is as yet too imperfect to warrant an attempt to place thermal units on a natural and mechanical basis".

Planck, 1912[3]. $k = 1$—the degree is defined by setting Boltzmann's constant equal to unity. This was part of a system of "natural units" in which the units for mass, length, and time were defined by setting the velocity of light, the gravitational constant, and Planck's constant equal to unity.

Lewis and Adams, 1914[4]. $k = 1$ defines the degree, as with Planck. This was part of a system of "ultimate rational units" in which the velocity of light, the charge on the electron, and the mass of the electron were made equal to unity. Lewis and Adams also considered the possibility of $R = 1$ (universal gas constant per mole), but preferred $k = 1$.

McAdie, 1917[5]. Melting ice at 1,000 kilobars is defined as 1,000° N (for New), with zero at absolute zero This scale was selected as convenient for meteorological data.

Worthing, 1940[6]. Discusses several possibilities and rejects them all.

Brown, 1942[7]. The temperature of a system is defined as the specific pressure-volume energy (in ergs/g) of hydrogen in thermal equilibrium with the system. With all measurements thus referred to a specific thermometric gas, temperature can be considered proportional to the mean square velocity of the molecules of this particular gas, and its dimensions can be taken as L^2T^{-2}.

Laws, 1962[8]. Let one degree be the temperature increase required to add one joule of energy to 1 g of water.

Georgian, 1964[1]. $R = 1$, in conjunction with the metre–kilogram–second (M.K.S.) system.

Overcoming our reluctance to add to a list which may already be too long, we now suggest that the degree be defined so that $R = 2$ (M.K.S. basis). In an ideal gas:

$$E = \frac{3}{2}R\theta \qquad (1)$$

where E is the internal energy per mole and θ is the absolute temperature. Since each molecule has three (translational) degrees of freedom, we choose $R = 2$, thereby making temperature the molal internal energy per molecular degree of freedom:

$$\theta = \frac{E}{3} \qquad (2)$$

We would express temperature in the M.K.S. system, (joules/kg mole)/(degrees of freedom/molecule) and, like Georgian, scale it up by 10^4 for convenience.

In a broad sense, the temperature thus defined is the molal internal energy per degree of freedom for any substance, because at thermal equilibrium the same quantity of energy is stored by each fully activated degree of freedom. Degrees of freedom other than translational are quantized, and will be only partly activated at low temperatures. This does not, however, destroy the utility of the concept of energy per degree of freedom. A well known application is the calculation of heat capacity from spectral data, which involves estimating the extent to which various degrees of freedom are activated.

Our view of the dimensions of temperature differs from that of Georgian. From equation (2) we assign temperature the same dimensions as E, and we consider that E has dimensions ML^2T^{-2}. Since E is energy per mole, we are thereby saying that n, the number of moles, is a dimensionless number. The problem of the dimensions of n has been discussed in detail by Duncanson[9]. Briefly, we think of the number of moles as being analogous to the number of molecules. A mole can be defined as a specified number of molecules, and for many purposes a mole can be treated as a macromolecule.

It is possible to give temperature the dimensions L^2T^{-2} by following the procedure of Brown[7]. Georgian seems to take this view, because in putting forward an argument in favour of these dimensions on the basis of the kinetic theory, he says[10]: " . . . the temperature is directly proportional to the mean square velocity of the molecules in an ideal gas". This can be so only if, like Brown, he considers a single specified gas, with a particular molecular weight, as a unique thermometric substance to which all measurements are referred. Otherwise two ideal gases of different molecular weights in thermal equilibrium would not have the same temperature, because their molecular velocities would be different.

On the other hand, if we say that temperature is proportional to the mean kinetic energy of the molecules, then all ideal gases of all molecular weights can serve equally well as thermometric substances.

The relative merits of ML^2T^{-2} and L^2T^{-2} as dimensions for temperature are discussed in more detail by Focken[11]. We find the arguments of Focken and of Duncanson[9,12] more convincing than those of Porter[13], cited by Georgian[14].

Finally, we would like to comment on Georgian's statement that henceforth "In using temperature in dimensional analysis we will not fall into the error of considering temperature endowed with a separate dimension". We believe that many problems in dimensional

analysis can be formulated with a separate dimension for temperature with no consequent error in the solution[15].

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Fine Structure in the Radial Distribution Function from a Random Packing of Spheres

EARLIER, we reported[1] the radial distribution function from a random packing of spheres covering the range of sphere separations from 1·0 to 1·3 diameters. Inspection of the data indicated some possible fine structure in the distribution function. The results supplemented those of Bernal[2] and Scott[3] which covered a far larger separation range.

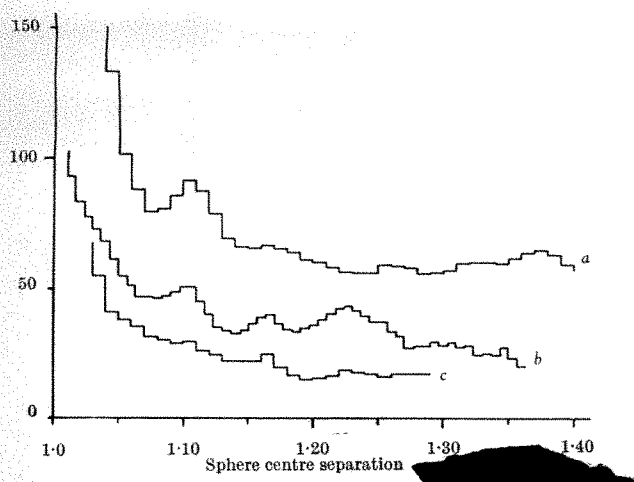


Fig. 1. Radial distribution function histograms; and Clark; *c*, Bernal.

The data were proc[illegible] two main separations

two spheres of the ring (Fig. 2*a* and *b*, 5–6). The sphere centre separation of this gap has been calculated as $\frac{4\sqrt{2}}{3\sqrt{3}} = 1{\cdot}0886\ldots$ diameters.

If another ring of five spheres is added to this first ring such that each sphere touches two adjacent spheres of the ring as well as one of the central pair, then five gaps are observed between these added spheres. Three of the gaps can be shown to be caused by a sphere centre separation of 1·0886 diameters (Fig. 2*c*, 12–8, 8–9 and 9–10), but the other two are greater. They are larger because one member of the new ring occupies a site above the original small gap and is therefore slightly below the plane of the other four new spheres. The sphere centre separation for these larger gaps can be shown to be $\frac{5}{\sqrt{19}} = 1{\cdot}14707\ldots$ diameters (Fig. 2*b*, 10–11 and 11–12).

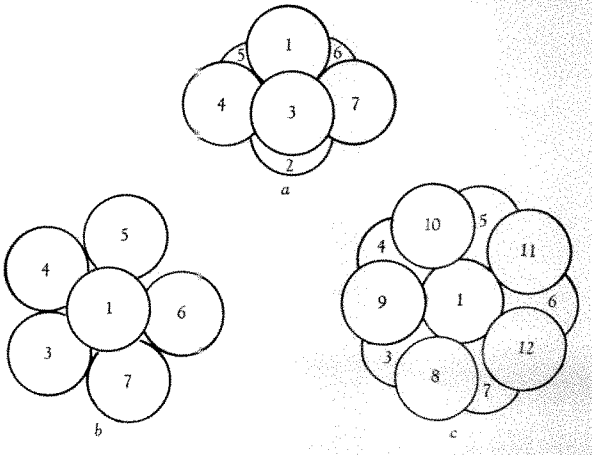


Fig. 2. Configurations containing small gaps.

These separations agree approximately with the first two small peaks of Fig. 1, but both are smaller than the actual separation peaks. It requires at least seven spheres and fifteen contacts to form the smallest gap. If one small separation is introduced somewhere in the fifteen contacts, then it is found that in five cases the 1·0866 diameters separation is reduced whereas in ten cases it is increased.

For the 1·14707 diameter separation it requires at least nine spheres and twenty-one contacts to form a configuration where the separation is possible. If each of these contacts is opened in turn it is found that five have little effect, six close the separation and ten open it. A rigorous [illegible] difficult than in the first case because [illegible] separations in the new layers but [illegible] introduced into the structure [illegible] en the two main separations

THE SOLID STATE

Oxygen-inhibited Grain Growth in Thin Films of Semiconducting Cuprous Iodide

THIN films, a few thousand angstroms thick or less, of various inorganic materials can be formed by the polycrystalline condensation of the low-pressure vapour on a cool substrate. Such films are gaining importance in optical elements, in electronic microcircuitry, and in devices for information storage.

It is common experience for the optical and/or electrical properties to change gradually with age after the film has been deposited, and numerous examples of such changes have been cited. It is evident, then, that the utility of thin films partly depends on limiting or controlling the changes of properties which occur on ageing. In a recent investigation of thin-film dielectric properties, Weaver[1] found recrystallization or grain growth to be a common result of ageing inorganic films. Grain growth caused extensive degradation of electrical properties. It is common practice nowadays when thin films are being deposited to temperature cycle or anneal the deposit in order to speed up ageing by encouraging changes in the growth of grains to occur more rapidly. Stabilizing the size of individual grains in a thin film is, therefore, a major problem in the search for stable optical and electrical properties.

Rutter and Aust[2] have shown that small amounts of soluble impurities can cause a considerable reduction in the rate of grain boundary movement in bulk metals. Ruhl[3] has suggested that impurities in a thin film of tin deposited at 20° K reduced the rate of grain growth during annealing at higher temperatures.

This report describes how small amounts of oxygen in thin films of cuprous iodide reduce the rate of grain boundary movement and stabilize the optical transmission for long periods of time. Previously, Herrick and Tevebaugh[4] reported the beneficial effect on the conductivity of thin films of cuprous iodide which resulted from the addition of small amounts of oxygen. Oxygen concentrations estimated at 10^{19} atoms per c.c. were produced by intentional burial of oxygen gas in the condensate during the vacuum condensation of copper vapour at room temperature. The thin condensate film was converted to cuprous iodide containing oxygen by brief treatment with iodine vapour. This treatment is not believed to alter [illegible]ntly the grain size of the film.

[illegible]prous iodide films 1000–1400 Å thick [illegible]terephthalate base have an average [illegible]0 Å. Spontaneous growth arising [illegible] of up to 4300 Å which are [illegible] growth can be followed

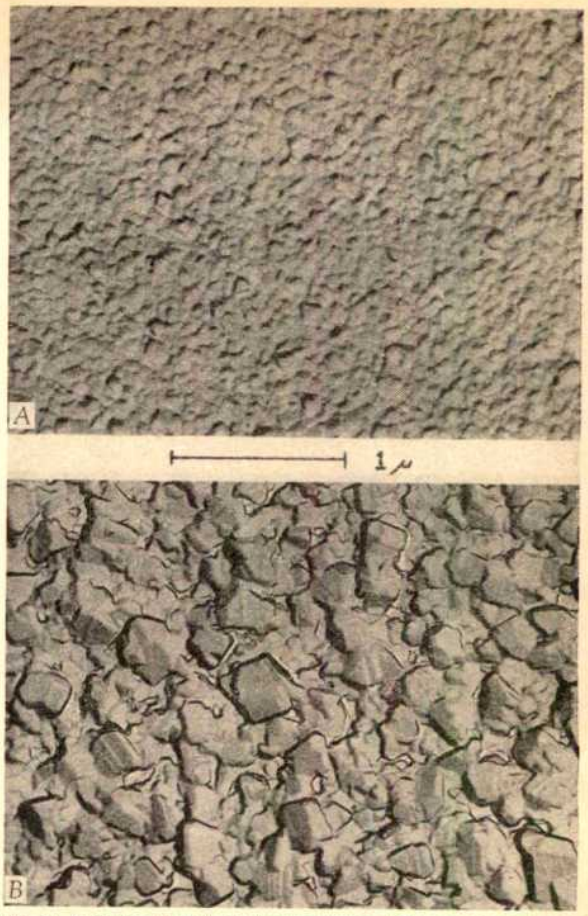


Fig. 2. Electron micrograph showing surface features of two cuprous iodide films from Fig. 1. Both films have aged 20 months at room temperature. *A*, 1 × 10^{-2} mm mercury oxygen pressure during copper condensation. *B*, 6 × 10^{-3} mm mercury oxygen pressure during copper condensation. (Platinum–carbon replicas, × 25,000.)

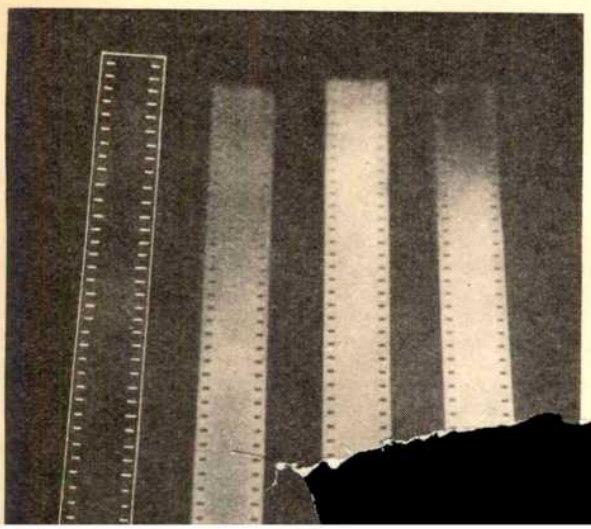


Fig. 2 shows electron micrographs of the surfaces of two films from Fig. 1—those having the greatest and the least oxygen contents. It is evident that the film with the highest oxygen content has almost maintained the original grain size after ageing 20 months. Over the same period, the film with least oxygen content has experienced extensive grain growth.

We conclude that the rate of grain growth was markedly slowed down in these experiments and that the effect probably resulted from the presence of oxygen in the film.

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Crystallization of Penicillin in the Solid Phase

DURING experiments on the hygroscopicity of penicillin salts, an unusual behaviour of substantially pure freeze-dried potassium benzylpenicillin was noted.

On exposure to air, the material absorbed moisture at a steady rate, up to a maximum content of 7–14 per cent, apparently remaining in the solid state. Then suddenly, the material shrank to a more compact form and began to lose weight. At first the loss was rapid and then it continued at a diminishing rate until the specimen returned to its original weight.

The time required for the uptake and subsequent loss of moisture was variable and depended on the temperature and relative humidity of the air, and on the weight and compactness of the specimen. A specimen weighing 0·1 g would usually take up moisture for about 4 h, and then completely lose it in about 18 h.

That the abrupt change which occurred at the point of maximum moisture content is a spontaneous crystallization of substantially amorphous material is evidenced by the changes in physical properties summarized in Table 1.

Table 1. CHANGES IN PHYSICAL PROPERTIES OF POTASSIUM BENZYLPENICILLIN ON EXPOSURE TO MOIST AIR

Property	Freeze-dried specimen		Control specimen*
	Before exposure	After exposure	
Stability on heating at 120° C	Decomposed within 16 h	Stable for 96 h	Stable for 96 h
Heat of solution (cal/mole) (at concentration 0·055 M)	4,550 ± 130	910 ± 90	850 ± 90
Aggregate refractive index	1·56	1·58	1·58
Microscopic appearance between crossed Nicols	Almost entirely isotropic flakes	Largely crystallites evenly distributed in a mainly isotropic groundmass†	Well-formed crystals showing uniform extinction
X-ray diffraction pattern	Faint diffraction lines with broad halo of scattering at low angles of diffraction	More intense diffraction lines with much less intense halo	Very intense diffraction lines

* Crystallized from aqueous butanol.
† Crystalline areas showing single crystal characteristics (that is, uniform extinction) also occurred in many flakes of exposed material, especially along the edges of the flakes.

A similar spontaneous crystallization was observed with freeze-dried specimens of sodium benzylpenicillin and potassium phenoxymethylpenicillin. No such phenomenon was observed when calcium or procaine benzylpenicillin, phenoxymethylpenicillin (free acid) or calcium phenoxymethylpenicillin was exposed to moist air.

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CHEMISTRY

Comparison of the Effect of Alpha- and Gamma-radiation on an Enzyme

EXTENSIVE investigations have been carried out on the effects of radiation on various organic polymers assuming that they could be regarded as model compounds for biological materials. Our previous work concerning the effects of gamma-rays on aqueous polymer solutions[1–3] revealed the existence of new effects resulting from the presence of water. Radiation-induced changes in the polymer may result either from the direct effect of the radiation on the solute molecules or from the indirect effect, that is, reactions of solute with reactive species such as hydrogen atoms, hydroxyl radicals or solvated electrons produced by irradiation of the water. The indirect effect becomes increasingly important as the concentration is reduced[4].

The radiation-induced reactions in solutions of macromolecules can be considerably affected by the presence of relatively small amounts of certain chemical additives. Some of the possible protection mechanisms can be envisaged to include the following: (*a*) Energy transfer between an excited macromolecule and the additive, the molecule being chemically unaltered. (*b*) Electron transfer from the additive to the polymer to replace an ejected electron before chemical changes in the molecule can occur. (*c*) True repair of a polymer radical by hydrogen transfer from the additive. (*d*) Stabilization repair of a solute radical by reaction with additive or additive radicals, the additive becoming incorporated into the molecular structure; the radical in this case becomes stabilized by modification as distinct from true repair. (*e*) The additive may react with radicals produced in the solvent before these can attack and modify the polymer; in this case protection is offered against the indirect effect and radiation damage is reduced without involving the macromolecule.

Our early research was carried out with γ-radiation; the effects of the more densely ionizing α-radiation on these systems is now being studied to provide a comparison between the effects of these two types of radiation and to point to possible differences in protection mechanisms. In the case of the densely ionizing α-radiation, track effects (reactions of primary reactive species with each other in the α-track before they have time to diffuse into the solution and react with the solute) have to be taken into account. This may lead to differences in the radiation effects on the macromolecules as well as on the protection.

The polymer solutions (polyvinylpyrrolidone, which is cross-linked in aqueous solution mainly by indirect action, and polyethyleneoxide, which is cross-linked mainly by the direct action of radiation) which we have investigated for γ-radiation do not lend themselves readily to a similar investigation using polonium-210 α-radiation. As an alternative system for this comparison, we have chosen solutions of yeast alcohol dehydrogenase. The irradiations were carried out with 0·1 ml. of 0·1 per cent solution in

special glass 'Melinex' cells at room temperature in the presence of air, with doses between 0·025 and 0·6 Mrads both with α- and γ-radiation. The residual activity of the irradiated alcohol dehydrogenase was determined spectrophotometrically at 340 mμ. Alcohol dehydrogenase, in the presence of nicotinamide adenine dinucleotide (NAD), catalyses the reaction to form acetaldehyde from ethanol, the nicotinamide adenine dinucleotide itself being reduced by this reaction. The method of analysis is to measure the velocity of the conversion of NAD to $NADH_2$ spectrophotometrically at a fixed wave-length (340 mμ). Yeast alcohol dehydrogenase has a reported molecular weight of 151,000 and contains about seven thiol groups per molecule on which it depends for its activity. The results shown in Fig. 1 indicate the usual exponential decrease in activity.

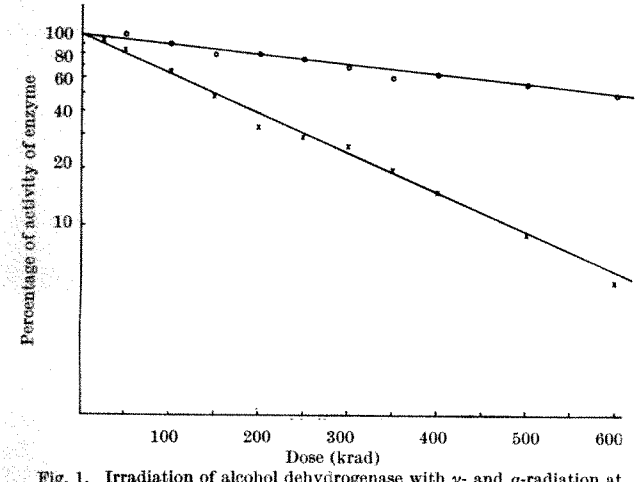


Fig. 1. Irradiation of alcohol dehydrogenase with γ- and α-radiation at room temperature in air. ○, Irradiation by polonium α-particles; ×, irradiation by cobalt-60 γ-rays. *p*H 7·9 to 8·0 adjusted with HCl.

It was found that the loss of activity of alcohol dehydrogenase was 44 per cent for a dose of 500 krads of α-radiation against 91 per cent for a dose of 500 krads γ-radiation. 44 per cent inactivation of the enzyme is brought about by a γ-dose of 125 krads, which means that γ-radiation is about four times as effective as α-radiation in inactivating alcohol dehydrogenase in aqueous solution.

For these 0·1 per cent solutions we can evaluate the average energy absorbed per enzyme molecule inactivated. If only the energy absorbed directly by the enzyme is considered, a dose of 500 krads of γ-rays given to a molecule of average molecular weight 151,000 amounts to:

$$E = \frac{5\times10^5\times0{\cdot}625\times10^{14}\times1{\cdot}51\times10^5}{6\times10^{23}} = 7{\cdot}8 \text{ eV}$$

However, this dose gives a considerable amount of deactivation (91 per cent). If r_0 is the average (37 per cent) inactivation dose,

$$\frac{n}{n_0} = \exp(-r/r_0)$$

where $n/n_0 = 0{\cdot}09$, $r = 5\times10^5$ rads so that $r_0 = 2{\cdot}07\times10^5$ rads; the average energy absorbed per enzyme molecule inactivation,

$$E_0 = 7{\cdot}8\,\frac{2{\cdot}07\times10^5}{5\times10^5} = 3{\cdot}2 \text{ eV}$$

This value is far lower than is common in the destruction of a homopolar bond. We must therefore assume an indirect effect, resulting from the radiolytic products formed in the water. If all the energy absorbed by the solution is transferred in this way, the value of E_0 increases to the high value of 3,200 eV energy absorbed in the solution per enzyme molecule inactivated. Such a high value may be reached owing to: (*a*) the very dilute nature of the solution; (*b*) self-protection of the enzyme owing to the thiol groups. Analogous measurements with very dilute solutions of γ-irradiated trypsin lead to a value of only 25 eV per trypsin molecule inactivated. Thus the former explanation cannot be sustained.

For α-radiation the average inactivation energy is four times greater, again emphasizing the recombination of particles within the α-track. Preliminary protection experiments have indicated that thiourea, cysteamine, cystamine, cystine and cysteine at low concentrations ($M\times10^{-4}$) protect the enzyme against both γ- and α-radiation. All these additives were found to be efficient protecting agents against γ-radiation in our polymer systems. This is being further investigated and more detailed results will be published later.

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Fluorescence Quantum Efficiencies of Octa-coordinated Europium Homogeneous and Mixed Chelates in Organic Solvents

Certain octa-coordinated europium chelates dissolved in organic solvents have been shown to be suitable liquid laser materials[1–4]. Determinations of fluorescence quantum efficiency for these compounds in solution are essential for their evaluation as potential candidates for efficient laser performance as well as for an understanding of the processes associated with the overall energy transfer.

The measurements of fluorescence quantum efficiency reported by Gudmundsen *et al.*[5] and Winston *et al.*[6] for europium theonyltrifluoroacetonate in ethanol, toluene and acetone were carried out with a chelate of undetermined composition. Bhaumik and Telk[7] reported fluorescence quantum yield values for europium complexes of three β-diketone ligands in actone, dimethylformamide (DMF), a mixture of ethyl alcohol and methyl alcohol, and a mixture of ether-isopentane-ethyl alcohol (EPA) at 300° K, 100° K and 77° K.

We have determined the relative fluorescence intensities of europium β-ketoenolates with different substituents attached to the chelate ring in solvents of various polarity in the freezing point–boiling point temperature range[8]. This communication reports the quantum efficiency of several octa-coordinated europium homogeneous (same ligand) and mixed (different ligand groups) chelates in a number of solvents at 25° C. These chelates were carefully prepared, purified, and well characterized by elemental analysis to correspond to the designated formula. Synthesis procedures for the ligands and their europium complexes have previously been described[9–11]. The following europium chelates were prepared: *tetrakis*(theonyltrifluoroacetonate)piperidinium (EuT_4P), *tris*(theonyltrifluoroacetonate)dipyridyl (EuT_3Dipy), *tris*(theonyltrifluoroacetonate)phenanthroline (EuT_3Phen), *tetrakis*(benzoylacetonate)piperidinium (EuB_4P), *tris*(benzoylacetonate)dipyridyl (EuB_3Dipy), *tris*(benzoylacetonate)phenanthroline (EuB_3Phen), *tetrakis*(dibenzoylmethide)piperidinium (EuD_4P), *tris*(dibenzoylmethide)dipyridyl (EuD_3Dipy), *tris*(dibenzoylmethide)phenanthroline (EuD_3Phen), *tetrakis*(benzoyltrifluoroacetonate)piperidinium ($EuBt_4P$), *tetrakis*(2-naphthoyltrifluoroacetonate)piperidinium ($EuNt_4P$), and *tetrakis*(dianisoylmethide)piperidinium ($EuDA_4P$). 'Spectro' grade acetonitrile, dimethylformamide and benzene were used without further purification. Absolute ethanol and butyronitrile were purified by repeated distillation from magnesium ethoxide

and anhydrous magnesium sulphate respectively. 10^{-3} molar solutions of chelate were freshly prepared before each determination. An Aminco-Bowman spectrophotofluorimeter was used to record the apparent fluorescence spectra in the front-surface arrangement. At the concentrations used, the exciting light (360 mμ) was completely absorbed for all chelate solutions in a thin layer near the front window of the cell. Correction factors were determined both for the exciting unit (xenon arc, monochromator, slits) and the detector system (slits, monochromator, *R*136 red-sensitive photomultiplier) by known procedures[12-14]. The corrected fluorescence spectra of chelate solutions were plotted against wave number and the area under the true fluorescence spectrum was calculated and compared with that obtained with freshly prepared solutions of rhodamine *B* in ethyl alcohol and eosin, in both ethyl alcohol and 10 N sodium hydroxide. The concentration of these two standards of known quantum efficiency was adjusted so that the optical density of the solutions was equal to that of the different chelates at the exciting wavelength. Rhodamine *B* in ethyl alcohol has a quantum efficiency of 0·69 (ref. 12), and eosin in ethyl alcohol or 10 N sodium hydroxide of 0·23 respectively[12].

Table 1 gives the results found for dimethylformamide (DMF) and acetonitrile (CH_3CN) for 10^{-3} M chelate solutions. The quantum efficiencies for the three chelates having a trifluoromethyl group were determined in addition to the two solvents mentioned in benzene (C_6H_6), ethanol (EtOH), and butyronitrile (C_3H_7CN). The values are given in Table 2.

Table 1

Chelate	Quantum efficiency in DMF	CH_3CN
EuT_4P	0 615	0 632
EuT_3Dipy	0 503	0·163
EuT_3Phen	0·365	0·123
EuB_4P	0·063	0 038
EuB_3Dipy	0 074	Insol.
EuB_3Phen	0 075	0·092
EuD_4P	0·074	0 067
EuD_3Dipy	0·058	Insol.
EuD_3Phen	0·040	0·049
$EuDA_4P$	0·035	Insol.

Table 2

Chelate	CH_3CN	Quantum efficiency in C_6H_6	C_3H_7CN	DMF	EtOH
EuT_4P	0·632	0 221	0·633	0 615	0·151
$EuBt_4P$	0·578	0·295	0·624	0·473	0·230
$EuNt_4P$	0·580	Insol.	0 585	0·561	0 354

The results were found to be reproducible to within 1 per cent. Removal of oxygen from solutions did not change the quantum efficiencies to any significant extent.

The quenching which results in decrease of the quantum efficiency is attributed to radiationless deactivation arising from vibronic coupling of the energy levels in both ligand and rare earth ion either intramolecularly or to the surrounding solvent molecules. The results in Table 2 are consistent with a higher quantum efficiency for the dissociated species $EuL_4^{\ominus}$ as compared with the neutral species EuL_4P (*L* is the ketoenolate anion). We have shown previously from molecular weight and conductivity measurements[15] that the fluorinated *tetrakis* chelates are essentially heterolytically dissociated in acetonitrile and DMF and undissociated in non-polar solvents such as benzene. This suggests strong intramolecular quenching at the organic cation site, probably through coupling of the electronic energy levels to vibrations of the two N—H bonds in the piperidinium cation. This is possible only in the undissociated *tetrakis* chelates. The low quantum efficiency in alcoholic solution has been attributed to the presence of the high energy vibrations of the O—H bonds in the solvent and confirmed by the significant improvement in quantum efficiency obtained with deuterated hydroxylic solvents[7,16,17]. In general, mixed chelates have lower quantum efficiency than the corresponding homogeneous *tetrakis* complexes. Butyronitrile is slightly superior to acetonitrile and DMF as a liquid laser host for europium ketoenolates.

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Correlation between Chemi-ionization in Flames containing Organic Fuels and the Heat of Oxidation of the Carbon in the Fuel Molecule

THE "chemi-ionization" which occurs when organic compounds are burned, either alone or mixed with hydrogen, in oxygen or air has received much attention. Data obtained by means of the flame ionization detector used in gas chromatographic work[1-4], and studies of ionization in flames at reduced pressure[5], show that when a variety (and sufficiently large number) of organic compounds is examined, certain regularities of behaviour become apparent. First, when traces of such a compound are introduced into a hydrogen/oxygen/nitrogen flame, the number of free electrons produced is proportional to the concentration of the compound in the flame gases. Secondly, for equimolar concentrations of compounds which are members of the same homologous series, the number of electrons varies linearly with the number of carbon atoms in the molecule. Thirdly, when the compound contains C=C or C≡C bonds[5], the number of electrons is increased with respect to the corresponding paraffin, and reduced when it contains oxygen.

These and other data of a similar nature have been used in the past to support specific reaction schemes for chemi-ionization, and the one which seems to have gained most support[5-7] is the reaction originally proposed by Calcote[8]: $CH + O \rightarrow CHO^+ + e^-$. On the other hand, workers interested primarily in the applications of the flame ionization detector have attempted to estimate atom and group responses[9] on the basis of the known responses of the detector to various compounds. Dewar[2] has also attempted to correlate the relative responses with the changes in bond energy involved in forming carbon dioxide from carbon atoms, disregarding completely the bond energies of any links broken in the oxidation.

The purpose of this communication is to direct attention to a striking correlation which seems to exist between

the relative number of electrons produced by a given organic compound $C_xH_yO_z$, and the heat of the reaction:

$$C_xH_yO_z + O_2 \rightarrow CO_2 + H_2 \quad (1)$$

(which is equivalent to the heat of combustion of the compound minus the heat of formation of any resulting water—at least for substances containing carbon, hydrogen and oxygen only). The heat of reaction (1), calculated from the heats of combustion and the heats of formation given in refs. 10 and 11, is plotted in Fig. 1 against the number of carbon atoms in the molecule for a selection of paraffins, olefines, acetylenes, benzenes, aliphatic alcohols, ketones, esters and acids. In Fig. 2 the relative steady-state numbers of free electrons obtained per fuel molecule burned in stoichiometric, premixed flames with oxygen at reduced pressure[5] are plotted in a similar fashion for most of the same compounds. The similarity between Figs. 1 and 2 is indeed marked.

An apparent point of difference is that the curves for the various types of compounds are more widely separated in Fig. 2 than in Fig. 1. It should be borne in mind, however, that the ordinates in Fig. 2 represent relative steady-state concentrations of free electrons under flame conditions, that is, they are partly determined by the process responsible for the removal of the charged species

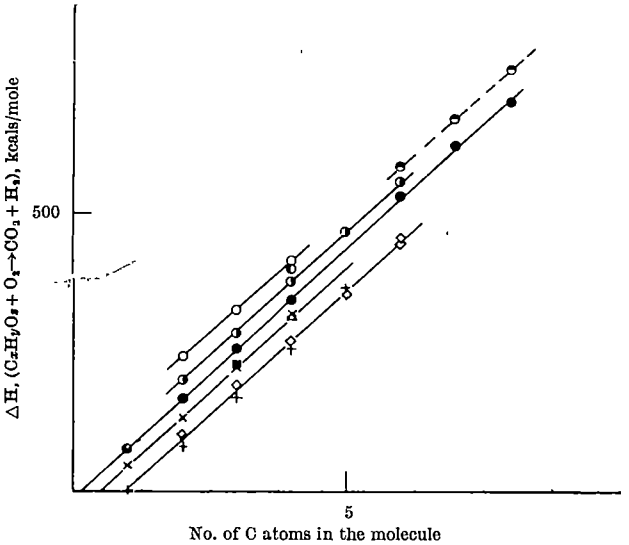


Fig. 1. Heats of oxidation of carbon in various molecules. ΔH, $(C_xH_yO_z + O_2 \rightarrow CO_2 + H_2)$ versus the number of carbon atoms in the molecule, for paraffins (●), olefines (◑), acetylenes (○), benzenes (◓), alcohols (×), ketones (■), ethers (△), esters (◇) and acids (+).

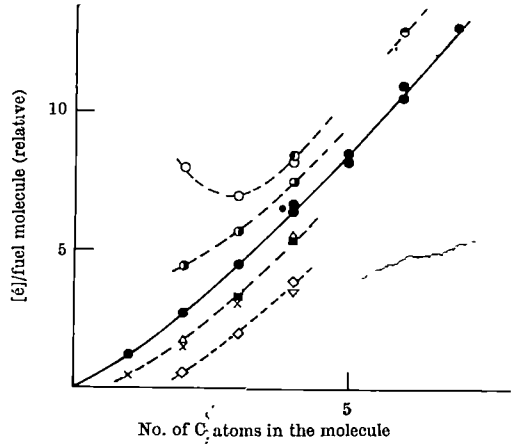


Fig. 2. Relative ionization in stoichiometric flames of various fuels (at reduced pressure). [e⁻]/fuel molecule (relative) versus the number of carbon atoms in the molecule, for paraffins (●), olefines (◑), butadiene (◐), acetylenes (○), benzene (◓), alcohols (×), ketones (■), ether (△), dioxane (▽) and esters (◇).

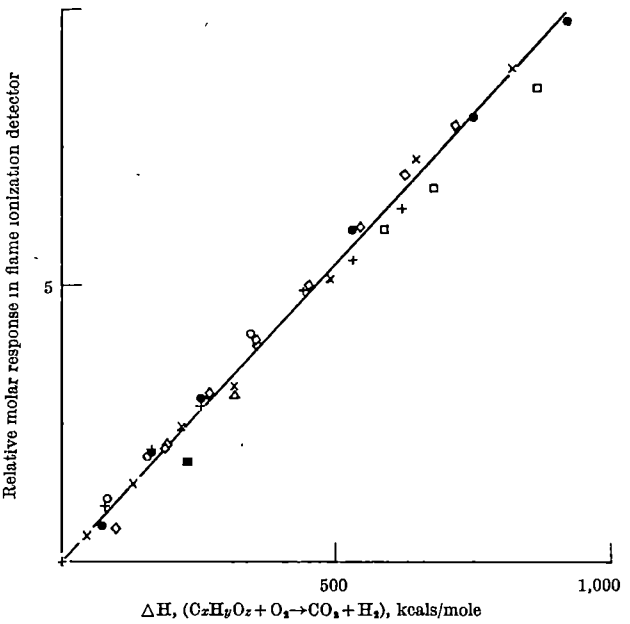


Fig. 3. Relative numbers of ions produced from various compounds as a function of the heat of oxidation of carbon in the molecule. Relative molar response in flame ionization detector versus ΔH, $(C_xH_yO_z + O_2 \rightarrow CO_2 + H_2)$, for paraffins (●), alcohols (×), ketones (■), ethers (△), esters (◇), acids (+), aldehydes (□) and glycols (○).

above the reaction zone. On this account it would no doubt be more satisfactory to use the relative responses of compounds in the flame ionization detector as ordinates in Fig. 2; such responses should represent the maximum rates of production of free electrons in a specified flame and be independent of the recombination processes. These ionization detector data were not used, however, because although they fall into the same pattern as the steady-state results for most classes of compounds, it appears that olefines and aromatic hydrocarbons do not give any more ions than the corresponding paraffins[3,4]. It is possible that this results from incomplete decomposition of these particular molecules on passage through the small diffusion flames[9] usually used in this type of work.

In Fig. 3 the relative ionization detector responses obtained by Perkins *et al.*[4] for paraffins, aliphatic alcohols, esters and acids are plotted against the heat of reaction (1). It can be seen that at least for these compounds there is a linear relationship between the two quantities.

The implications of this relationship will not be discussed fully here, except to note that it supports earlier conclusions[2,5] that from the point of view of ionization the molecule is shattered into single carbon atom units. It also suggests that the overall energy required to produce the ionization is related to that deriving from the complete oxidation of carbon in any particular molecule, without any apparent contribution from the oxidation of the hydrogen contained in the molecule.

Attention might also be directed to the fact that at least some carbon compounds which contain no hydrogen give rise to measurable responses in the ionization detector when added to a hydrogen flame—for example, carbon tetrachloride, dicarbon tetrachloride (ref. 3) and carbon disulphide (ref. 2). Moreover, if the relative responses for the chloromethanes and chloroethylenes[3] (scaled by comparison with *n*-heptane) are plotted against the heat of the hypothetical reaction:

$$C_xH_yCl_n + O_2 \rightarrow CO_2 + H_2 + Cl_2 \quad (2)$$

analogous to reaction (1), the points fit the straight line of Fig. 3 quite well. This would seem to eliminate the necessity for a C—H bond in the original molecule in order for the chemi-ionization to occur. The observation of

Sternberg *et al.*[9] that traces of carbon tetrachloride in carbon monoxide flames give rise to ions with an efficiency similar to methane appears to eliminate the suggestion that hydrogen participates in any form. However, this result must be regarded as inconclusive because apparently no precautions were taken to exclude all traces of moisture from the gases used.

I should like to acknowledge an I.C.I. Fellowship.

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Separation of Coumarins

SORPTION on cation exchange resins allows the complete separation of some coumarins[1] in dilute solution using a simple technique. A column of the styrene-divinylbenzene copolymer-type sulphonic acid cation exchange resin, Dowex 50 *W-X* 4 (−100, +200 mesh, Dow Chemical Co.), in the hydrogen form (bed length, 53 cm; bed volume, 131 c.c.) has been used for this purpose.

The coumarins investigated are divided into two groups. Group *A* includes coumarin, 7-hydroxycoumarin, 8-hydroxycoumarin and 7,8-dihydroxy-4-methyl coumarin; Group *B* includes 7-methylcoumarin, 3,4-dimethylcoumarin, 7-methoxycoumarin, 6-methoxy-4-methylcoumarin and 6,7-dimethoxy-4-methylcoumarin.

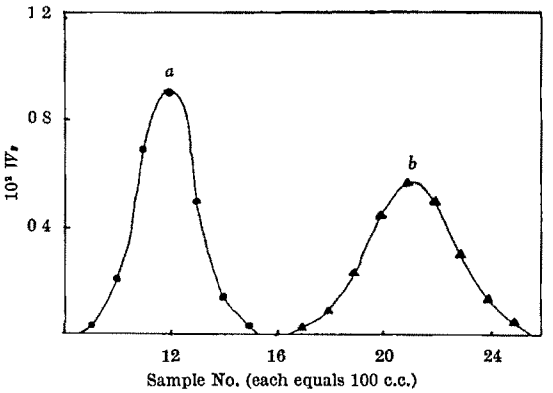


Fig. 1. Separation of (*a*) 7-hydroxycoumarin ($W = 2\,508 \times 10^{-5}$) and (*b*) 7-methoxycoumarin ($W = 2\,446 \times 10^{-5}$). W, solute content in m.equiv/100 c.c. of solution and sorbed on the resin bed. W_r, No. of m.equiv. of solute in 100 c.c. of effluent sample.

100 c.c. of solution (~5–6 × 10^{-4} M) in 10 per cent methanol (by volume), containing only one compound from each group, was passed through the column at the rate of 5 c.c./min and the run was then continued with 10 per cent methanol (by volume) as influent, to elute the sorbed solutes on the column. The effluent was collected in samples and estimated for solute content by ultraviolet absorption[2]. Each compound was eluted separately. Fig. 1 shows the separation obtained with a mixture of 7-hydroxycoumarin and 7-methoxycoumarin.

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Chloro-olefine Complexes of Platinum

ALTHOUGH the reactions of fluoro-olefines with transition metal complexes have yielded very stable fluoro-olefine and fluorocarbon complexes[1–3] analogous reactions with chloro-olefines have hitherto resulted in chlorination to form metal chlorine bonds[4–6]. We find, however, that on refluxing a benzene solution of $Pt(PPh_3)_4$ (ref. 7) with tetrachloroethylene the complex $(Ph_3P)_2PtC_2Cl_4$ is formed in high yield as white air stable crystals with a melting point of 278° C dec. This would seem to be the first example of a perchloro-olefine transition metal complex. The reaction of $Pt(PPh_3)_4$ with a number of halogeno-olefines, for example, tetrafluoroethylene, trichloroethylene, *trans*-dichloroethylene, 1,1,1-trifluoro-2,3,3-trichloro-2-propene, appears to be a general reaction for the preparation of complexes of the type $(Ph_3P)_2Pt$ olefine. The complexes prepared in this work and the recently described tetracyanoethylene[8] complex are considerably more stable than the olefine complexes of this type which were originally prepared with *trans*-stilbene, *trans* 4,4, dinitrostilbene and acenaphthylene[9].

The infra-red spectrum of the tetrachloroethylene complex shows bands owing to carbon chlorine vibrations at 910, 804 and 796 cm^{-1}. The other chloro-olefine complexes also show carbon chlorine bands in this region of the infra-red. No band that can be attributed to a C=C stretching frequency[10] is observed in the infra-red spectra of the tetrachloroethylene and trichloroethylene complexes between 2,000 and 1,500 cm^{-1}. The compounds are probably best regarded as complexes of Pt (II), the olefine forming a three membered ring with the platinum by way of two σ bonds.

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Doubly charged Negative Ions of Oxygen, Fluorine, Chlorine and Bromine

ELECTRON impact processes give rise to both positive and negative ions. The negative ions are, in general, only about 10^{-3} times as intense as the positive ions. In contrast with positive ions, only singly charged negative ions have previously been reported. Calculations involving the crystalline state, where doubly charged negative ions (such as $O^=$ and $S^=$) apparently do exist, indicate that the formation of $O^=$ from O^- requires 7·8 eV in the gas phase[1]. The lifetime of a doubly charged ion in the gas phase would be expected to be short and has been predicted to be 10^{-10} sec or less[2]—too short for observation

in the usual mass spectrometer. This communication reports the observation of doubly charged negative ions of oxygen, fluorine, chlorine and bromine, with indications that the lifetimes are of the order of 10^{-4} sec (the ion collection time in the mass spectrometer used).

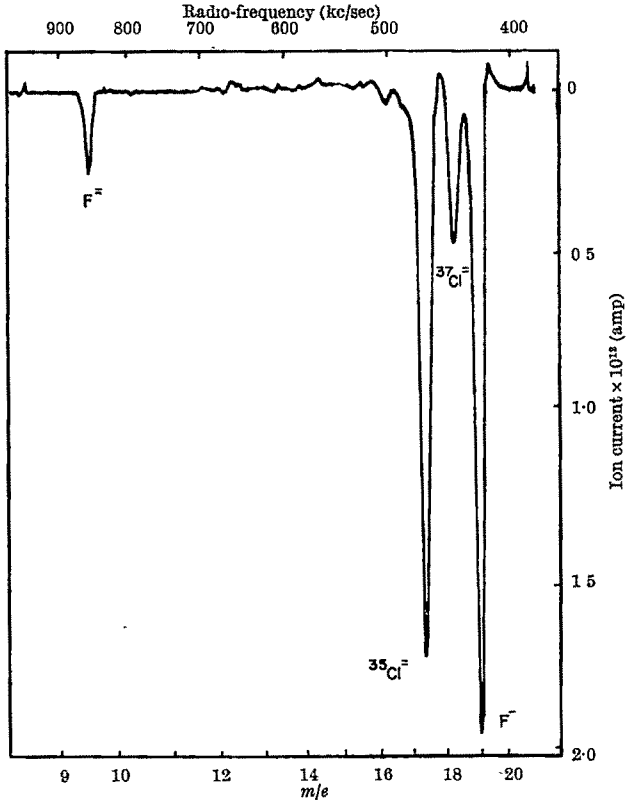


Fig. 1. Partial negative ion mass spectrum of CF_3Cl. The $^{35}Cl^-$ and $^{37}Cl^-$ peaks are not shown, but have an intensity of about ten times the $^{35}Cl^=$ and $^{37}Cl^=$ peaks. Electron energy, 70 V, trap current, 2 μamp; trapping potential, −0·75 V, radio-frequency voltage, 5 V/cm, 5 kG magnet.

We have observed these doubly charged negative ions in the mass spectra of carbon monoxide, oxygen, carbon tetrachloride, carbon tetrafluoride, CF_3Cl, CF_3Br and CF_2Cl_2, together with the expected singly charged negative ions oxygen, fluorine, chlorine and bromine. In the cases of CF_3Cl, CF_3Br and CF_2Cl_2, where both halogens are present, singly and doubly charged negative ions were observed for both halogens. A typical spectrum which shows F^-, $Cl^=$ and $F^=$ is presented in Fig. 1. Mass analysis was performed with an omegatron mass spectrometer. This instrument was developed for study at low masses[3], where resolution is usually very high. Resolution, however, is an inverse function of mass in the usual omegatron units—a fact which has limited the application of this unique instrument. The omegatron functions basically as a small cyclotron with the very important distinction that the radio-frequency electric field is applied across the entire analysing region and not just between the faces of two "D's" as in the cyclotron. This eliminates the possibility of harmonics in the omegatron operation[4]. (Our investigations confirm that the doubly charged negative ions which we observed do not arise from harmonics, but rather have the properties of a doubly charged species. This conclusion is based on studies of the effects of variations of pressure, trapping potential, electron beam current, and radio-frequency voltage. Peaks at multiples of the resonant frequency were not observed.) An ion the resonance frequency of which is equal to the applied radio-frequency, gains energy from the field continuously and generates an Archimedes spiral until the ion strikes the target electrode and is detected. A mass spectrum is obtained by recording the ion current as a function of the radio-frequency applied. Ions not detected are out of phase with the radio-frequency field, cannot gain energy continuously from the field, and therefore do not exceed a radius which is significantly less than the radius to the detector. Non-resonant ions thus generate complex rosettes[3]. A distinguishing characteristic of the omegatron is the fact that not all ions are extracted from the ion source.

The ion source used was essentially that of the General Electric ion resonance mass spectrometer. Measurements were made with a nominal electron energy of 70 eV at ion source pressures of the order of 10^{-5} torr and a trap current of 2 amp. Negative ions were selected for study by the application of a variable trapping voltage. By applying a negative d.c. voltage to two plates symmetrically placed on either side of the electron beam, instead of a positive charge which has been routinely used in the omegatron, not only are the negative ions held in the plane of the detector to increase sensitivity but also positive ions can be partially or completely removed (depending on the d.c. potential) from the ion beam. A General Radio type 1001*A* signal generator was used together with a Keithley model 610*A* electrometer and a permanent magnet of 5 kG.

It is apparent from the spectra of these compounds (see Fig. 1) that the doubly charged negative ions are fairly intense. No doubly charged negative species was noted in other negative ion mass spectrometric studies of these compounds; this eliminates consideration of the highly improbable process whereby an atom may capture two electrons simultaneously to form the doubly charged negative ion. However, it is possible for secondary processes to occur in the omegatron[5]. Consider the path of a non-resonant ion: after formation by electron impact, it is accelerated from the electron beam only to reach some maximum radius and then pass back through the electron beam again. Thus, for example, when a radio-frequency is selected which corresponds to an m/e value of 17·5 ($^{35}Cl^=$), the $^{35}Cl^-$ resonance frequency is not the same as the applied radio-frequency and $^{35}Cl^-$ would follow a non-resonant ion path. When the ion passes back through the electron beam, it is possible in the interaction of the singly charged negative ion and the electron beam to form a doubly charged negative ion. Alternatively, two non-resonant Cl^- ions may interact to form the observed $Cl^=$. The Cl^- ion intensity has been observed to increase linearly with the electron beam current while the $Cl^=$ ion intensity is very nearly a square function of the electron beam current. This is the dependency dictated by either of the mechanisms proposed here. Furthermore, these mechanisms give some indication of why doubly charged negative ions can readily be observed in an omegatron mass spectrometer.

We thank Esso Research and Engineering Co., Baton Rouge, Louisiana, for the gift of the ion resonance mass spectrometer which was modified and used in this investigation.

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Gas–Solid Chromatographic Separation of Atmospheric Gases on Activated Alumina

SEPARATION of oxygen, nitrogen and carbon dioxide by gas–solid chromatography has been accomplished on multiple columns of different adsorbents[1,2]. Greene *et al.*[3] almost achieved this separation on activated charcoal, but resolution of oxygen and nitrogen with this adsorbent was incomplete.

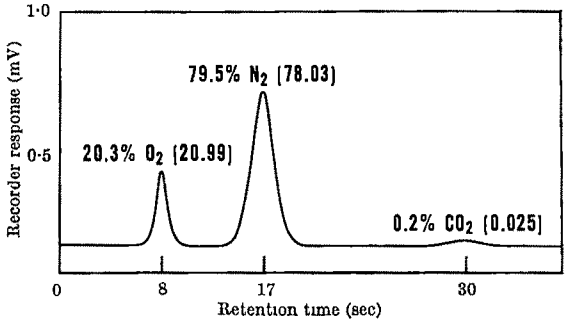


Fig. 1. Chromatogram of air gases from an alumina column purged with carbon dioxide. Figures in parentheses are values for the composition of dry air. Instrument: Beckman GC-4 Column, 4 ft. × 1/8 in. aluminium packed with 60/80 mesh *F*-20 'Microtek' alumina (activated by passing helium carrier through it for 1 h while at 350° C sample size, 15 μl. injected by way of sampling valve; carrier, helium, at 30 c.c./min; detector, thermal conductivity, operated at 25° C, 235 m.amp; attenuation, × 5; chart speed, 12 in./min.

Fig. 1 is a chromatogram of air gases from a single 4-ft. column of 'Microtek' alumina operated isothermally at 0° C. Because alumina at low temperatures tends to adsorb carbon dioxide irreversibly[4], the column was first purged with that gas for 1 h at 0° C to reduce adsorbent activity. Peak area percentages for both oxygen and nitrogen are in close agreement with the known composition of air. The somewhat high value for carbon dioxide probably resulted from dry ice used during the experiment. Column purge with carbon dioxide is necessary and important to its quantitative elution from alumina. Analysis of pure nitrogen, after the carbon dioxide purge, indicated no carbon dioxide displacement—only the nitrogen peak eluted. To ensure carbon dioxide peaks of reproducible area, it was necessary to carry out the purge often—preferably once every 3 h for about 15 min. Any gradual loss of carbon dioxide during column use would be shown by a slow rise in the chromatogram baseline. This was not observed when the purge was carried out regularly. Because irreversible adsorption effects are strong enough to remove carbon dioxide from air mixtures if purging is neglected, a carbon dioxide "peak" from a saturated column operated isothermally can only appear when carbon dioxide is introduced in amounts that exceed the adsorbed saturation concentration.

Although the Beckman *GC*-4 chromatograph was equipped with a low-temperature accessory for oven cooling, this attachment was not used. Instead, to check amenability of the separation technique to instruments of less refinement, dry ice in a paper cup was placed in the oven. The oven atmosphere was cooled as the airstream from the circulating fan was continuously passed over the surface of the dry ice. Inlet and exhaust ports, feed lines, and the detector compartment were operated at room temperature to maximize detector sensitivity[5]. Air samples of 15 μl. were routed to the column through a gas sampling valve, although chromatograms could be exactly duplicated by syringe injections of equivalent volumes.

Activated alumina offers a convenient means by which air gases can be analysed. Its use allows the analyst to operate his instrument under the best possible conditions of flow and temperature and also results in fast, reproducible chromatograms.

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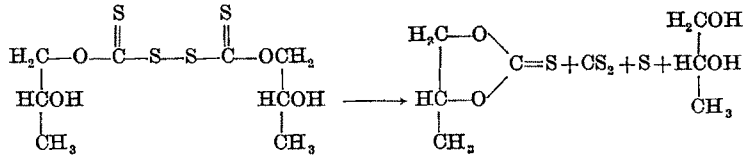
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Rearrangement of *Bis*(o-thiocarbonyl) Disulphides

IN recent work[1] we showed that *bis*(1,2-*o*-isopropylidene-3-*o*-thiocarbonyl-α-L-glucofuranose) disulphide rearranged on standing in basic organic solvents to give equimolar amounts of carbon disulphide, elemental sulphur, 1,2-*o*-isopropylidene-α-D-glucofuranose, and 1,2-*o*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate. That work has now been extended to an investigation of *bis*(*o*-thiocarbonyl) disulphide derivatives (referred to as xanthides) of 1,2-, 1,3-, 1,4-, L5-, and 1,6-dihydroxy alkanes.

The monoxanthates of the diols were prepared by treating the diol compound or its dioxane solution with 1 molar equivalent of sodium hydroxide (5 N) and excess carbon disulphide. The corresponding xanthides were prepared by treating the xanthates with iodine solution.

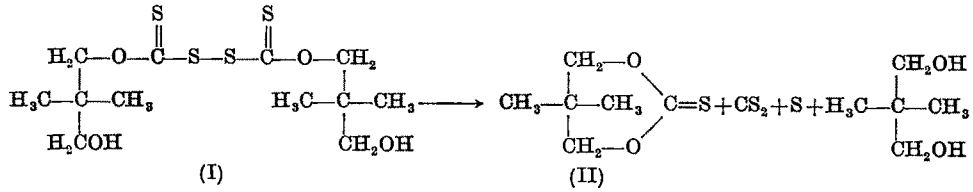
The monoxanthide of 1,2-propanediol on standing either at 25° C for 3 days or in anhydrous pyridine at 25° C for 3 h rearranged to yield equimolar amounts of 1,2-propanediol, 1,2-propane thionocarbonate, carbon disulphide, and elemental sulphur.



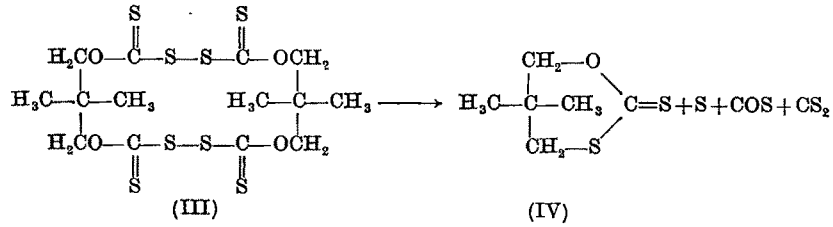
The thionocarbonate was recovered as an oil. Analysis showed a sulphur content of 27·6 per cent; the sulphur content calculated for $C_4H_6O_2S$ is 27·1. The ultra-violet spectrum showed λ_{max}^{MeOH} 234 mμ (ε 13,800). The extinction coefficient of 1,2-*o*-isopropylidene-α-D-glucofuranose 5,6-thionocarbonate at 234 mμ was 15,700.

The monoxanthide of 2,2-dimethyl-1,3-propanediol (I) was recovered in an overall yield of 43 per cent as a yellowish crystalline compound, m.p. 103–106°; λ_{max}^{MeOH} 240 mμ (ε 20,000), 285 mμ (ε 11,400). Analysis showed: carbon, 39·2; hydrogen, 6·1; sulphur, 36·0. The values calculated for $C_{12}H_{22}O_4S_4$ are: carbon, 40·2; hydrogen, 6·1; sulphur, 35·7. When (I) was allowed to stand in anhydrous pyridine at 45° C for 4 h, equimolar amounts of carbon disulphide, elemental sulphur, and the cyclic thionocarbonate derivative (II) of 2,2-dimethyl-1,3-propanediol were identified. Compound II was crystalline, m.p. 101°, λ_{max}^{MeOH} 244 mμ (ε 14,500) and its structure was formulated on the following basis: elemental analysis gave the empirical formula $C_6H_{10}O_2S$. Found: carbon, 49·5; hydrogen, 6·9; sulphur, 21·7; molecular weight, 138 (vapour pressure osmometry in acetone); calculated for $C_6H_{10}O_2S$: carbon, 49·3; hydrogen, 6·9; sulphur, 21·9; molecular weight, 146. The infra-red spectrum showed no hydroxyl absorption. The presence of a C=S group was shown by a positive iodine-sodium azide test[2] and by

converting II quantitatively to the known cyclic carbonate[3] on treatment with silver nitrate. The thionocarbonate II was prepared independently by treating a dioxane–pyridine solution of 2,2-dimethyl-1,3-propanediol with thiophosgene by a procedure reported previously[1].



(I) (II)



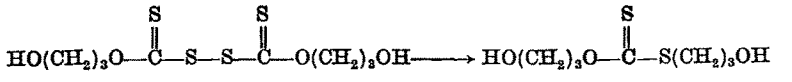
(III) (IV)

When a dioxane solution of 2,2-dimethyl-1,3-propanediol was treated with a large excess of sodium hydroxide (12 N) and carbon disulphide, the dixanthate was obtained. Treatment with iodine gave the corresponding crystalline dixanthide (III) in a 40 per cent yield, m.p. 183–185°, λ_{max}^{MeOH} 99 per cent–dioxane 1 per cent 240 mμ (ε 18,600), 285 mμ (ε 6,400). Found: carbon, 33·0; hydrogen, 3·9; sulphur, 49·0; molecular weight, 500 (Rast in camphor): calculated for $C_{14}H_{20}O_4S_8$: carbon, 33·0; hydrogen, 3·9; sulphur, 50·4; molecular weight, 508. The dixanthide was recovered unchanged on treating with pyridine at room temperature for 18 h, but on heating on a steam bath for 30 min, III decomposed with rearrangement to give elemental sulphur, carbon disulphide, carbonyl sulphide, and a cyclic dithiocarbonate derivative (IV).

The dithiocarbonate (IV) was recovered in 95 per cent yield as a crystalline solid, m.p. 62–63°; λ_{max}^{MeOH} 225 mμ (ε 5,300), 295 mμ (ε 12,000). Found: carbon, 44·4; hydrogen, 6·2; sulphur, 39·1; molecular weight, 162 (vapour pressure osmometry in acetone). Calculated for $C_6H_{10}OS_2$: carbon, 44·5; hydrogen, 6·2; sulphur, 39·6; molecular weight, 162.

The monothiocarbonate derivative of compound IV was obtained as a syrup on reflux of an acetone solution of IV with a saturated solution of potassium permanganate. Found: sulphur, 21·4; calculated for $C_6H_{10}O_2S$: sulphur, 21·9. The course of oxidation was followed spectrometrically and, by the end of the reaction, practically no absorption occurred at 295 mμ while a strong carbonyl peak showed at 5·9μ. Alkaline hydrolysis of an alcoholic solution of the monothiocarbonate derivative at 25° gave 1 molar equivalent of sulphydryl derivative as revealed by iodine titration.

Although (I) rearranged in pyridine to yield a thionocarbonate, 1,3-propanediol monoxanthide when treated under similar conditions underwent a different type of rearrangement-fragmentation. This reaction may be represented as follows:



The monoxanthides of 1,4-butanediol, 1,5-pentanediol, and 1,6-hexanediol rearrange similarly to form the *S*-alkyl dithiocarbonate esters in good yield. All the *S*-alkyl dithiocarbonate esters had characteristic absorption maxima at 225 and 280 mμ. A similar spectrum was obtained for the known 1,2 : 5,6-di-*o*-isopropylidene-3-*o*-[(methylthio)-thiocarbonyl]-α-D-glucofuranose. Infra-red analysis revealed nearly identical spectra for the esters with characteristic absorption bands at 7·2 and 8·4μ, whereas the corresponding xanthides had absorption maxima at 7·0 and 8·7μ.

The thermal decomposition of xanthides was reported by Twiss[4] to give *S*-alkyl dithiocarbonate esters along with carbonyl sulphide and elemental sulphur.

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Oxidation of Transition Metals in Molten Lithium Chloride–Potassium Chloride Eutectic by Mercuric Chloride

MOST investigations of ionic melts have been concerned with the electrochemical[1] or spectroscopic properties of the solution[2]; relatively little work has been carried out on specific chemical reactions in these media[3,4]. These systems provide obvious advantages over the more conventional liquid media normally used in the preparation of complexes. However, difficulties can occur in isolation of the product from the melts, but these reactions can be particularly useful for the preparation of complexes to be studied *in situ*. As part of the general study of chemical reactions in melts, this communication reports some oxidation studies which we have carried out in potassium chloride–lithium chloride eutectic (40·5 mole per cent potassium chloride) of transition metals, as this provides a useful means of producing a variety of metal chloro-species, some of which are extremely susceptible to hydrolysis. For these complexes the alternative preparations may lead to potential contamination and subsequent difficulty in studying the properties of these compounds in the melt.

From the available redox potentials in the lithium chloride/potassium chloride eutectic, in principle, a number of reactants are capable of oxidizing the transition metals and a preliminary survey using chlorine, mercuric chloride, mercurous chloride and silver chloride was carried out. All the reagents were found to react with a

variety of the metals; thus nickel metal, which is inert to the potassium chloride/lithium chloride eutectic, was rapidly oxidized to the characteristic blue tetrachloronickelate II anion, $[NiCl_4]^{2-}$, by all the reagents. We have confined our investigations chiefly to the mercuric chloride system, as this provides a suitable redox system [$E^\circ = -0{\cdot}5$ V]. In addition, the mercury formed in the reaction was isolated and used as a measure of the oxidation state of the metal ion in solution from the ratio of mercury recovered to metal lost. Using mercuric chloride, it has been possible to oxidize all the transition elements, with the exception of platinum, iridium, osmium and gold, and this technique provides a useful means of rapidly obtaining a solution of these metals which has analytical potential. We have been concerned with establishing both the oxidation state and stereochemistry of the resultant chloro-complexes both in solution and in the solid melts. The oxidation states of manganese, nickel and copper determined chemically are 2·09, 2·07 and 1·01 respectively. The spectra indicate that, in the solid, all the complexes with the exception of cobalt are octahedral, while in the melt iron, cobalt, nickel and possibly manganese are tetrahedral. Thus a change of stereochemistry has occurred on solidification of the melt. The oxidation states of the melts were established as titanium III, chromium III, iron II and cobalt II from the spectra in the melt.

Two sets of conditions can be used, corresponding to an excess of either the metal or the oxidizing agent. In the former case this can lead to a mixture of oxidation states; thus in the vanadium system the spectra and the computed oxidation state ($\sim 2{\cdot}5$) indicate a mixture of vanadium II and III chloro-complexes. It has been shown that vanadium III is reduced to vanadium II by vanadium in the melt[5]. With excess of mercuric chloride only vanadium III species were present. For the second and third row transition elements, it is possible that the intermediate oxidation states obtained may correspond to the formation of metal clusters which formally appear to contain mixed oxidation states[6]; this aspect is at present being investigated as a potential preparative method for these classes of compounds.

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BIOPHYSICS

Effect of Temperature on Bioelectric Oscillations of Bean Roots

It has been previously shown that roots of *Vicia faba* immersed in dilute salt solutions generate weak electric fields[1]. These cause potential differences that can be measured in regions adjacent to the root. Under certain conditions, these potentials undergo spontaneous oscillations of several millivolts amplitude, with a period of about 5 min (that is, 12 cycles per hour) at 25° C (ref. 2). If the bioelectric field of a root is not oscillating spontaneously it can be forced to oscillate by subjecting it to periodic oscillations of osmotic pressure, or of concentration of the growth hormone indolyl-3-acetic acid in the surrounding medium. Under these conditions, a characteristic resonance response is observed when the enforcing oscillation has a period close to the natural period of the spontaneous oscillation[3]. This behaviour has been described in terms of a feedback oscillator having three exponential delay elements for which the following sequence of operations has been proposed: the potential of the cellular membrane effects migration of growth hormone electrophoretically; the hormone alters permeability of the membrane, and the resulting change in ion movement modifies the electric field, thus completing the loop. The literature on this subject has been reviewed by Scott[4].

To define more precisely the interacting parts of the feedback loop, we have investigated the effect of temperature on the oscillation of the electric field of the root. This investigation shows that the frequency of the electric oscillation is dependent on the mean temperature of the medium surrounding the bean root.

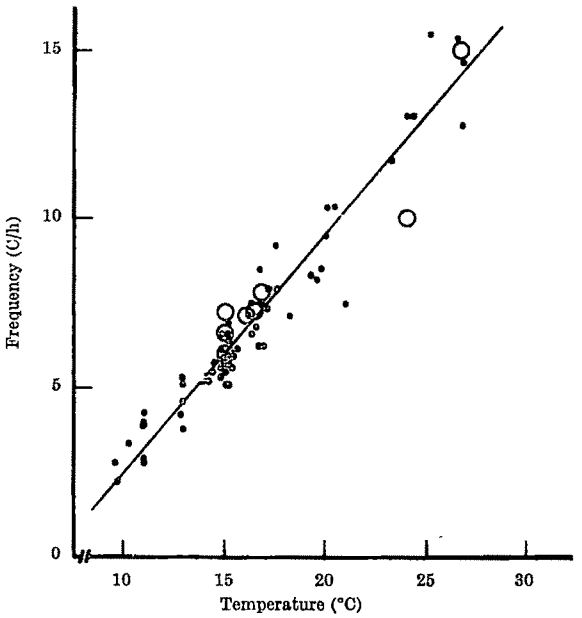


Fig. 1. Frequency of oscillation as a function of mean root temperature. Data obtained for two or more consistent cycles of oscillation. ●, Natural oscillation of twenty-nine roots grown two days at 19°–20° C; ○, resonant response to ± 0·15° C or ± 0·25° C oscillation of temperature.

Fig. 1 gives the average frequency of the natural oscillations of 2 day old roots at various temperatures. Each root was immersed in rapidly flowing 10^{-4} M potassium chloride solution the temperature of which was carefully controlled. Some roots the natural oscillations of which were heavily damped were pre-treated with small amplitude temperature oscillations at the approximate natural period; this reduced damping as described by Jenkinson[5].

The relationship shown in Fig. 1 is significantly linear from 10° C to 27° C (the ordinate of a second order polynomial regression differs by no more than 0·1 per cent from the linear regression to the data). This linearity is unexpected, as many of the processes involved in the postulated feedback loop, and their energy sources, are likely to be thermochemical. In thermochemical reactions one would expect the rate constants to be about proportional to the exponential of $(-E\ddagger/RT)$, where $E\ddagger$, the activation energy, is assumed constant. This relationship approaches an exponentially increasing function over small ranges of temperature variation, and if all three rate constants in the proposed loop varied in this way one would expect the frequency of the loop oscillation to vary with temperature in a similar way. Investigations made into the theoretical behaviour of simple arrangements of the loop components have not yet yielded a model which would have the observed linear frequency relationship. It is likely that more complex processes are involved than described by the simple three-component model—for example, involving non-linear or compensatory elements.

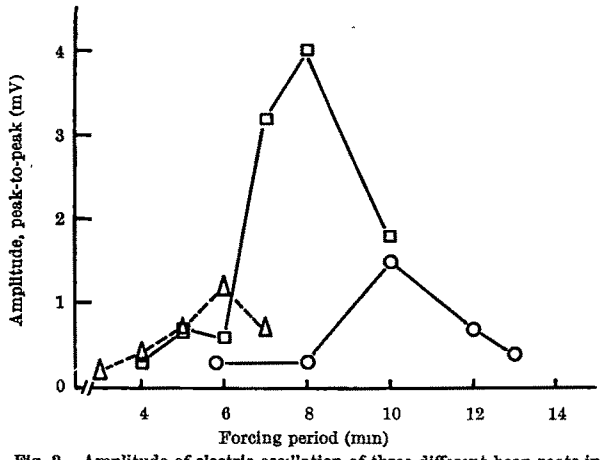


Fig. 2. Amplitude of electric oscillation of three different bean roots in response to ±0·25° C oscillation of temperature at various periods. Mean temperatures: ○, 15° C; □, 17° C; △, 24° C.

The roots also showed a resonant response to changes in the period of small oscillations of the temperature of the bathing medium (see Fig. 2). This behaviour is analogous to that found when osmotic pressure or concentration of indolyl-3-acetic acid is made to oscillate[3]. The amplitude of the applied temperature oscillation was in the range ±0·15° C to ±0·25° C; it was held constant within 0·02° C for each period. The resonant frequencies of some of the beans are shown as open circles in Fig. 1. The resonant frequency corresponds closely to the natural one. This lends further support to the feedback hypothesis, and suggests that the stimulus of small temperature oscillation does not unduly disturb the normal time constants of the feedback loop.

Jenkinson and Scott[3] have shown that the difference in phase between forcing oscillation and bioelectric response provides information on the sequence of operations forming the loop. They have defined the quantity total phase shift as the amount by which this phase difference changes when the period of the forcing oscillation is changed from very short to very long values. The present work has yielded a total phase shift of about 90° C for temperature oscillations. This suggests that the temperature oscillation may be affecting the feedback system at a different point in the loop from that stimulated by oscillation of osmotic pressure (270°) or of indolyl-3-acetic acid (180°).

Future work involves a more detailed investigation of the phase relationships, and of the effect of metabolic inhibitors, with a view to providing a clearer understanding of the nature of the feedback system.

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A Possible Explanation for the Effect of Magnetic Fields on Biological Systems

ALTHOUGH there is considerable controversy over the validity of any given example of the influence of a magnetic field on a biological system, the number of such reported phenomena[1] continue to make it a useful task to speculate on mechanisms which are reasonable on a molecular level. The direct influence of magnetic fields on chemical equilibrium or rate of reaction has occasionally been reported; however, this cannot usually be reproduced, and the general weight of evidence is that such effects are insignificant[2]. No satisfactory picture of what a weak magnetic field can do to molecular-level phenomena seems to have been advanced.

To the best of our knowledge the explanation offered here has been overlooked, and appears to offer a consistent and reasoned basis for suggesting that a magnetic field, of the order of 1,000 gauss (G) or more, influences the phenomena of charge transport, both ionic and electronic, mass transport (diffusion rate), and reaction rate in a biological medium. The argument is based on the combination of two rather well-known and well-documented observations: (1) the rod-like molecules in a liquid crystal can orient themselves in a magnetic field; (2) liquid crystalline materials have been identified in a host of biological systems.

Reports on liquid crystals[3] have included several examples of the influence of weak magnetic fields on the orientation of the rod-like molecules and of the consequences of this phenomenon. Forty years ago, using relatively weak magnetic fields (1,000–5,000 G), Svedberg investigated the rates of reactions carried out in mesomorphic states as solvents[4]. He studied the unimolecular thermal decomposition of picric acid, pyrogallol and trinitroresorcinol in *p*-azoxyphenetole, and found that the rate decreased significantly when the liquid crystal was oriented in an external magnetic field. Svedberg also studied rates of diffusion of *m*-nitrophenol in a mixed liquid crystal system of *p*-azoxyanisole and *p*-azoxyphenetole[5]. He found that magnetic fields affected the rate of diffusion—the rate rose when the magnetic fields were parallel to the direction of flow, and fell when the field was applied across the direction of flow.

There have been many reports of the effect of magnetic fields on the dielectric properties of liquid crystals[3]. For example, Maier, Barth and Wiehl[6] found that the difference in the transverse and the longitudinal dielectric constant of 4,4′-di-*p*-methoxyazobenzene increases rapidly up to 1,000 G, and is then independent of field strength. The picture which has developed is that the rod-like molecules, or ellipsoidal swarms of these molecules, are oriented by magnetic fields of the order of 1,000 G and that many of their properties are markedly influenced.

That biological systems are replete with liquid crystalline material has also frequently been reported. For example, Stewart[7] showed that the complex lipids present in the adrenal cortex, ovaries and myelin exist at body temperature in a characteristic mesophase. Robinson[8] has shown a widespread occurrence of the cholesteric phase in polypeptide solutions and biological structures. Reviews of much of this work were presented at the 1965 International Liquid Crystal Conference[9].

This explanation might apply to both "field sensitive" and "gradient sensitive" phenomena[1]. The orientation of rods parallel to a magnetic field occurs for both diamagnetic and paramagnetic materials[10].

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Simple Allosteric Model for Membrane Pumps

THE list of plausible hypothetical schemes which have been advanced to account for active transport is so long that any hope of extending it might appear unwarranted. However, very few specific molecular mechanisms have been considered. It therefore appears timely to point out that transport contingent on a chemical reaction can be understood in terms of a very simple molecular model, for which a noteworthy precedent can be found among recent crystal structure determinations.

The proposed pump has some properties in common with earlier proposals of Glynn[1], Burgen[2] and Post[3]. It differs from them in the specific assumption of an allosteric rearrangement as a mechanism for imparting direction to the diffusion process. Its superficial similarity to other proposals[4–8] is outweighed by further reaching dissimilarities.

To function as a pump a polymer molecule must meet three structural conditions only. (1) It must contain a slit or a cavity in the interior of the molecule, large enough to admit a small molecule. (2) It must be able to assume two different configurations, such that the molecular cavity is open to one side of the membrane in one configuration and to the opposite side in the other. (3) It must contain a binding site for the transported species in its molecular cavity, the affinity of which for the transported species is different in the two configurations.

The additional condition that the molecule be oriented within a membrane is not strictly relevant to its ability to function, but is implied, since it must be met if the directional properties of this function are to be observable on a macroscopic scale.

The energy required for the allosteric rearrangement between the two configurations could be derived from any one of many sources. One can conceive of molecular pumps activated electrically, chemically, photochemically, by pressure, and in other ways. The special case of the chemically activated pump is of primary interest here.

The pump is most conveniently discussed in terms of two movable polypeptide chains, shown in Fig. 1, with sodium ion as the transported solute. The number of chains, their exact chemical nature and the nature of the transported species are not critical to the argument.

The two alternative configurations of the pump are represented in Fig. 1, *A* and *B* respectively. The allosteric rearrangement $A \rightarrow B$ results from a chemical reaction, depicted as a phosphorylation of one of the chains, with *A* as the stable form of the unphosphorylated and *B* the stable form of the phosphorylated molecule. The sodium binding site in the molecular cavity has a high affinity for sodium in the *A* form, and a low affinity in the *B* form.

The essential sequence of events in the process of active transport is then the following.

The sodium ion is adsorbed at the binding site in configuration *A* from the left compartment, where the sodium concentration is relatively low. Its adsorption triggers a phosphorylation of the pump (at either a nearby or a distant site); this, in turn, triggers the allosteric rearrangement $A \rightarrow B$, as a result of which the affinity of the binding site for the sodium ion is lowered, the opening to the left compartment closed and that to the right compartment opened. Since the volume of the molecular cavity is small, the sodium concentration within it is in effect larger than in the compartment on the right, providing the chemical potential gradient necessary for diffusion out of the membrane. Dephosphorylation and repetition of the cycle could be triggered either by the outward diffusion of sodium, or by a subsequent adsorption of potassium.

An exceedingly subtle allosteric rearrangement will suffice for the present purpose. A change of 2–3 Å in the relative position of the two chains will determine whether the gate to the molecular cavity is open or closed to a given molecular species. Estimates of the displacements of chemical groups required to alter the affinity of a binding site by one or more orders of magnitude lead to comparable values. Differences of the order of 1–7 Å in the relative positions of haemoglobin chains between crystals of reduced and oxygenated haemoglobin have been found by Muirhead and Perutz[9]. If the current interpretation that these differences result from oxygenation alone is correct, the haemoglobin molecule provides a precedent for the type of molecular mechanism which is required to operate an allosteric pump.

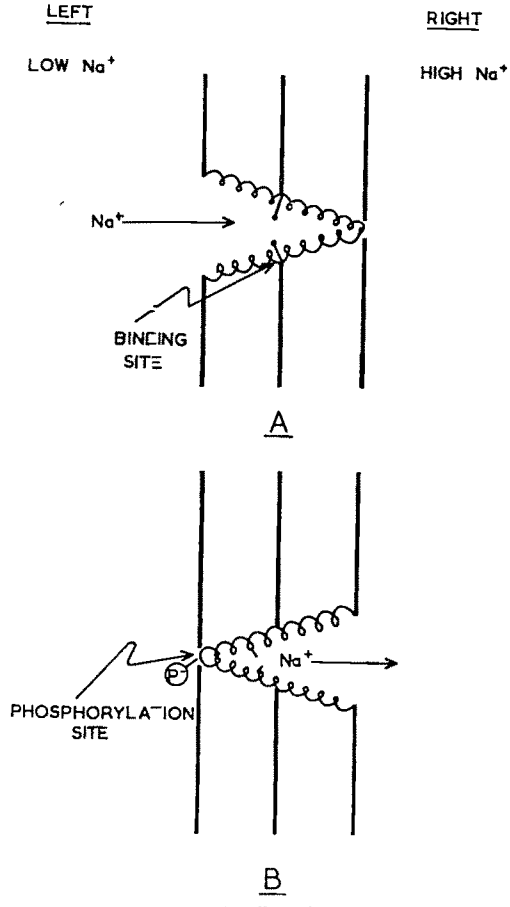


Fig. 1. Longitudinal section of the allosteric pump *A*, Sodium form, *B*, potassium form

Assuming reasonable values for the affinity constant of the binding site and for the dimensions of the molecular cavity, the competence of the allosteric pump is seen to be adequate to sustain most experimentally observed gradients. Thus a pump with three sites for sodium, an affinity constant of the order of 10^3 in the *A* and of the order of 0 in the *B* configuration, and a cavity volume as large as 1000 Å^3, would pump sodium ions from a solution containing 10^{-3} M (or more) sodium into a solution containing 3 M (or less) sodium, either "up" or "down" hill.

Pumps for different molecules need differ only in the specificity of binding sites, and the same pump molecule can be adapted to transport more than one molecular species. Only details of the mechanism need to be altered to account for differences in specificity and for the occurrence of coupled transport. Thus, the stoichiometry of the sodium pump in red blood cells, believed to be an exchange of three sodium for two potassium ions[3,10] for each molecule of ATP, can be accounted for by the scheme shown in Fig. 2.

Each pump unit is thought to consist of three pairs of chains, forming three binding sites specific for sodium in

configuration *A*. Occupation of the middle site triggers the phosphorylation, which, in turn, triggers the molecular rearrangement to *B* and a simultaneous transformation of two of the sites into sites specific for potassium, in the manner shown. Adsorption of potassium triggers the dephosphorylation and rearrangement to *A*, which is accompanied by a loss of affinity for potassium and reappearance of a configuration favourable for a repetition of the cycle.

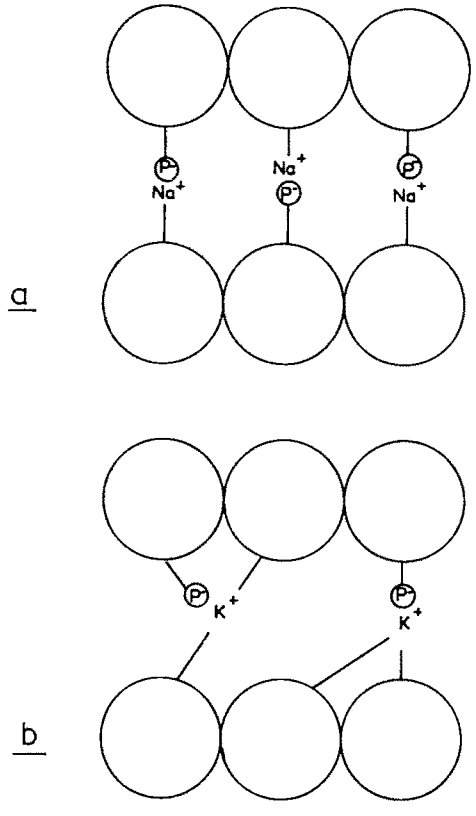


Fig. 2. Transverse section of the coupled sodium-potassium pump *a*, Sodium form; *b*, potassium form.

It should be emphasized that transport by the allosteric pump is in no sense carrier-mediated. Unlike the translocator of Mitchell[4,5] or the rotating carrier of Danielli[11], it requires neither translational nor rotational motion of the binding site. It operates on the same principle as the locks in overland waterways, by altering the potential energy of the transported species. Its kinetics can be described by the diffusion equation applied to a three compartment system (inside/pump/outside), if provision is made for the time-dependence of the intercompartment diffusion coefficients and the chemical potential within the pump. Explicit dependence on the rate of the chemical reaction can be introduced in the conventional manner[12]. The model is not adequately described by a vectorial coupling coefficient, linking the observed flux to the affinity of the chemical reaction[13]. Such a formulation implies complete independence of the rate of transport from the chemical potential gradient across the membrane, which the model clearly does not.

Perhaps the principal virtue of the allosteric model is the simplicity with which it accounts for the principal experimental findings—the ability of very thin (50–100 Å) membranes to concentrate solutes, the localization of the phosphorylation reaction on one side of the membrane[3] and the dependence of pump synthesis on a single genetic locus[14]. In addition it suggests that pump molecules should be amenable not merely to isolation but also to design and synthesis, and that it should be possible to generate reaction-dependent transport in artificial monolayers or crystals formed from them.

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BIOCHEMISTRY

Ecological Significance of Anthocyanins in the Seed Coats of the Phaseoleae

THERE is a wide variation in the flavonoids of the seed coat of different cultivars of *Phaseolus vulgaris*[1]. In 'Canadian Wonder', a cultivar which is grown widely both in temperate and tropical zones, we have found the quantities of anthocyanins shown in Table 1. Other cultivars contain these anthocyanins in different proportions, or the compounds may be absent, as in 'Comtesse de Chamborde' for example.

Table 1. APPROXIMATE PERCENTAGE OF WEIGHTS OF ANTHOCYANIN PIGMENT IN DRY SEED-COAT OF 'CANADIAN WONDER'

Flavonoid	Percentage of dry seed-coat
Pelargonidin-3-monoglucoside	1·0
Cyanidin-3-monoglucoside	1·0–1·5
Pelargonidin-3 : 5-diglucoside (P3 : 5g)	0·5 in all: mainly P3 . 5g
Delphinidin-3-monoglucoside	
Cyanidin-3 : 5-diglucoside	

The total quantity of flavonoids may be as high as 5 per cent, but it varies according to the conditions under which the seed is grown and harvested. Because of the variation in the results of investigation of their biological activity, various theories have emerged concerning the part played by the compounds. They were considered to have no, or very little, biostatic activity[2], but this view has recently been revised[3,4]. It would seem that the choice of organism and the test conditions are critical and, in our survey of the activity of sections of paper chromatograms prepared from flavonoid extracts[1] of seed coats, stimulation and inhibition occurred. These tests were carried out by placing 1 cm² chromatogram "cut-outs" on a "lawn" of organisms seeded on Petri dishes. A summary of the results is given in Table 2.

A result of particular interest was the reactivity of pelargonidin-3-glucoside (P-3-G), in view of the high concentration of this compound in the seed coat. Further tests were, therefore, conducted using 0·2 ml. portions of liquid medium in which the spores were suspended, to which P-3-G was added in the form of a chromatographically purified preparation obtained from the original material. These results are presented in Table 3. Organism counts were made using the Miles and Misra technique.

The results show that, at the level of 1,000 p.p.m., P-3-G is biostatic to at least one of the yeasts tested. The observations on bacteria are similar to those of Powers[4], but are not in complete agreement with the hypothesis of Masquelier[3], who suggested that organisms which inhabit soil are less sensitive to inhibition by flavonoids than related pathogenic organisms.

With the *Rhizobium* species, which are known to vary in their output of indolyl-3-acetic acid[5,6], these observa-

Table 2. REACTION OF A RANGE OF SOIL MICRO-ORGANISMS TO SELECTED ZONES OF CHROMATOGRAMS FROM SEED-COAT EXTRACTS OF *P. vulgaris*

Organism sp. and strain	R_F values:	Mean inhibition (*I*) and stimulation (*S*) zones in relation to R_F values[¶]							
		0·10		0·20		0·30			0·40
		Approximate location of bands							
Fungi									
Aspergillus flavus Link. *MR*008*	*SS*	[*II*]	—[*II*]	[*I*]	*SSS I*—	*SSS*	*S*	*I*	*SS*
Penicillium citrinum Sopp. *MR*001*	*I*	—	—[*I*]	[*II*]	*S I*	*S*	—	—	—
Yeasts									
Kloeckera africana (Klöcker) Janke N.C.Y.C. 26†	*SS*	(*I*)	—[*I*]	[*II*]	*SSS I*	*S*	*I*	*I*	—
Hansenula mrakii Wick, N.C.Y.C. 500†	—	—	—	[*I*]	*SSS I*	*I*	*II*	*III*	—
Debaryomyces phaffii Capriotti N.C.Y.C. 665†	*SS*	—	—	[*I*]	*SSS*—	*I*	—	—	—
Bacteria									
Pseudomonas phaseolicola (Burk) Dowson 605†	*II*	—	—[*II*]	[*I*]	*SS*—	(*I*/*S*)	*II*	—	—
" 1341‡	*II*	—	—	—	*SSS*—	(*I*/*S*)	*II*	—	—
Rhizobium trifolii§ Dangeard *Rh.* 5	*SS*	(*I*/*S*)	—[*I*]	[*II*]	*SS I*	(*I*/*S*)	*SSS*	*SS*	*SS*
Rhizobium phaseoli§ Dangeard *Rh.* 3601	*SS*	[*I*]	—[*II*]	[*S*]	*SSS*—	*II*	*SSS*	(*S*)	*II*
				Cyanidin-3,5 diglucoside			Pelargonidin-3-monoglucoside		

All cultures grown at 25° C, but recorded at 1–5 days according to strain of organism.
* Tropical Products Institute, London.
† Brewing Research Institute, Nutfield.
‡ A.R.C. Plant Pathology Laboratory, Harpenden.
§ Soil Microbiology Department, Rothamsted.
¶ Using solvent *BAW* 612. The location of spots is semi-diagrammatic.
Brackets indicate: ( ), variable reaction about the inhibition/stimulation threhold; [ ], restricted to 'Mont d'Or Waxpod' extracts, probably owing to coumaric acids.

Table 3. GROWTH OF THREE SOIL YEASTS (*Y*26, *Y*500, *Y*665), IN MICRO-CULTURE, IN RELATION TO ADDED P-3-G AND SUCROSE

Expressed as log-ratio* of the number of viable organisms at the start of the experiment to those present at three successive sampling times (T_1, T_2, T_3)

Conc. of P-3-G in p.p.m.	5 per cent sucrose present								
	*Y*26†			*Y*500†			*Y*665†		
Time‡	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
1,000	0·05	0·45	0·15	$\bar{1}$·80	$\bar{1}$ 40	$\bar{1}$ 60	1·20	1 30	1·50
100	0·45	0·45	0·35	0·50	0 60	1·10	1·50	1·80	1·60
0	1·00	1·15	1·05	0·40	0·90	0 80	1·20	1 40	1·20
	Sucrose absent								
1,000	$\bar{1}$·80§	$\bar{1}$·95	$\bar{1}$ 75	0·15	0 70	0·20	1·20	1·00	1·70
100	$\bar{1}$·95	0·15	$\bar{1}$·85	0·20	0·40	0 20	0·85	1·65	$\bar{1}$·75
0	0·45	0·25	$\bar{1}$·85	$\bar{1}$·90	0·40	0·30	0·50	0·75	$\bar{1}$·70

* The original inoculum was about 5×10^4 organisms per culture.
† Species as in Table 2.
‡ Incubation for 3, 6, 12 days. Maximum development occurred between 3 and 6 days, and the number of viable organisms decreased from then.
§ The bar values indicate depression of reproduction.

tions suggest a possible mechanism for the interaction between host species and symbiont, which determines specificity. It is not suggested that P-3-G is the only, or even the most important, of the flavonoids responsible for this interaction[7], but it is suggested that further investigation of the effects of this group of materials on various species of *Rhizobium* is warranted[8,9].

We consider this type of mechanism is also important in influencing the development of the micro-flora on the leguminous seed-coat[10]; preliminary investigations in this laboratory show that it affects the relations between the groundnut and the toxin-producing species of *Aspergillus*. One reason why its significance has been overlooked is that the width of the inhibition zone around a test spot is not proportional to concentration, in Petri dish tests, as it is with many antibiotics.

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Inhibition of the Biosynthesis of Steviol by a Growth Retardant

THE glycoside stevioside, which occurs naturally in *Stevia rebaudiana* (a South American member of the Compositae), is responsible for the remarkable sweet taste of the leaves of that plant. According to the *Merck Index*, it is 300 times sweeter than sugar. The aglycone of stevioside is the tetracyclic diterpenoic acid steviol. Structurally, it is closely related to gibberellin and shows growth promoting activity in some test plants which were hitherto thought only to be promoted by gibberellin[1–3]. Steviol is also converted by the fungus *Fusarium moniliforme* into a gibberellin-like substance which is apparently not identical with gibberellins A_1–A_9 (ref. 4).

When *F. moniliforme* was grown in the presence of growth retardants such as *AMO*-1618 (2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylate methyl chloride) or CCC (2-chloroethyl trimethylammonium chloride), less gibberellin was produced, but growth of the mycelia was not retarded[5]. It was concluded that this reduction in the amount of gibberellin produced resulted from inhibition of gibberellin biosynthesis rather than from destruction or inactivation of gibberellin[6]. This conclusion was substantiated by the finding that mevalonic acid, the precursor of gibberellin, was not incorporated into gibberellin by *F. moniliforme* in the presence of *AMO*-1618 (ref. 7). There is good reason to believe that the growth-retarding effect of *AMO*-1618 and CCC in higher plants can be explained in terms of the inhibition of the biosynthesis of gibberellins*. The reduction of the gibberellin content of pea seeds developing in detached pods was reduced by 60 per cent before inhibition of growth became obvious. This clearly indicates that a lowered gibberellin level is the cause rather than the consequence of growth retardation[8]. Using a series of CCC analogues, Harada *et al.* found a complete correlation between the inhibition of gibberellin biosynthesis in *Fusarium* and the suppression of growth in higher plants[9].

We noted that *Stevia* plants treated with *AMO*-1618 failed to exhibit the characteristic sweet taste. By extending this observation and by utilizing the incorporation of acetate into steviol, we were able to show that *AMO*-1618 was indeed responsible for the suppression of the biosynthesis of steviol.

A group of four *S. rebaudiana* plants were treated twice weekly with 16·6 μc. sodium acetate-2-^{14}C dissolved in 0·05 per cent aqueous solution of 'Tween 20'. The solution was applied in 50 μl. drops to the apex and youngest leaves. A total of 250 μc. were administered during the 4 week experimental period. Three of the plants also

* *Note added in proof.* *AMO*-1618 was found to inhibit the cyclization of geranyl-geraniolpyrophosphate in an endosperm preparation from *Echinocystis macrocarpa* (ref. 11).

received 20 ml. of a 4,000 p.p.m. solution of *AMO*-1618 in the form of a soil drench. The growth retardant was also applied twice weekly and for the same period of time as the labelled acetate, but it was applied 1 day before treatment with acetate. The plants were maintained in a controlled environment and subjected daily to 10 h of light at 24° C and to 14 h darkness at 17° C; these conditions applied before and during the experiments. The parts of the plant that formed after the application of the chemicals were separated from the older parts and lyophilized, extracted and hydrolysed according to a previously described procedure[10].

Ninety-three milligrams of residue were obtained from the *AMO*-treated plants and 37 mg from control plants (no *AMO*): 1·86 mg of the former and 1·91 mg of the latter were spotted as 2 cm wide strips 15 mm from the edge on a 50 × 200 × 0·25 mm layer of silica gel *H* (Brinkmann Instr.). These were then developed over 170 mm in $CHCl_3$–EtOAc–HOAc (12 : 8 : 1), and scanned for radioactivity in an adapted Actigraph II (Nuclear Chicago). The results, as shown in Fig. 1, indicated that the radioactivity associated with steviol from the *AMO*-treated plants was about half that of the controls. The fluorescence of steviol under ultra-violet light after charring the thin-layer chromatogram with 50 per cent sulphuric acid on a hot plate also indicated that less steviol was present after treatment with *AMO*.

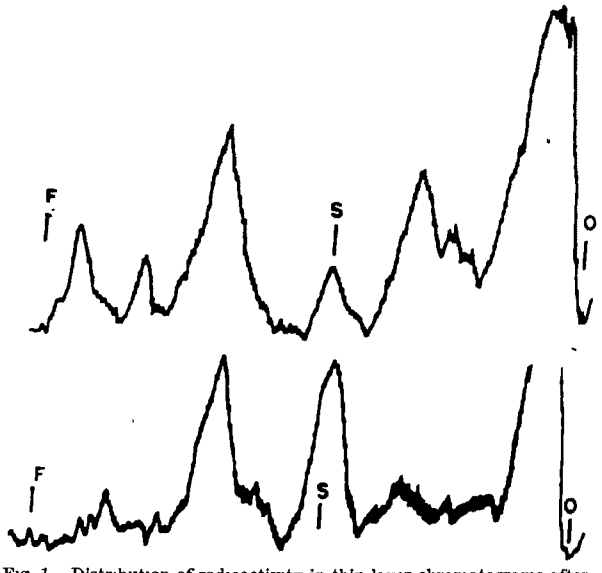


Fig. 1. Distribution of radioactivity in thin-layer chromatograms after treatment of *S. rebaudiana* with radioactive acetate. Upper curve: plants received *AMO*–1618. Lower curve: control, no *AMO*. Chromatogram scanned at 0·75 in./h, full-scale 300 c.p.m., time constant 40 sec, slit width 0·0625 in. *S*, Steviol, as shown by means of co-chromatographing a sample of authentic steviol, and also demonstrated in earlier work (ref. 4) by chemical means (crystallization to constant specific activity, melting point, and preparation of a derivative).

As in other plants, treatment with *AMO* causes a reduction in internode length in *S. rebaudiana*. Gibberellic acid completely counteracted the growth retardation; however, the lowered contents of steviol appeared not to be affected. The results suggest that *AMO* interferes not only with the biosynthesis of gibberellin, but also with that of the cyclic diterpenes in general. These findings are significant in two respects. *AMO*, and perhaps also other growth retardants, can be used for blocking the synthesis of certain plant terpenes and can thus serve as a tool to elucidate further the biosynthetic pathways. Furthermore, cyclic diterpenes can be used as "model substances" for studying certain parts of the biosynthesis of gibberellins in higher plants. This is of considerable interest because the gibberellin content of most higher plants, except certain seeds, is very low, which renders the isolation and identification of intermediates a difficult task. In contrast, the level of diterpenes in some plants is quite high. Stevioside amounts to as much as 7 per cent of the dry weight of *Stevia rebaudiana* plants.

The steviol biosynthesis was not completely suppressed, even though a high concentration of *AMO* was used. This could have resulted from the fact that separation of the parts formed after the commencement of the *AMO* treatment from those which had been present before was not complete. Another reason could be that 24 h between *AMO* and the application of acetate was not sufficient time to allow adequate amounts of *AMO* to be translocated to the biosynthetic sites of steviol, which are apparently the youngest leaves.

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Ribonucleic Acid Turnover in Tobacco Leaves infected with Tobacco Mosaic Virus

A LARGE number of investigations using different techniques have shown that the nucleus and the chloroplasts of infected leaf cells are implicated in the synthesis of tobacco mosaic virus (TMV). The source of materials for the synthesis of virus protein and ribonucleic acid (RNA) has been shown to be low molecular weight residues which are in metabolic sequence with carbon dioxide, ammonia and inorganic phosphate[1]. Recently, however, Reddi[2] reported that the uridine, but not the phosphate, component of TMV–RNA was derived from the degradation of ribosomal RNA, which occurred as a result of infection. This communication describes some results of a study of the effect of TMV synthesis on the RNA content and turnover of mainly two fractions of tobacco leaves. Half-leaves of *Nicotiana tabacum* var. White Burley, about 20 cm long, were inoculated with TMV, the opposite leaves serving as control, except when the sampling was to continue for more than three days after inoculation when separate plants were used. Leaves were sampled by taking leaf pieces of equal wet weight for each sample in such a way that each part of the leaf was represented in each sample. Incubation of the leaf pieces in phosphorus-32 was achieved by infiltrating them with 1–5 μc./ml. of carrier-free phosphorus-32 in distilled water at *p*H 6·5, and leaving them at 23·5° C under illumination. The leaf tissue was ground in 25 mM *tris*-hydrochloric acid at *p*H 7·6, containing 5 mM magnesium chloride, 1 mM calcium chloride, 0·5 M sucrose, and 10 mM β-mercaptoethanol at 0°–4° C. Debris was removed by centrifugation at 100*g* for 30 sec. The supernatant was fractionated into a nuclear-chloroplast fraction (2,000*g* for 5 min), containing also starch grains and some cell wall material; a mitochondrial fraction (42,000*g* for 12 min), containing also cell membranes, some green material and, in the case of infected leaves, some virus; and a ribosomal fraction (140,000*g* for 90 min) containing ribosomes and the remaining virus. Ribosomes and virus were separated by dissolving the ribosomal pellet in 0·3 ml.

tris buffer, 25 mM, at *p*H 7·6, containing 5 mM magnesium chloride, and centrifuging the solution at 120,000*g* for 70 min on a 5–20 per cent sucrose density gradient column prepared in 25 mM *tris* buffer containing 5 mM magnesium chloride. The column was sampled by collecting drops; the drops constituting the ribosomal zone were pooled and the concentration determined by spectrophotometry. RNA was extracted with phenol and an equal volume of 25 mM *tris* buffer at *p*H 7·6 by shaking in the cold for 10 min. The water phase was re-extracted with phenol and excess phenol was removed with two ether extractions. Ribonucleic acid was precipitated with three volumes of ethanol and three drops of 3 M sodium acetate overnight at −17° C, and the precipitate was dissolved in distilled water. Precipitation with ethanol was repeated twice. When RNA free of radioactive impurities was required, the ethanol precipitations were followed by three cycles of solution in 6 M guanidine chloride and 10 mM ethylenediamine tetraacetic acid at *p*H 7·2 and precipitation with 1·2 volumes ethanol in the cold. Finally, the RNA was washed with 70 per cent ethanol, defatted, and hydrolysed in 0·2 ml. 1 N potassium hydroxide at 30° C for 16 h. The hydrolysate was adjusted to *p*H 3·5 with perchloric acid, and the nucleotides were separated by high voltage electrophoresis in 50 mM potassium citrate buffer at *p*H 3·50. Radioactivity was counted using a liquid scintillator in a 'Packard Tricarb' spectrometer.

The RNA content per unit area of the leaf fractions decreased with leaf expansion. The ribosomal RNA decreased faster than all other fractions, but there was no difference in the rate of degradation between infected and control leaves of plants grown under three different growth conditions. The mitochondrial fraction of the infected leaves exhibited a less pronounced rate of degradation, because some TMV sedimented with this fraction. In accord with other findings[3], the nuclear-chloroplast (N–C) fraction of infected leaves 2–3 days after inoculation showed a small (about 15 per cent) increase relative to control leaves, which corresponded to a third of the RNA incorporated in whole virus at that time. Attempts were made to find whether this RNA was in any way connected with the synthesis of TMV–RNA. Incubation of leaf tissue with phosphorus-32 followed by fractionation showed that the specific activity of the N–C fraction, not unexpectedly, was eight times higher than that of the ribosomes. Incorporation of phosphorus-32 by the N–C fraction of the infected leaves increased continuously with incubation time, whereas the same fraction of the control leaves reached a plateau after about 80 min (Fig. 1). The stimulation of RNA synthesis after 120 min was usually 40 per cent. After 4 h of incubation stimulation was apparent. The incorporation of phosphorus-32 by ribosomes showed a similar increase beginning after 90 min, but it was less evident after shorter incubation times, and varied between 10 and 30 per cent (Fig. 2).

Extraction of the RNA of the N–C fraction with phenol left about 10 per cent of the RNA unextracted. This was extracted by adding sodium dodecyl sulphate at a final concentration of 0·3 per cent to the phenol phase and extracting the RNA using *tris* buffer at *p*H 7·6 for 15 min at 50° C. The specific activity of the sodium dodecyl sulphate–RNA was three times higher than that of the phenol RNA, but there was no difference in specific activity between those of infected and control leaves irrespective of incubation time.

Determination of the nucleotide composition of the RNA of the N–C fraction, obtained from the relative radioactivity of the nucleotides, did not indicate that TMV–RNA (cytidine monophosphate (CMP) 19·4, adenosine monophosphate (AMP) 29·1, guanosine monophosphate (GMP) 24·4, uridine monophosphate (UMP) 27·1), or its complementary strand[4], was involved. The shift of composition of this fraction towards high AMP and UMP content (CMP 21·2, AMP 26·2, GMP 29·4, UMP 23·2), compared with the composition derived from spectrophotometric methods (CMP 22·3, AMP 23·0, GMP 31·9, UMP 22·7), and the variability of determinations in different experiments possibly obliterated any real difference. The composition of the ribosomal RNA was more consistent (CMP 21·8, AMP 24·9, GMP 30·3, UMP 23·0), but there was no significant difference between infected and control leaves.

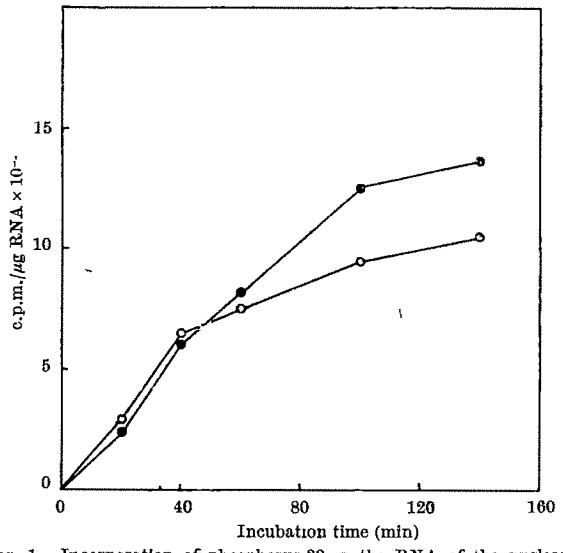


Fig 1. Incorporation of phosphorus-32 in the RNA of the nuclear-chloroplast fraction of healthy and TMV infected tobacco leaf cells ●, Infected; ○, healthy.

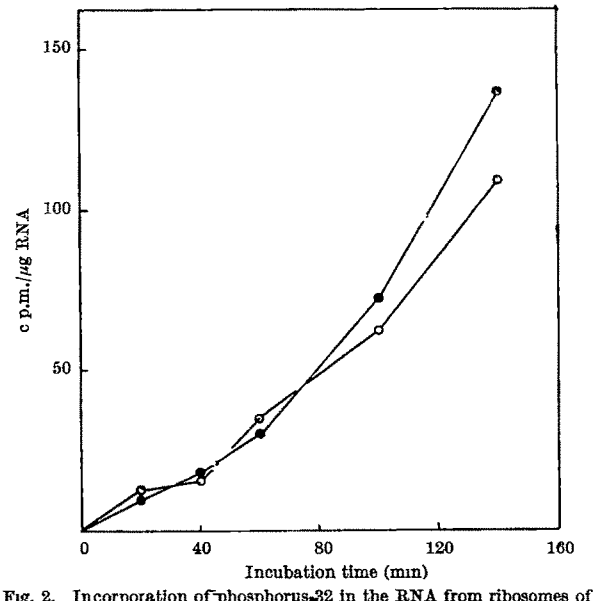


Fig. 2. Incorporation of phosphorus-32 in the RNA from ribosomes of healthy and TMV infected tobacco leaf cells. ●, Infected; ○, healthy.

It was concluded that infection of tobacco leaves with TMV stimulates synthesis of RNA in the nuclear-chloroplast fraction, which may be responsible for the increased incorporation of phosphorus-32. It can easily be extracted, which suggests that it is not firmly bound to any cell component. The nucleotide composition did not reveal similarity to TMV–RNA. The higher specific activity of the RNA from ribosomes of infected leaves only after long periods of incubation time suggests that the extra radioactivity is not incorporated into the ribosomal RNA, but into an extraneous RNA bound to ribosomes. This

RNA may participate in the production of some protein necessary in virus multiplication.

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Porphyria induced in Rats by Orotic Acid and the Effect of Adenine-5′-monophosphoric Acid

IN previous work we showed that adenine nucleosides and nucleotides play an important part in the quantitative regulation of porphyrin biosynthesis. Administration of inosine or adenosine monophosphate (AMP) reduced significantly the excess porphyrin formation in rats caused by different toxic agents (for example, allylisopropylacetylcarbamide, hexachlorobenzene, 3,5-dicarbethoxy-1,4-dihydrocollidine, lead acetate and griseofulvin)[1–3]. On the other hand, porphyrin synthesis by illuminated suspensions of *Rhodopseudomonas spheroides* in Lascelles medium I was strongly inhibited by AMP, adenosine diphosphate (ADP) and adenosine triphosphate (ATP). ATP was found to provoke more than 90 per cent inhibition at a concentration of 3 mM.

These observations have led us to study the reverse problem, namely, the effect of the decrease in the biosynthesis of ATP on porphyrin formation. Orotic acid seemed to be particularly suitable for this purpose, as it was recently shown by Von Euler *et al.*[5] that a balanced diet containing 1 per cent orotic acid caused a 50 per cent decrease in the hepatic level of acidosoluble nucleotides in rats.

Our experiments with rats maintained for 1–3 weeks on the diet used by Von Euler *et al.* confirmed these findings. Using the luciferase method[6] we found a highly significant reduction in the level of hepatic ATP. Unexpectedly, a similarly significant increase was observed in the concentration of ATP in the red blood cells. At the same time, we noted significant hepatic steatosis (Table 1).

Table 1

	Rats intoxicated by orotic acid during 3 weeks	Controls	Rats intoxicated by orotic acid during 1 week
Liver ATP level (μmol/100 g net weight)	60 ± 8·7 (mean of 10 rats) $t = 4·6$ $P < 0·001$	164·5 ± 11·0 (mean of 10 rats)	98 ± 10·7 (mean of 8 rats) $t = 3·03$ $P < 0·01$
Erythrocytic ATP level (μmol/100 ml.)	83 ± 8·5 (mean of 9 rats) $t = 7·7$ $P < 0·001$	46 ± 1·6 (mean of 11 rats)	89 ± 7·1 (mean of 8 rats) $t = 7·5$ $P < 0·001$
Total liver lipids (g/100 g net weight)	8·4 ± 1·2 (mean of 8 rats) $t = 3·6$ $P < 0·01$	3·8 ± 0·23 (mean of 10 rats)	— —

In order to study the ensuing disturbances of porphyrin metabolism, three series of experiments were followed, namely, administration of the diet containing 1 per cent orotic acid for 1, 2 and 3 weeks. Urinary and faecal porphyrins and porphyrin precursors, δ-aminolaevulic acid and porphobilinogen (ALA and PBG) were studied every 3–4 days. The level of etherosoluble porphyrins (copro + protoporphyrin) in the liver and red blood cells was determined immediately after death provoked by bleeding following mild ether anaesthesia. After 2 weeks of orotic acid administration, we also studied the level of etherosoluble porphyrins in the pooled bone marrow of eight rats and the activity of ALA synthetase in mitochondria isolated from the liver of four rats, using the method of Granick and Urata[7].

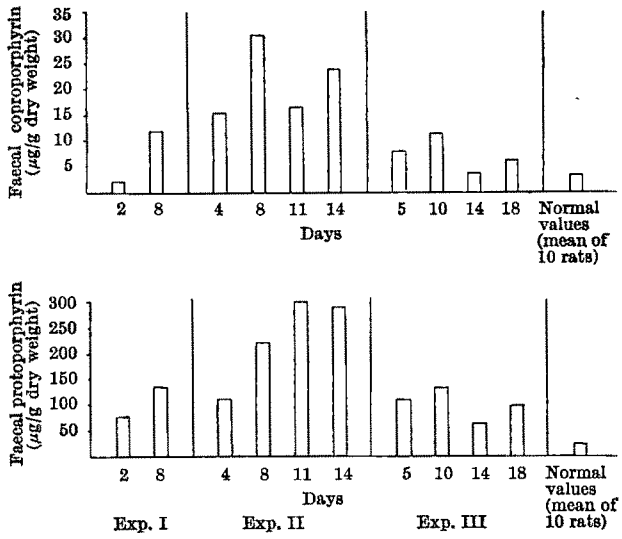


Fig. 1. Balanced diet containing 1 per cent orotic acid maintained for: 1 week (Exp. I); 2 weeks (Exp II); 3 weeks (Exp. III).

The urinary excretion of porphyrins and porphyrin precursors remained at almost normal levels between practically normal limits in all our experiments. On the other hand, there was a very marked increase in faecal porphyrin excretion (Fig. 1). There was a similar increase in the concentration of etherosoluble porphyrins in the liver and red blood cells when orotic acid was administered for at least 1 week, and furthermore, a significantly high level of these porphyrins was found in the bone marrow. After two weeks' intoxication with orotic acid enhanced activity of ALA synthetase was discovered in liver mitochondria (Table 2).

Table 2

	Rats intoxicated by orotic acid for 2 weeks	Controls	Rats intoxicated by orotic acid for 1 week
Hepatic level of etherosoluble porphyrins (μg/100 g net weight)	16·2 ± 0·9 (mean of 8 rats) $t = 4·0$ $P < 0·01$	8·4 ± 1·4 (mean of 6 rats)	12·4 ± 0·4 (mean of 9 rats) $t = 2·3$ $P < 0·05$
Erythrocytic level of etherosoluble porphyrin (μg/100 ml.)	69·0 ± 3·5 (mean of 8 rats) $t = 5·6$ $P < 0·001$	50·6 ± 1·95 (mean of 10 rats)	78·0 ± 3·3 (mean of 9 rats) $t = 6·4$ $P < 0·001$
Etherosoluble porphyrins in pooled bone marrow (μg/10^{10} red cells)	38 (mean of 8 rats) —	23 (mean of 8 rats)	— —
ALA synthetase activity in liver mitochondria (mμmol ALA formed / mg protein of mitochondria)	19·8 ± 3·5 (mean of 4 rats) —	0–4·5 (mean of 10 rats)	—

Our findings show that the administration of orotic acid to rats provokes a highly significant increase in the faecal, erythrocytic and medullary level of etherosoluble porphyrins and only a mild increase in the liver concentration of these pigments. As the urinary excretion of porphyrins and porphyrin precursors was virtually unmodified, the observed disturbances of porphyrin metabolism appear to be similar to those found in human erythropoietic protoporphyria[8].

The increased activity of ALA synthetase in liver mitochondria of rats intoxicated with orotic acid suggests that the accumulation of porphyrins can be attributed to the stimulation of their synthesis by the toxic agent. Granick has shown this to be so for other compounds capable of inducing experimental porphyria[9].

Thus we now have some evidence that the enhancement of the porphyrin biosynthesis by orotic acid is related to a decrease in the formation of ATP by the liver. Intramuscular administration of AMP at the dose of 20 mg per rat per day from the beginning of the period of intoxication with orotic acid was found to reduce very significantly

excess porphyrin formation as well as the increased ALA synthetase activity of liver mitochondria (Table 3).

Table 3

	Intoxication with orotic acid for 2 weeks	
	Rats treated with AMP (8 rats)	Rats not treated with AMP (8 rats)
Faecal etherosoluble porphyrins 4th day	106·5	124·7
(μg/g dry weight) 8th day	216·7	252·5
11th day	259·5	320·0
14th day	257·5	313 0
Etherosoluble porphyrins in liver tissue (μg/100 ml. net weight)	11·6 ± 0·7 $t = 5·2$	16·2 ± 0 9 $P < 0$ 001
Etherosoluble porphyrins in red blood cells (μg/100 ml.)	43 ± 1·7 $t = 6·0$	69·0 ± 354 $P < 0$ 01
Etherosoluble porphyrins in pooled bone marrow (μg/10^6 erythroblasts)	26	38
ALA synthetase activity in liver mitochondria (mμmol ALA formed per mg protein of the mitochondria preparation)	19 8 ± 3·5 $t = 3·1$	35·8 ± 4·1 $P < 0$ 02

These results confirm our earlier observations concerning the experimental production of porphyria by a toxic adenine analogue, 6-mercaptopurine. This compound was also found to reduce the biosynthesis of ATP. The metabolic disorders of this porphyria were significantly alleviated by the administration of inosine[10].

An experimentally induced porphyria was observed in mice following the administration of griseofulvin (by de Matteis and Rimington[11]), and in rats (by ourselves[3]). MacNall[12] has emphasized the structural similarity of griseofulvin with the purine ribosides and suggests that this antibiotic may act as a competitive analogue. In this connexion, it is interesting that we found that the administration of AMP or inosine improved the disturbances of porphyrin metabolism induced by griseofulvin in rats[3].

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Gonadotrophin-inhibiting Action of Certain Carbohydrates

A GONADOTROPHIN-INHIBITING substance has been reported to occur in the urine of children[1] and adults[2,3] and in certain samples of human serum[4]. It inhibits the action of human chorionic gonadotrophins (HCG) and luteinizing hormone (LH), but is ineffective against the action of follicle-stimulating hormone (FSH). This communication reports the observation of certain similar effects using *N*-acetyl-D-neuraminic acid (D-NANA)—a constituent of both HCG and FSH, and using the related compound *N*-acetyl-D-mannosamine (D-NAM).

Synthetic D-NANA was obtained from the Koch-Light laboratories and related compounds were synthesized in this Department. Before they were injected into animals, these compounds were dissolved in water and carefully neutralized with dilute sodium hydroxide if necessary. The uterine weight assay[5] and the ovulation assay[6] were carried out using immature mice.

In a typical experiment, D-NANA and HCG were mixed in the proportion of 1 mg to 1 I.U. The regression of the log response on the log dose was significant, and in a three by two assay the potency of the mixture was only 39 per cent (95 per cent fiducial limit 14–67) compared with that of HCG alone.

The effect of D-NANA was also investigated using proportions of 1 mg to 3 I.U. HCG and 1 mg to 32 I.U. HCG with dosages ranging from 0·5 I.U. to 16 I.U. HCG. There was some reduction of uterine weight in mice given the first mixture, but when the uterine weights of animals given a higher proportion of HCG to D-NANA were determined, no significant reduction was found. The compound *N*-acetyl-D-mannosamine was also tested; it is biochemically related to D-NANA by means of a reversible aldolase reaction involving pyruvic acid and alternative pathways[7]. When mixed in the proportion of 1 mg to 1 I.U. HCG, it reduced the mean uterine weight to 62 (48–80) per cent of the mean uterine weight of mice given the same doses of HCG alone. With respect to HCG it was also effective when given at twice this dosage. The inhibitory action seems to be relatively specific as the isomeric compound *N*-acetyl-D-glucosamine was without significant effect (uterine weights 88 (62–124) per cent of the controls). Furthermore, L-NANA, *N*-acetyl-L-mannosamine and fourteen other closely related derivatives were inert.

This assay is not specific for HCG. For this reason the effects of D-NANA and other compounds were investigated in the ovulation test in mice[6] which is relatively specific for HCG and LH. Both D-NANA and D-NAM reduced the activity of HCG in this test. When mixed in the proportion of 1 mg to 1 I.U. HCG the potencies (compared with controls in 2 : 2 assays) were 68·9 (13·3–106) per cent for D-NANA, and 61 (42–96) per cent for D-NAM. These results extend and confirm the preliminary observations of Ladman and Soper[8], who used a similar assay technique but who had only a very crude preparation of NANA. They found that increasing dosages of this material progressively decreased the number of ova produced in mice given 0·5 I.U. HCG. Some toxicity was noted at the highest dose used (50 mg). It is probable that this resulted from impurities in the preparation, or from the *p*H of the final solution which was not recorded. During the course of our work we have not noted any toxic effects when D-NANA has been administered in neutral solution. Finally, neither D-NANA nor D-NAM was found to have any effect on the uterine-stimulating activity of oestradiol-17β, or on human pituitary LH as determined by the ovulation assay (relative potency 100 (66–152) per cent). The latter observation is surprising in view of the close similarity between the biological activities of LH and HCG. However, it is known that pituitary LH differs chemically from HCG as it does not contain appreciable amounts of NANA and does not suffer loss of activity on incubation with neuraminidase[9].

The action of hormones depends on their structure and their ability to reach their target cells. It is tempting to suggest that any entity which inhibits the action of HCG is concerned with one or more of the following features: (i) an ability to compete for a site of hormone action (compare with the well known haemagglutination phenomena of the influenza virus where NANA-containing mucoproteins and polysaccharides are competitive inhibitors[10]); (ii) an ability to undergo conversion into a compound exhibiting (i). These features may be operative in the inhibition of HCG activity by D-NANA and D-NAM and they appear all the more significant when it is appreciated that human pituitary LH is not inhibited in the same way.

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Administration of *N*-Acetyl-D-mannosamine to Mammals

N-ACETYL-D-mannosamine (NAM) is biochemically related to *N*-acetyl-D-neuraminic acid (NANA), a constituent of both human follicle stimulating hormone and human chorionic gonadotrophin (HCG). The compound itself is devoid of biological activity, but when mixed with HCG it reduces the activity of this hormone in both the uterine weight and ovulation assays[1]. We therefore considered it to be of interest to study the fate of NAM after the administration of it to mammals.

N-Acetyl-D-mannosamine has a pleasant, bland, sweetish taste. It has not been found free in human serum but has been reported to occur in human semen[2]. In mammals it is interconvertible with its 6-phosphate[3] and with NANA[4] by way of condensation with pyruvate. It can be assayed in aqueous solution using the Morgan-Elson reagents[5].

In determining NAM in plasma (3–150 μg/ml.) it has been found preferable to dialyse the plasma (1 ml.) against water (100 ml.) at 2° C overnight and then to pass the dialysate down a 'Dowex 50H+' column (30 × 1·5 cm). The first 150 ml. which was collected was freeze-dried, redissolved in water (1·2 ml.) and the NAM estimated on a suitable portion (1 ml.). The method was calibrated by adding known amounts of NAM to samples of serum and submitting them to the same procedure and reading the optical densities against a serum blank.

After oral administration of NAM to human subjects, the levels detected in serum were extremely variable. There are several possible reasons for this; one important factor appeared to be the relative blood glucose level while another was the presence of bacteria in the saliva which synthesized induced enzymes capable of destroying NAM[6].

The maximum levels of NAM which could be detected in five starving subjects were 78 μg/ml. (4 h after 5 g NAM), 205 μg/ml. (1 h after 5 g NAM), 104 μg/ml. (4 h after 10 g NAM), 20·8 μg/ml. (8 h after 10 g NAM), while in the fifth no NAM was detectable in the serum up to 8 h after 10 g NAM had been administered. A sixth subject took ampicillin (1·5 g) during the previous 36 h to destroy possible salivary bacteria and showed a maximum serum level of 30 μg NAM/ml. 4·5 h after taking 10 g. No NAM could be detected in the urine of two subjects given 5 g and 10 g oral doses of NAM. In the subject with the highest level of NAM (205 μg/ml.) the concentration had dropped to 10 μg/ml. in a further 2·75 h. During this time there was also a fall in blood glucose from 69 mg/ml. to 49 mg/ml. The blood glucose (75 mg/ml.) was determined in the third subject when the level of NAM was 104 μg/ml. After a midday meal, the level of NAM slowly fell to 78 μg/ml. after a period of 1·5 h while the blood glucose rose to 100 mg/ml. 1·5 h later the level of NAM was still 72·2 μg/ml. and the blood glucose 88 mg/ml.

It would appear that much of the NAM administered orally is converted to glucose as there is a rapid rise in blood glucose in the starving subjects. Thus administration of 10 g NAM to one subject caused a rise in the blood glucose from 76 mg/ml. at 1 h to 108 mg/ml. at 4 h, while in a second it rose from 51 mg/ml. at 0·5 h to 100 mg/ml. at 1 h. In two subjects, when NAM (10 g) was administered 30 min after 10 g glucose had been given, extremely low levels of NAM resulted, the maximum after 4 h being only 16·6 μg/ml. Oral administration of NAM (10 g) to two other subjects 2 h after breakfast resulted in similar low levels of NAM, the maximum after 4 h being 11 μg/ml. and 14·6 μg/ml. of NAM respectively.

Tablets of NAM (5 g) taken sublingually over 15 min were ineffective in one subject who was later found to have salivary bacteria active against NAM. Enteric coated (cellulose phthalate acetate) NAM (5 g) swallowed by the same subject produced a NAM level of 56 μg/ml. after 5 h while in a second subject who took 2·5 g the serum level of NAM rose to 30 μg/ml. after 8 h.

Parallel experiments were performed in sheep. Two sheep given 1 g NAM intravenously rapidly metabolized it giving serum levels of 43 and 47 μg/ml. (0·75 h), 25, 33 (1·5 h), 6, 7 (4·5 h), 5, 12 (6 h) and 9, 4 (24 h). In the first animal the serum level at 0·25 h had reached 72 μg/ml. Rather similar results were obtained in two sheep given 1 g NANA intravenously and serum levels were 53, 79 μg NANA/ml. (0·25 h), 38, 24 (0·75 h), 21, 21 (1·5 h) and 7, 8 (4·5 h).

Portions (39·4 mg) of (*N*-acetyl-1-^{14}C)-D-mannosamine (0·269 g) were prepared[7] by treatment of D-mannosamine hydrochloride (0·4 g) with (acetic-1-^{14}C) anhydride (0·2 mc.) dissolved in acetic anhydride (0·25 ml.). These were injected into two female rats. One rat was dissected after 1 h and the other after 3 h. The extraction of the test compound from each organ was carried out overnight at room temperature. Five millilitres of acetone were used for all organs except for the liver, which required 10 ml. and small organs (pituitary, gut, heart, ovaries, uterus, thyroid) which were given 2 ml. only. Thereafter all organs were extracted three times with acetone (5 ml.). The dry weights of the organ residues were determined. The activity of each extract was determined with a Packard 'Tricarb' liquid scintillation counter and expressed as total 100 sec count of bulked acetone extracts/mg dry weight. The major site of radioactivity was the kidneys (528 after 1 h; 62 after 3 h) rather than the liver (85 after 1 h; 28 after 3 h) or spleen (54 after 1 h; 11 after 3 h). Epithelial tissues were richer in activity (skin, 207, 29; gut, 174, 35; lung, 208, 17) than muscle (67, 13), heart (110, 13) or brain (20, 9). The ovaries (159, 39) and uterus (144, 47) were also noticeably active in contrast to adipose tissue (77, 21) with its slow rate of turnover. Activity rapidly disappeared from the blood; some was recovered in the urine and faeces, but the major amount of NAM was presumably metabolized by way of the Krebs cycle.

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Experimental Porphyria induced in Rats by Ethionine

Our earlier findings have shown that administration of adenine nucleosides or nucleotides to rats with different experimental porphyrias was followed by a decrease in excess porphyrin formation[1-4]. These observations have led us to the conclusion that these purine derivatives and particularly ATP play an important part in the quantitative regulation of porphyrin biosynthesis[1]. Moreover, it occurred to us that if this hypothesis is correct, administration of compounds capable of causing a decrease in the biosynthesis of adenosine triphosphate (ATP) might well bring about the formation of excess porphyrins and porphyrin precursors. Our observations with 6-mercaptopurine[3] and orotic acid[4] have confirmed this working hypothesis.

Ethionine seemed to be a particularly interesting compound in this respect, as several authors have already shown that ethionine is a powerful inhibitor of hepatic ATP synthesis, even after a single dose[5-7]. On the other hand, Gibson *et al.*[8] have reported that ethionine stimulates porphyrin synthesis in *Rhodopseudomonas spheroides*.

We have therefore made this investigation of the effect of ethionine on porphyrin metabolism and the level of ATP in the liver and red blood cells of the rat. Three different types of experimental condition were set up: (1) The rats were maintained on a low protein diet (8 per cent casein containing 0·5 per cent ethionine) for 3 weeks. (2) Another group of rats received a daily dose of 50 mg ethionine (administered by gastric tube) for 1 week. The diet of these animals contained 12 per cent casein. (3) A third group of animals received 100 mg ethionine per day (administered by gastric tube) for two successive days. These rats were maintained on a balanced diet.

Controls were fed the same diet as the rats treated with ethionine. Whenever ethionine was introduced through the gastric tube, the controls received a corresponding volume of solvent (physiological saline) by the same means.

Urinary and faecal porphyrins and porphyrin precursors (ALA and PBG) were determined every 3–4 days. The level of etherosoluble porphyrins (coproporphyrin + protoporphyrin) was determined in the liver and red blood cells immediately after death provoked by bleeding following mild ether-anaesthesia. Where ethionine had been administered for 2 days only, a porphyrin analysis of the red blood cells was omitted. The luciferase method[9] was used for the determination of ATP levels in liver and red blood cells. In some animals, the activity of ALA-synthetase in liver mitochondria was also determined, using the method devised by Granick and Urata[10]. In order to test the effect of adenine nucleosides or nucleotides on the disturbances of porphyrin metabolism, brought about by ethionine, inosine or adenosine monophosphate (AMP) or ATP was administered intramuscularly at a daily dose of 20 mg from the beginning of the period of ethionine intoxication.

Urine analyses showed a clear-cut increase in ALA and coproporphyrin excretion in rats intoxicated with ethionine. This increase was important even in our shortest experiments (2 days). Porphobilinogen appeared in the urine of some rats only during the first days of ethionine administration and uroporphyrin was not detected (Fig. 1). Our findings concerning the faecal excretion of copro- and proto-porphyrin were somewhat complicated by the side effects which resulted from administration of a low protein diet. In our first and second series of experiments (diets containing, respectively, 8 per cent and 12 per cent casein), the intestinal excretion of porphyrins was very significantly increased even in the control. Nevertheless, during the first week of ethionine administration, the increase was clearly higher in the intoxicated rats than in the controls. In the short experiments (2 days) where the animals were maintained on a balanced diet, the stimulating effect of ethionine on the accumulation of faecal porphyrins was fairly evident (Fig. 2).

As is shown in Table 1, the level of etherosoluble porphyrins in the liver and red blood cells was found to be significantly increased except in rats of the first experimental series, where ethionine had been administered for 3 weeks. This last fact, together with the decline in the level of faecal porphyrins in these animals during the last week of the experimental period, can probably be attributed to a general inhibition of biosynthetic processes provoked by the prolonged ethionine intoxication. We noted, just as Hutterer *et al.*[11] had, that at the end of the longer experiments there was no significant difference in

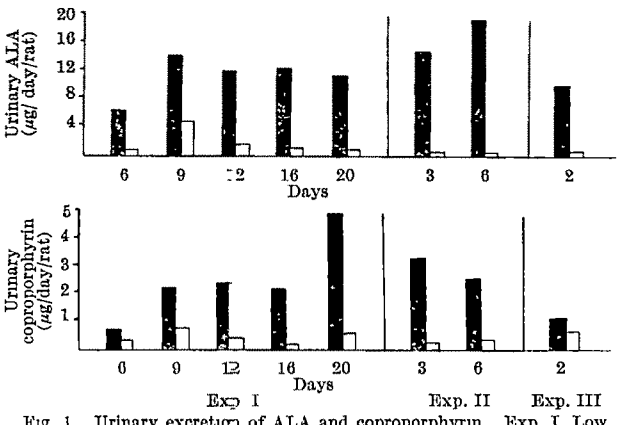


Fig. 1. Urinary excretion of ALA and coproporphyrin. Exp. I, Low protein diet (8 per cent casein) containing 0·5 per cent ethionine for 3 weeks. Mean value of six intoxicated and six control rats. Exp II, Low protein diet (12 per cent casein), 50 mg ethionine daily by gastric tube for 1 week. Means of twelve intoxicated and twelve control rats. Exp. III, Balanced diet, 100 mg ethionine daily by gastric tube for two successive days. ■, Rats intoxicated by ethionine; □, controls.

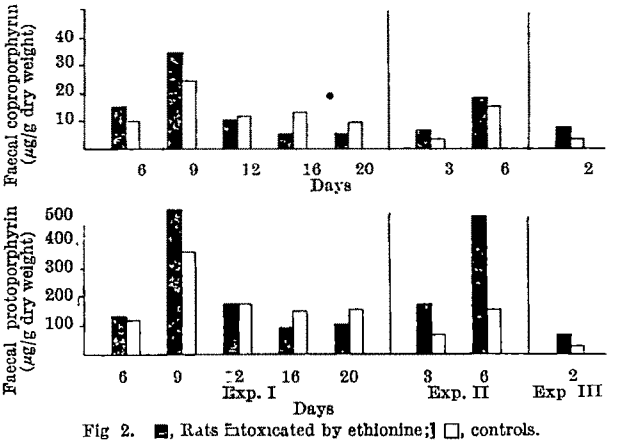


Fig 2. ■, Rats intoxicated by ethionine; □, controls.

the lipid content of the liver between ethionine-treated and control rats. However, we observed a decrease in the level of hepatic ATP after administration of ethionine for 1 week or 2 days; the concentration of ATP in the red blood cells remained unchanged (Table 2). No increase was observed in the activity of ALA-synthetase in the liver of mitochondria of rats intoxicated with ethionine for 3 weeks. Administration of inosine, or AMP or ATP, did not appear to have any effect on the disturbance of porphyrin metabolism caused by ethionine.

Thus our findings clearly show that ethionine intoxication for 3 weeks, 7 days or even 2 days causes a statistically significant accumulation of porphyrins in urine, faeces, hepatic tissue and red blood cells on one hand, and an important decrease in the hepatic level of ATP on the other. These experiments agree with our earlier observations on the effects of 6-mercaptopurine[3] and orotic acid[4], which suggested that the experimental inhibition of ATP biosynthesis is accompanied by the appearance of porphyria. Granick[12] has recently observed that compounds capable of inducing an enhancement of porphyrin synthesis cause an increase in the activity of ALA-synthetase in liver mitochondria by stimulating a *de novo* formation of the enzyme. We did not note this phenomenon after the administration of ethionine, but it is possible that protein synthesis is inhibited by this toxin, as was suggested by Farber *et al.*[13]. On the other hand, Weber *et al.*[14] reported that ethionine is able to reduce the increased synthesis of certain enzymes occurring in liver influenced by inducing agents.

There are at least two ways by which we could explain the lack of a beneficial effect of adenine derivatives (inosine, AMP, ATP) on the disturbances of porphyrin metabolism caused by ethionine. First, as had previously been established under different experimental conditions[1,15,16], the effect of purine derivatives on excess porphyrin synthesis is directed on the synthesis of ALA. For example, in an illuminated suspension of *Rhodopseudomonas spheroides* in Lascelles mixture I (containing glycine and succinate as substrates), ATP at a concentration of 3 mM causes almost total inhibition of porphyrin synthesis. On the other hand, ATP is without effect when the micro-organisms are suspended in Lascelles mixture II (containing ALA as substrate[15]). It was reported by Gibson *et al.*[8] that ethionine stimulates porphyrin biosynthesis by *Rhodopseudomonas spheroides* in mixtures I and II to an equal extent. Thus, in ethionine intoxication part of the raised level of porphyrin synthesis may escape the inhibitory effect of adenine nucleosides and nucleotides. The second mechanism, which has arisen from our findings, is that the inhibition of excess porphyrin formation by these adenine derivatives results from the stimulation of the biosynthesis of a physiological inhibitor—which seems to be a protein and probably an enzyme[17]. The inhibitory effect of ethionine on protein synthesis and on the neoformation of adaptative enzymes in the hepatic tissue might thus explain, at least partly, the inefficacy of the adenine derivatives in alleviating the disturbances of porphyrin metabolism in rats with ethionine poisoning. This hypothesis is consistent with the observations of Farber *et al.*[13], who have recently emphasized that ATP administration prevents or reverses only some of the effects of ethionine intoxication. Furthermore, as they have shown, acute hepatic ATP deficiency leads to a complex biochemical disturbance involving several other metabolic pathways as well as those leading to an accumulation of porphyrins. Our present and earlier findings confirm this hypothesis.

Table 1

Mean values of etherosoluble porphyrins (copro + proto)

	Liver (μg/100 g liver wet weight) Ethionine intoxication	Liver (μg/100 g liver wet weight) Controls	Red blood cells (μg/100 ml.) Ethionine intoxication	Red blood cells (μg/100 ml.) Controls
(1) After 3 weeks of low protein diet (8 per cent casein) containing 0·5 per cent ethionine (4 intoxicated rats, 4 controls)	29·0	27·6 Difference not significant	116·5 ± 9·7 $t = 4·9$ $P < 0·01$	41·0 ± 8·8
(2) After 1 week of low protein diet (12 per cent casein), 50 mg ethionine daily by gastric tube (12 intoxicated rats, 12 controls)	17·1 ± 2·3 $t = 2·3$ $P < 0·05$	11·0 ± 1·0	62·0 ± 7·2 $t = 3·6$ $P < 0·01$	33·0 ± 3·5
(3) After 48 h of balanced diet, 100 mg ethionine daily by gastric tube (12 intoxicated rats, 6 controls)	13·6 ± 1·6 $t = 2·2$ $P < 0·05$	8·4 ± 1·4	—	—

Table 2

	Liver (μmol/100 g wet weight) Ethionine intoxication	Liver (μmol/100 g wet weight) Control	Red blood cells (μmol/100 ml.) Ethionine intoxication	Red blood cells (μmol/100 ml.) Control
(1) Low protein diet (12 per cent casein) 50 mg ethionine daily by gastric tube during 1 week	79·0 ± 8·6 (mean of 14 rats) $t = 6·3$ $P < 0·001$	168·0 ± 10·2 (mean of 16 rats)	84·6 ± 9·9 (mean of 9 rats) $t = 0·9$ $P > 0·05$	72·0 ± 3·0 (mean of 11 rats)
(2) Balanced diet 100 mg ethionine daily by gastric tube for 2 successive days	61·0 ± 8·3 (mean of 12 rats) $t = 7·9$ $P < 0·001$	164·0 ± 11·0 (mean of 10 rats)	—	—

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Use of Carbon-14-labelled Arginine to measure the Catabolic Rate of Serum and Liver Proteins and the Extent of Amino-acid Recycling

THERE is evidence that in both man[1] and rat[2,3] protein deficiency causes a decrease in the catabolic rate of plasma albumin, which may plausibly be regarded as an adaptive reaction. To demonstrate this, it is necessary either to use a label which is not re-utilized, such as radio-iodine, or to reduce re-utilization to a minimum by injecting proteins that have been biosynthetically labelled in a donor animal. Tissue proteins cannot be studied by these methods, and therefore it is not known whether they react in the same way as plasma albumin to changes in dietary protein intake.

It has been shown by several workers that when labelled amino-acids are given, the rate of decay of radioactivity of both liver and plasma proteins is reduced in animals on a low protein diet[4,5]. This has been taken as evidence of a reduced catabolic rate of liver proteins, paralleling that of plasma proteins, but it could also result from recycling or re-utilization of the labelled amino-acid. To the extent that recycling occurs, the apparent half-life of any protein, calculated from the decay curve after

the administration of a labelled amino-acid, must be longer than the true half-life. The term "apparent half-life" is, of course, an approximation, because the decay curve is not strictly exponential.

In the experiments described here an attempt was made to separate these two phenomena—recycling and catabolism—and to measure simultaneously the true and apparent half-life of liver and serum proteins in normal and protein-deficient rats. Advantage was taken of the observation made by Swick[6] that the guanidine carbon of arginine, which is exchanged in the urea cycle, cannot be re-utilized. Assuming that virtually all the free arginine that enters the amino-acid pool goes through the urea cycle before it is incorporated in protein[7], when uniformly labelled arginine is incorporated into protein, the rate of decay of radioactivity in the 6-carbon (6-C) atom of arginine will be a measure of the true half-life of the protein, and the rate of decay in any or all of the other carbon atoms will give the apparent half-life.

Rats were given intraperitoneally 10 μc. of U-^{14}C-L-arginine, followed 18 h later by 100 μc. of carbon-14-labelled sodium carbonate in order to label the 6-C atom of arginine[8]. Two hours after this second injection a sample of liver (200–300 mg) was removed under ether anaesthesia. Blood samples (about 500 μl.) were taken from the tail daily for 3 or 4 days. At the end of this time the rat was killed and a second liver specimen obtained. Serum and liver proteins were precipitated with picric acid, and hydrolysed with 6 N hydrochloric acid. The specific activity of the 1-C atom of arginine was measured by treating one part of the hydrolysate with arginine decarboxylase in a micro-Conway unit. The carbon dioxide liberated was trapped in alkali in a planchet placed in the centre well, and counted after drying. The amount of arginine was measured with the same enzyme in the Cartesian diver microrespirometer[9]. The specific activity of the 6-C atom was measured by treating another portion of the hydrolysate with arginase. The urea produced was estimated by the method of Ceriotti and Spandrio[10]; carbon dioxide was liberated by reacting with urease, and collected and counted as before.

The true and apparent half-lives of mixed serum proteins were calculated from the slopes of the semilogarithmic plots of specific activity against time. The lines were fitted by the method of least squares. No correction was made for blood removed by sampling. The first sample used in the calculation was that obtained 24 h after the liver biopsy. Before that, equilibration between extra- and intra-vascular protein pools would probably not be complete. For calculation of the half-life of mixed liver proteins only two points were available, separated by an interval of 3 or 4 days.

Two experiments were carried out. In the first, two adult rats were given a protein-free diet for 3 days before the injection of labelled arginine, and kept on this diet for a further 4 days until the end of the experiment. In the second experiment three young rats (each weighing about 100 g) were given the protein-free diet for 10 days before the injection, and then for 5 more days until they were killed. In both experiments an equal number of rats of comparable weight were kept on a stock diet. With one exception the rats seemed to show no ill effect from the biopsy. In one rat (No. 26, Expt. 2) there was excessive trauma at the biopsy, and the rat lost weight afterwards, although it was on a normal diet. The results are shown in Table 1. In the five normal rats the average value for the true half-life of mixed serum proteins was 2·0 days. To study the mixed proteins is admittedly a crude approach, but with the amounts of serum available it was not possible to do the analyses on the separate protein fractions. In rats fed a high-protein diet, Jeffay and Winzler[2] found values of about 2·5, 0·7 and 5 days for the half-life of albumin, α-globulin and γ-globulin. Our figure for mixed proteins must represent a weighted average, and seems quite consistent with these findings. In the protein-deficient rats it seems that the longer period of depletion (experiment 2) did cause a reduction in the catabolic rate of the mixed proteins. The mean value for the true half-life in this group was 3·36 days. In the experiments of Jeffay and Winzler[2], a protein-free diet caused an increase in the half-life of albumin to about 4·5 days, but no increase in that of the globulins. Therefore, the increase in the half-life of the mixed serum proteins found in the present experiments must underestimate the real change in the catabolic rate of albumin. Excluding No. 26, which lost weight, in the normal rats the average for the apparent half-life is 2·7 days. In the protein-deficient rats it was doubled (mean 5·5 days).

Table 1

	Rat No.	Diet*	Weight† change (per cent)	Serum protein half-life (days) True	Serum protein half-life (days) Apparent	Liver protein half-life (days) True	Liver protein half-life (days) Apparent	Isotope‡ replacement ratio Serum	Isotope‡ replacement ratio Liver
Expt. 1	10	*N*	—	2·22	2·83	1·67	3·7	22	55
	14	*N*	—	1·52	2·35	1·85	3·8	36	52
Expt. 2	27	*N*	+2[illegible]	1·98	2·83	2·08	4·3	30	52
	28	*N*	+1[illegible]	2·13	2·78	2·10	10·2	23	81
	26	*N*	−5·5	2·18	5·25	1·85	42	59	97
Expt. 1	15	*PF* 3	−1[illegible]	1·57	4·88	2·30	6·85	68	67
	16	*PF* 3	−[illegible]	2·79	7·28	2·42	4·35	62	44
Expt. 2	29	*PF* 10	−[illegible]	2·67	4·75	2·50	10·2	44	76
	30	*PF* 10	−[illegible]	3·72	5·55	1·86	6·7	33	67
	31	*PF* 10	−4	3·70	6·23	2·52	10·8	41	77

* *N*, Stock diet. *PF*, protein-free diet, 3 or 10 days.
† Percentage of weight change during the experimental period.
‡ For definition see text.

The true half-life of liver protein in the normal rats averaged 1·9 days, and in the protein-depleted rats 2·3 days. This difference is statistically significant ($0·05 > P > 0·01$). It seems, therefore, that in protein deficiency there is a fall in the catabolic rate of mixed liver proteins, although the reduction is smaller than that found for plasma albumin by other workers[2,3]. The values for the apparent half-life are rather irregular, perhaps because of the disturbing effect of the liver biopsy. In all cases it is longer than the true half-life, and in some much longer.

The question arises as to whether from these results it is possible to define quantitatively the extent of "re-utilization". By this term is meant the incorporation into protein of amino-acids derived from the catabolism of body protein and not from food. Thus on a protein-free diet all synthesis must represent re-utilization. The specific activity of liver and serum proteins nevertheless falls because some of the amino-acid incorporated is derived from tissues such as muscle, which are poorly labelled. It seems probable that most of the isotopic amino-acid that is re-incorporated must have come from tissues which are highly labelled and are turning over rapidly. If so, the extent of re-utilization of isotope will be a measure of what might be called "internal" recycling within a pool of highly labelled protein. This pool roughly corresponds to the protein of the viscera and plasma; it contains about 20 per cent of the total protein of the body, but accounts for about 50 per cent of total protein turnover[11]. An index of the extent of this recycling of isotope can be calculated from the present results. If $F1$ is the true fractional rate of turnover of serum or liver protein, and $F2$ the apparent fractional rate, and if a steady state is assumed, the ratio $\frac{F1-F2}{F1}$ represents the proportion of isotope lost by catabolism that is replaced in the same time interval. The calculated values of this isotope replacement ratio are shown in the last column of Table 1. For serum proteins the ratio seems to increase with protein depletion, particularly in the early stage, before there is a reduction in catabolic rate. In liver the striking point is the high rate of replacement even in the animals on a normal diet, particularly in

view of the fact that arginine is only a semi-essential amino-acid. The results of Penn, Mandeles and Anker[12] suggest that the rate of re-utilization must be different for each individual amino-acid.

On the basis of kinetic data, Swick[6] was able to give a more precise definition of the extent of amino-acid re-utilization in the liver. He defines it as the probability that the carboxyl-carbon atom of arginine, liberated from liver protein by catabolism, will be re-incorporated in liver protein. Under his experimental conditions this probability was about 50 per cent. If in the present experiments all the arginine entering the liver pool from different sources had had the same specific activity, the "isotope replacement ratio" would be identical with the probability ratio calculated by Swick. The fact that they are of the same order of magnitude suggests that the isotope replacement ratio may be a useful indication of the extent of amino-acid re-utilization in the strict sense defined by Swick. Because an increase in the extent of re-utilization may be an important feature of the process of adaptation to low protein intakes, even an approximate quantitative measurement of the effect may be of value.

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Intracellular Repressor of Alkaline Phosphatase

THE repression of β-galactosidase in *Escherichia coli* has been demonstrated to be sensitive to 2,4-dinitrophenol[1]. This presumably indicates that high energy phosphate bonds are involved. Alkaline phosphatase is repressed by *ortho*-phosphate in the medium. We have used this as a test to determine whether high energy phosphate bonds are essential to the mechanism of enzyme repression. Is the intracellular repressing phosphate *ortho*-phosphate or must it participate in a high energy compound for the formation of "active repressor"?

Cells of *E. coli B* were grown and alkaline phosphatase assayed as previously reported[2]. Addition of 0·001 M 2,4-dinitrophenol had no effect on enzyme production or repression. 0·01 M abolished enzyme synthesis. We were unable to find any concentration that stimulated alkaline phosphatase production. A similar result was obtained with arsenate. If, then, *ortho*-phosphate is itself the intracellular repressor, a phosphate acceptor which would reduce the intracellular concentration of *ortho*-phosphate should stimulate enzyme synthesis. Various nucleosides were tested, and it was found that inosine stimulated normal enzyme production and also reduced the amount of time required for recovery from repression by added phosphate (Fig. 1). A similar but less pronounced effect occurred with guanosine and adenosine.

Manson *et al.*[3] have discovered a mechanism for repression of alkaline phosphatase synthesis by *ortho*-phosphate in a particulate fraction of *E. coli*. Our observations make it more probable that the intracellular repressor is indeed *ortho*-phosphate and that their mechanism is the one operative *in vivo*. In view of this, the function

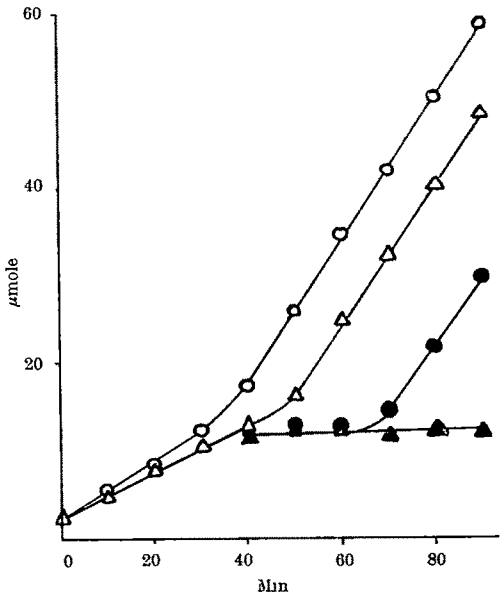


Fig. 1. *E. coli* was grown and the enzyme induced as previously described in run 1 (△). In run 2 (○) the induction medium was fortified with 0·01 M inosine. After 30 min, 0·001 M sodium monohydrogen phosphate was added to portions of run 1 (▲) and run 2 (●). 0·5 ml. portions were withdrawn at the times indicated and the enzyme assayed. The vertical scale represents micromoles of *p*-nitrophenyl phosphate hydrolysed in 60 min.

of the organic repressor the properties of which have been inferred[4] becomes even more obscure.

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A Sex-determined Renal Calcification in Rats

AN examination of several hundred white Wistar rats from the only source of laboratory animals available to this institution revealed a 100 per cent incidence of renal calcification in female animals and a complete absence of this condition in males of the species. This situation also applied to pure line white Wistar, black Wistar and black and white Wistar strains but was not observed in several species of mice from the same source and fed the identical diet.

These animals were bred and maintained on a commercially produced feed pellet with a calcium and phosphorus content of 2·0 per cent and 1·6 per cent of the dry weight respectively. The relatively high level of these two elements was found to result from the use of bonemeal in the pellets. The renal calcification, however, could not simply be related to the intake of these minerals as female rats bred and raised on synthetic diets[1] (calcium 0·31 per cent, phosphorus 0·27 per cent) also showed invariable occurrence of the nephrocalcinosis. A satisfactory control diet was provided by the use of 'Farex' (Glaxo Laboratories, Ltd.), a supplemented, mixed cereal baby food, and milk (1 : 5 w/v). This diet had a content of 0·88 per cent calcium and 0·68 per cent phosphorus on a dry weight basis, and female rats were free of kidney deposits after even 6 months on this diet. The condition also did not occur in female animals raised on a low phosphorus rachitogenic diet[2] (calcium 1·42 per cent, phosphorus 0·28 per cent), which is not surprising in view

of its effect on mineralization and growth. With the exception of the rachitogenic diet, growth rates were high and comparable on these diets, with no difference observed between male and female rats.

Renal deposits of calcium were histologically demonstrable within 3 weeks of weaning female rats on to the standard pellets. The calcification commenced at the cortico-medullary junction, where it remained most dense throughout life, but later spread into the medulla. The cortex remained unaffected although there was some enlargement of the deposits with increasing age. Electron microscopy revealed the deposits to be usually lamellated and to be both intracellular and intra-tubular in location. A few deposits had the typical appearance of hydroxyapatite.

Table 1. RENAL CALCIFICATION IN WHITE WISTAR RATS

Sex	Age (weeks)	Diet	Treatment	Calcium	Phosphorus	Histology
M	26	*P*	—	0·38 ± 0·03	12·20 ± 0·46	Negative
F	6–8	*P*	—	1·12 ± 0·56	13·60 ± 0·80	Positive
F	26	*P*	—	12·10 ± 14·00	16·20 ± 6·0	Positive
F	11–18	*C*	—	0·37 ± 0·01	12·60 ± 0·35	Negative
M	5–11	*P*	Castrated	0·39 ± 0·05	13·10 ± 1·1	Negative
M	9–12	*P*	DHT	3·41 ± 2·80	14·20 ± 0·98	Positive
F	5–11	*P*	Ovariectomized	0·39 ± 0·05	13·60 ± 0·80	Negative
M and F	10	*P*	Gonadectomized + oestroform	3·96 ± 2·33	13·80 ± 0·32	Positive

P, Commercial pellet diet; *C*, control 'Farex' diet; DHT, dihydrotachysterol, 1 mg per 100 g body-weight by intraperitoneal injection; analytical data as means with standard deviations in mg/g dry weight of kidney, minimum of seven animals in each group.

Kidney mineralization following administration of hypercalcaemic agents such as parathyroid hormone, dihydrotachysterol and vitamin D is well known. With nephrocalcinosis induced by these agents there is a diffuse distribution of mineral throughout the kidney, the cortex being most affected[3]; death from renal failure is a common event with these treatments. A feature of the female-specific renal calcification described in this report is that the affected animals were apparently healthy, with normal weight gain, ability to withstand multiple pregnancies, normal serum calcium and phosphorus levels and no impairment of renal function as judged by blood urea measurements.

Experimental work has shown that the sex specificity of this nutritional nephrocalcinosis is related to oestrogen level. A group of female rats was ovariectomized and weaned at 30 g weight on to the standard pellet diet. Periodic examination of kidneys from these animals showed the absence of renal deposits over the 8 weeks of observation. In a similar experiment both male and female rats were gonadectomized and received 100 μg of oestradiol benzoate ('Oestroform', British Drug Houses, Ltd.) twice weekly by intramuscular injection while fed on standard pellets. Within 3 weeks all animals had developed the typical renal deposits. The results of some of these treatments are summarized in Table 1, which also gives analyses for total calcium and phosphorus in the decapsulated kidneys. These quantitative data give an index of the magnitude of the calcification and illustrate its progression with age in the female rats.

Both oestrogens and a dietary factor are implicated in the aetiology of the condition described. The nutritional factor remains undetermined, but it would seem that it is not related to absolute levels of calcium and phosphorus, vitamin D or magnesium in the diet.

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Isolation of Fluorescent Components from Ox-bone Human Dentine and Gelatine

EARLIER investigations[1–4] on the fluorescent material in calcified tissues have indicated that it is associated with the collagenous organic matrix of these tissues. Observations on soluble tissue matrix preparations showed they had excitation maxima at 330 mμ with corresponding emission maxima about 400 mμ.

Using a 'Sephadex' ion-exchange column (C.25 *CM* at *p*H 4·2) we have now successfully separated the fluorescent material from alkaline hydrolysates of ox bone, human dentine and gelatine preparations with 80–90 per cent recovery. The isolated material possessed a characteristic ultra-violet absorption spectrum with maxima at 264 mμ and 325 mμ. The fluorescent excitation and emission maxima of the isolated material were 330 mμ and 400 mμ, respectively, and were identical to those obtained from both solid and solution preparations of the corresponding intact matrices. Fluorescence was destroyed by bromination and permanganate oxidation. The isolated material fractionated on a 'Sephadex *G*-10' gel filtration column into two components, with identical fluorescence characteristics.

Consideration has been given to the concepts that the fluorescence associated with calcified tissue proteins may arise either from a separate substance bound to the matrix or, alternatively, be the property of some intrinsic structural feature of the collagen molecule itself. Our results indicate that the fluorescent material survives alkaline hydrolysis of the total matrix, and can apparently exist as a separate entity, with the same excitation and emission characteristics in the isolated state as when associated with the matrix. It presumably possesses oxidation and bromination sensitive centres. From the gel-filtration results a tentative estimate of a molecular weight of less than 700 for the material may be made, the results also indicating that the fluorescence is associated with two fractions of differing molecular weight.

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Freezing of Bound Water and Protein Aggregation in Frozen Cod Muscle stored near its Melting Point

THE muscle proteins of frozen fish slowly aggregate at a rate depending on the temperature. Since the reaction causes the flesh to become tough to eat, and eventually unacceptable to the consumer, a considerable research effort has been directed towards means of slowing it down—the usual method being to keep the temperature as low as possible.

In cod (*Gadus morhua* L.) air-blast frozen at −30° C the change proceeds most rapidly between −1° and −2° C (ref. 1), and it was therefore surprising to learn recently that fish landed in Portugal after storage on a catching vessel at temperatures just below their freezing point ("superchilled") had suffered little damage to their proteins[2]. The trials were repeated under laboratory conditions in this country[3] and, after thawing, the samples

were shown both by subjective examination and by the "Cell Fragility" technique[4] (a physical method for measuring cold-storage changes in fish muscle) to be almost unaltered, although the conditions were apparently such as to cause maximum change.

It seemed likely at first that the fish had never actually frozen: the proteins of cod muscle at −1·5° C in the supercooled condition (without ice present) do not aggregate[1]. However, calorimetry showed[3] that about half the tissue water was in fact frozen out after 8 days at an ambient temperature of −3° C.

The only difference between the fish showing the maximum rate of change just below their melting-point and the "superchilled" samples appeared to be the temperature at which each was initially frozen. The present report describes the results of keeping frozen cod at −1·6° C after freezing at different temperatures.

The anterior halves of fillets from cod about 60 cm long, caught by trawling near Aberdeen in August 1965, were chopped into seven or eight pieces. "Mixed samples" were made by placing fifteen such pieces, each weighing about 50 g, and each from a different fish, into a polythene bag and sealing under vacuum. Half of these "mixed samples" were frozen by placing in an air-blast freezer at −30° C, and the other half by placing in a large insulated bath of sodium chloride solution, continuously stirred and maintained at −3° C by adding solid carbon dioxide from time to time. The samples were removed from the blast-freezer after 3 h, and from the brine bath after ascertaining that ice was present in all the samples. Further time would elapse before the ice had completely permeated the brine-cooled samples; the present procedure was merely intended to guard against supercooling. The samples at −30° C were left in a room at −7° C for some hours before mixing with those frozen at −3° C. All were then placed in a small cabinet with internal temperature maintained at −1·6° C as already described[1], and a mixed sample of each kind was removed every 2 or 3 days for testing by the cell fragility method[4] after thawing at about 18° C.

The results are shown in Fig. 1, and it is clear that the samples frozen at −3° C deteriorated much more slowly than those air-blast frozen at −30° C, though both were stored at the same temperature.

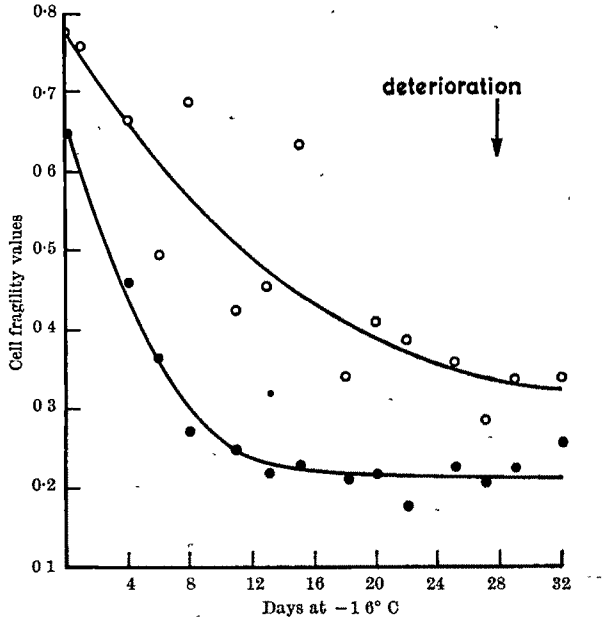


Fig. 1. Deterioration of the protein of frozen cod muscle stored at −1·6° C, as measured by the cell fragility method. Each point is the mean of fifteen determinations. ●, Fish frozen at −30° C and then warmed up to −1·6°. ○, Freezing initiated in a bath at −3° C.

Fish muscle appears to bind water with a variety of bond energies[5]. Progressively lowering the temperature causes the detachment of more and more bound water and its crystallization as ice, with a resultant increasing damage to the protein[6]. Rewarming the tissue does not reverse the situation—much of the extra ice remains at higher sub-zero temperatures[6], so that more is present in tissue warmed up to −1·6° C than in that cooled down to it. Because a greater proportion of the water is frozen, the tissue salts are more concentrated in the blast-frozen samples, and it has long been thought that concentrated tissue salts are, at least partly, responsible for the changes that occur in the proteins of frozen fish[7,8]. Herein probably lies the explanation for the effect shown in Fig. 1.

In his review of the freezing of tissue, Meryman[9] suggested that ". . . considerably more water remains frozen during warming than would have frozen at the same temperature during cooling". The effect was demonstrated experimentally by Love and Elerian[6], who found differences between the specific volumes of tissue cooled to, and warmed up to, −14° C. The findings of the present report seem to be another manifestation of the same phenomenon.

Discussing the new "superchilling" process, Partmann[10] concluded that the tissue would rapidly deteriorate unless it could be prevented from freezing. In the light of the present information, one can be more optimistic, especially where a relatively short storage time is contemplated.

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Studies on the Proteins from Chromaffin Granules of Ox, Horse and Pig

CHROMAFFIN granules of the adrenal medulla are cell organelles which contain catecholamines in a high concentration. It has been suggested that the proteins of these granules are involved in the storage of these catecholamines. So far only the proteins from the granules of ox adrenal medulla have been characterized. These proteins are highly soluble in water, and the purification as well as some properties of the main component of the soluble fraction have been reported[1–3]. Recently, it was shown that the secretion of catecholamines from the perfused ox adrenal medulla is accompanied by a release of this specific component[4].

In the present investigation we compared the solubility of the proteins in chromaffin granules from ox, horse and pig and the behaviour of the soluble fraction in starch-gel and polyacrylamide disc electrophoresis. This comparative investigation was thought to provide further evidence for the significance of these proteins for chromaffin granules.

Adrenal medullae of ox, horse and pig were homogenized in ice-cold 0·3 M sucrose. Nuclei and cell debris were removed by centrifugation at 12×10^3 *g*-min. The supernatant was centrifuged at $2{\cdot}4 \times 10^5$ *g*-min to

sediment the so-called "large granule" fraction which contains mitochondria, lysosomes and chromaffin granules[5]. 1·5 ml. of the resuspended sediment were layered on 5 ml. of 1·6 M sucrose and centrifuged at 8·4 × 10^6 *g*-min. By this method chromaffin granules were obtained as a sediment. 42–47 per cent of the total catecholamines present in the homogenate were recovered in this final sediment. For the determination of the solubility of the proteins the chromaffin granules and the mitochondrial fraction were lysed in 0·005 M citrate buffer (*p*H 6·3) and the insoluble material was spun down at 9 × 10^5 *g*-min. After addition of trichloroacetic acid (TCA, final concentration 5 per cent) to both sediment and supernatant the precipitates obtained were extracted with boiling alcohol–ether (3 : 1 v/μ) and their protein nitrogen was determined by the micro-Kjeldahl method.

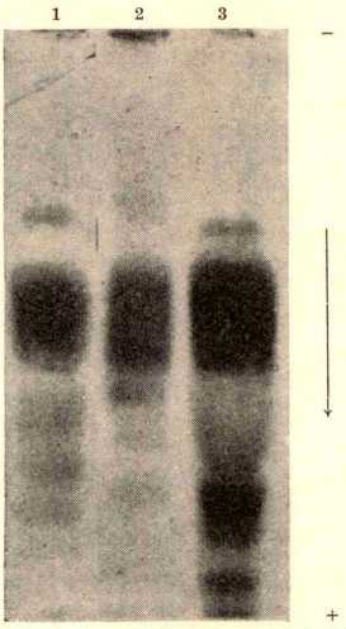


Fig. 1. Vertical starch-gel electrophoresis (ref. 6) of the soluble proteins of chromaffin granules from the adrenal medulla of (1) ox; (2) pig; (3) horse.

Under these conditions 70 ± 1 per cent of the proteins in ox granules, 74 ± 2 per cent in horse granules and 57 ± 1 per cent in pig granules were water-soluble. The corresponding values for the mitochondrial fraction were between 20 and 32 per cent. A resolution of the soluble proteins of chromaffin granules into several bands was obtained with vertical starch-gel electrophoresis[6]. The result of one experiment is shown in Fig. 1. It is evident that in all three species there is a main protein component which migrates to the same position. Several smaller components do not show any correspondence. Comparable results were obtained by polyacrylamide disc electrophoresis. Scanning (by 'Chromoscan') of the starch-gel patterns showed that the main component represents 49 ± 6 per cent of the total soluble proteins from ox granules, 59·5 ± 6 per cent from horse granules and 73·5 ± 5 per cent from pig granules.

We conclude that a high solubility and the presence of a main component which migrates to the same position in starch-gel electrophoresis are characteristics of the proteins of chromaffin granules of all three species examined. If these soluble proteins are essential to the function of chromaffin granules, then the main protein component which was found to be common to all species examined is most likely to be involved.

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Effect of Glucose on the Denaturation of Glucose Dehydrogenase by Urea

GLUCOSE dehydrogenase from spores of *Bacillus cereus* is heat stable in solutions of sodium chloride at *p*H 6·5 (ref. 1). Increasing concentrations of sodium chloride also protect the enzyme from inactivation by 8 M urea. The enzyme has a half-life of 7 min in 8 M urea, *p*H 6·5, but in 8 M urea : 1 M solutions of sodium chloride, the half-life of the enzyme is 192 min. The rate of decay of glucose dehydrogenase activity in the presence of urea follows second order kinetics[2]. A number of enzymes can be protected from urea inactivation by their specific substrates[3–8]. In this communication we report the effect of glucose in stabilizing glucose dehydrogenase and partially reversing the inactivation resulting from urea.

The preparation, purification, specificity and assay of glucose dehydrogenase have been reported elsewhere[1,9]. All experiments were performed at 0° C in 0·05 M phosphate buffer, *p*H 6·5. Fresh urea solutions (Baker, C. P.) were prepared for each experiment in order to eliminate the possibility of cyanate formation and its subsequent interaction with the protein[10]. The enzyme was incubated with 8 M solutions of urea containing 0–1·0 M glucose, and at various times samples were taken and assayed for enzyme activity. The reciprocal fraction activity remaining was plotted against time as shown in Fig. 1.

Concentrations of glucose as low as 0·1 M increased the half-life of the enzyme from 7 min to 90 min and further protection was achieved as the concentration of glucose was increased from 0·1 to 1 M. The decay of the enzyme at all concentrations of glucose was a second order function. Furthermore, the enhancement of stability of the enzyme was half-power with respect to glucose. We interpreted these data to mean that glucose binds to two "urea sensitive" groups on the protein. The denaturation

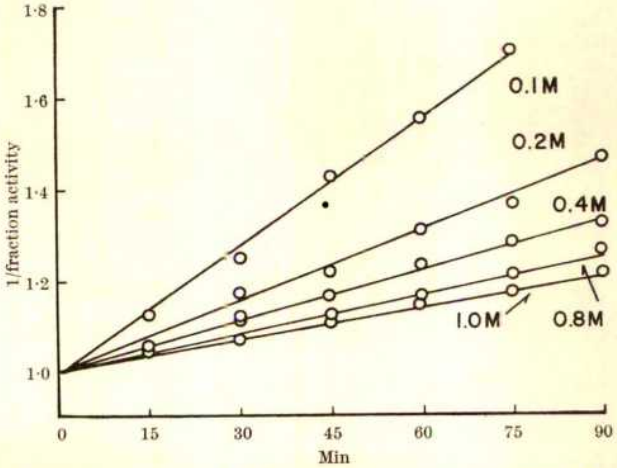


Fig. 1. Inactivation of glucose dehydrogenase by 8 M urea in the presence of various concentrations of glucose. The reaction was carried out in 0·05 M phosphate buffer, *p*H 6·5, at 0° C.

caused by 8 M urea could be partially reversed. Glucose dehydrogenase was treated with 8 M urea for 25 and 45 min and the loss of enzyme activity was determined. After this, the samples were dialysed against phosphate buffer, phosphate buffer + 2·5 M sodium chloride and phosphate buffer + 1 M glucose. In each instance 3 ml. of the enzyme mixture in 8 M urea was dialysed against 500 ml. of solution for 3 h at 4° C. The initial activity of the enzyme without urea was 8,000 U. The results are shown in Table 1.

Table 1. DENATURATION OF GLUCOSE DEHYDROGENASE BY 8 M UREA AND ITS PARTIAL RECOVERY FOLLOWING DIALYSIS

Time of incubation with 8 M urea	Activity of enzyme after incubation with 8 M urea (U)	Activity after dialysis against phosphate buffer (U)	Activity after dialysis against phosphate buffer + 2·5 M sodium chloride (U)	Activity after dialysis against phosphate buffer + 1 M glucose (U)
25 min	4,500	4,500	4,600	6,250
45 min	3,000	4,400	4,200	6,200

In both treatments (25 min and 45 min) the degree to which renaturation of the enzyme occurred after dialysis against 1 M glucose was identical. After 45 min incubation with 8 M urea the enzyme activity was reduced to 37 per cent, but after dialysis against 1 M glucose, the level of enzyme activity increased to 77 per cent of the original activity. There was thus a partial recovery of 40 per cent. However, only 12 per cent of the enzyme was renatured when the urea inactivated enzyme was dialysed against phosphate buffer or phosphate buffer + 2·5 M sodium chloride.

The following experiment was designed to determine whether prolonged exposure of the enzyme to urea, which results in complete denaturation of the enzyme, would still enable partial recovery to take place when the denatured enzyme was dialysed against 1 M glucose (Table 2).

Table 2. EFFECT OF PROLONGED EXPOSURE TO UREA FOLLOWED BY DIALYSIS AGAINST 1 M GLUCOSE

Concentration of urea	Activity remaining after 90 min (per cent)	Activity after dialysis against 1 M glucose (per cent)
5 M	67	100
8 M	3	0

The enzyme was incubated with 5 M and 8 M urea for 90 min. The reaction mixtures were dialysed against 1 M glucose in 0·05 M phosphate buffer, *p*H 6·5 at 4° C for 20 h. The prolonged exposure to 8 M urea resulted in irreversible denaturation of the enzyme (Table 2). Thus, the extent of reversibility appeared to be a function of the exposure time and concentration of urea. Table 1 shows that some irreversible denaturation also occurred when the enzyme was treated with 8 M urea for 25 min. At a lower concentration of urea (5 M) there was complete reversibility even after a prolonged exposure of 90 min. The denatured enzyme (8 M urea for 30 min) was also dialysed against 1 M solutions of galactose, fructose, α-methylglucoside, mannose and sucrose, but these were no more effective than phosphate buffer in reversing the inactivation.

Rajagopalan, Fridovich and Handler[5] have investigated the inhibition of a variety of enzymes by urea. They found that urea inactivation was most pronounced in enzymes utilizing large organic molecules as substrates. The inhibition of urea inactivation by the substrate was invariably competitive for such enzymes but not for those utilizing inorganic substrates. Our investigations show that the inhibition of urea inactivation of glucose dehydrogenase by glucose was also competitive. The substrate effect was specific and other monosaccharides, differing only in the configuration of a single carbon atom, afforded no protection. This was also true for the inactivation of the enzyme by 5-dimethylaminonaphthalene sulphonyl chloride where only glucose or glucose-6-phosphate would protect the enzyme[1].

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PATHOLOGY

Antitumour Activity of *N*-Hydroxyurethane

N-HYDROXYURETHANE ($C_2H_5O—\overset{\overset{O}{\|}}{C}—NHOH$) is known to be a carcinogen and to induce certain chromosomal aberrations[1,2]. It contains the hydroxamic acid group, a moiety apparently essential for the antitumour activity of hydroxyurea ($NH_2—\overset{\overset{O}{\|}}{C}—NHOH$) and its congeners[3,4]. For this reason, it was considered of interest to determine whether *N*-hydroxyurethane was active against various transplantable mouse tumours.

Daily doses of *N*-hydroxyurethane were administered subcutaneously to mice bearing various tumours. Table 1 presents the optimal dose for each tumour. A variant of the transplantable acute lymphoid leukaemia *L*1210 resistant to *N*-hydroxyurethane (*L*1210/*N*-hydroxyurethane) was produced by treating *L*1210 mouse leukaemic cells for several generations with daily doses of 400 mg *N*-hydroxyurethane/kg.

N-Hydroxyurethane was active against leukaemia *L*1210 and a variant of *L*1210 resistant to cytosine arabinoside (*L*1210/cytosine arabinoside). *N*-Hydroxyurethane was less active against the mast cell tumour *P*815 and leukaemia *P*288 and showed little or no activity against the plasma cell tumour *YPC*-1. Hydroxyurea, which gives a 50–65 per cent increase in median survival against advanced leukaemia *L*1210 at daily optimal doses of

Table 1. ANTITUMOUR ACTIVITY OF *N*-HYDROXYURETHANE IN VARIOUS MOUSE TUMOURS

Tumour	Range of doses (mg/kg)	Daily optimal dose	Day treatment initiated	Median survival time Control	Median survival time Treated	Increase in survival over control (per cent)
Leukaemia *L*1210	50–1,000	400–500	6	9·0	12·0	34*
*L*1210/cytosine arabinoside	200–600	400–600	6	9·5	14·0	47
*L*1210/*N*-hydroxyurethane	100–500	400	6	9·0	9·0	0
*P*815 mast cell	100–500	500	1	10·0	12·5	25
		300–400	6	10·0	11·5	15
*P*288 leukaemia	100–600	400–600	5	9·5	11·5	21
YPC-1 plasma cell	100–600	400–500	6	13·0	13·5	4

* Range of 25–42 per cent in five experiments.

250–500 mg/kg, seemed to be cross-resistant to *L*1210/*N*-hydroxyurethane (increase in survival time of 17 per cent at daily optimal doses of 600–750 mg/kg). *N*-Hydroxyurethane was inactive in mice bearing this resistant variant. Thus, it appears that these two antitumour agents have the same mechanism of action. *N*-Hydroxyurethane is carcinogenic and active against leukaemia *L*1210 whereas hydroxyurea is not carcinogenic[5] but active against *L*1210. Urethane ($C_2H_5O—\overset{\overset{O}{\|}}{C}—NH_2$) is carcinogenic but ineffective against advanced leukaemia *L*1210. These data suggest that the ethyoxy group (present in both urethane and *N*-hydroxyurethane) may be involved in carcinogenesis whereas the hydroxamic acid group is responsible for antitumour activity.

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PHYSIOLOGY

Standing Waves Inside the Heart

SINCE Wetterer and Pieper[1] introduced their miniature high-resolution electronic pressure transducers, it has been possible to measure accurately phasic pressure changes inside the ventricular and atrial cavities of the mammalian heart beating *in situ*.

We measured pressure changes in the right ventricle and the right atrium of the heart of dogs which had been anaesthetized with morphine sulphate (2·5 mg/kg body-weight), sodium diallylbarbituric acid (25 mg/kg body-weight), urethane (200 mg/kg body-weight) and sodium pentobarbital (10 mg/kg body-weight).

The electronic pressure transducer used in our study was constructed according to the technique devised by Wetterer and Pieper[1] and mounted on the tip of a plastic catheter; pressure changes were measured by means of three lateral holes in a stainless steel cap covering the pressure-sensitive membrane. This manometer, together with the amplifier and the recording instrument, had a resonant frequency far greater than 100 c/s.

The chest of a dog was opened under anaesthesia and, maintaining the animal in this condition, the miniature catheter tip manometer was placed inside the apical region of the right ventricle by way of the right jugular vein with the aid of a fluoroscope. The right ventricular pressure was continuously measured at 100 mm/sec recorder speed, while the manometer was drawn slowly back into the right atrium while under fluoroscopic observation. At the onset of diastolic relaxation a sudden fall in pressure occurred, which indicated a transient pressure oscillation, probably corresponding with diastolic suction of blood from the atrium into the ventricular cavity (Fig. 1). We found that the amplitude of this negative-pressure oscillation varied continuously along the apex to base axis, and that it was maximal at the apex and zero in the region of the atrio-ventricular valve (Fig. 1). Moreover, a damped pressure oscillation over 1·5 wavelength can be observed at the apex of the right ventricle.

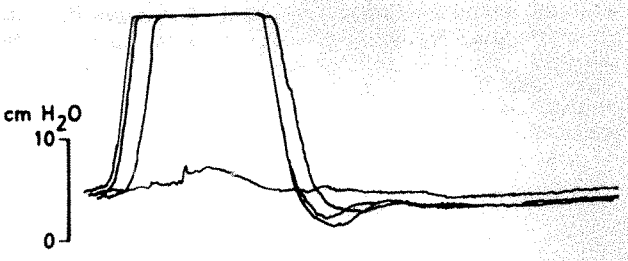


Fig. 1. Superimposed pressure curves recorded within the right heart of a dog, when a pressure transducer is continuously withdrawn from the ventricular apex into the atrium. The curves have been shifted slightly to the right in order to emphasize changes in amplitude of the early diastolic pressure dips.

These observations suggested that the heart chamber acts as a resonating cavity where standing waves occur, during diastole, between the apex and the fairly open central venous system. The wavelength associated with the ground frequency of these early diastolic oscillations would be four times the apex to base length, measured along the central axis of the heart (Fig. 2).

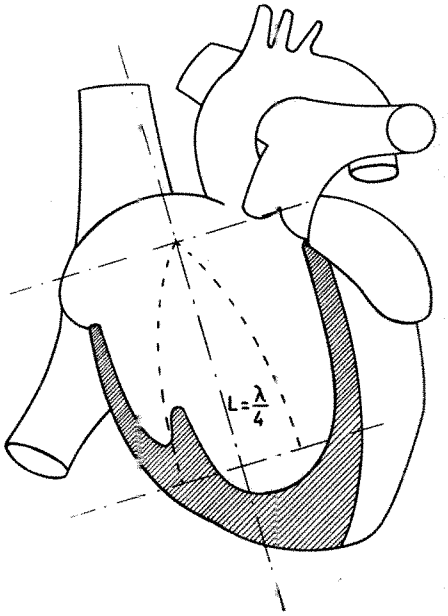


Fig. 2. Schematic representation of the right ventricle during diastolic time.

The relation between the apex to base length (L), the period of the oscillation (T) and the pressure wave velocity (v) is given by

$$\frac{4L}{T} = v$$

Such a relation provides a means for estimating the dimensions of the heart at diastole once the wave velocity is known.

If we consider the heart chamber to be an elastic cylindrical reservoir of known volume with a modulus of elasticity K, we can calculate the wave velocity from a variant of the Moens–Korteweg equation[2,3]

$$v = \left(\frac{K}{\rho}\right)^{\frac{1}{2}} \quad c = 0{\cdot}974 \ (K)^{\frac{1}{2}}$$

where $K = V\frac{dP}{dV}$, P, the pressure inside the ventricle (dynes cm^{-2}), V is the ventricular volume (cm^3), and ρ is the density of blood (1·055 g cm^{-3}).

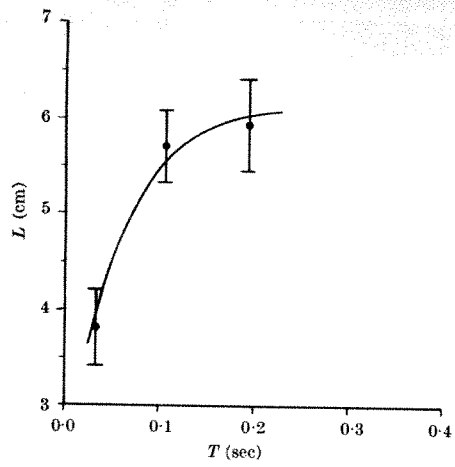


Fig. 3. Apex to base length of the right ventricle in function of early diastolic time. Averages are calculated over ten consecutive heart beats.

Because precise data for the volume elasticity of the right ventricle of dogs were not available, we calculated K from data given by Gauer[4] and Sandler[5] for man. This gives a value for the wave velocity of about 128 cm sec^{-1}. The average axial length L calculated from each of the three half-periods, which can be observed at the apex, is represented as a function of time in Fig. 3. The rapidly converging curve provides further evidence for the suggestion that diastolic filling is nearly complete at early diastole.

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Dependence of Velocity of Structural Breakdown of Injured Cross-striated Muscle Fibres on Time of Denervation

THIS communication reports some results of investigations of the structural breakdown after denervation of muscle. The experiments indicate the extent to which intracellular factors play a part in the process of structural breakdown of injured muscle fibres.

Research into the process of structural breakdown in injured cells is of interest in the study of muscular pathology and for an explanation of elementary mechanisms of muscle contraction. Previous investigations dealt mainly with the influence of extracellular factors on structural breakdown. According to Rothschuh[1], and our earlier findings[2], the process of structural breakdown is based on at least two processes: (1) a contraction which may vary in its intensity; (2) a decay of organized structures. It is probable that proteolytic enzymes play some part in the second process.

It has been shown that the activity of myosin-ATPase decreases after denervation. According to Fischer[3], after denervation the activity of myosin-ATPase decreases only a little at first, but after a fortnight the decrease becomes more marked, probably because of weaker binding of ATP to myosin. At the same time, a change in the molecular shape of proteins seems to occur, as a result of which myosin and actin combine to form a stable complex[4]. The diminished ability to contract may be a result of the change in activity of ATPase and the molecular changes of the muscle proteins. Further investigations showed an increase in proteolytic activity[5,6].

The question remained of whether these changes were also reflected in the processes of structural breakdown. In our experiments we used male white rats weighing, on average, 200 g. Denervation of the right caudofemoralis muscle was accomplished by splitting the sciatic nerve near the vertebral column and excising a 1·5 cm piece of the nerve with all its branches. The corresponding muscle of the other leg served as a control. Preparation of the muscle fibres was carried out 10, 21 and 42 days after denervation. The distance from the nerve of the muscle fibres which decayed in unit time was used as a measure of the velocity of structural breakdown: measurements were made by means of microphotographs. Bundles of muscle fibres with four to five fibres were used, and these were obtained by carefully picking the muscle to pieces.

Fig. 1 shows the change in the velocity of structural breakdown as compared with that in the control muscle. Ten days after denervation there are no important changes in the velocity of structural breakdown. The velocity of breakdown in the denervated muscle amounts to $17{\cdot}44 \pm 1{\cdot}44\mu$/min. In the control the velocity is $15{\cdot}50 \pm 1{\cdot}43\mu$/min. The differences between the denervated muscle and the control are within the range of variation and are not significant ($P > 0{\cdot}25$, $t = 0{\cdot}95$).

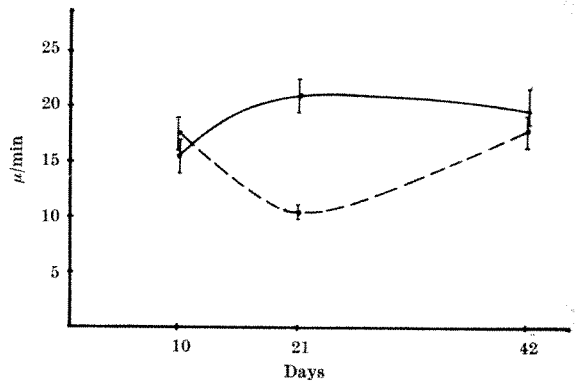


Fig. 1. Changes in velocity of structural breakdown dependent on denervation time compared with the control muscle. — — — — —, Denervated muscle; ———, control muscle.

By comparison with the control, a decrease in the velocity of structural breakdown becomes obvious after 21 days. The velocity of structural breakdown of the denervated muscle is $10{\cdot}55 \pm 0{\cdot}59\mu$/min whereas for the control it is $20{\cdot}87 \pm 1{\cdot}64\mu$/min. These results were obtained with many animals at different times. The difference between the velocity of structural breakdown of denervated muscle and control muscle can be considered significant ($P < 0{\cdot}001$, $t = 7{\cdot}15$). The different velocity of structural breakdown of control muscles after 10 and 21 days of denervation is not statistically significant. Further investigations are required of the possible velocity change in structural breakdown of control muscle. The measurement of velocity of structural breakdown 42 days after denervation is difficult owing to considerable atrophy of the muscle. This may injure the fibres and thus influence the measurements obtained. The result of this investigation does not show a significant difference ($P > 0{\cdot}25$, $t = 0{\cdot}83$) between the velocity of structural breakdown in the denervated muscle ($17{\cdot}82 \pm 0{\cdot}65\mu$/min) and the control ($19{\cdot}83 \pm 1{\cdot}76\mu$/min). The results of previous investigations on structural breakdown, and those of our own, considered in the context of the aforementioned hypothesis of the elementary processes of structural breakdown, lend support to the idea of successive contraction and structural breakdown.

Twenty-one days after denervation, the ability to contract is decreased. If it is accepted that contraction and proteolysis are separate and successive processes in structural breakdown, it is clear that, with a decreased ATPase activity and a diminished contractability, proteolysis will not be effective in breaking down the structure.

Fig. 1 shows an increase in the velocity of structural breakdown 42 days after denervation. Further investigations must be carried out to determine that this is not an experimental artefact and to find whether this increase is caused by secondary factors acting during this period or by primary factors.

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In vitro Maintenance of Rabbit Sperm at Room Temperature

MAMMALIAN germ cells have been utilized by several investigators for the investigation of the biochemical and physiological mechanisms of senescence in living cells[1]. Functionally and metabolically active bovine sperm have been successfully maintained and studied at room temperature for a period of 6 days or more[2]. The results of these studies led to the present work in which two chemically defined media were used to maintain large numbers of vigorously motile rabbit sperm. The two media tested here were 'Coconut Milk Extender' (CME)[3] and Norman-Johnson solution No. 2 (N-J-2)[4], a chemically defined medium. Over a 6 day period of storage at room temperature (24° ± 2° C), N-J-2 proved to be the more satisfactory medium for supporting survival and activity of the sperm.

The acrosome of the mammalian sperm is a relatively unstable structure which may become detached from the sperm. Bedford[5] reported that, as a result of *in vivo* ageing in the female reproductive tract, there was an increase in the loss of the acrosome cap from rabbit sperm. In our investigation it was found that *in vitro* ageing alone at room temperature in the two media did not increase acrosome cap loss from rabbit sperm.

The procedures used in this work were as follows: ejaculated sperm were obtained with an artificial vagina from ten hybrid male rabbits; the semen was pooled and the sperm were washed free of seminal plasma by gentle centrifugation (700*g* for 15 min) within 30 min of collection. The washed sperm were divided into two parts; one part was diluted with N-J-2 and the other part with sodium citrate buffered medium containing 15 per cent coconut milk, to give a final concentration of 10–12 × 10^6 sperm per c.c. Plastic vials (2 c.c. capacity) were filled completely with the suspended cells and tightly capped to maintain a relatively anaerobic state during a 6 day period of storage. The vials were kept in the dark[3] at ambient room temperature (22°–26° C). Fresh vials of extended sperm were removed every day for 6 consecutive days, thoroughly mixed and then examined and rated according to the following: (1) sperm motility, which was qualitatively rated by means of an arbitrary scale described by Norman *et al.*[6]; (2) concentration of the motile cells and concentration of the stored sperm—these were determined with the use of a haemocytometer; (3) loss of acrosome cap. To determine the number of cells which had lost their caps, samples of sperm were prepared as smears, fixed in formal-saline and stained in buffered Giemsa stain[7].

Table 1 summarizes the results of five replicates in the two different dilution media. The number of live cells appeared to be greater in CME after 6 days of storage than in N-J-2; however, a greater number of cells were found to be motile in N-J-2. The quality of motility of the cells dropped drastically after the second day of storage in CME but not in N-J-2. This may be the result of the presence of chloride ion in N-J-2, which may serve as a metabolic inhibitor, enabling the cells to conserve some of their energy for a later activity. CME contains calcium nitrate and it is known that nitrate enhances rabbit sperm metabolism[8]. It seems evident that in CME most of the energy source was used up during the first few days after which motility and the number of live cells decreased quickly.

Table 1. EFFECT OF NORMAN–JOHNSON SOLUTION NO. 2 AND 'COCONUT MILK EXTENDER' ON MOTILITY, SURVIVAL AND LOSS OF ACROSOME CAP OF RABBIT SPERM KEPT AT ROOM TEMPERATURE FOR 6 DAYS

Diluent	Sperm age in days	Percentage of sperm alive	Percentage of sperm motile	Quality of motility*	Percentage of sperm which lost acrosome cap
CME	0	91	92	5·0	10
	3	82	77	2·1	11
	6	72	64	1·3	13
N-J-2	0	89	91	4·9	9
	3	83	83	3·1	10
	6	69	72	1·8	12

* 0 (non-motile), 1 (vibratory), 2 (poor progressive), 3 (fair progressive) 4 (good progressive), 5 (excellent progressive).

After 6 days of storage some of the cells from both media were centrifuged and resuspended in fresh CME containing calcium nitrate and 5 per cent egg yolk. It was found that the motility of cells stored in CME could not be restored to better than "fair progressive". However, the motility of sperm stored in N-J-2 and later resuspended was restored to better than "good progressive". It was also found that a much higher percentage of the cells stored in N-J-2 responded favourably to resuspension than cells stored in CME. It was concluded that the prolonged maintenance of an economically usable number of functionally active rabbit sperm, at room temperature in N-J-2, can be of advantage in artificial insemination. N-J-2 was found to be a better diluent for storing rabbit sperm at room temperature because a greater number of cells remained motile over the storage period; and because a considerably larger percentage of the cells could be reactivated by resuspension.

In vitro ageing increased acrosome cap loss by only 3 per cent in both diluents; but this increase was not found to be significant ($P > 0.05$). These observations seem to contradict those of Bedford. However, it is possible that the increase in acrosome cap loss that Bedford observed did not result from ageing alone, but possibly from the combined effects of ageing and the physiological environment of the female reproductive tract.

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Vascular Migration of Transplanted Trophoblast in the Golden Hamster

THIS communication presents some preliminary results of an investigation of the invasive properties of hamster trophoblast transplanted to extra-uterine sites. The hamster is of particular interest in this respect, for in the course of normal gestation the trophoblastic giant cells exhibit a remarkable migratory activity not described in any other species[1]. The tertiary trophoblastic giant cells are extremely migratory and deeply invade the maternal arterial system. They are found in the mesometrial arteries for as long as 3 weeks *post partum*.

Donor hamsters were killed 6·5 days *post coitum* and the ectoplacental trophoblast dissected from the embryonic pole of the conceptuses. The hamster differs from the mouse and rat in that the ectoplacental tissue is not in the form of a cone but sits like a cap on top of the embryonic sac. A few hamsters were killed 3·5 days *post coitum* and the blastocysts flushed from the uterine horns with saline. The testis was initially chosen as the site for transplantation because it has been shown in the mouse to provide a very favourable environment for the development of eggs and ectoplacental cones[2,3].

Two days after transplantation beneath the tunica albuginea of the testis the ectoplacental cap proliferates into a nodule of trophoblast tissue at the site of inoculation. This proliferation opens up the blood system in the testis and produces haemorrhagic channels between the seminiferous tubules at the periphery of the graft. The first migratory movements of the trophoblast cells occur down these channels, in the interstitial cell areas. From 2–4 days after transplantation trophoblast cells can be seen in various stages of migration away from the original site of inoculation. They take up residence within the vascular system of the testis and line the wall of the vessels with their cytoplasm flattened in close contact with the endothelial surface. The way in which penetration of the blood vessels occurs is not yet apparent. It is possible that there is only one site of breakdown and all the trophoblast cells migrate in at this point. Trophoblast is potentially capable of invading tissues both by phagocytosis[4] and cytolysis[2,3], and it seems likely that one or both of these mechanisms is effecting this penetration.

During the early stages following transplantation there is some degeneration of surrounding seminiferous tubules, but not to the extent reported during trophoblast invasion of the testis in the mouse[3]. The presence of the developing hamster trophoblast induces numerous multinucleate spermatids in the tubules surrounding the graft, a phenomenon also seen in the mouse[2] and the guinea-pig[5]. Six days after transplantation almost all the trophoblast cells have left the original site of inoculation, which is marked only by a small patch of degenerate seminiferous tubules. The trophoblast cells are seen forming an almost continuous lining to the vessels nearby (Fig. 1), and a few are present in vessels further away. By the twelfth day (the longest period yet studied) the cells have become distributed throughout much of the vascular system of the testis. They still retain their characteristic morphology and staining properties. Whether they can survive longer, and migrate outside the testis itself, has yet to be discovered.

The migratory activity of the trophoblast is not limited to cells derived from the ectoplacental cap because transplantation of the hamster blastocyst to the testis produces trophoblast cells which follow a similar invasive pattern. This appears to be the first report of successful extra-uterine development of the hamster egg.

To test whether this ectopic migratory behaviour of hamster trophoblast is limited to the environment of the hamster testis, ectoplacental caps from the hamster were transplanted to the testes of adult hybrid mice. Autopsy after 6 days revealed hamster trophoblast cells in the vascular system of the mouse testis. The reciprocal experiment of transplanting mouse trophoblast to the hamster testis showed the development of a nodule of normal trophoblast tissue, but no migratory activity. It would seem that migration is an independent property of hamster trophoblast and is largely uninfluenced by the environment. It is also of interest that in these trans-specific transplantations there is no apparent immunological reaction against the trophoblast at the stages examined.

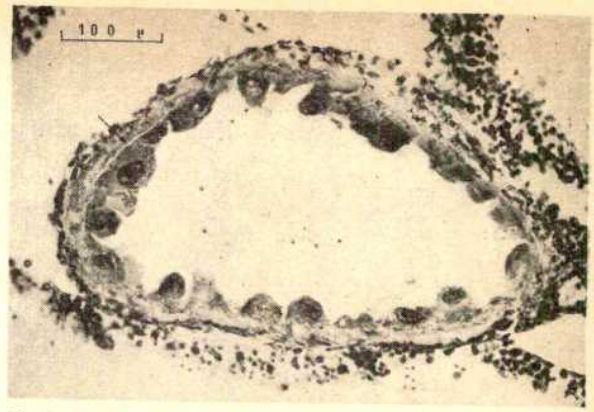


Fig. 1. Trophoblast cells lining the endothelial wall of an artery in the hamster testis. The endothelium is intact, but shown in clear focus only in the section arrowed. Seminiferous tubules and interstitial cells surround the artery.

The only other extra-uterine site to which hamster trophoblast has yet been transplanted is the kidney. The ectoplacental cap, placed beneath the kidney capsule, proliferates to produce a nodule of trophoblast tissue which also undergoes some migratory movement. Eight days after transplantation a number of trophoblast cells can be seen attached to the endothelial wall of blood vessels in the boundary zone of the kidney. However, a large number of cells still remain under the kidney capsule after this period. It may be that the more compact nature of kidney tissue limits the migratory movements of the trophoblast.

In the testes examined so far, the trophoblast cells appear to have migrated into the arterial network. This is based on observation of the characteristic histological appearance of arteries. However, in some instances, particularly the small capillaries, it is more difficult to identify the arterial nature of the vessels. If the migration is solely arterial this phenomenon is directly comparable with the situation in normal hamster pregnancy, where the tertiary trophoblastic giant cells show selective invasion of the arteries and are never found in veins. Orsini has found that these giant cells bring about pronounced changes in the arteries, which become dilated and thin-walled and lose their characteristic structural appearance[1]. These changes are probably brought about by some secretion of the giant cells. An exhaustive examination of serial sections of the testes is necessary before it is possible to say whether such an effect is produced in the testicular arteries.

The hamster trophoblast cells must possess some tropism, possibly towards oxygen tension, in order to invade preferentially the arterial system. Once inside the artery they have to migrate against the flow of blood, and this seems to be achieved by movement along the endothelial wall. The function of the trophoblast cells in this situation is entirely obscure. Their function during early gestation is primarily an invasive one to establish intimate connexion between mother and embryo, and it is difficult to understand the advantage of continuing an invasion into the maternal blood system. The only other known occasion when trophoblast tissue is found normally in the maternal blood system is in man. During the latter half of normal pregnancy, fragments of syncytial trophoblast break away from the placenta and are deported to the uterine veins[6]. Their function is unknown, but

it has been suggested that they may be of immunological importance[6]. They are probably normally broken down by some lytic mechanism in the circulating blood, although large numbers may reach the lungs. The phenomenon of trophoblast deportation to the lungs during normal pregnancy has also been reported for the chinchilla[7] (also personal observation).

These results show that extra-uterine transplantation, particularly to the testis, may be a profitable technique to investigate further the remarkable migratory behaviour of hamster trophoblast cells.

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Effect of Degraded Carrageenin on Gastric Secretion Stimulated by Histamine and 'Histalog'

THERE is some evidence that in the guinea-pig, the oral administration of degraded carrageenin, a sulphated polysaccharide, causes a slight temporary reduction of gastric acidity in response to histamine[1]. We have had occasion to study the effect on gastric secretion when degraded carrageenin is administered subcutaneously. The results indicate that carrageenin parenterally has a pronounced inhibitory action on the acid secretory response to histamine but, somewhat paradoxically, not on the secretory response to 'Histalog' (betazole hydrochloride), a structural isomer of histamine.

Gastric juices were obtained from fasted unanaesthetized adult male guinea-pigs by intubation 1 h after the intramuscular injection of an aqueous solution of histamine acid phosphate (1 mg/kg) or 'Histalog' (100 mg/kg). Degraded carrageenin (5 per cent aqueous solution) had been injected subcutaneously 9 h previously in a dose of 400 mg/kg body weight. The results are shown in Table 1.

Table 1. EFFECT ON STIMULATED GASTRIC SECRETION OF DEGRADED CARRAGEENIN ADMINISTERED SUBCUTANEOUSLY

(Means ±1 *S.D.*)

Treatment and No. of animals	Gastric secretion: Volume (ml.)		Gastric secretion: Total acid conc. (m.equiv/l.)	
Histamine only (10)	8·9 ± 2·2	$P < 0.01$	128·6 ± 8·2	$P < 0.001$
Carrageenin + histamine (10)	3·7 ± 3·3		106·1 ± 13·1	
'Histalog' only (8)	16·2 ± 2·5	$P > 0.10$	132·5 ± 10·3	$P > 0.60$
Carrageenin + 'Histalog' (10)	14·0 ± 3·5		134·6 ± 8·0	

Carrageenin markedly reduces the volume and total acid concentration of the gastric juice secreted in response to histamine. How it produces this effect is not known. It may be that it renders histamine inactive by forming a complex with histamine as in the case of heparin[2], which is also a sulphated polysaccharide. By the use of 'Histalog' it has, however, been possible to establish that carrageenin does not directly interfere with the acid secretory mechanism. These results are of interest in relation to the role of histamine in gastric secretion.

We thank Dr. G. B. Shirlaw of Laboratories Glaxo-Evans, Paris, for supplying degraded carrageenin ('Ebimar'), and Dr. E. A. G. Cook of Ely Lilly and Co., Basingstoke, for supplies of 'Histalog'.

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HAEMATOLOGY

Enhancement of the Activity of Optimum Concentrations of Urokinase by the Venom of *Echis carinata*

THE venom of *Echis carinata* (saw-scaled or carpet viper) is known to have powerful haemotoxic actions, promoting coagulation in whole blood or plasma and digesting fibrin[1]. We have recently investigated the action of this venom on the fibrinolytic enzyme system using the caseinolytic assay system of Remmert and Cohen[2] as modified by Alkjaersig[3]. Urokinase (2,000 U/ml.) was substituted for streptokinase, and 0·1 molar phosphate buffer for acid and alkali. Results are expressed in casein units/ml. Where *Echis* venom was used, 0·5 ml. of a 1 mg/ml. solution of a desiccator-dried preparation reconstituted in 0·9 per cent saline was incorporated in the system in place of 0·5 ml. of the phosphate buffer used in the assay. Similarly, buffer was substituted for urokinase and plasminogen in the experiments in which these reagents were omitted.

Table 1. CASEINOLYTIC ASSAYS

Urokinase	Venom	Plasminogen	Casein units/ml.
+	−	+	1·7 ± 0·3
+	+	+	5·1 ± 0·4
−	+	+	2·9 ± 0·2
−	−	+	0·5 ± 0·1
−	+	−	2·0 ± 0·1

The results are shown in Table 1, which presents the mean values and standard deviations for seven assays performed in duplicate. It will be seen that the venom of *Echis carinata* has direct caseinolytic activity (2·0 ± 0·1 units of activity); it also has the ability to produce partial activation of plasminogen, the increase in proteolytic activity of a venom-plasminogen mixture compared with venom alone being 0·4 ± 0·2 units ($t = 6.28$, $P < 0.001$). This compares with an activation of 1·7 ± 0·3 units produced by urokinase, due allowance being made in all cases for the spontaneous activity of plasminogen (0·5 ± 0·1 units). Of more importance, however, the results also show that the venom can augment the activity of an optimum concentration (2,000 U/ml.) of urokinase as a plasminogen activator, the activity produced by incubation together of urokinase, venom and plasminogen being 5·1 ± 0·4 U/ml., this being 1·4 ± 0·3 U more than that to be anticipated from the separate incubation of venom and plasminogen/urokinase with casein ($t = 11.96$, $P < 0.001$).

It has also been shown that the venom does not augment the activity of spontaneously activated plasmin and it would therefore appear that the augmentation of caseinolytic activity results from an action of the venom on plasminogen or on urokinase; but as incubation of venom and urokinase results in striking loss of urokinase activity it would seem probable that the venom acts directly on plasminogen. It may be that the action of the venom on the plasminogen molecule is to produce an alteration in its tertiary structure so that more arginine and lysine bonds are made available to attack by urokinase.

The inhibitor of plasminogen activation (ε-aminocaproic acid) has been added to the system but has no effect on the ability of the venom to augment plasminogen activation by urokinase. However, addition of a specific antivenom (Behringwerke A.G.) to the test system results in inhibition of proteolytic activity, plasminogen activation and also augmentation of the action of urokinase.

If the fraction of the venom which produces this unique effect can be isolated from the coagulant fraction it may have an important place in thrombolytic therapy as an adjuvant to urokinase and streptokinase, producing

augmentation of thrombolytic activity and thus accelerated vascular clearing.

Further investigation of the phenomenon may make available more detailed knowledge of the mechanism of plasminogen activation.

Plasminogen, streptokinase and ε-aminocaproic acid were provided by Dr. Hans Dahlstrom of A. B. Kabi, Stockholm, and urokinase by Dr. R. Herting of Abbott Laboratories, Illinois. The work was supported financially by the Ministry of Overseas Development, Ministry of Health, Kenya, and Merck, Sharp and Dohme Research Laboratories (Dr. M. Tishler).

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BIOLOGY

Caulerpicin, a Toxic Constituent of *Caulerpa*

IT has long been known that the marine green alga *Caulerpa* has a peppery taste. Yet varieties of this species are used as a salad delicacy in the Philippines and lack it. We have attempted to discover the nature of the flavour-giving substance, having in mind its recognition for chemotaxonomic purposes. One of us (G. A.-S.) was able to obtain from ether extracts adsorbed on and then eluted from alumina crystalline material which melts at 95° C. We have named this material "caulerpicin". Mass spectrometry [illegible] that caulerpicin has a molecular weight of 649 with a possible formula of $C_{43}H_{87}O_2N$ and properties unlike those of any other compound of which we are aware. The infra-red spectrum indicates that caulerpicin may be a long-chain saturated hydroxy amide. So far it has been found in several *Caulerpa* species or varieties in different concentrations; as much as 600 mg have been isolated from 1,500 g dry weight of one variety of *Caulerpa racemosa* (Forsskal) J. Agardh.

Different people respond differently to caulerpicin. Some merely obtain a mild anaesthetizing sensation which is not immediate but is delayed for a minute or two. Others also obtain a numbness of the tongue or lips. In one subject exposed to the substance at various times truly toxic symptoms have become stronger and stronger following each contact. Almost immediately on chewing the raw dried *Caulerpa* material, the subject felt a numbness at the tip of the tongue. This has developed to a point at which the reaction is one of numbness of the extremities coupled with a cold sensation in the feet and fingers, rapid and difficult breathing, slight depression and, finally, loss of balance requiring the subject to lie down. The symptoms wear off, depending on the dosage, in a few hours to a day. Coupled with these reactions to the impure and pure substance, the same subject has developed a sensitivity to oysters and crabs and eating them produces the same symptoms.

The toxic effects are somewhat similar to those reported[1] for ciguatera fish poisoning in the Tropical Pacific. According to Banner's investigations ciguatera poisoning is caused most frequently by eating the red snapper (*Lutjanus bohar* Forsskal) which in turn feeds on herbivorous acanthurids such as *Acanthurus triostegus* Linnaeus. Dawson *et al.*[2] reported that *Caulerpa serrulata* (Forsskal) J. Agardh was found in the alimentary tracts of reef fishes from Palmyra Island in the Central Pacific. One of these, *Arothron hispidus* Linnaeus, was found to be poisonous; another, *Acanthurus triostegus*, is often poisonous.

In so far as we have been able to determine from Banner and Helfrich[3] and from questioning, ciguatera is unknown in the Philippines, although it is widespread in the Tropical Pacific. However, it is possible that the toxic response to shellfish on the part of our subject in the Philippines was due to substances such as caulerpicin being passed to the human food organisms, oysters and crabs, through a detritus food chain rather than through the herbivore fish to carnivorous fish food chain. We do not believe the effects of caulerpicin which we observed to be identical to those of ciguatera, but there is little information on this point.

The fishes and algae mentioned are common and generally distributed throughout the Philippines. Forsskal's authorship of so many of their names is an indication of their abundance and presence at the Red Sea end of their general biogeographic distribution across the Pacific. Since *Caulerpa* is a genus very widely spread in the tropics and also occurs in cold water in South Australia, for example, it would seem that if it is related to fish poisoning this would already be known. That *Caulerpa* is not so conspicuous a genus in the Eastern Pacific and perhaps the Eastern Atlantic as it is in the western reaches of these same oceans, may account for the lack of literature on this subject. We would appreciate any information relating *Caulerpa* to toxicity in marine products or to food chains either as detritus or directly-grazed material.

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Flower Apices cultured *in vitro*

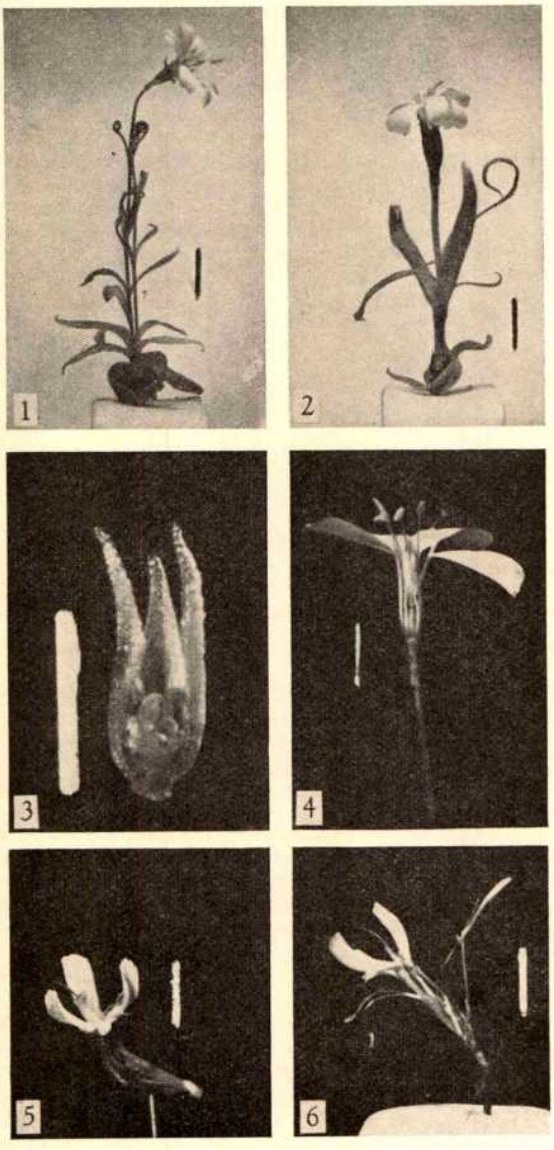
ALTHOUGH there are numerous reports dealing with the culture of plant tissues *in vitro*, there have been few successful attempts to culture isolated flower primordia. A suitable technique would, however, be valuable for many morphogenetic investigations, and therefore attempts have been made to culture buds of two species of *Viscaria*.

Dissection of flower apices was carried out by aseptic methods which yielded sterile cultures without difficulty. The excised apex was placed on a filter paper bridge[1] dipping into liquid nutrient medium in a 1 in. diameter 'Monax' tube covered with polypropylene film. The cultures were grown at 24° C under a 16 h daylength at an intensity of about 600 lumens per sq. ft. The nutrient medium was based on that of Murashige and Skoog[2]; the concentrations of the major elements and the ferrous sulphate–ethylenediamine tetraacetic acid (EDTA) complex were those recommended and, of the minor elements, one-tenth of those quoted. Other constituents (of which some were derived from Goodwin[3] and some from Vasil[4]) were as follows (quantities per litre); sucrose, 20 g; coconut milk, 100 ml.; casein hydrolysate, 1 g; inositol,

100 mg; cysteine, 5 mg; ribonucleic acid, 25 mg; kinetin, 1 mg; 8-OH-quinoline, 0·3 mg; EDTA, 37·3 mg; thiamine, 4 mg; nicotinic acid, pyridoxine, calcium pantothenate, riboflavine, biotin, choline, L-ascorbic acid, adenine and menadione, all at 1 mg; gibberellic acid and β-indolyl-3-acetic acid, both at 1 mg. The medium was adjusted to *p*H 5·7–5·8 and autoclaved.

Two long-day plants, *Viscaria candida* and *V. cardinalis*, have been used in this study. Both species flowered with from seven to ten pairs of leaves under continuous long day conditions and remained vegetative almost indefinitely under short day conditions. About 15 long days were required for full induction of flowering.

When vegetative apices about 1 mm in size were taken with one or two pairs of leaf primordia and grown under long days, they generally grew without rooting for a considerable time, and flowered after producing about the same number of leaves as a pot-grown plant. The flowers in such cultures were normal in all parts (Fig. 1).



Figs. 1–6. (1) Growth from a 1 mm vegetative apex after 10 weeks (scale equals 5 mm). (2) Growth after 5 weeks from a 500μ apex excised after minimal photoinduction (scale equals 5 mm). (3) State of flower bud when excised at 1–2 mm (scale equals 1 mm). (4) Normal flower (with calyx, two petals and one stamen removed) grown on plant under the same conditions as the cultured flower in Fig. 5 (scale equals 5 mm). (5) Flower cultured from a 2 mm bud, photographed 18 days after excision (scale equals 5 mm). (6) As Fig. 5, with calyx removed to show stamens and ovary (scale equals 5 mm).

Apices removed from plants which had received minimal photo-induction sometimes developed vegetatively and sometimes formed flowers when placed in culture. Fig. 2 shows a culture grown from an explant taken at 500μ in size with three leaf primordia. A further pair of leaves was laid down in culture followed by a full set of flower primordia, which developed normally in all respects.

If an apex were excised with sepal primordia only, or with petal and sepal primordia, the succeeding flower parts were all initiated and no treatment has so far disturbed the normal sequence. With such small apices, however, full development of the flower to maturity rarely occurred unless a pair of leaves was present. When buds were taken without leaves but with all parts just initiated (that is, at 500μ–2,000μ in size) normal development of the flower parts occurred (Figs. 5 and 6). In buds of less than 2 mm the calyx was well developed, the petals very small and the ovary at an early stage of initiation in the centre of the bud (Fig. 3). The stamens were the most advanced, but archesporial tissue was not differentiated in the anthers. In the complex medium used in this study normal growth of calyx, ovary and ovules was usually obtained. Petals were pigmented and fairly normal in shape, but sometimes they tended to be rather strap-shaped and sometimes lacked a corona. Stamen development was variable; in many cases normal development occurred and up to 50 per cent of the cultures produced one or more (sometimes ten) stamens with pollen which was sculptured and stained with acetic-orcein. Larger buds taken at 4–5 mm and containing pre-meiotic archesporial tissue tended to produce pollen more easily. The stamen filaments sometimes elongated normally but were often much reduced in length, though this also tends to occur in normal flowers grown at 24° C (Fig. 4).

This work has shown that cultures grown with leaves generally produced normal flowers, as Baldev[5], Chailakhian[6] and Raghavan and Jacobs[7] have also observed, the latter obtaining some viable seed. In previous work, culture of flower buds without leaves has proved more difficult. Tepfer[8] did not obtain development unless the calyx was removed; he did not achieve normal stamen development[9] and was unable to take a flower bud from the early stages of initiation through to maturity and flower opening. Galun[10] obtained ovary development in the cucumber, but he did not obtain[11] satisfactory stamen development. The complex medium used in this investigation has enabled flower buds to be grown without leaves from the early stages of initiation to maturity. Meiosis has been shown to occur in the stamens, and normal pollen and ovules have frequently been produced. Work is proceeding to reduce the complexity of the nutrient medium and define the conditions under which a fully normal flower is developed.

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A Culture Method of growing *Entamoeba histolytica* and Other Anaerobic Amoebae for the Study of their Nuclear Division

THE mode of nuclear division in *Entamoeba histolytica* and other anaerobic amoebae is not clearly understood. There are two main reasons for this: first, difficulty is encountered in obtaining the various normal stages of nuclear division; second, unsuitable cytological techniques are used to distinguish between chromatic and non-chromatic substances. Dobell[1] developed a culture method of obtaining large numbers of *E. histolytica* trophozoites in an actively growing condition. He grew amoebae by the Boeck–Drbohlav technique in a tube into which a coverglass was placed. The amoebae crept over the coverglass, where they continued their development, fed on the accompanying bacteria and rice starch and multiplied. Because they adhered to the glass, they could be readily obtained from the culture by removing the coverglass, and then fixed and stained. It should be emphasized that by this method large numbers of bacteria and rice starch particles also remain attached to the coverglass. This makes any study of nuclear structure and nuclear division difficult.

Singh[2,3] developed a method for the culture of small free-living amoebae; this enabled him to obtain without any difficulty all stages of nuclear division in nine species of amoebae. The amoebae were grown on thin films of non-nutrient agar on slides. They were then fixed according to normal cytological methods and the slides brought to water. At this stage the film of agar was removed by giving the slide a gentle shake in water; this left the majority of the amoebae attached to the slide.

The agar film culture method of Singh[2] has been modified to provide large numbers of trophozoites of *E. histolytica*, *E. invadens* and *E. moshkovskii* in a normal and actively growing condition. Thin films of non-nutrient agar (0·8–1·0 per cent agar depending on the quality of agar; 0·85 per cent sodium chloride) were made on coverslips. One or two small drops of hot melted agar were placed on 22 × 22 mm number '0' coverslips and quickly covered by round coverslips (16–18 mm diameter). When the agar had solidified, the top coverslip was gently pushed from one side and removed, leaving the film of agar on the lower coverslip. The films were kept in moist Petri dishes until required.

Twenty-four to forty-eight hour old amoebae growing in modified Boeck and Drbohlav medium (M/40 phosphate buffer in 0·85 per cent sodium chloride was used to dilute inactivated horse serum 1/7; *p*H 7·2) with mixed bacterial flora and rice starch were pooled and inoculated in large numbers in hollow ground slides (ground glass slides with a circular cavity 16 mm in diameter and 4 mm deep). The cavities were filled with diluted horse serum and a small quantity of rice starch (Difco) was added. They were then sealed with coverslips containing agar films, keeping the film downwards. Care was taken to avoid air bubbles. Any extra fluid around the coverslip was removed by filter paper. The cavity slides were then inverted and incubated at 37° C. There was profuse growth of amoebae on the agar films. After various intervals of time, ranging from 16 h to 24 h, the slides were inverted and the coverslips with the films gently removed with a pair of forceps. The amoebae were quickly fixed in Carnoy's solution (absolute alcohol six parts, chloroform 3 parts and glacial acetic acid one part) for 30–45 min. They were then placed in 90 per cent alcohol for 24 h and gradually brought to water. At this stage the films were gently detached around the periphery with a fine needle and removed by gently shaking the coverslips in water. The majority of the amoebae remained attached to the coverslips, and were stained with iron alum haematoxylin. It seems that amoebae sink through the agar, as observed by Singh[2] in the case of free-living amoebae, and remain adhered to the glass after the film has been removed.

It is hoped that by this method it will be possible to find normal, young and actively dividing amoebae. As the amoebae are confined to a small area, it should be easy to locate dividing individuals under an oil-immersion lens. The amoebae can be stained by suitable techniques so as to show up the chromatanic and non-chromatanic substances at various stages in the nuclear division.

Shaffer[4] has recently shown that the trophozoites of *E. histolytica* in Shaffer–Frye or CLG medium[5] possess two or more nuclei. From 2 to 25 per cent of the amoebae were binucleate between 8 h and 16 h and continued for 120 h of incubation. No binucleate amoebae were present at 4 h or 8 h of incubation. A few trophozoites which contained from three to five nuclei were found without any apparent relation to the growth cycle of the culture. Based on these observations, Shaffer[4] has suggested that nuclear division and cell division may be separate phenomena that do not necessarily proceed in parallel.

We have found that on a single coverslip preparation made from 16–24 h old cultures of *E. histolytica* and *E. invadens* there were plenty of trophozoites with two or three nuclei. Amoebae with four or five nuclei were less frequent. Amoebae containing six, seven and eight nuclei have also been observed; multinucleate amoebae also occurred with *E. moshkovskii*. The nuclei and cell cytoplasm in multinucleate *E. histolytica*, *E. invadens* and *E. moshkovskii* appeared to be quite normal. Our observations support Shaffer's[4] suggestion that nuclear and cell division are probably two separate phenomena.

We thank Dr. B. N. Singh, head of the Division of Microbiology, for suggesting the problem and for helpful advice.

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Effect of Spinal Lesions on the Colour Change of the Minnow (*Phoxinus phoxinus* L.)

THE dorsal and lateral surfaces of the minnow (*Phoxinus phoxinus* L.) change colour on pale and dark backgrounds. This colour change is effected by changes in the condition of the melanophores which are found on these surfaces of the minnow. Large macromelanophores and small micromelanophores can be seen under the binocular microscope, and the differential distribution of these two types gives rise to a prominent pattern which is to be observed particularly during the paling and darkening of the fish. Black-adapted minnows taken from a black background and placed on a white one pale rapidly during the first few minutes under nervous control, then continue to pale at a slower rate under hormonal control[1]—maximum pallor is reached after some hours. The macromelanophore pattern pales more slowly than the micromelanophores and it is still visible after the fish has been paling for one minute but has more or less disappeared after 3 min. On a black background the fish darkens rapidly during the first few minutes and maximum darkness is attained after some hours.

A melanophore aggregating centre exists in the medulla oblongata of the minnow from which autonomic pigmentomotor fibres pass caudally to their spinal outflow levels

and then pass cephalically and caudally along the sympathetic chain and innervate the melanophores by means of the spinal and trigeminal nerves[2]. The spinal pigmento-motor fibres are located dorsomedially within the tissues which include the substantia gelatinosa Rolandi and the dorsal part of the corpus commune posterius[3,4].

Kappers *et al.*[5] claimed that knowledge of the sympathetic nervous system of fish was very meagre especially in regard to finer details. This still holds to-day for the spinal autonomic tracts. The experiments described here are concerned with the effect on the colour responses of cutting a proportion of the spinal pigmento-motor fibres.

Lesions were placed ventrally, dorsally and laterally by means of micro-dissection instruments at vertebrae 3–4 and 10–11 and at the spinal outflow levels between vertebrae 12 and 14 (refs. 3 and 4) in different fish. The colour responses to black and white backgrounds were tested after the fish had recovered from anaesthesia and compared with their pre-operational colour responses. The extent of each lesion was determined histologically from serial sections stained with Mallory's trichrome.

The effect on the colour responses of lesions placed at vertebrae 3–4 and at vertebrae 10–11 depended on whether the dorsomedial spinal tissues at these levels were damaged. Total destruction of these tissues eliminated the rapid, neurally controlled colour responses of the fish to different backgrounds whereas the colour responses were unaffected if the dorsomedial tissues were intact. Partial damage to the dorsomedial tissues at these levels resulted in a prominent dark mottled pattern which was distributed over the dorsal and lateral surfaces of the fish and was observed when the fish were allowed to pale on a white background. This pattern appeared to arise from a differential behaviour of the melanophores and it persisted when the fish was left on a white background for 3 min.

This effect was observed in fifteen fish in which spinal lesions had been placed at vertebrae 3–4 or 10–11 and in all cases there was appreciable damage to the dorsomedial tissues, but there was no correlation between the development of this pattern and the aspect of the lesion. In particular, dextral and sinistral hemisections did not result in a unilateral response but produced a similar bilaterally distributed mottled pattern.

A similar mottled pattern was observed in eighteen minnows in which spinal lesions were placed between vertebrae 12 and 14. At these levels there appears to be no correlation between the extent of the lesion and its effect. The results seem to indicate that at these levels the spinal pigmento-motor fibres run ventrally from the dorsomedial spinal tissues and are widely dispersed throughout the cross-sectional area of the spinal cord. Furthermore, lesions placed in the posterior part of the outflow region, that is at vertebrae 13 and 14, did not affect the colour response of only the caudal region of the fish but, instead, a mottled pattern completely covering the dorsal and lateral surfaces was observed, thus indicating that pigmento-motor fibres from these spinal levels run both cephalically and caudally along the sympathetic chain. The macromelanophores of the lateral stripes and of the dorsolateral pattern appear to be more readily affected by damage to the pigmento-motor fibres than the micromelanophores, but, in spite of this, the post-operational mottled pattern has a somewhat different appearance from the pre-operational macromelanophore pattern which is observed during paling and the precise nature of the post-operational mottled pattern is worthy of further investigation.

The mottled pattern in some of the specimens was examined under the binocular microscope by means of a method adapted from that described by Hogben and Slome[6] and Healey[1]. Some of the melanophores showed asymmetrical dispersion and it is suggested that this observation supports the conclusion of Gray[7,8] that individual melanophores are innervated by more than one neurone. Thus the asymmetrical response would indicate that some of the melanophore innervating fibres are associated with spinal pigmento-motor fibres which have been cut while other fibres innervating the same melanophore were associated with intact spinal pigmento-motor fibres.

It is suggested, on the basis of the observations described here, that cutting a proportion of the spinal pigmento-motor fibres results in a differential behaviour of the melanophores in response to background reversals from a black to a white background. It would appear that the fibres of any specific region of the spinal pigmento-motor tract are associated with melanophore innervating fibres having a wide cutaneous distribution. It is also tentatively suggested that postganglionic melanophore innervating fibres retain their individual integrity rather than form a continuous diffuse cutaneous nerve net. This latter suggestion requires substantiation by a closer study of the precise nature of the post-operational melanophore pattern and further study of melanophores as effectors.

This investigation was conducted under the supervision of Dr. E. G. Healey during the tenure of a Department of Scientific and Industrial Research studentship.

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Relation of Temperature to the Duration of the Development of Insects

THE relationship between temperature and the mean hatching time of eggs is of the same pattern for most insect species. The mean hatching time decreases as the temperature increases until the so-called peak temperature is reached; this is the temperature at which the mean hatching time attains its minimal value. At temperatures above the peak, the observed mean hatching time increases with increasing temperature.

The majority of the attempts that have been made to give a mathematical description of this relationship represent the hatching time as undergoing a steady decrease with increasing temperature; the data at temperatures above the peak are ignored. A noteworthy and invariable feature of the experiments is the increase in the proportion of eggs which fail to hatch at temperatures above the peak. It is my conjecture that the temperature–hatching time relationship estimated from experimental data is distorted by a bias introduced by the high mortality at high temperatures. This bias may be so large as to explain the increase in hatching time at temperatures above the peak.

Although the cause of death at high temperatures is unknown, it probably results, not from the direct action of heat, but from an upset in the balance of some metabolic process[1]. As the temperature rises, the metabolism of the egg increases; the extra energy which results is expended on accelerating the period of development. At some point the metabolic processes break down and the egg fails to develop. It is probably this factor—the breakdown of metabolism—that is the direct cause of death. The effect of temperature on survival is merely indirect by making the rate of metabolism fall within the limits of tolerance.

It is plausible to infer from this description of growth and mortality that eggs developing at the same metabolic rate have a common hatching time and a common survival rate; the hatching time and survival rate both decrease with increasing metabolism. Because metabolism increases with increasing temperature, the model outlined here is apparently at variance with the increase in mean hatching time at high temperatures observed in experimental work. The explanation for this apparent disagreement can be found in the wide variability in hatching times of individual eggs kept at the same constant temperature. A typical instance of this variability is provided by Stanley's data[2] on hatching times of the eggs of *Tribolium confusum* maintained at 28° C; the smoothed frequency distribution is shown in Fig. 1; it has a mean of 143 h and a range extending from 129 h to 226 h. Stanley obtained data which had distributions of the same shape and order of variability, but with progressively smaller means (at 29° C, 31° C and 33° C). The high mortality at 35° C left too few successful hatchings on which to base a smoothed distribution. The effect of increases in temperature is to shift the distribution of hatching times progressively towards the left until the point at which mortality begins to operate. At this point, the individuals on the left of the distribution (those with the more rapid development and shorter hatching time) suffer a higher rate of mortality than those with longer hatching times. Consequently the mean hatching time of the survivors is greater than that which would have been obtained if there had been no mortality; thus the relationship between the observed mean hatching time and temperature does not follow the same pattern as that between the inherent hatching time of an individual egg and temperature. In the former case the observed mean is greater than the theoretical one. As the mortality progressively increases beyond the peak value and only the individuals in the long tail of the skew distribution hatch successfully, the divergence between the observed mean and the theoretical value becomes larger.

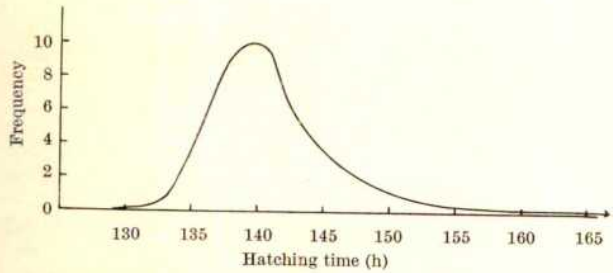


Fig. 1. Smoothed distribution of hatching times of *Tribolium confusum* eggs kept at 28° C. Data from Stanley (ref. 2).

Even if the inherent hatching times of individual eggs continue to decrease with increasing temperature up to the point of death, provided that the degree of skewness of the distribution is sufficiently pronounced, the observed mean hatching time may well show an increase at high temperatures.

As yet no assessment can be made of the validity of this conjectural description of egg development; because of the heavy mortality at high temperatures, the data available are insufficient to provide a basis for criticism. A clear picture can only be obtained from a much larger number of data.

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Chromosomes of the Coypu

THE coypu, *Myocastor coypus* Molina (or *Myopotamus coypus*, *Myopotamus bonariensis* Commers, *Hydromys coypus* Geoff.), is a South American rodent that belongs to the family Octodentidae and sub-family Capromyidae. To-day it is found in all continents, living in swamps and fish ponds as a feral animal or raised in captivity for its fur[1]. So far as we know, no previous investigation of chromosome number and morphology has been carried out on this animal.

This communication reports the observations we made on number and morphology of the chromosomes in the coypu using the bone marrow tissue culture method. Preparations of cultures from both sexes were analysed. The diploid chromosome number of the coypu, belonging to the standard brown type, was found to be forty-two ($n = 21$).

Several methods for preparing cells were tested including the aceto orcein treatment of testes[3], the cornea method[4,5] and the phytohaemagglutinin blood culture[6]. For unknown reasons the last method failed to give any mitoses. A modified form of the bone marrow culture method[6] was found to give the best results. Three animals, one male and two females, belonging to the farm-bred standard brown type were used. Each animal was injected intraperitoneally with 0·2 ml. of 0·1 per cent 'Colcemid' solution. After 3 h the animal was killed by an overdosage of 'Nembutal' (10 ml.) and the femurs were immediately dissected out. The epiphyses were cut off and the marrow was washed out with warm (37° C)

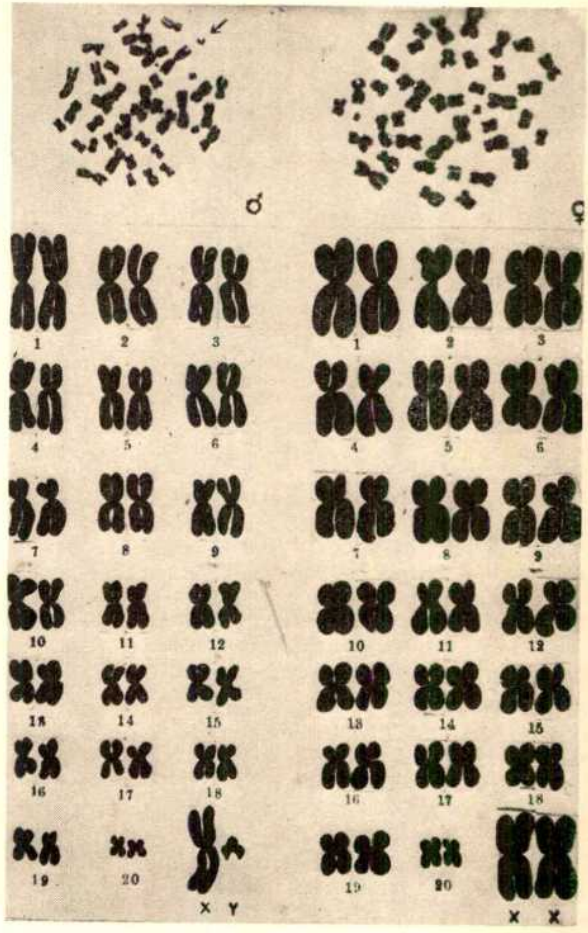


Fig. 1. Coypu karyograms. Male karyogram on the left and female on the right. In the upper left corner the Y chromosome is marked by an arrow. The original photos are magnified × 378, and the chromosomes have been arranged according to their size and the position of the centromere.

1 per cent sodium citrate solution. After 30 min the cells were centrifuged at 700 r.p.m. for 10 min, the supernatant removed and 3 ml. of fixative (three parts absolute alcohol to one part glacial acetic acid) was carefully added, so that the cells were suspended in it. After 10 min the material was centrifuged at 400 r.p.m. for 5 min, the supernatant was removed except for one drop in which the cells were re-suspended, and freshly prepared fixative was added. Slides were immersed in 20 per cent ethanol and after 2–3 drops of the suspension had been pipetted on to each one, they were heated over a flame for a few seconds. The cells were stained with 'Giemsa' solution.

By this method we were able to obtain two hundred cells in mitotic metaphase suitable for analysis. From the counts it was found that the number of chromosomes in the coypu is forty-two ($n = 21$). Very few deviations from this number were observed, and these were most probably the result of technical errors. A few polyploid cells were also observed.

In typical cells (Fig. 1), out of 21 pairs there is one very large (No. 1) and one very small (No. 20). The rest of the chromosomes are a continuous range of sizes between these two extremes. The two X's are second to largest and the Y is the smallest but one. The only telocentric chromosome is the Y chromosome. The chromosomes of pairs Nos. 1, 2, 10, 13, 14, 17 and 18 can be considered metacentric and the remainder sub-metacentric. No chromosomes with satellites were observed.

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RADIOBIOLOGY

Correlation of Lead-210 with Strontium-90 in Human Bones

THE radioactive gas radon-222, produced by the decay of radium-226, diffuses from the ground and is transported by atmospheric turbulence far from its origin through the troposphere and into the stratosphere[1]. The radioactive decay of radon-222 and its short-lived descendants produces measurable quantities of lead-210 in the atmosphere[1–3]. Lead atoms so formed become attached to dust particles and eventually return to the Earth's surface, principally in rainfall[1,4,5]. Hill's[4] report of a correlation coefficient of 0·87 between the quantity of rainfall and the concentration of lead-210 in grass clearly indicates a linear relationship. Holtzman[6] reported a relationship between the concentration of lead-210 in the human skeleton and in the atmosphere. Whether the lead-210 enters the body principally through precipitation on foodstuffs that are later eaten[5] or through direct inhalation, or by both means[7], is at present uncertain. It is therefore feasible to view the occurrence of lead-210 as a "natural fall-out" product, similar, in this respect, to fission product fall-out, artificially produced from a nuclear detonation. In fact, very strong similarities between lead-210 and fission product activities have been reported with respect to their behaviour in the atmosphere[1], to their rate and mode of deposition[1,5], and to their concentration in grasses[4]. The similarities between lead-210 and fission product activities do not imply any relationship in their production. The formation of lead-210 in nuclear weapons explosions is improbable, and if any were so formed it would be negligible in comparison with the amount formed by natural processes[8]. The observed similarities between lead-210 and fall-out are caused by their similar behaviour in the atmosphere.

When lead or strontium is ingested, it is deposited predominantly in the skeleton. Thus, a correlation may exist in the human skeleton between lead-210 and strontium-90, a long-lived fission product. To investigate this possibility, we analysed for lead-210 samples of vertebra from fourteen infant children residing in the Cincinnati, Ohio, area, who died at the ages of 3 days to 12 months. The strontium-90 concentrations were determined at the U.S. Public Health Service, Northeastern Radiological Health Laboratory in Winchester, Massachusetts, and have been published[9]. The lead-210 content in the bone samples was determined by dissolving a 5 to 10 g sample of bone ash in 0·1 N hydrochloric acid, depositing the polonium-210 from the sample on a 1–0·5-in. diameter polished nickel planchet[10,11]. The lead-210 activity was then calculated from the polonium-210 α-activity after correction for incomplete polonium-210 ingrowth had been made. The calcium content of the bone samples was obtained by precipitating the calcium as the oxalate with oxalic acid at a pH of 2·5–3, dissolving the precipitate in sulphuric acid and titrating the calcium with a standardized solution of potassium permanganate[12]. The results are given in Table 1. The uncertainty in the lead-210 and strontium values, calculated for a one standard deviation counting error, is 5 and 6 per cent, respectively. The average lead-210 and strontium-90 concentrations in these skeletal samples are 0·133 and 2·19 pc./g calcium, respectively. The average lead-210/strontium-90 ratio was 0·068. The vertebra of the 3 day old infants contained lower concentrations of both lead-210 and strontium-90, but the ratios of the two nuclides were high in all three cases. These concentrations probably reflect contributions from the mother during pregnancy, and may imply less placental discrimination against lead than against strontium.

Table 1. THE LEAD-210 AND STRONTIUM-90 SKELETAL CONTENT OF FOURTEEN INFANTS FROM CINCINNATI, OHIO

Age Months	Age Days	Sex	Date of death	^{210}Pb (pc /g Ca)	^{90}Sr* (pc./g Ca)	^{210}Pb/^{90}Sr
0	3	F	2/62	0·082	0 87	0·094
0	3	M	4/62	0 079	0·95	0 083
0	3	F	4/62	0 101	1 23	0 082
1	29	M	7/62	0·129	1 70	0 076
3	13	M	1/63	0 129	3·37	0 038
5	8	M	12/62	0 164	2 97	0 055
7	0	M	4/62	0·130	2 10	0 062
7	4	M	9/62	0·170	2 80	0 061
8	0	F	6/62	0·135	2 45	0 055
10	0	M	1/62	0·122	1 90	0 064
10	11	M	10/62	0·239	4 52	0·053
11	6	M	12/62	0 122	2 52	0·048
11	6	M	6/62	0 124	1·62	0 076
12	0	M	2/62	0·131	1·74	0 075
Average				0·133	2 19	0 068

* ^{90}Sr values from *Radiat. Health Data*, **5**, 620 (1964).

In Fig. 1 the lead-210 concentrations are plotted against the strontium-90 concentrations in pc./g calcium. A linear relationship is clearly suggested. The value of the linear correlation coefficient here was determined as 0·89. Such a strong correlation lends support to the hypothesis that lead-210 is an airborne contaminant, and

is related to fission product fall-out through its similar behaviour in the atmosphere. The fact that these samples were obtained from residents of a limited age group in one area, who subsisted on similar foods (primarily milk), was without doubt partially responsible for the observed correlation. The principal source of strontium in children is milk, which is concentrated by the cow from a diet consisting mainly of grass. Because a strong relationship has been reported between strontium-90 and lead-210 in grass, and because cows' milk has been observed to reflect lead uptake by the cow[13], this correlation between strontium-90 and lead-210 in infants is to be expected.

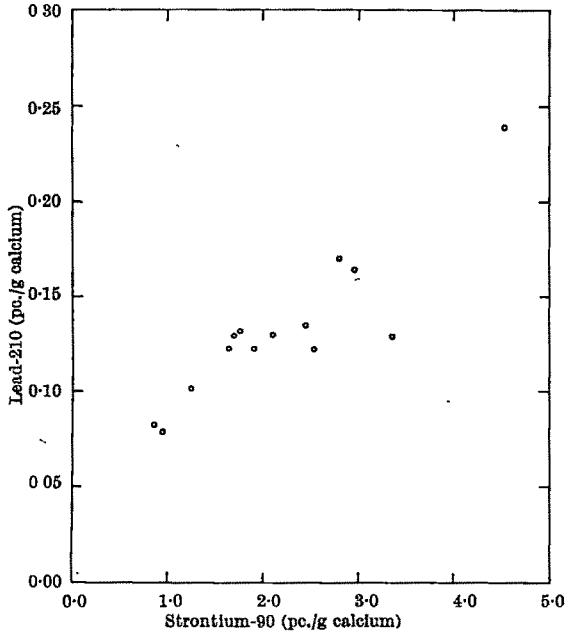


Fig. 1. Concentration of lead-210 versus strontium-90 in bones of infants from Cincinnati, Ohio.

Lead-210 and strontium-90 concentrations were also obtained for a set of eighteen vertebra samples of persons between 5 and 22 years of age who had resided in New Hampshire, Vermont and Massachusetts. For this set of samples the linear correlation coefficient was calculated to be 0·67. Although the linear correlation is not nearly so satisfactory as that found for the infant samples, a linear relationship is recognizable. The varied eating habits of the older group may be responsible for the smaller correlation. Persons of this age group consume a wide variety of foods grown in different localities, which undoubtedly contain different levels of strontium-90 because the distribution of fall-out from atmospheric nuclear tests is not uniform. Thus the fission product fall-out, following shortly after an atmospheric nuclear explosion, predominates over fall-out returning to ground from the stratosphere where a close lead-210 fission product relationship has been observed[1]. No correlation could be detected between lead-210 and radium-226 in our samples. A similar lack of correlation was reported by Holtzman[7]. The different distribution pattern of radium-226 and lead-210 in bone again indicates that lead-210 enters the environment as a product of fall-out, and is not related to its place of origin.

The relative dose to the infant bones delivered by lead-210 and strontium-90 can be calculated from the observed average activity ratio, 0·068. Because the principal dose contributed by the lead-210 to polonium-210 chain is delivered by polonium-210, the equilibrium that exists between lead-210 and polonium-210 in bone must be known. In conflict with the value of 0·1 for the polonium-210/lead-210 activity ratio in bone presented by Committee II of the International Commission on Radiological Protection[14], recent investigators have shown this value to be close to unity[7,15–18]. Assuming, therefore, that radioactive equilibrium exists in bone between lead-210 and polonium-210, and that there is a relative biological effectiveness (RBE) of 10 and a uniform distribution of lead-210 and strontium-90 within the bone, the dose delivered by lead-210 and daughter elements to these infant bones is 3·4 times that delivered by strontium-90 and its daughter, yttrium-90.

The results of this study indicate that a correlation exists between lead-210 and strontium-90 in the human skeleton, and also support the fall-out hypothesis which postulates contamination of the environment with lead-210. Furthermore, they demonstrate the similar behaviour of lead-210 and fission products in our environment, as noted in earlier reports. The relative dose, lead-210/strontium-90, delivered to the infant bones, based on the average lead-210/strontium-90 activity ratio, was calculated to be 3·4. If the nuclear test ban treaty continues, the lead-210/strontium-90 ratio observed in this study will increase because the lead-210 concentration should remain relatively constant while the strontium-90 concentration should slowly decrease.

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Response to X-irradiation of a Tumour recurring after a TCD_{95} Radiation Dose

CHINESE hamster cells which survived 1,500 rads (a survival fraction of $\approx 2 \times 10^{-3}$) were found to have several heritable altered biological properties. These included a slower cell population doubling time, a smaller cell size, an abnormal chromosome number and morphology, and an increased radio-sensitivity—namely a D_0 value of $\approx$135 rads instead of the 205 rads for the unirradiated or control cell line. Furthermore, these characteristics persisted in a number of the sub-lines for many months of continuous culture[1]. That changes of this type should occur in cells of tissue irradiated *in vivo* is plausible, and if they do occur such alterations would have important implications for tumour radiobiology and for clinical radiotherapy. This report describes an initial experiment that was designed to test for such an effect.

A radiation dose–tumour control response was performed on second generation isotransplants of a *C3H* mouse mammary carcinoma growing in the subcutaneous tissue of the flank of *C3H* mice (female, mammary tumour agent positive); the carcinomata were about 70 mm³ at the time of irradiation. The tumours were treated with one of five dose levels (single dose treatment) and irradiation was administered under conditions of local tissue anoxia (for additional details, see refs. 2 and 3). The dose that yielded local control of tumour in half the treated lesions (TCD_{50}) was 5,990 rads at 240 days after treatment.

A recurrent tumour was noted in one of eight animals at 240 days following 6,600 rads, or a dose corresponding to TCD_{95}. This tumour was in the centre of the irradiated area, that is to say, it was not a marginal recurrence. It is highly probable that the lesion developed from a single surviving cell and, therefore, might be an *in vivo* counterpart of the "small colony" of Sinclair. This recurrent tumour had a volume doubling time of about 17 days as compared with 3–4 days for original unirradiated tumours. The small recurrent tumour (3 mm diameter) was excised and isotransplants were prepared. The resultant tumours also grew quite slowly and had reached a diameter of about 15 mm by 6 months. From this tissue, isotransplants were prepared using inbred animals from the same source as those used in the original dose response investigation. The "take rate" of the tumour transplant was 98 per cent for both series. For the original dose response assay, transplantation was made 24 h after the host animal had received 400 rads of total body irradiation. The investigation of which that assay was a part[2] showed that the TCD_{50} for tumours growing in animals which had received total body irradiation was similar to that for tumours in untreated animals, and also was the same as for autotransplants (all treatments given to 4 mm³ tumours and under conditions of local tissue anoxia). Because of those findings, the dose response assay of the recurrent tumour was made on tumour tissue transplanted into unirradiated mice.

Table 1. TCD_{50} VALUES FOR TUMOURS PREVIOUSLY UNIRRADIATED AND FOR TUMOURS RECURRENT FOLLOWING A TCD_{95} RADIATION DOSE. THE FIGURES INDICATE THE 95 PER CENT CONFIDENCE LIMITS AROUND THE TCD_{50} ESTIMATE

Previously unirradiated tumour	Recurrent tumour
TCD_{50} in rads at 240 days	TCD_{50} in rads at 240 days
5600 ... 5990 ... 6390	4800 ... 5130 ... 5480

The results of these experiments are given in Table 1, and show that the TCD_{50} at 240 days was significantly less for the recurrent lesion, that is, 5,080 rads instead of 5,990 rads.

The difference in TCD_{50} values was not so marked as it would have been had the change in D_0 been comparable with that observed by Sinclair. However, the *in vitro* investigations showed a gradual reversion toward the normal or control value with prolonged culture. The irradiation of the tumour tissue derived from the recurrent lesion occurred about 18 months following the treatment of the initial tumour. The results reported here suggest that the changes described by Sinclair for cells investigated *in vitro* may occur in cells of tumour tissue irradiated *in vivo*. Further and more detailed investigations of this effect are planned.

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AGRICULTURE

Fleece Length and the Reactions of Sheep to Wet and Dry Heat

THE experiments of Lee[1] indicate that shearing sheep may, depending on climatic conditions, either increase or decrease the animal's reaction to heat, and that atmospheric temperature and humidity conditions can be divided into two distinct groups on this basis. This work has been confirmed by Klemm[2], whose results clearly demonstrate that shorn sheep tolerate hot wet atmospheres better than unshorn sheep, the reverse situation applying under hot dry conditions. In view of these differences, it was of interest to examine the influence of a range of fleece lengths on the reactions of sheep to wet and dry heat.

Ten 6 year old Merino wethers, each of which weighed 42·3–51·4 kg, were used in the experiment. The wethers were of a fine woolled strain and at appropriate intervals randomly selected pairs of them were shorn so that at the beginning of the experiment the range of fleece lengths shown in Table 1 was represented. During a pre-experimental period of 14 days, and when not in the hotroom, the sheep were housed indoors (temperature range: 9°–26° C) and communally fed a maintenance ration of equal parts lucerne chaff and concentrates at 5 p.m. daily. Drinking water was available *ad lib.* at all times except during hotroom exposure. During the pre-experimental period the animals were accustomed to the experimental conditions and procedures.

Table 1. INFLUENCE OF FLEECE LENGTH ON THE REACTIONS OF SHEEP TO HOT WET AND HOT DRY ATMOSPHERES

Fleece length (cm)	Test atmosphere: Hot dry 0*	Hot dry 1	Hot dry 3	Hot dry 6	Hot wet 0	Hot wet 1	Hot wet 3	Hot wet 6
0·5	101·1†	103·5	104·0	104·1	101·0	103·8	104·8	105·5
	17·2‡	104 2	178·7	188·5	15·2	159·0	219·7	227·0
1·0	101·6	103·9	104·3	104·4	100·9	103·5	104·9	104·9
	19·2	131 2	181.2	175·0	17·2	150·7	214·5	225·0
2·0	101·3	103·4	103·4	103·2	101·5	104·0	104·9	105·5
	26·7	112·0	160·2	161·7	35·5	173·5	220·2	223·7
4·0	101·3	103·4	103·6	103·6	101·3	104·1	105 0	105·8
	41·0	143·0	151 0	159 0	43·7	191·5	253·7	245 7
8·0	101·4	103·5	103·3	103·2	101·4	103·7	104·6	105 4
	65·7	155 2	151·7	145·0	69·7	193·5	216·7	207·2

* Hours of exposure.
† Rectal temperature, °F.
‡ Respirations per mn.

Tests of the animals' reactions to hot wet (41·0° C, 30 mm mercury) and hot dry (41·0° C, 10 mm mercury) atmospheres were made over an 11 day period commencing on April 21, 1966. In all, four tests were made with each animal, two alternate exposures to both hot wet and hot dry conditions. To minimize acclimatization to heat, two full days were allowed between successive tests on each animal. One wether from each of the five pairs was tested one day and the remaining five animals the following day.

On each test day the five wethers to be used were placed in the preheated hotroom at 9.30 a.m. and remained there for 7 h or until their rectal temperature exceeded 41·7° C (107·0° F). Measurements of rectal temperature (by thermocouple inserted 4 in.) and respiratory rate (flank movements counted over two 30 sec intervals) were made immediately before the animals entered the hotroom and at hourly intervals during the subsequent exposure. In the interests of brevity, only the results from 0, 1, 3 and 6 h of exposure to test conditions are presented in Table 1. In all cases, however, these results are fully representative of the hourly recordings actually taken.

The results from exposure of the test wethers to a hot dry atmosphere substantiate previous reports[1,2] that such conditions are best tolerated by unshorn sheep. Further examination of the data in Table 1 reveals that, while there is a progressive decline in response with increasing fleece length, the animals may be divided into two distinct

groups according to their response to the hot dry conditions. Wethers with fleeces 0·5 and 1·0 cm long exhibited similar reactions and these were distinctly higher than those of wethers with a fleece length of 2, 4 or 8 cm. It would seem that a fleece about 2 cm long affords a substantial degree of insulation against a hot dry atmosphere in the absence of a radiant heat load, and that fleeces in excess of this length provide little additional protection. Under more severe conditions when radiant heating is superimposed on a hot dry atmosphere it has been shown[3,4] that 4 cm represents a similar critical fleece length.

Under the hot wet conditions used in this experiment, fleece length had no apparent effect on the heat tolerance of the test animals. The 30 mm mercury water vapour pressure used was dictated by the capabilities of the hot-room at the time of the experiment and, although not extremely high, could have been expected to elicit a fleece length effect on heat tolerance[2], especially with regard to respiratory rate. The fact that the superior heat tolerance which was exhibited by the woolly sheep under hot dry conditions is no longer apparent under the hot wet atmosphere suggests that the latter was, under the conditions of the experiment, only marginally wet.

Klemm[2] has attempted to explain the relative intolerance of unshorn sheep to hot wet atmospheres in terms of the hygroscopic properties of wool, the resultant exothermic reaction giving rise to a wool-skin temperature gradient. If this is so then *in vitro* studies with sheep skins which were carried out concurrently with the experiment described here add further weight to the suggestion that the hot wet atmosphere used was only marginally wet. These skins gained weight only very gradually when exposed to hot wet conditions. For example, a skin which clipping showed to carry a 7 cm fleece weighing 2·81 kg in the ante-room gained about 30 g during each of the first 2 h of exposure and only a further 40 g during the remaining 5 h.

Comparison of the results from the two test atmospheres illustrates the influence of humidity on heat tolerance in sheep and the ability of this species to tolerate all but the most severe environments.

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Effect of Stocking Rate and Lambing Time on Gestation Length in Sheep

ALEXANDER[1] suggested that severe undernutrition of sheep may reduce the gestation length in sheep and he reported reductions of up to 3·6 days in gestation length in ewes transferred to a low plane of nutrition.

In an experiment varying stocking rate and lambing time[2], the length of gestation was recorded in a flock of 378 ewes over a three year period. In the extreme Mediterranean environment in which this work was carried out (Kojonup, Western Australia) the withholding of supplementary feed can result in severe nutritional stress to autumn (April–May) lambing ewes that are being depastured at a high stocking rate.

The mean gestation lengths for the several treatments in the 3 years of the experiment are shown in Table 1, while the liveweight of the ewes in relation to the number of days pregnant is shown in Fig. 1.

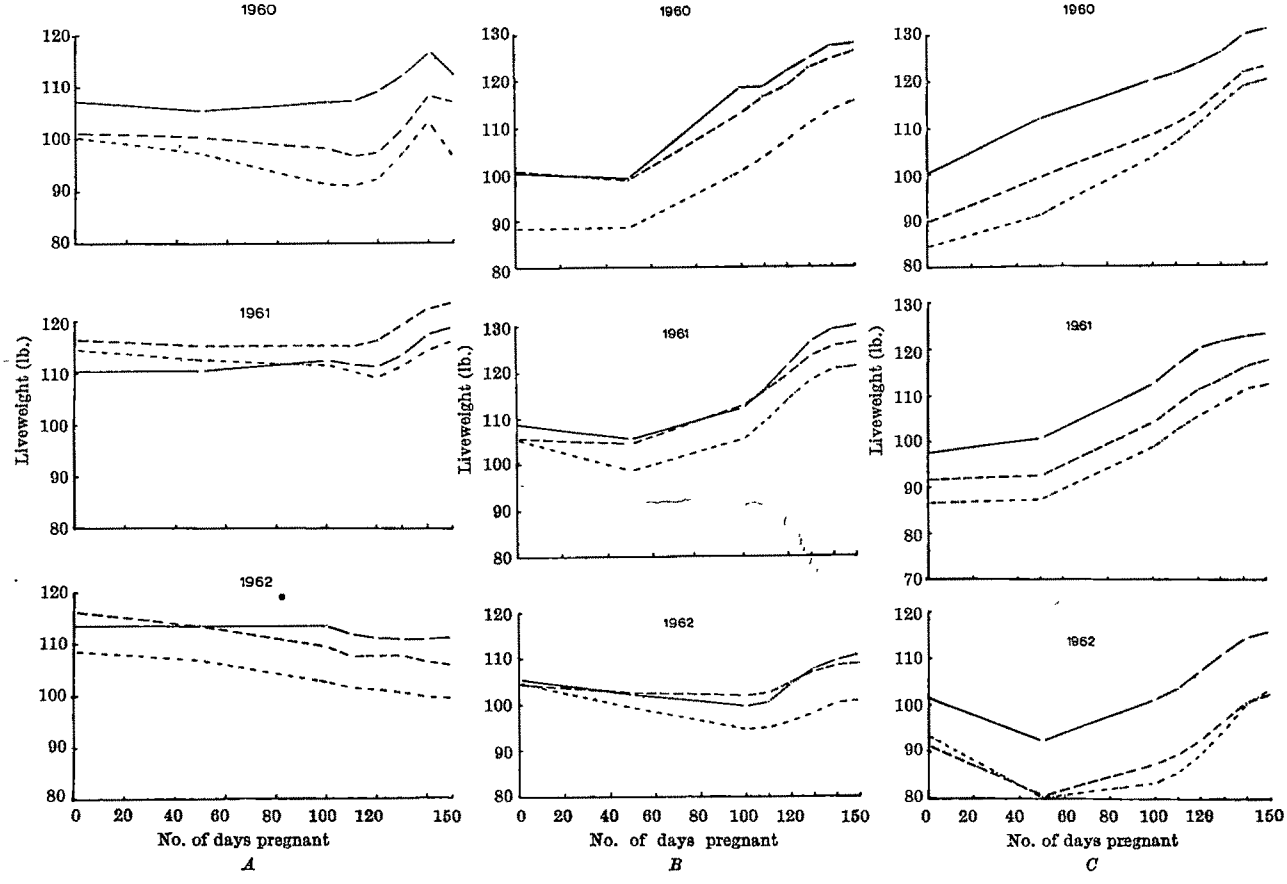


Fig. 1. Liveweight change of ewes in relation to number of days pregnant. *A*, Autumn lambing; *B*, winter lambing; *C*, spring lambing. ——, 1·5 ewes per acre; – – – –, 3·0 ewes per acre; — · — · —, 5·0 ewes per acre.

Table 1. MEAN DURATION OF GESTATION IN DAYS OF EWES LAMBING IN TIME AGAINST STOCKING RATE

(1960–1962)

Year	Lambing time	Mean gestation length Stocking rate 1·5	3·0	5·0
1960	Autumn	150·6	152 0	151·1
	Winter	151·7	151·4	150·5
	Spring	151·4	150·8	150·8
1961	Autumn	152·7	153 2	153·7
	Winter	152·6	151·2	152 2
	Spring	150·9	150·8	152·2
1962	Autumn	151·7	152·1	151·4
	Winter	153·4	152·7	153·7
	Spring	153·1	152·2	153·3

Analysis of variance

Source of variation	Degrees of freedom	Mean square	Variance ratio
Years	2	5·154	
Lambing time	2	0·463	
Stocking rate	2	0·181	
Years × lambing time	4	2·126	13·17*
Lambing time × stocking rate	4	0·638	3 95†
Stocking rate × years	4	0·511	3·16 N.S.
Years × lambing time × stocking rate	8	0·161	
Total	26		

* Denotes $P < 0·01$.
† Denotes $P < 0·05$.
N.S., denotes $P > 0·05$.

Fig. 1 shows that the range of liveweight change during pregnancy was from a loss of 9·0 lb. (indicative of severe nutritional stress) to a gain of 29·0 lb. The range of liveweight changes obtained should be sufficient to determine the possible effect of nutrition on gestation length under field conditions. Although the greatest mean loss in weight for any treatment was 9 lb., individual sheep lost up to 20 lb.—a figure comparable with those of Alexander.

In the three seasons studied there were considerable differences in the pattern of liveweight change of the ewes in late pregnancy. Nevertheless, there was no evidence that gestation length was related to plane of nutrition. Twin lambs, of course, increase the nutritional stress of pregnancy[3]. In the present experiment, very few twin lambs were born, and in all cases gestation length was greater than 150 days.

The apparent effect of lambing time on gestation length in 1961, but not in the other seasons (as shown by the highly significant interaction in Table 1), needs explanation. It was thought that it might be accounted for by varying nutritional effects in the 3 years, of which maternal liveweight change during pregnancy could provide an index. The change from 100 to 140 days was accordingly used as the independent variable in an analysis of covariance of the gestation length data. The regression in the error term was not significant, and the effect of lambing time on gestation length in 1961 remained significant after a possible nutritional factor had been eliminated in this way. Climatic differences might provide a possible explanation.

No acceptable interpretation of the interaction of lambing time and stocking rate can be proffered.

The small and statistically non-significant difference of 0·6 days[4] in the gestation length of ewes bearing a single lamb on a high plane of nutrition compared with ewes bearing a single lamb on a low plane gives some support to the present findings.

The differences between our findings and those of Alexander are probably due to dissimilar experimental conditions. In Alexander's experiment, the under-nutrition was induced abruptly by giving different feed levels to ewes which before the experimental period had either been maintaining or gaining weight. In our case, where the ewes on the low plane of nutrition were kept continuously on the experimental plots, under-nutrition was prolonged, and became progressively more severe. Thus, when the plane of nutrition has been steadily and slowly deteriorating, insufficiency of foetal nutrition need not be a factor involved in the initiation of parturition as postulated by Spiegelberg[5].

When the plane of nutrition is deliberately reduced in middle or late pregnancy, as in the case reported by Alexander[1], then insufficient foetal nutrition may well reduce gestation length.

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IMMUNOLOGY

Maintenance of Histocompatibility Polymorphisms

THE transplantation antigens present a striking example of genetic polymorphism. Medawar[1] has stated that "Although there are no factual grounds for supposing that antigenic diversity is anything but an unfortunate consequence of constitutional differences between individuals of a species, yet one is under some obligation to rack one's brains for evidence of any good it might conceivably do. Only thus can antigenic polymorphism be made genetically respectable". Because during gestation the foetus is a homograft to the mother and would therefore seem liable to rejection, this variability would at first sight appear to be a distinct disadvantage to a viviparous animal. The hypotheses put forward to account for the survival of the foetal homograft have recently been reviewed by Billingham[2]. Most of them have not withstood experimental verification, but there is good reason to believe that one, which assumes the presence of a physical barrier between mother and foetus, is correct[3,4]. Antigenic differences between mother and offspring are not, however, without effect; the consequences of blood-group incompatibility, for example, are well known. Recently, it has been shown that other antigenic differences may affect the growth of the placenta[5], and also the size of the foetus itself[6,7]. If one inbred strain of mice is mated with another, the hybrid foetus is significantly larger just before birth than a pure bred foetus of either parental strain. This increase in size does not arise from classical heterosis alone, as the effect can be enhanced by making the mother immune to the tissues of her partner or diminished by making her tolerant of them[6]. In these experiments it seems probable that the foetuses which are unlike their mothers, and therefore larger at birth, have a greater chance of survival. These observations suggest a system for maintaining balanced polymorphism; it can be represented by the following model.

We assume a random-mating population in which there are two alleles, H_1 and H_2, at frequencies p and q, respectively ($p + q = 1$). We consider that the reproductive abilities of the females of all three genotypes are equal, but that offspring against which the mother can form antibodies enjoy a selective advantage. We also assume that the frequencies of the three genotypes in each generation are approximately in Hardy–Weinberg equilibrium. Strictly, the last mentioned assumption is only valid if the selective differences between genotypes are small, but even large differences will not significantly alter our conclusions. The frequencies of matings and offspring are set out in Table 1. It can be seen that the heterozygote shows a net selective advantage over both homozygotes.

Table 1

Mating ♀	♂	Frequency of offspring H_1H_1	H_1H_2	H_2H_2
H_1H_1	× H_1H_1	p^4		
H_1H_1	× H_1H_2	p^3q	$p^3q(1+s)$	
H_1H_1	× H_2H_2		$p^2q^2(1+s)$	
H_1H_2	× H_1H_1	p^3q	p^3q	
H_1H_2	× H_1H_2	p^2q^2	$2p^2q^2$	p^2q^2
H_1H_2	× H_2H_2		pq^3	pq^3
H_2H_2	× H_1H_1		$p^3q^2(1+t)$	
H_2H_2	× H_1H_2		$pq^3(1+t)$	pq^3
H_2H_2	× H_2H_2			q^4
Total		p^2	$2pq(1+\frac{1}{2}sp+\frac{1}{2}tq)$	q^2

Genotype	H_1H_1	H_1H_2	H_2H_2
Selective value	$a = 1$	$b = 1 + \frac{1}{2}sp + \frac{1}{2}tq$	$c = 1$

From Fisher's[8] formula we find that there is a stable equilibrium when:

$$q = \frac{b-a}{2b-a-c} = \frac{\frac{1}{2}sp+\frac{1}{2}tq}{sp+tq} = 0{\cdot}50$$

Differences in survival between the two homozygotes will, of course, alter the point of balance. Our model resembles one given by Hull[9], except that we assume no selective disadvantage of the heterozygous offspring from heterozygous mothers. It also differs from Hull's in giving stability over a wider range of conditions. Hull[9,10] puts forward his model to represent the results of experiments in which he crossed two inbred strains of mice (*C3Hf/He Ha* agouti and *HG/Hu* black-and-tan). He found that litters were deficient in young whose coat colour genotype resembled that of the mother. As he pointed out, this deficiency may not be the result of the action of the coat colour genes themselves, but of the action of loci sufficiently closely linked to remain associated with them through several meiotic divisions. It is significant in this context that the histocompatibility locus *H3* is known to be closely linked with the locus which controls the agouti character[11]. However, an explanation of Hull's results in terms of histocompatibility must account for his finding that there is a deficiency of heterozygous offspring from heterozygous mothers. There are two possibilities: either there is antigenic recognition by the foetus as well as by the mother, or the heterozygous young suffer a disadvantage unrelated to their immunological status—a disadvantage which is overcome when the mother can react against them.

The experiments reviewed in this communication show that immunological interactions between mother and foetus can influence foetal size and probably foetal survival. The effects of transplanting paternal tissues into the mother suggest that histocompatibility antigens are involved in these interactions. The data indicate a selective system capable of maintaining balanced polymorphism, and they provide a possible explanation of the great genetic diversity of transplantation antigens within species of viviparous animals.

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Effect of Thymectomy on Transplantation Resistance induced by Polyoma Tumour Homografts

POLYOMA virus will induce neoplasms in most strains of newborn mice but not usually in mice older than 2 weeks[1,2]. Adult mice infected with polyoma virus are relatively more resistant to challenge of syngeneic polyoma-type tumours[3]. Such resistance can also be induced by homografts of polyoma tumours[4]. This resistance is not dependent on circulating viral antibodies and can apparently be transferred to normal recipients by lymphoid cells from immunized mice. To interpret these findings, Habel[5] and Sjögren *et al.*[6] proposed that polyoma virus transforms normal cells to neoplastic cells in the newborn and in the adult mouse, and that the transformed cells now contain a new foreign cell antigen which will provoke an immune response in the adult but not in the newborn animal. Thus, ordinarily, neoplasms will arise in newborn animals, whereas adult animals are capable of rejecting tumour cells containing foreign antigen. This tumour antigen is apparently common for most polyoma tumours induced by the same strain of virus[7]. This concept is supported by the finding that thymectomy during the immediate postnatal period—known to effect maturation of immunological faculty—can alter the course of polyoma virus oncogenesis. Thymectomy at 3 days of age rendered normally highly resistant (*C57BL*) mice susceptible to the oncogenic action of polyoma virus[8]. The same effect was found when the virus was injected into mice 1 month old[9]. Mice thymectomized when 3 days old and then infected with polyoma virus were unable to develop transplantation resistance against a challenge of syngeneic polyoma tumour cells[10]. Additional evidence is now reported that *C57BL* mice, thymectomized at 3 days of age and then immunized with homografts of polyoma tumour cells when 1 month old, are unable to develop resistance against challenge by syngeneic polyoma tumour cells.

Polyoma tumour 4198 originated *in vitro* in tissues derived from a *C3Hf/Hen* strain of mouse[10], and polyoma tumour-89 originated in a *C57BL/KaLw* thymectomized mouse. Both tumours were induced by the LID_1 strain of polyoma virus. They are both transplantable tumours and grow progressively until they kill their syngeneic hosts; they have been cultivated *in vitro* for many generations. Both tumours are free from demonstrable virus as determined by the haemagglutination inhibition test and by plaque formation, but the polyoma tumour specific antigen is known to be present as determined by transplantation resistance tests.

Adult mice (1–2 months old) were given homografts of polyoma tumours (4,198 cells to *C57BL* mice or 89 cells to *C3H* mice) as follows: 2×10^6 trypsinized cells suspended in *tris* buffer were injected subcutaneously. At the first inoculation an initial outgrowth was often seen, followed by regression as the result of a homograft reaction. On complete regression of the first homograft a second inoculation of 2×10^6 cells was made. No temporary growth was observed on this occasion. A third inoculation of 2×10^6 cells was given 2 weeks later. Two to three weeks after this all mice were challenged subcutaneously with syngeneic polyoma tumour cells. The animals were observed for a minimum of 60 days after challenge.

The results in Table 1 show that a homograft of polyoma tumour cells indeed induces transplantation resistance. *C57BL* mice were better recipients than *C3H* mice. This is because *C57BL* mice are immunologically more competent early in life[11]. Similar results have been obtained for transplantation resistance developed against Moloney lymphoma. After they had been immunized by homografts of Moloney lymphoma, *C57BL* mice developed stronger resistance to syngeneic challenge of Moloney lymphoma than other strains of mice such as *BALB/c*

Table 1. R_F AND SPECTRAL PROPERTIES OF PRODUCTS FROM *E. coli*

Experiment No.	Spot designation	R_F	*A* ratio (265/355)
1	*A*	0·68	–
	B	0·78	–
2	*C*	0·66	3·83
	D	0·78	3·31
3	*E*	0 95	–
	F	0 69	–
	G	0·75	–
Standard	OTC	0 76	1·24
Standard	Tetracycline	0·78	1·11
Standard	Desdimethylamino-oxytetracycline	–	1·32

The experiments were run as follows: Cells from 50 ml. of nutrient broth culture incubated for 15 h at 37° C were collected by centrifugation and washed twice with water. They were resuspended in 50 ml. of a salts and glucose medium containing OTC (50 μg/ml.), and incubated for 105 min at 37° C with shaking. The cells were recovered by centrifugation (yield about 100 mg wet weight). The cells were extracted with 10 per cent TCA at 0° C for 1 h. The TCA was removed by three extractions with ether. The extract was freeze dried to concentrate the material, then resuspended in a small volume of water, and analysed by ascending paper chromatography in a solvent of isobutyric acid—0·5 N ammonium hydroxide (5 : 3). Chromatography was extended over 15 h at room temperature (about 25° C).

Except for experiment No. 2, it has not been possible to elute the spots from the paper after chromatography; this has greatly limited the scope of the investigations so far. The results given in Table 1 would seem to demonstrate that three new compounds are formed from OTC during the incubation period. These are, respectively, a material of slower R_F, faster R_F, and the same R_F as OTC. The spectral properties of the R_F 0·78 material suggest that it is not the parent compound. A control exposure of 5 mg/ml. of OTC to 9 per cent TCA for 1 h at 0° C resulted in only one spot on paper chromatography, of R_F 0·78 and an absorbancy ratio (265/355) of about 0·96. It was apparently unchanged OTC. Another control experiment showed that no fluorescent spots were extracted from *E. coli* carried through this procedure when OTC was omitted from the incubation mixture. Immersion of cells in a boiling water bath for 15 min before exposure to OTC completely eliminated their ability to convert the drug to these new compounds. The ability of the *E. coli* to concentrate OTC from the external medium[2] was destroyed concomitantly. Although alternative explanations are possible for these observations, it would appear that bacterial viability is essential for the observed changes in the OTC to occur. These changes are presumably the result of enzyme catalysed reactions within the bacteria.

When bioautographs were prepared from the paper chromatograms (experiment No. 3), using *Staphylococcus aureus* as the test organism, clearly defined zones of inhibition were apparent for all three spots. Thus these compounds retain antibacterial properties.

Note added in proof. After the submission of this manuscript, Izaki and Arima (*J. Bacteriol.*, 89, 1335; 1965) reported further details of the uptake of OTC by *E. coli*.

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Specific Activity of Phenylalanine Deaminase in Extracts of the *Proteus–Providence* Group

THE possession of a phenylalanine deaminase which converts phenylalanine to phenylpyruvic acid is one of a combination of five properties unique for the *Proteus–Providence* group of organisms[1]. Of a total of 185 *P. hauseri* (*P. mirabilis* + *P. vulgaris*), 155 *P. morganii*, 29 *P. rettgeri* and 239 *Providence* strains qualitatively examined for the presence of this enzyme[2–4], all but two *Providence* strains[3] were positive, although *P. morganii* strains have been reported to yield on occasions weak or negative results[5].

As part of an investigation of this enzyme, its specific activity in extracts of the *P. hauseri*, *P. morganii* and *P. rettgeri* strains previously used[6] was investigated. An additional fifty-seven strains of *P. morganii* were also examined. The strains were grown overnight on Difco nutrient agar supplemented with 0·3 per cent extracts of yeast. Organisms were collected and placed in 0·8 per cent saline and sonicated at 9 kc/s for 20 min before they were centrifuged in the cold at 198,000*g* for 30 min. Protein concentration in the cell-free supernatant was measured by the biuret method and crystalline bovine albumin was used as the standard. The specific activity of phenylalanine deaminase in the supernatant was measured by incubating 1·0 ml. with 0·2 M *tris*-hydrochloric acid buffer at *p*H 7·4 (1·0 ml.) and 0·08 M D,L-phenylalanine (1·0 ml.) for 60 min at 37° C after which the reaction was stopped by the addition of 40 per cent trichloroacetic acid (1 0 ml.). A blank was included for each sample with the trichloroacetic acid added and mixed before addition of the phenylalanine. After centrifugation, 1·0 mL samples of the supernatant were adjusted to *p*H 2 and assayed with ferric chloride for phenylpyruvate content by the method of Rowsell[7].

Table 1. PHENYLALANINE DEAMINASE ACTIVITY OF *Proteus* AND *Providence* ORGANISMS

	No. strains	< 50	50–2,000	2,000–4,000	4,000–10,000
P. mirabilis	19	—	—	—	19
P. vulgaris	9	—	—	—	9
P. rettgeri	22	4	18	—	—
P. morganii	74	—	5	69	—
Providence	24	—	6	18	—

Overnight nutrient agar growths were collected and placed in 0·8 per cent saline and sonicated in the cold. After centrifugation at 4° C, the phenylalanine deaminase activity of the clear supernatants was determined and expressed as μmoles phenylpyruvate formed/mg of protein/min.

Results are presented in Table 1. Results were not affected by the incorporation of 0·2 per cent D,L-phenylalanine in the growth medium and the striking feature is the high deaminase activity of *P. hauseri* extracts. *P. hauseri* differs from the other groups of organisms investigated here in its pronounced swarming capacity and the ability to ferment xylose, to produce hydrogen sulphide and to liquefy gelatine. The quantitative difference in phenylalanine deaminase activity demonstrated may support claims[1,8] that biochemical differences among these groups of organisms are at generic level within a tribe Proteae.

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Department of Microbiology,
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[1] Rauss, K., *Intern. Bull. Bact. Nom. Tax.*, 12, 53 (1962).
[2] Henriksen, S. D., *J. Bact.*, 60, 225 (1950).
[3] Shaw, C., and Clarke, P. H., *J. Gen. Microbiol.*, 13, 155 (1955).
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GENERAL

Origin of the Theory of Errors

J. H. LAMBERT should be given precedence over Gauss as the originator of the theory of errors. Galle[1] stated that Gauss arrived at the idea of the principle of least squares while reading Lambert, but he did not mention the essence of Lambert's work on the subject. Furthermore, no mention has been made of this in the various works on Lambert[2–4]

The three main works by Lambert are *Photometria*[5], *Theorie der Zuverlässigkeit der Beobachtung und Versuche*[6], and *Aumerkungen und Zusätze zur practischen Geometrie*[7]. The first of these is available in the Ostwalds' *Klassiker* series, but the relevant portion was omitted from the translation. Moreover, the translator inserted a special comment on this portion asserting that it contained nothing of interest to readers.

These works contain:

(*a*) The first general outline to be published after Galilei[8] of the properties of observational errors.

(*b*) A rule for estimating the precision of measurements by comparing the means taken with and without the most extreme observation. This was the first numerical rule for such estimations, but was inadequate for a comparative estimation of various series of data which differed in the number of their individual observations.

(*c*) A somewhat loose proof of the preference for an arithmetical mean as compared with individual observations (ref. 7, art. 443–5).

(*d*) A derivation (ref. 7, art. 429–30) of a curve named later by Laplace "a curve of facility of errors", which because there were "no reasons" for supposing the existence of an "angle" (*eckige*) form took the shape of a semicircumference. The same manner of reasoning was used in similar circumstances by Laplace in 1774:

$$d\varphi(x+dx)/d\varphi(x) = \varphi(x+dx)/\varphi(x)$$

as there was "no reason" to suppose that there were different laws for $\varphi(x)$ and $d\varphi(x)$ (ref. 9).

(*e*) A definition of the objectives of the theory of errors. Possibly even the term itself should be credited to Lambert (see foreword in ref. 6), although in this connexion Lambert often mentions Marinoni's *De re ichnographica* (see ref. 6, art. 12). As to the aims, these are (ref. 6, art. 321) to find connexions between errors, the consequences of errors, circumstances of observations and the accuracy of instruments (*Verhältnis zwischen den Fehlern, ihren Folgen, den Umstanden der Ausmessung und Güte des Instruments*). The *Theorie d. Folgen* was classified independently as having as its objective the study of errors of functions of observed elements. But Lambert then introduced (ref. 6, art. 1) reliability (*Zuverlässigkeit*) of observations and implied that the aim of the corresponding theory was to deduce the "true value" and to estimate the accuracy of observations. The classification now seems rather clumsy; however, taken as a whole, a "united" theory of errors emerges.

(*f*) An enunciation of the "minimax" principle (minimization of the maximum residual error in geodetic adjustments—the minimum being sought among all possible solutions (ref. 7, art. 420)). But Lambert confessed that he did not know how to use this principle "in a general manner, without many devious ways" (*auf eine allgemeine Art, und ohne viele Umwege*). The use of this principle in a rudimentary form for solving a redundant system of linear algebraic equations should be credited to Euler[10].

(*g*) Rules for fitting straight lines and curves—the observations being divided into groups and the centres of gravity of these taken instead of original observations (ref. 6, art. 20, and others).

(*h*) The use of the principle of maximum likelihood (ref. 5, art. 299–303). Lambert started from a curve *NMLK* (Fig. 1), the equation of which was unspecified but corresponded with the following properties of observational errors: $\varphi(x)=\varphi(-x)$, $\varphi(|x_2|)<\varphi(|x_1|)$ *if* $|x_2|>|x_1|)>\alpha$. He calculated the number of ways in which the observations P, Q, R and S would occur n, m, l and k times, respectively, as:

$$\frac{(n+m+l+k)!}{n!\,m!\,l!\,k!}PN^n\,QM^m\,RL^l\,SK^k \sim PN^n\,QM^m\,RL^l\,SK^k$$

with NP, MQ, etc., equal to the ordinates of the curve. He then gives a graphical method of maximizing the last product using subtangents. He considered the ordinates to be equal to the corresponding "true number" of observations: in this he was almost copied by Lagrange[11] (problem 10), who held that in a continuous case the "facility of errors" was the ratio of the number of cases of the specified type to the total number of cases.

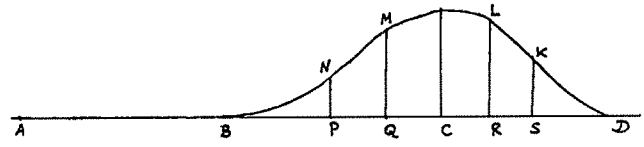


Fig. 1.

Lambert did not specify that his solution was equivalent to differentiating with respect to the unknown mode x_0 of a disintegration $\varphi(x-x_0)$.

It should be noted that Lambert did not regard this principle as a useful one in practice, and he did not return to it.

The χ^2 distribution is due to the German optician, Ernst Abbe[12], and his work is said[13,14] to have been printed in 1863.

Abbe considered the case of normally distributed errors of observations $x_1, \ldots, x_n$ by means of two functions: $\Delta=x_1^2+\ldots+x_n^2$ and $\Theta = (x_1-x_2)^2+\ldots+(x_n-x_1)^2$. Actually he calculated the integral:

$$\frac{h^n}{\pi^{n/2}}\int\ldots\int dx_1\ldots dx_n\, e^{-h^2(x_1^2+\ldots+x_n^2)}$$

(where h is a measure of the precision of the observations), taking (i) $0 < x_1^2+\ldots+x_n^2 < \Delta$; (ii) $0 < (x_1-x_2)^2+\ldots+(x_n-x_1)^2 < \Theta$; (iii) $\Theta < \mu\Delta$, where $0 < \mu <$ by definition of Θ and Δ. He finally arrived at a criterion for rejecting observations with "systematic influences", as they are called in the theory of errors, and noted that departures from the normal distribution correspond to departures of Θ/Δ from 2. But the main point of his work is to be found in (i), where he actually arrived at the χ^2 distribution:

$$P(\Sigma x_i^2\in[\Delta,\Delta+d\Delta]) = \varphi(\Delta) = \left\{h^n/\Gamma(n/2)\right\} e^{-h^2\Delta}\Delta^{\frac{n}{2}-1}\,d\Delta$$

Helmert[15] introduced into geodetic literature what he called a "modified" Abbe's criterion; however, he never mentioned Abbe's deduction of the χ^2 distribution, and this is one of the reasons why the origin of this distribution is usually ascribed to Helmert[16].

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Institute for the History of Natural Science and Technology,
Academy of Sciences of the U.S.S.R., Moscow.

[1] Galle, A., *Über die Geodätische Arbeiten von Gauss, C. F. Gauss*, Werke, 11, Abt., 2 (1924-9).
[2] Lambert, J. H., *Nach seinem Leben und Wirken* (Herausgegeben D. Huber, Basle, 1829).
[3] Barthel, E., *Archiv fur Geschichte der Mathematik, der Naturwissenschaften und der Technik*, 11, N1-2 (1928).
[4] Lambert, J. H., *Opera Mathematica*, 1-2 (Zürich, 1946-8).
[5] Lambert, J. H., *Photometria, Sive di Mensura et Gradibus Luminis Colorum et Umbrae* (Angustae Vindelicorum, 1760, art. 271-306).
[6] Lambert, J. H., *Theorie der Zuverlässigkeit der Beobachtung und Versuche (Beytrage zum Gebrauche der Mathematik und Deren Anwendung*, 1, B, 1765).
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[9] Laplace, P. S., *Mémoire sur la Probabilité des Causes par les Événements, Oeuvres Completes*, 8, P (1891).
[10] Euler, L., *Piece qui a remporté le Prix de l'Acad. Roy. Sci. en* 1748, P, art. 122-3 (1749).
[11] Lagrange, J. L., *Misc. Taurinensia*, 5, 1770–1773, s.a. (Oeuvres, t. 2).
[12] Abbe, E., *Dissertation zur Erlangung der Venia Docendi bei der Philos. Fakultät Jena*, 1863 (Gesammelte Abhandlungen, 2, Jena, 1906). (*Beytrage zum Gebrauche der Mathematik und Deren Anwendung*, 1, B,
[13] Gore, G. E., *A Bibliography of Geodesy* (U.S. Coast and Geodetic Survey Report for 1887, 1889).
[14] Helmert, F. R., *Die Ausgleichung Nach der Methode der Kleinsten Quadrate* (1924).
[15] Cramer, H., *Mathematical Methods of Statistics*, art. 18, 1 (Princeton, 1946).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Wednesday, August 31—Wednesday, September 7

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Nottingham)—128th Annual Meeting.

Wednesday, August 31

At 7.30 p.m.—Prof. Sir Joseph Hutchinson, C.M.G., F.R.S.: "Land and Human Populations" (Presidential Address).

Thursday, September 1

At 10 a.m.—Prof. R. S. Nyholm, F.R.S.: "Intermetallic Bonding in Metals and Chemical Compounds" (Presidential Address, Section B).

At 10 a.m.—Prof. J. W. S. Pringle, M.B.E., F.R.S.: "The Treasure-House of Nature" (Presidential Address, Section D).

At 10 a.m.—Prof. G. L. S. Shackle: "Policy, Poetry and Success" (Presidential Address, Section F).

At 10 a.m.—Prof. J. Greig. "Technique" (Presidential Address, Section G).

At 10 a.m.—Prof. J. D. Matthews: "Forestry and the Landscape" (Chairman's Address, Section K*).

At 10 a.m.—Mr. J. Scupham, O.B.E.: "The Social Factor in Education" (Presidential Address, Section L).

At 10 a.m.—Prof. W. Ellison: "Agriculture and Changes in Land Use" (Presidential Address, Section M).

At 2.30 p.m.—Mr. H. G. Conway, C.B.E.: "The Concord: An International Project in Supersonic Transport" (Young People's Lecture).

Friday, September 2

At 10 a.m.—Prof. W. H. McCrea, F.R.S.: "Cosmical Physics" (Presidential Address, Section A).

At 10 a.m.—Prof. L. R. Moore: "Frontiers in Geology—Geo-Microbiology" (Presidential Address, Section C).

At 10 a.m.—Prof. R. W. Steel. "Geography and the Developing World" (Presidential Address, Section E).

At 10 a.m.—Prof. A. Neuberger, C.B.E., F.R.S.: "The Operation of Control Mechanisms at the Cellular Level" (Presidential Address, Section I).

At 10 a.m.—Sir James Cook, F.R.S.: "The Science Information Problem" (Presidential Address, Section X).

At 11.30 a.m.—Dr. N. A. B. Wilson: "Psychology and Military Proficiency" (Presidential Address, Section J).

At 11.30 a.m.—Prof. A. R. Clapham, F.R.S.: "Nature Conservation and Botanical Research" (Presidential Address, Section K).

At 2.30 p.m.—Prof. F. G. Bailey: "The Peasant View of the Bad Life" (Presidential Address, Section N).

At 2.30 p.m.—Mr. R. L. Gregory: "Illusions in Space" (Young People's Lecture).

At 8 p.m.—Dr. A. H. Cottrell, F.R.S.. "Science and Economic Growth in the United Kingdom" (Evening Discourse).

Sunday, September 4

At 10.45 a.m.—Official Service in St. Mary's Church. Preacher: Canon D. R. Feaver.

Monday, September 5

At 10 a.m.—Prof. J. S. Weiner: "Aspects of Human Adaptation" (Presidential Address, Section H).

At 10 a.m.—Prof. J. N. Bradley: "Shock Waves in Chemistry and Physics" (Kelvin Lecture).

At 10 a.m.—Dr. D. Noble: "How the Heart Beats" (Darwin Lecture).

At 2.30 p.m.—Prof. R.L. F. Boyd: "The Rocket Age" (Young People's Lecture).

At 8 p.m.—Dr. N. Tinbergen, F.R.S.: "Behaviour as Equipment for Survival" (Evening Discourse).

Tuesday, September 6

At 11.30 a.m.—Prof. R. J. Ball: "Economic Model Building for Control" (Lister Lecture).

At 2.30 p.m.—Prof. A. R. Gemmell: "Biology and Crime" (Young People's Lecture).

Monday, September 5—Friday, September 9

BRITISH PHARMACEUTICAL CONFERENCE (at Manchester)—103rd Annual Meeting.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER (with some interest in physical or dynamical oceanography) IN THE DEPARTMENT OF OCEANOGRAPHY—The Deputy Secretary, The University, Southampton (August 31).

TEMPORARY LECTURERS or TEMPORARY ASSISTANT LECTURERS (2) IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, University Senate House, Bristol, 2 (September 1).

VICE-PRINCIPAL (with proven administrative ability, high academic and/or professional qualifications, and responsible experience in education and/or industry, commerce) OF THE COLLEGE OF TECHNOLOGY, Southend-on-Sea—Chief Education Officer, Civic Centre, Victoria Avenue, Southend-on-Sea (September 1).

RESEARCH ASSISTANT (chemistry graduate) for a biological and chemical survey of the River Wear—The Registrar and Secretary, University of Durham, Old Shire Hall, Durham (September 3).

SENIOR RESEARCH FELLOW (with a veterinary qualification and preferably postgraduate experience in virology or microbiology) IN THE SECTION OF POULTRY DISEASE, DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE, to join a team undertaking research into respiratory diseases of poultry in a new building to be available later this year at the Veterinary Field Station in Wirral, Cheshire—The Registrar, The University, Liverpool, 3, quoting Ref. CV/198 (September 3).

TUTOR IN MEDICINE to give instruction in clinical medicine and to take part in the clinical and research activities of the Department of Medicine—The Registrar, The University, Manchester, 13, quoting Ref. 188/66 (September 3).

LECTURER or ASSISTANT LECTURER in SOCIOLOGY—The Secretary, The University, Aberdeen (September 4).

RESEARCH ASSISTANT (with a degree in geography or agricultural economics or similar qualifications, and preferably a knowledge of agricultural statistics and of statistical mapping) IN THE DEPARTMENT OF GEOGRAPHY to assist Prof. J. T. Coppock in the preparation of an agricultural atlas of Scotland—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 5).

RESEARCH ASSISTANT (mycologist or plant pathologist) IN PLANT PATHOLOGY IN THE DEPARTMENT OF BOTANY to join a group investigating obligately parasitic fungi—The Secretary of the University Court, The University, Glasgow (September 9).

CHAIR OF CHEMISTRY—The Assistant Registrar, University College of Cape Coast, 15 Gordon Square, London, W.C.1; or The Registrar, University College of Cape Coast, University Post Office, Cape Coast, Ghana (September 10).

RESEARCH ASSISTANT (veterinary graduate) IN THE DEPARTMENT OF VETERINARY PREVENTIVE MEDICINE at the Veterinary Field Station, Wirral, Cheshire—The Registrar, The University, Liverpool, quoting Ref. CV/204 (September 10).

TUTORIAL RESEARCH STUDENT (man or woman) IN PHYSICS (experimental or theoretical)—The Secretary, Royal Holloway College (University of London), Englefield Green, Surrey (September 10).

LECTURER or ASSISTANT LECTURER (mathematician with research interests in any branch of pure mathematics or mathematical statistics) IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (September 17).

LECTURER (preferably with a special interest in stress analysis) IN THE POSTGRADUATE DEPARTMENT OF APPLIED MECHANICS—The Registrar, The University, Sheffield (September 17).

EXPERIMENTAL OFFICER (with H.N.C. or a university ordinary degree in science, and specially experienced and interested in histological techniques) IN THE DEPARTMENT OF MICROBIOLOGY, for duties which will consist of assisting with and developing methods of studying tissue infected with bacteria, fungi and viruses using techniques for conventional, immunofluorescence and electron microscopy—The Professor of Microbiology, The Queen's University of Belfast, Belfast, Northern Ireland (September 15).

RESEARCH SCIENTIST (statistician) (with a Ph.D. degree in arts or science with mathematics and mathematical statistics as major subjects, or postgraduate research experience or equivalent standard and duration, supported by satisfactory evidence of research ability) IN THE DIVISION OF MATHEMATICS, C.S.I.R.O., to take part in the respective Divisional research programmes of the Division of Protein Chemistry and the Division of Animal Health (Animal Health Research Laboratory) which are closely located in MELBOURNE—The Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 440/191 (September 23).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with a good honours degree in botany or biology and preferably postgraduate experience in ecological research) to take part in the ecological studies of the Nature Conservancy's Conservation Research Division at its Monks Wood Experimental Station, Abbots Ripton, Huntingdon—The Natural Environment Research Council (E), State House, High Holborn, London, W.C.1 (September 24).

LECTURERS/SENIOR LECTURERS (2) IN BIOLOGY (in the field of genetics); and a LECTURER IN BIOLOGY (in the field of whole plant physiology) in the School of Biological Sciences, University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 26).

READER IN OBSTETRICS AND GYNAECOLOGY at Charing Cross Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (September 19).

CHAIR OF PHILOSOPHY at the University of New England, Armidale, New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

JUNIOR DEMONSTRATOR (non medical, preferably with experience in some branch of endocrinology) IN THE DEPARTMENT OF PHYSIOLOGY—Prof. A. A. Harper, The Department of Physiology, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, 1 (September 30).

READER, SENIOR LECTURER or LECTURER IN MICROBIOLOGY at Monash University, Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, September 30).

RESEARCH FELLOW (organic chemist, with a Ph.D. or equivalent research experience) IN THE DEPARTMENT OF MEDICAL CHEMISTRY, The John Curtin School of Medical Research, Institute of Advanced Studies, Australian National University, to work on the synthesis and reactions of nitrogen heterocycles—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

LECTURER (preferably with experience in the application of physical chemistry to metallurgical problems) IN METALLURGY—The Registrar, The University, Sheffield (October 1).

READER IN GEOGRAPHY at King's College—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 3).

LECTURER (specialist in one or more branches of human geography) IN GEOGRAPHY at the University of Tasmania, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 14).

CHAIR OF BIOCHEMISTRY at the University of Melbourne Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 31).

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (if aged under 22, five passes in the G.C.E. examination including English at "A" level; if aged over 22, H.N.C. or a scientific degree, preferably in biochemistry or microbiology) AT THE FOOD RESEARCH INSTITUTE, NORWICH, for work on

the biochemistry and physiology of micro-organisms—The Secretary, Low Temperature Research Station, A.R.C., Downing Street, Cambridge, quoting Ref. 66/14.

GRADUATE RESEARCH ASSISTANT (preferably with a knowledge of statistics) to participate in a survey of the careers and previous academic discipline and performance, of engineering and science graduates of Imperial College—The Registrar, Imperial College of Science and Technology, London, S.W.7.

HISTOLOGIST (with an honours degree in physiology or zoology or an equivalent qualification and preferably previous experience in histological techniques) IN THE PHYSIOLOGY DEPARTMENT, to be in charge of the histological work of the Department and to collaborate in research, mainly in the field of endocrinology—The Secretary, National Institute for Research in Dairying, Shinfield, Reading, Berkshire, quoting Ref. 66/N/21.

SENIOR/CHIEF (Physics) TECHNICIAN (female) IN THE PHYSICS DEPARTMENT for duties which mainly involve the use of radioactive isotopes in clinical diagnosis—The Secretary, Dudley Road Hospital, Birmingham, 18.

SENIOR LECTURER (preferably with special interests in physiology and research) IN ZOOLOGY/PHYSIOLOGY—The Principal, Barking Regional College of Technology, Longbridge Road, Dagenham, Essex.

SENIOR TECHNICIAN I (with experience in chemical pathology) to work in the new Renal Transplant Unit in conjunction with the Medical Unit—The House Governor, St. Mary's Hospital, Paddington, London, W.2.

SUB-LIBRARIAN (honours graduate with a librarianship qualification, and preferably experienced in the field of science and technology) IN THE ANDERSONIAN LIBRARY—The Registrar, University of Strathclyde, George Street, Glasgow, C.1, quoting Ref. 52/66.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

The Mathematical Association. School Library Mathematics List. Compiled for the Mathematical Association by A. P. Rollett. Pp. 28. (London: G. Bell and Sons, Ltd., 1966.) 1*s.* 6*d.* net. [206

Master Plan for the Loughborough University of Technology. Pp. 143. (Loughborough: The University of Technology, 1966.) 42*s.* [206

Water Resources Board: Hydrometry Division. Crump Weir Design. Pp. ii+92. (Reading: Water Resources Board, 1966.) [216

EMI Electronics, Ltd. Materials Handling Systems. Pp. 13. (Hayes, Middx.: EMI Electronics, Ltd., 1966.) [216

Northern Ireland: Ministry of Agriculture. Leaflet No. 25: Sheep—Management of Upland Flocks. Pp. 12. Leaflet No. 50: Pig Diseases. Pp. 14. (Belfast: Ministry of Agriculture, 1966.) [226

Northern Ireland: Ministry of Agriculture. Leaflet No. 3: Controlled Lighting for Egg Production. Pp. 3. Leaflet No. 26: Sheep Diseases. Pp. 16. Leaflet No. 31: Coccidiosis in Poultry. Pp. 4. Leaflet No. 35: Feeding Bacon Pigs. Pp. 11. Leaflet No. 47: Sheep—Management of Lowland Flocks. Pp. 16. Leaflet No. 132: Farm Planning. Pp. 14. (Belfast: Ministry of Agriculture, 1966.) [236

Ministry of Overseas Development. Report of the Tropical Products Institute 1965: The Report of the Director of the Tropical Products Institute. Pp. viii+31. (London: H.M. Stationery Office, 1966.) 5*s.* net. [236

Department of Education and Science. A Compendium of Teacher Training Courses in England and Wales, 1967–68. (List 172.) Pp. ii+41. (London: H.M. Stationery Office, 1966.) 7*s.* 6*d.* net. [236

General Register Office. Census 1961—England and Wales. Industry Tables. Part 1: Pp. lii+1–220. 48*s.* net. Part 2: Pp. xxx+221–371. 33*s.* net. (London: H.M. Stationery Office, 1966.) [236

Auguste Comte and the Making of Sociology. By Prof. Ronald Fletcher. (Auguste Comte Memorial Trust Lecture, No. 7.) Pp 31. (London: The Athlone Press, University of London, 1966. Distributed by Constable and Co., Ltd.). 5*s.* [276

Forestry Commission Leaflet No. 52: The Fallow Deer. By W. A. Cadman. Pp. 39. (London: H.M. Stationery Office, 1966.) 2*s* 6*d.* net. [276

The Houses of the Royal Society of Arts: a History and a Guide. By D. G. C. Allan. Pp. x+53. (London: The Royal Society of Arts, 1966.) 5*s.* [276

The Scottish Field Studies Association. Annual Report, 1965. A Visiting Biologist's View of Kindrogan Field Centre. By Patrick Coker. The Bryophytes of the Kindrogan Area. By Ursula K. Duncan. Small Mammals at Kindrogan. By Paul A. Moxey. Hoverflies. By Martin Speight. Pp. 27+4 plates. (Glasgow: The Scottish Field Studies Association, 1966.) 5*s.* [276

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FORTY MILLION ENGLISHMEN?

SIR JOSEPH HUTCHINSON will have helped enormously to awaken interest in the problem of how people are to continue living in crowded countries. It is tempting, and easy, for the members of industrialized societies to persuade themselves that imbalance between the land and the population it must support is an affliction of remote parts of Asia and Africa. In his presidential address to the British Association (see page 1053, this issue), Sir Joseph has made it plain that advanced societies such as the United Kingdom are as much in peril. Moreover, he has shown that the task of bringing land and population into harmony is not simply one of matching two large numbers comfortably together, but that it is also necessary for the nature of a nation's agricultural system to be harmoniously in character with the structure of its society. It is true that agriculturalists are diligent in pointing out what risks imbalance may entail, but that is no reason to disregard what Sir Joseph Hutchinson had to say on Wednesday. But what of his attempt to say where the balance between the population and the land should be established in the particular circumstances of Britain? Is the ideal population one of 40 million people, or should it be more, or even less? To say that there should be a balance is one thing. To say precisely where it should be struck is quite another. In particular, it is not a task which agriculturalists by themselves can usefully attempt.

To be fair, Sir Joseph Hutchinson did not invest his figure of forty million with moral rectitude, but, rather, he described it as a "target figure". He arrived at it by general arguments, not arithmetic. He was concerned about pollution of the environment and the water supplies, the increasing competition for somewhere to spend a holiday, the encroachments of the conurbations on the countryside, and the supposed "stress and disorientation" of young people forced to live in cities. It is true that if only the population were smaller, some (though not all) of these problems would disappear. But is that all there is to say? Are these problems so serious that the British population should deliberately seek to shrink in numbers? And are there no benefits to be set off against the evident inconveniences of a densely packed population? Is modern life such a gloomy business?

The admitted nuisances of modern life are, of course, a challenge for societies that wish to go on being known as civilized, but the problems of pollution and waste disposal could be tackled with at least as much success as the Victorian banishment of cholera from London. Shortages of water and other natural resources are often exaggerated, as the committee of the U.S. National Academy of Sciences makes plain (see page 1008, this issue). The scarcity of beaches is a function of the cost of air travel, and it is always possible that twentieth century fashion which sends people rushing to the beaches will be replaced by one that is socially more convenient. (In any case, it would always of course be possible to manufacture extra beaches if the need for them were great enough.) In other words, these nuisances and the scarcities are not absolute but relative. One way of dealing with them is for the population of Britain, and of similar countries, to contract; another is to tackle them directly, and to get rid of them.

City life is a ready scapegoat, and nobody will fail to shrink from the kind of encroachment on the countryside, and the ugliness which accompanies it, which is all too plain on the outskirts of most large cities. Yet this, too, is a relative, not an absolute, problem. There is nothing but indifference to decree that a modern city must be an offence against the countryside. Indeed, there is no reason why modern cities should take up so much space. As things are, the largest cities tend to be hollow at the centre, with the result that the social benefits which could flow from the interaction of large groups of people are somehow dissipated in the diurnal flow of the population in and out of the suburbs. It could well be that many of the social problems dangerously apparent in cities such as New York are consequences not of the large size of their populations but of the lack of care with which the populations have been bundled together.

But who is to see that reason is applied? And how? These are the questions that urgently need asking. The answer is not to create more social scientists, whatever that term may imply, for the problems are at once bigger and simpler. What needs to be accepted is that the development of modern technology has created a range of new opportunities, not an array of strait-jackets. In particular, if society wants clean rivers, it can elect to have them. Clean air is also to be had, if at a price. If the countryside is being ruined, that is because the people who use it—farmers included—have not collectively decided that the countryside is worth keeping. Cities would not be choked with traffic if those who live and work in them were really anxious to have a more rational system of transport. And the great cities of the world could be allowed to become even more populous (though not necessarily bigger on the map) and at the same time still more rewarding to live in if the social complexities of city life were properly acknowledged and dealt with.

Agriculture is important but not central. Sheer starvation is not the only consequence of the rapid increase of the population of the world, and may not even be the most serious. For every stomach that needs to be filled with food, there is a head that must be given some sense that the future will be better than the present. It is not easy to persuade such populations that they must recapitulate the evolutionary steps which more advanced societies have left behind them. In other words, the advice that they should first create a manual agricultural system on the pattern of English agriculture in the eighteenth century, wise though it may be, is likely to smack of what is called neo-colonialism. For this reason if for no other, it is com-

forting that the steady rise of agricultural productivity in the temperate zone of the Northern Hemisphere shows no sign of coming to a halt. For at least some time to come, the war against famine will have to be fought on the prairies of North America. In the long run, countries now undeveloped may practise novel forms of agriculture—or even buy wheat by exporting manufactured goods. At this stage there is no knowing just what will happen, but disaster and a repetition of familiar patterns are not the only possibilities.

MEDICAL RESEARCH

THE Medical Research Council is one of the most successful of the research councils responsible for the support of research in Britain, and the annual report for 1965–66 now published (see page 1009, this issue) is another cheerful story. Good works continue. New projects are being started, and promise to fill glaring gaps in the pattern of what is being done. The clinical research centre now being built will obviously do well, for example, even if it is not entirely clear why it should not have been created by the development of a teaching hospital.

Why does the Medical Research Council persist in getting such good value for its money ? Much probably depends on the way in which the council organizes its grant giving. More than half the annual budget last year was spent on supporting what are called the research units—groups of people ranging in size from a handful to a hundred—who are employed full-time by the council, kept supplied from the same source with equipment, yet free to play a full part in the intellectual life of the university or hospital in which they are sited. Although units like these are not unknown in other fields, the Medical Research Council has made by far the fullest use of them. A part of the reason for this, no doubt, is that it is often easier to specify a field of study than a particular objective, but the council has also been shamelessly but successfully living by the principle that it is better to back a man than a problem. Obviously there is a model here for other research councils to study if not to copy.

That said, however, it should be recognized that there may be snags. One is that the council may not be able to spend as much as it should on the support of research by young graduates, medically qualified and otherwise. Another is that the first charge on the council's budget is bound to be the salary bill of its full-time staff, not to mention their pension rights and other needs. An awkward consequence is that the budget must be less flexible than its total size would indicate. There is possibly a case for saying that the council would be happier with more elbow room within the framework of its budget. And there is certainly a case for asking that in future annual reports the council should substitute for the scant half-dozen pages now devoted to administrative matters a thorough discussion of the evolution of ways in its policies on these and other matters.

NEWS AND VIEWS

Fresh Thoughts on Water

PRESIDENT KENNEDY said that whoever solved the problems of water deserved two Nobel prizes—one for science, one for peace. The committee of the National Academy of Sciences which has just taken a cold look at water resources in the United States will certainly not get the latter for its frank analysis of what is wrong with American policies for managing water. The trouble is, in brief, the "quick fix" approach. Enthusiasts spring up, hot to build desaturation plants, hydroelectric dams near the Grand Canyon or—the champion here is President Johnson himself—clean whole rivers such as the Potomac from top to toe. Each of these plans, the N.A.S. report says flatly, is short-sighted. What is needed is intelligence and an end to rigid, departmental thinking.

There is no question that it is possible to make drinking water by removing the salt from sea water. But it is expensive and, once elaborate installations are set up for the purpose, little thought is given to alternatives which may be cheaper. The President's plan to purify the Potomac is fine—but prepared without sufficient attention to the changes that its proposals would bring to the soil, plant and animal and even human life along the sweep of the river. And, while conservationists see no lack of arguments for opposing the proposed dams on the Colorado River, the N.A.S. report makes a point that will hurt the advocates of water power; not enough thought has been given to the possibility that hydroelectricity will lose its value in the years to come.

In general, the committee found that the United States would have enough water to drink for the rest of the century, even though regional scarcities will persist. But there are big gaps in research into water resources, and the committee hopes that these will be filled. More should be known about the ways in which the environment—particularly when it is overlaid with tarmac—affects water. New techniques should be developed for treating waste water and, above all, people should start thinking about how to tap the underground supplies which constitute 97 per cent of the world's usable water.

Nothing the N.A.S. report said will dampen spirits in California, where mutual congratulations are being exchanged over the completion of a complicated agreement between the federal government, local authorities in Los Angeles and private electricity companies to build the world's largest desalination plant. A quick fix could not be applied in a better spot than parched populous southern California, in a constant wrangle with neighbouring states to use their water. (It now has eyes on the Columbia River far to the north-west.) The Los Angeles station, to be built on a man-made island offshore, will use nuclear power and do double duty, generating 1·8 million kWh of electricity a day while refining 150 million gallons of sea water as well, and at a cost which, if estimates are borne out in practice, will be as little as 23 cents a thousand gallons. The plant will cost $438 million, of which the federal share will be $72 million, and should go into action in 1971.

More Medical Research

THE budget of the Medical Research Council for 1965–66 was comfortably greater than that in the preceding year. According to the annual report now published, £11 million were spent in 1965–66 compared with £9·6 million in 1964–65, an increase of roughly 15 per cent. More than 90 per cent of the council's income consists of a parliamentary grant and another £770,000 is provided by government departments and public bodies in return for services rendered. The council spends most of its income on the current expenses of research units and laboratories throughout the United Kingdom and a comparatively small amount—£373,000 in 1965–66—on new buildings. The National Institute for Medical Research at Mill Hill, London, accounts for one-eighth of the total budget, roughly a half of which, or more than £5 million, is spent on research units and external scientific staff.

The council is clearly pleased with developments at the Clinical Research Centre at Northwick Park in North London. The annual report recalls that in the late fifties the council decided there was a need for a clinical research centre in Britain bringing together "clinical and para-clinical workers". The Northwick Park centre is being built in collaboration with the North West Metropolitan Regional Hospital Board. It will consist of a research institute, a laboratory, lecture hall, and accommodation for patients. The research institute itself will be a substantial building with 100,000 sq. ft. of space, and there will also be supporting facilities—an animal house, workshops, and so on. One wing of the hospital ward block, containing 140 beds, has been specially designed to meet the needs of research workers, principally by incorporating research laboratories into the main structure of the hospital. The annual report for 1965–66 records that Professor G. M. Bull of Queen's University, Belfast, has been appointed director-designate of the centre and that Dr. Richard Doll, of the M.R.C. Statistical Research Unit, has been appointed deputy director. Professor Bull is principally interested in renal and tropical diseases and the study of the balance of electrolytes within the body. Dr. Doll has won a considerable reputation by his statistical analysis of the aetiology of various kinds of cancer and of peptic ulcers.

As in previous years, the annual report also includes a review of the work of the various laboratories and research centres which the council supports. There is also a record of the council's involvement with public affairs, and in particular a reminder that it was primarily responsible for providing the Home Office with the information and advice on which it was actually decided to introduce legislation about the permissible content of alcohol in the blood of car drivers.

According to the report, the total staff of the Medical Research Council was 3,410 at the beginning of 1966. There were 809 scientific staff and 1,402 technical staff, and the total includes the personnel of the 79 research units (four of them abroad) which were in being at the end of the financial year. In the course of 1965–66 one unit (for obstetric medicine at Aberdeen) was closed on the retirement of the director, and three were established at Newcastle (the Reproduction and Growth Research Unit), Aberdeen (the Medical Sociology Research Unit) and at Hammersmith (the Cardiovascular Research Unit). During the same year the council undertook to support ten new research groups, bringing the total to 40. A research group differs from a unit, in the council's terminology, in that its staff is employed by the university or hospital concerned, which also agrees to relieve the council of financial responsibility within five years.

Porter of the R.I.

PROFESSOR GEORGE PORTER formally took up his post as Director of the Royal Institution on September 1, and his chemical research group is expected to take up quarters in the Davy–Faraday Laboratory within a week or so. Preparations for the arrival of Professor Porter and his team have been under way for some time, and substantial alterations have been made to the laboratories and the domestic rooms at the building in Albemarle Street. The members of the institution plan to welcome Professor Porter and to say goodbye to the retiring director, Sir Lawrence Bragg, when the latter returns from the United States in the autumn.

The immediate effect of the change of directors will be a shift in the emphasis of the research programme at the laboratory away from X-ray crystallography, which has been dominant there for several decades. Since the War the Royal Institution has housed the work of Professer J. D. Bernal on the structure of silicates, that of Dame Kathleen Lonsdale on the structure of ice and diamonds and, recently, that of Professor D. C. Phillips on lysozyme. This tradition of X-ray crystallography has, of course, been fostered in the past seven years by the distinction of Sir Lawrence Bragg in that field. Professor Porter has been concerned with flash photolysis at the University of Sheffield, though his career has been varied. In the mid-fifties he spent some years as deputy director of the British Rayon Research Association in between academic appointments at Cambridge and Sheffield.

The question of how the change of directors will affect the outward activity of the Royal Institution is probably more absorbing and more important, though at this stage there is only guesswork to go on. Sir Lawrence Bragg has sought to carry out the task of conveying science to the public by building up a series of lectures on science for schoolchildren—without, of course, diminishing the importance attached by the members of the Royal Institution to the ceremonial lectures on Friday evenings in season. It would be in character if Professor Porter aimed at an older age group. The fact that he does well on television may be a clue to what will happen, although Sir Lawrence Bragg has been no mean performer. But these are only details. The central question is the one that has faced directors at the Royal Institution for the past thirty years—how to make full use of the plant, and make a real bite on public awareness of science, without seriously disturbing the traditions of the place, jealously guarded as they are

Research in Japan

JAPAN maintains itself as a major industrial power even though research expenditure there falls short of that of any other highly industrialized nation. Expenditure in 1964 was £438 million, representing

1·9 per cent of the national income, compared with spending in Britain in the same year of £750 million (2·9 per cent). Of the total more than a quarter was spent by the vigorous chemical and chemical engineering industries. A survey in 1962–63 by the Japanese Ministry of International Trade and Industry showed that, in one sense, Japan is running at a deficit, spending more in royalties on foreign patents than she earns on her own patents; in 1964 the royalties paid on foreign patents amounted to nearly £50 million. Japan's defence estimates for 1966 are paltry in comparison with those of comparable countries (£336 million against nearly £2,500 million for the United Kingdom), but savings in defence do not seem to have released money for research. How has Japan managed to run a successful economy without the expenditure on science and technology which is nowadays regarded as a prerequisite ?

The answer may be that Japan squeezes the maximum value out of each yen spent on research. An example of this is her admittedly unsophisticated space research programme, in which eight satellites are to be launched for a total cost of less than £22 million, spread over a long period of time. Somehow Japanese scientists have managed to carry out the programme at an annual expenditure of less than £3 million, and without the escalation of costs that has bedevilled British projects and joint European endeavours such as ELDO. In 1964 the outlay for each research worker in Japanese research was £2,250, the total budget of £438 million being divided among 195,000 research workers. This is a remarkably low figure, to be compared with a figure of about £10,000 per annum in the United Kingdom. While the figures may not be strictly comparable—it is not quite clear what definition of a qualified research worker is applied in Japan—they do indicate a wide disparity. It would seem that Japan maintains industrial momentum by an understanding of cost-effectiveness in research. There is no doubt, too, that in the short term the speed at which scientific research can be translated from the laboratory bench to the factory floor is more important than the volume of work in progress. There is, for example, a flourishing Japanese electronics industry.

New Zealand Science

EXPENDITURE on science in New Zealand is increasing steadily, both absolutely and as a percentage of the Gross National Product. This is plain from the report of the National Research Advisory Council for the year ended March 31, 1966. The report says that expenditure has increased by a factor of 3·2 since the year ending in 1955, although the increase in prices since then implies a real increase in expenditure by a factor of 2·3. During this period the number of scientific staff employed in government departments has increased by only 55 per cent. Table 1 shows the actual expenditure on science in New Zealand in alternate years in the past decade (excluding sums of money spent on buildings). The table also shows the way in which the number of scientists employed in government departments has increased.

Table 1

	Actual expenditure £(000)	As percentage of G.N.P.	Scientific staff in government departments March 31	Expenditure at 1966 prices
1955	2,733	0·27	568	100
1957	3,547	0·35	592	123
1959	4,110	0·36	628	133
1961	4,912	0 38	684	153
1963	5,759	0 40	754	172
1965	7,428	0·42	819	209
1966	8,709*	0·46*	882*	233*

* Estimated.

Of the total expenditure on science in the current financial year, the council estimates that 74 per cent will be spent by government departments, of which the Department of Scientific and Industrial Research takes slightly more than half. Universities spend £800,000, or between 8 and 9 per cent of the total in New Zealand on research.

As a result of a detailed review of research policy carried out in previous years, the council has recommended to the Government of New Zealand a considerable increase in the scale of building research, which is estimated to cost £330,000 in the current year and which will be increased by some 18 per cent in the course of the following three years. Advice for industry and research in transport are also to be increased, and an attempt is to be made to bring the meteorological service up to strength. Work at the Institute of Nuclear Sciences (of the Department of Industrial and Scientific Research) will be extended, principally by the purchase of a 20,000-c. cobalt source.

Foot in the Door

THE Committee of Vice-Chancellors and Principals of England and Wales is delighted with a modest step towards rationalizing entrance requirements to universities now agreed upon by 29 out of 35 universities. The universities concerned have agreed to adopt one or other of two patterns of examination qualifications as a condition for university entrance. One pattern includes five passes in General Certificate of Education examinations, with at least two passes at the Advanced level; the other, intended to cater for students at technical colleges and other such institutions, involves four passes in GCE examinations, three of them at the Advanced level. Particular universities will continue to accept students with various other patterns of qualifications in GCE examinations but, apparently, the number of different patterns acceptable to the universities of England and Wales has been reduced from 19 a year ago to 9 this year.

The universities which have chosen to stand aloof are not all black sheep. In fact, the University of Lancaster requires less of intending university students than either of the two patterns now uniformly adopted. The Universities of Warwick, Keele and Exeter differ from the general pattern in requiring either a language or English qualification from those wishing to become scientists. The universities of Oxford and Cambridge, predictably, have not yet modified their matriculation requirements sufficiently to conform with the general pattern, although there is optimistic talk that Cambridge is considering relaxing the requirements of two language qualifications for intending scientists.

None of this, of course, diminishes the strongest pressure of the universities on the schools—the way in which particular departments or faculties in British universities require particular subjects to have been studied at school by intending students. It remains possible for a student to satisfy entrance requirements

without being able to persuade a university department to teach him what he wants to learn. (The continuing complexity of course requirements is in the edition for 1967–68 of *A Compendium of University Entrance Requirements for First Degree Courses in the United Kingdom*, Association of Commonwealth Universities, 14*s*. 6*d*.) But the Committee of Vice-Chancellors has embarked on a study of that problem too, even though a commonly agreed solution cannot be reached for two years or more. Five sub-committees of the vice-chancellors' committee are studying the course requirements of universities in the physical sciences and mathematics, biological sciences, medicine, humanities and the social sciences. Those serving on these committees are said to be surprised at the way in which universities are prepared to relax previously stringent requirements, but it remains to be seen whether this tendency will be sufficient to allow the schools, in their turn, to liberalize curricula.

Too Much Room at the Top

THE general impression in British universities that the shortage of laboratory technicians is most acute at the top of the salary scale has been confirmed by a study carried out by the Edinburgh branch of the Association of University Teachers. While most of the research workers in the various departments of the university were dissatisfied with the number of technicians available, a "considerable body" was convinced that the number of junior technicians was too high compared with that of more senior people. Among the reasons for this imbalance is the lack of a suitable career structure for university laboratory technicians. In particular, salary scales are too low and, the authors of the study infer, "the university is at present becoming a training ground for technicians which is increasingly valued by outside employers". This conclusion, based on a systematic survey of seventeen departments at the university, is borne out by the impressions of universities elsewhere.

One of the recommendations which arise from the survey at Edinburgh is that there should be a general improvement of salary scales and that in particular the Whitley Council scale should be adopted by all and not merely by some departments. Another suggestion is that the more senior people should in future salary reviews be more generously treated than those at the bottom of the ladder. It is also suggested that promotion to senior technician should not be determined by vacancies at this level, but that this grade should be recognized as one to which qualified and capable technicians would in due course automatically graduate.

The authors of the survey also have a number of suggestions to make about the training of technicians. In the first place it was thought that more could be done by the teaching staff of departments to help with the training of technical staff. Apparently, at Edinburgh day release for junior technicians is habitual and many departments even allow day release to technicians who are older than 21. In the long run it is thought that much could be done to bring more technicians into research work, especially during vacations, and also that those public bodies which award research grants should take fuller account of the need of technical assistance and the need that technical assistants should have some promise of a secure job, possibly beyond the duration of the research grant concerned.

These findings and suggestions will no doubt be widely echoed in other British universities. More is likely to be heard of this issue in the autumn, when the Committee on Scientific Manpower under Sir Willis Jackson is due to report on this and other matters.

Use More Wood

THE Forest Products Research Laboratory of the Ministry of Technology has been devoting more of its time and resources to the considerable problems of wastage in the use of timber. In the introduction to the annual report for 1965, Mr. B. K. Blount, chairman of the laboratory's Steering Committee, says that timber and timber products represent 10 per cent, or £500 million, of all imports into the United Kingdom in a year, and that imports of sawn timber and plywood alone account for more than £200 million. While the supply of home grown timber is increasing, so is the demand; and it is likely that Britain will continue to depend on imported timber for most of its supply. The report says, however, that "economies could be achieved by improvements in processing methods, by the wider application of correct methods of timber usage and by the utilization of low-quality wood which is at present neglected or discarded".

The laboratory plans to work in close collaboration with the public authority recently set up by the Ministry of Public Buildings and Works to evaluate new building components and materials; test work on timber materials will be carried out at the Forest Products Laboratory where it is thought that the interest of the authority will complement its own increasing appreciation of the structural value of wood in building. New methods of construction with timber provide the substance of several investigations being undertaken for industry.

Computing Centre

THE National Computing Centre is slowly building up steam, and has just ordered its first computer, an English Electric Leo Marconi *KDF*9, which should be installed by next April. In the meantime, recruiting is under way and work has begun on the site at Manchester.

The present cost of the centre, which was established as a public company in June, is being borne by the Ministry of Technology, but the Ministry's grant of £290,000 for the first year of operation will not go far when the *KDF*9 alone costs £400,000. Professor Gordon Black, the director of the centre, hopes that it will be independent of the government within a few years, through dues from member organizations and payment for services.

One of the centre's first acts has been to support a one-year course for systems analysts being started this October at Imperial College, London. Professor Black considers that this course, which will combine computer techniques with optional subjects ranging from accountancy to operational research, fulfils a major need. There is a possibility of another course being offered at the London School of Economics early in 1967. Professor Black also sees the centre as advising industry at large on the use of computers. He hopes

to be able to set up a catalogue of computer programmes to include all the programmes available. Professor Black is not interested in doing specific jobs for industry but rather in "getting the industry off the ground". With this in mind he hopes to provide software for hospitals, for the gas and electricity boards, and for industry at large in cases where a number of firms are working on the same problem. He also believes that a major task of the centre will be to work towards a standardization of computer languages—there are 12 in use in Britain at present which he hopes to reduce to two by the time the regional computing centres are established.

University News:

London

DR. BRIAN DONOVAN has been appointed professor of physics at Westfield College. Prof. R. W. Tiffen has been appointed to the chair of mathematics, tenable at Birkbeck College. The title of professor has been conferred on the following members of the teaching staff of Chelsea College of Science and Technology, which was admitted as a School of the University of London as from August 1: Dr. A. H. Beckett (pharmaceutical chemistry); Dr. G. M. Bell (applied mathematics); Dr. S. E. Dicker (physiology); Dr. J. F. Dippy (chemistry); Dr. A. K. Jonscher (solid state electronics); Dr. K. W. Keohane (physics); Dr. A. G. Morton (botany); Dr. R. D. Purchon (zoology); Dr. H. J. V. Tyrrell (physical and inorganic chemistry). The title of professor has been conferred on the following: Dr. L. S. Bosanquet (mathematics, in respect of his post at University College); Dr. S. P. Datta (medical biochemistry, in respect of his post at University College); Dr. F. F. Heymann (physics, in respect of his post at University College); Dr. R. M. H. McMinn (anatomy, in respect of his post at King's College); Dr. J. L. Mongar (pharmacology, in respect of his post at University College).

Announcements

THE Enrico Fermi Award of the United States Atomic Energy Commission has been awarded for the first time to scientists outside the United States. The Atomic Energy Commission announced last month that Professor Otto Hahn, Professor Lise Meitner, and Professor Fritz Strassmann, of Germany, have each been awarded a third share in the $50,000 prize together with the customary gold medal. Professor Meitner, who worked with Hahn and Strassmann at the Kaiser Wilhelm Institute on the fission of uranium, now lives in Cambridge, England.

Two new journals, *Chemistry and Physics of Lipids* and *Cell Biology Communications*, are being published at the beginning of January 1967 by the North-Holland Publishing Company. The former will appear once a quarter, and the latter will be bi-monthly but may later become a monthly.

A COURSE on gas chromatography–mass spectrometry has been organized by LKB Instruments, Ltd., in collaboration with the Gas Chromatography–Mass Spectrometry Unit of the University of Glasgow, and is to be held in the University of Glasgow from October 3. Further information can be obtained from Dr. P. R. Masek, LKB Instruments, Ltd., LKB House, 137 Anerley Road, London, S.E.20.

THE autumn meeting of the Chemical Society will be held at the University of Sussex during September 21–22. Further information can be obtained from the General Secretary of the Chemical Society, Burlington House, London, W.1.

THE seventh International Astronautical Congress will be held in Madrid during October 10–15. Further information can be obtained from the International Astronautical Federation, 250 Rue Saint-Jacques, Paris 5.

AN international technical meeting on "The Chemistry of the Aquatic Habitat" will be held at the Hydrobiological Institute, Nieuwersluis/Amsterdam, during October 10–16. Further information can be obtained from Dr. H. L. Golterman, Hydrobiological Institute, Rijksstraatweg 6, Nieuwersluis.

THE twelfth annual meeting of the Health Physics Society will be held in Washington, D.C., during June 18–22, 1967. Further information can be obtained from J. C. Villforth, Radiological Health Laboratory, 1901 Chapman Boulevard, Rockville, Maryland.

AN international meeting on "Liver Regeneration" will be held in Montecatini Terme during October 29–30. Further information can be obtained from the Scientific Secretary Office of Milan, 6 via Modica, Milan.

A SYMPOSIUM on "Electrode Processes", sponsored by the University Grants Commission, will be held in the University of Jodhpur during November 1–5. Further information can be obtained from Prof. R. C. Kapoor, Department of Chemistry, University of Jodhpur, India.

THE twenty-fourth annual Pittsburgh Diffraction Conference will be held at the Mellon Institute, Pittsburgh, during November 9–11. Further information can be obtained from Dr. Sidney Diamond, Applied Research Laboratory, United States Steel Corporation, Monroeville, Pennsylvania.

A CONFERENCE on "Small-angle Scattering of Electrons and X-rays", arranged jointly by the Electron Microscopy and X-ray Analysis Groups of the Institute of Physics and the Physical Society, will be held in the Institution of Electrical Engineers, London, during November 17–18. Further information can be obtained from the Meetings Officer of the Institute of Physics and the Physical Society, 47 Belgrave Square, London, S.W.1.

A SYMPOSIUM on "Fundamentals of Gas-Surface Interactions", co-sponsored by the U.S. Air Force Office of Scientific Research of the Office of Aerospace Research and the General Atomic Division of General Dynamics Corporation, will be held in San Diego, California, during December 14–16. The symposium will be concerned with the results of theoretical and experimental investigations of the following general topics: the surface and its characteristics; adsorption and reaction of gases on or with surfaces; scattering processes, including energy and momentum transfer at the gas-surface interface. Further information can be obtained from Dr. H. Saltsburg, chairman, General Dynamics—General Atomic Division, P.O. Box 608, San Diego 12, California.

ERRATUM. Figs. 2 and 3 accompanying the article "Extracellular Recording of Converging Input on Cortical Neurones using a Flexible Microelectrode", by L. T. Rutledge and J. A. Duncan (*Nature*, 210, 737; 1966), did not adequately show full details of the wave forms obtained in this experiment. Reprints with a clearer reproduction of the diagrams are to be had from Prof. L. T. Rutledge, Department of Physiology, University of Michigan, Ann Arbor, Michigan.

ERRATUM. In the article entitled "An Operator Model of Crossing-over", by Dr. H. L. K. Whitehouse (*Nature*, 211, 708; 1966), line 40 of page 711 should read "$\left(\frac{A}{A+B} = 0\right)$ shows the frequency with which hybrid".

JOHN DALTON AS A HISTORICAL FIGURE

By FRANK GREENAWAY

Deputy Keeper, Department of Chemistry, The Science Museum, South Kensington, London

ENDS are often beginnings as well, in the study of the history of science as in anything else.

The formal celebration in Manchester in September of the bicentenary of the birth of John Dalton (1766–1844) is likely to mark not only the end of a period of re-assessment of Dalton's life and work, but also the beginning of a new phase in the study of the chemistry of the nineteenth century. Other commemorations of men far surpassing Dalton in intellect and output have not promised so distinct a change in historical studies. Why make so bold a prediction ?

I suggest three reasons. First, the history of chemistry is in need of a radical re-appraisal. Two recent generations of scholars have gone back to the origins of scientific chemistry in the eighteenth century and have set out in some detail the work of the first men to bring high genius to bear on the problems of the constitution of matter: Black, Priestley, Bergman, Cavendish and the like, with the figure of Lavoisier nearly always somewhere in the picture. The composite picture has some completeness, and although a great deal of work remains to be done in correcting perspectives (as Smeaton has shown in his study of Fourcroy[1]) and in the editing of manuscripts (as Uno Boklund has done with Scheele[2]), there is no longer as much justification as there was for the one-sided concentration on eighteenth century chemistry of recent years. The chemistry of the past thirty years (that is to say, chemistry based on a unified quantum-mechanical view of matter coupled to new physical methods of observation, and new concepts of manufacture and social use) has apparently little obvious connexion with nineteenth century chemistry. The historical continuity needs to be re-presented if the modern chemist and the modern historian are to find any interest in new work in the history of chemistry between 1800 and 1930.

The second reason behind my claim for the importance of the Dalton commemoration is directly linked with the first. The effect of Dalton's atomic theory on nineteenth century chemistry was profound. By studying its interpretation by his contemporaries, its consequences, its modification at the hands of others, its competitors, its evolution into a comprehensive structural system, we can hope to follow at least one (possibly the only) continuous thread of thought through the chemistry of the nineteenth century. Moreover, and paradoxically as I shall show, it opens up new lines of sight on an almost unexplored field: the social and economic history of chemistry.

The third reason for the modern relevance of Dalton's life is the increasing interest in the methods of the scientist, work on which has by and large neglected the special problems of chemical thought. Students of scientific method have examined very closely patterns of enquiry in physics, astronomy and biology. The unthinking dismissal of chemistry as a rough and ready pursuit destined to be swallowed up in a generalized physics has been made quite out of date by new adventures of ideas in fields like molecular biology, where concepts of organization springing directly from an older chemistry have shown that chemical organization of matter has a significance which abstract physical thought cannot penetrate. If the state of chemistry in 1800 is compared with that in 1850 the differences are enormous, and nearly all have something to do with the factors which made up Dalton's theory of combination. Yet we cannot be clear or specific about how these changes were initiated in the minds of individuals. It might be argued that the mental processes of the eighteenth century chemists, few in number, remote in experimental resources from modern chemistry, could give us little help in understanding present-day chemical discovery. This is not true of the nineteenth century chemists who were making discoveries under intellectual and economic pressures which have many features similar to those of to-day. The chemical thought of the nineteenth century lies ready to be narrated and analysed just as much as does its practical achievement. Right at the beginning, provocative, even exasperating, is the mental development of John Dalton, putting to us the twin problems: what prepares a man to turn a picture of organization into a quantitative method, and what turns a quantitative formulation into a visual device ?

Dalton's achievement was a simple one: he showed how it was possible to relate microscopic measurements on substances entering into combination to a quantitative theory of microscopic organization. That is to say: elements consist of characteristic atoms; these atoms combine according to simple laws; therefore combination on the large scale will reflect these simple laws, and, vice versa, the examination of the actual massive behaviour of elements in combination will give information about the constituent atoms. The ultimate importance of this idea was not seen in Dalton's day, and its powerful secondary effects, which were not fully felt until well into the twentieth century, are not adequately recognized even to-day. It did, however, alter the whole of chemical thought within a few years of its widespread publication.

Four years elapsed between the time Dalton reported to a meeting of the Manchester Literary and Philosophical Society on October 21, 1803 (ref. 3), that he had achieved some success in a new speculation, and the time Thomas Thomson set out Dalton's contentions in his own *System of Chemistry*[4]. In the next year, 1808, Dalton published Part 1 of Volume 1 of his *New System of Chemical Philosophy*, that strange, ill-balanced book, which began as an attempt to emulate the amateur piece-meal physics of the eighteenth century and ended as a clumsy unfinished attempt to systematize the new professional chemistry of the nineteenth. Scattered through the later parts (Volume 1, Part 2 of 1810, and Volume 2, Part 1 of 1824) are some good observations, showing that Dalton had learned his chemistry tolerably well. The rest is of moderate merit, and already old-fashioned in style. But Chapter 3, "On Chemical Synthesis", of the 1808 part, in which Dalton explains his rules of combination, is the work of genius. Or at least, it seems so now. To Dalton's contemporaries, the atomic hypothesis was one to be approached carefully and critically.

The idea of the atom was as old as the Greeks; it had never really been lost to sight, and attempts to interpret the behaviour of matter in terms of particles endowed with appropriate mechanical properties had been made over and over again. Newton and Daniel Bernoulli had been able to explain a good deal in different ways by mechanical atomic hypotheses and there were a score of good chemists who had offered variants on an old theme. None, however, linked quantitative chemical behaviour with the essential corollary: the uniqueness of chemical species. Until Lavoisier, they could not. Nearest to success were the partly quantitative notions of Bryan Higgins and his nephew, William Higgins, but neither came anywhere near the universality of Dalton's theory[5].

The issues of the time were by no means clear or simple. Were compounds always formed in definite proportions ? Were organic compounds subject to the same laws as inorganic ? Were the so-called elements, the number of which continued to increase beyond belief as new discoveries were made, really distinct, or was there some

underlying simplicity? Was the behaviour of gases in any way connected with chemical constitution and, if so, what were its laws?

These were only a few of the problems. Dalton's theory did not solve them all, but it did give them a common basis, and although Davy, Wollaston, Berzelius and other leaders chose to adopt modifications of Dalton's ideas, rather than swallow them whole, the debates now had a point of reference. Supremely important was the fact that chemical analysis could now, through a system of proportions related to atomic weights, be given a unity it had never possessed before.

The laws of combination were so closely linked with atomism that it was easy to suggest that the atomic theory was directly inferred from them. Thomas Thomson had believed this, and for fifty years after Dalton's death it was good enough for most histories. Then in 1898, Henry Roscoe and Arthur Harden[6] examined the remains of Dalton's manuscripts and began a second phase in Dalton studies, by relating Dalton's published statements to the chronology of his notebooks. Their chief service was to show how important were Dalton's speculations about the nature of gases. Soon afterwards A. N. Meldrum[7] in a long series of papers urged that chemical evidence had in fact been in Dalton's mind, but that it was the result of experiments on the reaction between nitric oxide and oxygen which Dalton had carried out in his work on the composition of the atmosphere.

Nothing important was added to this in the next forty years. Historians of chemistry continued to treat the origin of Dalton's theory as a problem of no great importance, until in 1939 Partington[8] attempted to repeat Dalton's nitric oxide experiments, and showed that Meldrum's reliance on them was unjustified. Again little followed until J. B. Conant created the opportunity for a new enquiry by including the atomic-molecular problem in his series of *Harvard Case Histories*. L. K. Nash[9] wrote this case-history, basing the Dalton section on the older evidence. Soon after, in 1956, Nash[10] brought the Dalton problem back to the forefront by rejecting his earlier appraisal in favour of a novel and fertile suggestion. He analysed Dalton's work on gas-solubility, the work which has given rise to "Dalton's Law of Partial Pressures", had brought Dalton his early reputation, but had never been treated by historians of chemistry as at all closely connected with Dalton's chemical thought. Nash thus made Dalton the physicist the antecedent of Dalton the chemist, and in so doing made Dalton once again into a human figure, of unknown mental character, worthy of complete re-examination.

The material for a new biographical approach to the Dalton problem was reviewed in 1958[11], the stimulus given by Nash to consideration of Dalton the physicist suggesting that more attention be paid to Dalton's early work in meteorology, and on other phenomena such as the hydrological cycle.

Since that time many scholars have been preparing the ground for the commemoration to be held this bicentenary year. Much new work has been published already. Guerlac[12] has asked for reconsideration of the influence of Richter on Dalton, Brock and Knight[13] have examined the controversies over atomic theory in the mid-nineteenth century, Thackray[14] has shown that we need not be put off by the loss of much Dalton manuscript by enemy action in 1940, and has brought to light many unexamined Dalton sources. Dalton does not play the same kind of central part in early nineteenth century chemistry as Lavoisier in the generation before him, because the changes he brought about were neither so personal, so revolutionary, nor so clear-cut, nor was the main body of his work so comprehensive. Dalton was more of a catalyst than a reagent, but the several conceptual reactions catalysed by his ideas could well occupy scholars for a long time.

Some tasks are obvious: Partington's *History of Chemistry* has gathered a mass of facts which it will be the work of years to digest into critical specialist histories. The judgments and perspectives which this monumental work fails to provide are still to come, in almost every part of the vast territory which Partington has covered detail by card-index detail. There is still, however, another territory, wide open for exploration: that of the history of applied chemistry. Sherwood Taylor[15] tried to relate theoretical progress to industrial techniques, but his work is no more than an elementary introduction. Although there have been other works on some technical or economic aspects of industrial chemistry, no deep and detailed work has been written showing the relationships of theory, practice and social influence.

The irony is that Dalton made virtually no personal contribution to industrial chemistry, either by any discovery of manufacturing or economic significance at large, or by any service to the industries of Manchester, a fact which his prosperous commercial friends sadly regretted[16]. In another field Dalton unknowingly contributed to social change. As society was affected more and more by technical change, chemical factors came closer to daily life, in articles in common use and in the urban environment. The Pure Food Acts (1860 onwards) and the Alkali Acts (1864 onwards) are examples of the legislation which was made possible by the rise of analytical and control methods based on these same quantitative methods. One could follow this argument through to the present day, showing how the stoichiometric systems of nineteenth century chemistry still underlie most large scale practical chemistry.

I have said nothing about the rise of the theory of valency implicit in Dalton, which survives in so much of chemistry except at the theoretical frontier. The architectural chemistry which serves the molecular biologists is in direct line from Dalton and Wollaston. If physicists and biologists are rather over-fond of philosophizing about their most recent findings, chemists seem to philosophize too little. This is a pity, because the organization of matter, which is what chemistry is all about, is the key to so much that puzzles us in our material existence and, if research on the biochemical interpretation of mental processes comes to anything, may be the key to some of the operations of the mind.

Philosophically, the emergence of theories of the organization of matter at several levels and the study of the kind of discovery which must be made to stimulate thinking about organization present problems which bear on modern science as much as on older science. Dalton stands at the beginning of a new phase in the appreciation of material phenomena, and his thought, and the interpretation of his thought by others, deserves a deep study. It might well modify our ideas about the scope of scientific thought through the nineteenth century and even help us to understand our present thinking about matter at deeper levels than the atomic-molecular.

Be that as it may, the figure of John Dalton is likely to be looked at more closely now than ever before, to the great benefit of the yet unwritten history of chemistry as a major activity of thinking men.

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JOHN DALTON THE ATOMIST

By DR. J. ROSE
Blackburn College of Technology and Design

"Thou knows . . . no man can split the atom"—DALTON

JOHN DALTON was born at Eaglesfield in Cumberland on September 6, 1766, of Quaker parentage. He himself remained a Quaker all his life, and his teachers, and friends during his childhood and early manhood, such as Elihu Robinson and John Gough, were Quakers. His father taught him arithmetic and navigation, while the wealthy Robinson interested him in meteorology and the blind Gough helped him with the studies of languages and mathematics. Dalton had the urge to teach at an early age. At the age of twelve he set up a school and at the age of fifteen he was working as an assistant in a Kendal school. He delivered, when twenty-one, a series of lectures on natural philosophy and was appointed in 1793 tutor in mathematics and natural philosophy at New College, Manchester, which was a dissenting institution fathered by the Warrington Academy. There he taught chemistry, using as his textbooks Lavoisier's *Elements* and Chaptal's *Chemistry*. He resigned from this post in 1800, and supported himself for the rest of his life by giving lectures and private tuition, while carrying out research.

To his research work Dalton applied simplicity of mind, boldness of imagination and systematic thinking. As an experimenter he was self-taught, and he used cheap materials and simple apparatus. His attitude was coloured by his preoccupation with meteorology. He kept a meteorological journal, beginning with observations at Kendal in 1788 and continuing until his death on July 27, 1844. It was this study of climate, water and air that eventually led him to the atomistic concept of matter. In his work which led towards his atomic theory Dalton worked quite independently from William Higgins (1762–1825), who was to make substantial contributions to an understanding of the forces between particles, the concept of multiple proportions and some modern aspects of reaction mechanism. Higgins did not attach any importance to atomic weights, which were to be a fundamental feature of Dalton's theory, nor did he propose any methods for their determination.

In order to follow the trend of reasoning and experimentation that led from a study of air and water to the atomic theory, it is necessary to consider certain observations made by Dalton in his *Meteorological Observations and Essays*, the first edition of which was published in 1793. Dalton asserted that pure air is an intimate mixture of "gasses" and the aqueous vapour is not chemically combined in the atmosphere, while vapour pressure is determined by the temperature alone. These concepts were quite novel and original, since hitherto air had been considered a chemical compound of nitrogen and oxygen, while water vapour had been considered a state of solution in the atmosphere, similar to that of salt in water, and that in some cases it could actually be transformed into air.

In his *New Theory of the Constitution of Mixed Aeriform Fluid and particularly of the Atmosphere*, Dalton criticizes Lavoisier's statement[1] that "the atmosphere is a compound of all the fluids, which are susceptible of the vaporous or permanently elastic state, in the usual temperature, and under the common pressure". According to Dalton, the last five words should be omitted. He elaborated his theory of mixed gases in four experimental essays read at fortnightly intervals to the Manchester Philosophical Society beginning on October 2, 1801, the starting point being Newton's concept of repulsive forces between particles.

In the first essay, he propounded a theory which is equivalent to what is now called the "Law of Partial Pressures" (William Henry restated this in 1804 as "every gas is a vacuum to every other gas"); he did not, however, use the term "atom" but instead "ultimate particle". The second essay was concerned *inter alia* with measurements of vapour pressures of water from 32° to 212° F, and calculations of these above 212° F by means of a rule; the former results lacked precision, while the latter were erroneous because they were based on an unsound rule. Of considerable significance was Dalton's finding that water vapour in the atmosphere behaves like any other gas in a mixture of gases.

In the third essay he showed that the quantity of water evaporating in a given time was proportional to the vapour pressure, while in the fourth essay he dealt with the expansion of different gases by heat and stated that all gases expand in the same way on heating. (It should be noted, however, that Charles, Gay-Lussac and Dalton share the honour of this discovery[2].) The ideas propounded by Dalton in the material published in 1801 were supplemented by a paper presented in 1803 on the diffusion of gases in which he showed that different gases mix uniformly. The quantitative aspect of what was to become known as Dalton's law of diffusion was not given until more than 25 years later by Graham.

Despite the ingenious and forceful presentation of the new theory of mixed gases, most of the leading scientists and textbook writers of the day reacted violently to Dalton's new ideas. Even his old teacher and friend John Gough was a persistent critic of Dalton's theory. Dalton's theory did indeed contain a serious flaw, because it was partly based on Newton's statical theory of repulsions; this accounts for the discrepancy between Graham's law of diffusion and the theory of mixed gases. The introduction of the kinetic theory of gases, as a result of the work of Dalton's pupil, J. P. Joule, removed some of the objections and clarified the subject of mixed gases. The important step in the evolution of the atomic theory was Dalton's attempt to adapt Newton's theory, in which air was considered as an element, by considering air as a mixture of gases, in order to explain gaseous diffusion. While in 1801 Dalton did not concern himself with particle sizes, two years later on the basis of chemical evidence—the formation of steam—he arrived at the conclusion which he later stated in his *New System of Chemical Philosophy* in the form[3] " . . . every species of pure elastic fluid has its particles globular and all of a size; but that no two species agree in the size of their particles, the pressure and temperature being the same". Oddly enough, Dalton anticipated Avogadro's hypothesis in 1801, but abandoned it[4], basing his change of mind on Davy's and Priestley's experimental results[5]. An entry in his notebook of 1803 contains an explicit denial of the "hypothesis". Despite this, the stage was now set for further developments culminating in the atomic theory.

It is clear from Dalton's notebook relating to September, 1803, as analysed by Partington[6], that he was aware of the following principles:

(*a*) Matter consists of atoms that are indivisible and indestructible.

(*b*) All atoms of a given element have the same invariable weight, whatever their combination with atoms of other elements or conditions, while atoms of different elements have different weights.

(*c*) The weight of a particle of a compound is the sum of those of its constituent atoms.

(*d*) The laws of fixed multiple and reciprocal proportions underlie the formation of particles composed of atoms of various elements.

(*e*) On the basis of the atomic weight of hydrogen taken as unity, it is possible to arrange those of other elements in an order of increasing values.

Indeed, on page 244 of his notebook an entry for September 6, 1803, gives the earliest known table of atomic weights. A printed report of a lecture given by Dalton to the Manchester Philosophical Society on October 21, 1803, gives another table of atomic weights on the hydrogen scale.

There were, however, a number of erroneous statements in Dalton's accounts of the atomic theory, such as that "nitrous acid" (N_2O_3) is a compound composed of NO and NO_2, while NO_2 represents "nitric acid". Apart from these and other relatively minor blemishes, the entries in his notebook in 1803 contained correct versions of the main part of his atomic theory; he was the first to use letter symbols for elements only. (Earlier William Higgins had assigned single letters to elements and compounds, but had been inconsistent in his notation[7].) For example, on page 361 of Dalton's notebook the characters for the elements are shown as follows: ⓗ or ○ for hydrogen; ○ for oxygen; Ⓐ or ⊙ for nitrogen; Ⓢ or ⊕ for sulphur. The symbols for compounds were: ○○ for water; ○⊙ for ammonia; ⊕○ for "sulphurated hydrogen"; ○⊕○ for sulphuric acid. As a result of Davy's intervention, Dalton gave a fairly complete account of his theory to the Royal Institution on December 22, 1803.

It is thus evident that Dalton was able to formulate his chemical atomic theory in 1803. The question arises, however, as to how Dalton actually arrived at his atomic theory. In his lectures to the Royal Institution in 1810, he attributed the origin of the theory to the study of mixed gases. But Meldrum[8] showed that Dalton had proposed a number of different accounts of the origin of the theory. He sometimes ascribed it to his study of Richter's table of equivalents, on other occasions he attributed it to his work on mixed gases, oxides of nitrogen, or olefiant gas and "carburetted hydrogen". A recent view[9] based on the discovery of a lecture syllabus of 1805 is that the study of the properties of water vapour and the calculation of the atomic weights of its constituent elements led Dalton to atomic weights of other substances.

Another plausible view, suggested by Nash[10], is that the immediate point of departure was the study of the solubility of gases in liquids, particularly since Dalton tried to explain why different gases have different solubilities. This opinion is supported by the fact that Dalton's paper on solubility, published in 1805, contains a table of atomic weights of elements and particle weights of compounds. This volume also contains a paper on the composition of the atmosphere, which implies the law of definite and multiple proportions based on the study of the oxides of nitrogen. Dalton's second book, *A New System of Chemical Philosophy*, published in three parts in 1808, 1810 and 1827, summarizes among other things his findings in connexion with the new atomic theory. The actual description of the atomic theory is given on pp. 211–220 of Part 1 which was printed in Manchester in 1808; the section is entitled "On Chemical Synthesis". The object of the book[11] is "to show the importance and advantage of ascertaining *the relative weights of the ultimate particles, both of simple and compound bodies, the number of simple elementary particles which constitute one compound particle, and the number of less compound particles which enter into the formation of one more compound particle*" (ibid., p. 213). He was successful in achieving his aims with the exception of the determination of formulae, particularly those of the oxides of nitrogen, as he admitted in 1827 (ref. 12). The reason for this was that Avogadro's hypothesis was required to obtain "an accurate knowledge of combining proportions".

The crowning achievement of Dalton's life was without doubt the formulation of the "chemical" atomic theory. He brought together quantitative chemistry and the atomic concept of matter, inferring microscopic properties from macroscopic measurements—the latter are themselves a reflexion of the atomic theory. Inorganic chemical analysis was given a rational basis by the prediction of theoretical compositions of substances and by a comparison of such with those experimentally determined. These ideas were later used in the realm of organic analysis and chemistry.

Dalton made other substantial contributions to science. In his lectures to the Manchester Literary and Philosophical Society (115 papers) he dealt with numerous topics including the elements of chemical philosophy, respiration and animal heat, aurora borealis, the absolute zero of temperature, steam engines, thermal expansion of gases, acids and bases, alkalinity of water, composition of steel and colour blindness. He was the first not only to prepare ammonium carbonate and to introduce the use of standard solutions in volumetric analysis, but also to demonstrate that the solubility of calcium hydroxide decreases with rise of temperature. One of the earliest definitions of the dew point was given by Dalton in 1799. He was able to disprove Rumford's theory that heat circulates in fluids by convection only, and showed that water is a poor conductor of heat. His procedure of adiabatic expansion was adapted in 1819 by Clement and Desormes to the determination of the ratio of specific heats of a gas. He contributed substantially to the elucidation of the composition of "inflammable air" by distinguishing between methane and ethylene (olefiant gas), and is also considered to be the discoverer of butylene. There is some inkling in his papers of polymerism (ethylene and butylene) and isomerism; he devised a tetrahedral formula for oxamide and postulated two different structural formulae for gelatine and albumin. The paper given on April 16, 1844, the last in his life, dealt with rainfall in Manchester during the preceding 50 years. In fact the last entry in his diary on July 27, 1844—the day of his death—was "little rain this day".

Dalton's services to chemistry were recognized and rewarded during his lifetime. In 1822 he was elected a Fellow of the Royal Society, and received the Royal Medal in 1826 with a eulogy from the then president, Sir Humphry Davy. Four years later Dalton was awarded Davy's place as one of the eight foreign associates of the Institut de Paris. In 1832 he became D.C.L. of Oxford, and a year later he was awarded a Civil Pension of £150 per annum (increased to £300 in 1836) and presented at Court. The University of Edinburgh conferred on him the degree of LL.D. in 1834, on the occasion of the meeting of the British Association for the Advancement of Science. The public contributed £2,000 to a marble statue of Dalton during his lifetime. He was the president of the Manchester Literary and Philosophical Society for 27 years until his death in 1844. He was given a public funeral, all the shops and warehouses in Manchester on the route to the Ardwick Cemetery being closed as a mark of respect.

[1] Lavoisier, A. L., *Traité de Chimie*, i, 31 (1789).
[2] Partington, J. R., *A History of Chemistry*, **3**, 771 (Macmillan, London, 1962).
[3] Dalton, J., *A New System of Chemical Philosophy*, Part 1, 187 (S. Russell (for R. Bickerstaff), London and Manchester, 1808).
[4] Dalton, J. (ref 3), 70–71.
[5] Partington, J. R. (ref. 2), 778–782.
[6] Partington, J. R., *Scientia*, **49**, 221 (1955).
[7] Higgins, W., *Comparative View*, 46, 48, 192 and 262 (J. Murray, London, 1789).
[8] Meldrum, A. N., *Chemical News*, **102**, 1 (1910).
[9] Greenaway, F., *Endeavour*, **25**, 73 (1966).
[10] Nash, L. K., *Isis*, **47**, 101 (1956).
[11] Dalton, J. (ref. 3), 213.
[12] Dalton, J., *A New System of Chemical Philosophy*, Part 1, 2, 351 (G. Wilson, London, 1827).

BOOK REVIEWS

MEN WITH THEIR K'S

Memoirs of a New Man

By William Cooper. Pp. viii + 277. (London : Macmillan and Co., Ltd., 1966.) 25*s*. net.

THE heroes of the novels of the scientific administrator who writes under the name of William Cooper have now got to the top. No longer provincial schoolmasters or ambitious red-brick scientists, they have now all got their K's. Whereas *The Struggle of Albert Woods* showed the author as a scientific John Braine, except that he seemed anxious to demonstrate that scientists fight their way to the top in a more urbane fashion than arts men, his latest book is concerned with the intrigues that take place in Oxbridge colleges and the corridors of power. But again the author's irony and humanity civilize the struggles he portrays.

The narrator is a metallurgist, a professor and Fellow of Clarendon College, Oxford—a mirror image of a recent foundation elsewhere. He is also a part-time member of the National Power Board, a vast amalgam of the A.E.A. and the nationalized fuel and power industries. In its London headquarters, and in its research stations, the intrigues among the members of the Board are the outcome of the rivalry between scientists, engineers, and arts-trained administrators. The chairman is an ex-Civil Servant, extremely tall, brilliant, and quite ruthless, but with a contempt for the scientists and engineers that precludes attempts at simple identification.

At Oxford the intrigue is about the appointment of a research fellow, and about the part that non-scientific fellows should play in a college primarily founded to support science and technology. It is slightly complicated by a love affair between the successful candidate and the narrator's daughter. The latter's ambivalent attitude towards this affair and his understanding relationship with his daughter are portrayed with the author's characteristic charm, and so are the scenes of his family life with the usual aristocratic-born wife. Somehow even a description of the middle-aged couple, slightly drunk after a party, experimenting with one of the more difficult positions of the *Kama Sutra*, neither offends nor invokes any psycho-analytic speculation.

Unfortunately men at the top are not as interesting as men on the way up. The dynamism of the author's previous novels is lacking, and, although the conflicts of the new men and their methods of resolving them will amuse the "in group" of those who would have had the chance to participate in or observe such conflicts in real life, this novel will not be likely to have a wide appeal. It is well written, as all the author's novels are, but the story develops slowly and the characters, recognizable as types though they are, except for the narrator hardly come to life. The introduction at the end of a coronary as a *deus ex machina* to solve the Power Board's conflicts of personal power leaves unsatisfied the interest that has slowly been built up.

This is not so good a novel as William Cooper's previous novels and it is difficult to see where his hero can go from the top. But those who enjoy his writing and are fascinated by the subjects with which he deals will nevertheless eagerly await the next one.

AUSTEN ALBU

CHARGED PARTICLES

The Special Theory of Relativity

By J. Aharoni. Second edition. Pp. x + 331. (Oxford: Clarendon Press; London: Oxford University Press, 1965.) 70*s*. net.

THIS is the second edition of a book which was first published as recently as 1959. The new edition contains a much expanded treatment of relativistic charged particle electrodynamics, but is otherwise unchanged.

Aharoni's avowed aim is to make available to students with modest mathematical background the entire mathematical machinery which has grown up around the special theory of relativity and the Lorentz transformation. He has in mind the calculus of tensors, including the spinor calculus, and throws in some group theoretical aspects for good measure. In addition, he covers the formal aspects of the classical theory of fields, scalar and vector, together with the variational principle in its most general form, that is, allowing for the variation of the boundaries and the conservation laws which follow. Here and there, one finds allusion to quantum concepts; so in particular in the last, big chapter on spinors where such concepts as "Hilbert space" and "observable" are quite casually introduced.

The dust cover flap states that "as regards the physical content, it is assumed that the reader is already acquainted to some extent with classical dynamics, Maxwell's theory, and elementary quantum theory". One is tempted to add "knowledge of the physical content of the special relativity theory is also assumed". To be sure, there is an introductory chapter on space and time in inertial systems, in which the formalism of the Lorentz transformation and the standard implications is set up in some painstaking detail, but even here the emphasis is on formal aspects: discussion of the Michelson experiment, the Doppler effect, etc., is relegated to an appendix in small print. From there on, everything is formal and mathematical. The pace is quite leisurely and all calculations are certainly performed in very great detail. First we have the tensor representations of the three-dimensional rotation group, the covariant version of Maxwell's theory, and this is followed by a chapter on general field theory. Next is a chapter on relativistic particle dynamics which lays emphasis on electron theory, radiation reaction, and the Dirac model, and covers somewhat sketchily the Feynmann–Wheeler absorber model for radiative reaction. Rohrlich's recent contributions to the old self-energy and stability problem for the point electron are quite adequately covered, but it seems odd that the truly covariant criterion for radiation is missed out. I found this much the best part of the book.

There is a brief chapter on relativistic hydrodynamics and then a long chapter on spinors. The relationship of the unimodular group to the Lorentz group is spelled out without previous careful elucidation of its relationship to the rotation group in three dimensions. This is worked in piecemeal. The discussion of ray representations is rather unsatisfactory and the "infinitesimal" developments are sketchy. Still, the technique of the relativistic spinor calculus ("dotted and undotted" spinors) deserves wider dissemination, and this book covers the ground in its formal aspects. Many readers perhaps may still ask "What is it all about?"

There will probably be students who will find this book very helpful, and for others it may act as a curtain raiser. I find myself at variance with the author's declared sentiment that painstaking attention to the calculational aspects of the formalism *ipso facto* paves the way to an understanding of physical theory.

Layout and printing are as one has come to expect of the Clarendon Press.

S. ZIENAU

GEOMORPHOLOGY MEDLEY

Précis de Géomorphologie

Par M. Derruau. Quatrième edition. Pp. 415+61 planches. (Paris: Masson et Cie., 1965.) 45 francs.

Principes et Méthodes de la Géomorphologie

Par J. Tricart. Pp. 496. (Paris: Masson et Cie., 1965.) 84 francs.

Techniques in Geomorphology

By C. A. M. King. Pp. ix+342. (London: Edward Arnold (Publishers), Ltd., 1966.) 40*s*. net.

THE name of a science is no doubt best understood as what scientists working in the field do; but it is notorious that "geomorphology" in its roots lends itself to an amorphous spread that spills over into a number of orthodox sciences, and not all geomorphologists speak the same language. Tricart and King are scarcely in direct line of descent from Derruau, or quite in harmony with one another.

Derruau's book, in its fourth and much revised edition, accepts geomorphology to be in essence physical geology. With only slight changes in balance it might well be recommended as an introductory text for would-be geologists. It begins with problems of crustal tectonics, including discussion of isostasy, continental drift, cratonic and geosynclinal contrasts, and orogenic cycles, as a basis for detailed study of the growth of landforms—although in doing so it assumes a knowledge of Earth structure that perhaps is too much to expect in junior students. Nearly half the book is very thoroughly descriptive, with some analysis, of the processes of erosion, particularized in chapters on the work of rain and rivers (Davis a little obtrusive), ice, wind, and the sea. An attractive feature of the description is the way in which kinds of landforms are correlated with the variable interaction of agents, when climate controls process. The influence of rock-foundation on relief is dealt with in a section that combines the elements of petrology with primary igneous forms, basins of sedimentation and classes of sediments, metamorphism, and tectonic structure; and the author does not overlook the significance of geological history, revealed in stratigraphy, in an understanding of the evolution of landscape. The many illustrations are orthodox but excellent in relevance and quality; and altogether the book is impressively attractive and readable, if not quite what a geologist would be prepared to call geomorphology.

Tricart, not always unemotional in his dialectic—he is bitterly critical of the malign influence exerted by the inductive speculations of W. M. Davis—defines the unexceptionable central concern of geomorphology as the study of the relief of the Earth's surface; but in doing so he relates geomorphology so closely to other disciplines that he takes over from them topics that normally would interest pedologists, hydrologists, sedimentologists, and even botanists. He writes as a practising geologist who takes for granted the foundations of physical geology on which a growing geomorphology rests. The relief of the Earth's surface includes relief in past times, and palaeo-geomorphology embraces stratigraphical correlation, including correlation esoteric in palynological, tephrological, and geochronological techniques.

This said, Tricart is more concerned with principle and method than with fields to be covered, his central theme being the need for system and order in the organization and explanation of what the geomorphologist observes. System, however, while acceptably justified by argument on what ought to be done, tends to be imposed and formal. This is notably so in a "taxonomic" classification of the units of geomorphological study into eight grades of magnitude from continents and oceans to yardangs and joint-partings and mineral decay, and in a gradation of events (secular orogenies, catastrophic landslips) in segments of a time-scale ranging from geological eras to hours or minutes. He lays much emphasis on the uses of maps and aerial photographs, and on ways of constructing maps so as to increase their usefulness and content of information, and map drawing is formalized in an exhaustive array of symbols to cover almost all possible kinds of landform elements. Statistics and morphometry are given a place as new techniques, but the treatment is slight.

Tricart is stimulating, not to say provocative, in his polemics. He builds a framework into which geomorphology may be put. He brings his own kind of order to what has hitherto been a certain waywardness in method. He rightly directs attention to the need for discipline in geomorphological practice and theory. And he summarizes methods appropriate to a variety of geomorphological problems. It is disappointing that in his analysis of actual examples and in the use of illustrations he does not always reach the measure of his intention or discriminate finely enough between the important and the relatively trivial, and that in specifying the bounds and contacts of geomorphology he follows trails beyond its limits and blurs its field by making it accretory and diffuse.

King's book, although covering part of the same ground, is far more modest in both manner and content. In the result it is far more instructive and substantial. Its bias is towards a geomorphology that on the one hand does not lean unduly on a limited physical geology, and on the other avoids plunging too deeply into geophysics and stratigraphy and petrology. It brings out the relationship of structure and process with great clarity, constantly doing so by reference to appropriate examples that give reality to theoretical interpretation. Geomorphology emerges as a tracing of the evolution of the existing landscape through the integrative correlation of a great variety of landforms, although the main objective of the book is neither to define the science nor to provide a conspective summary of its range. At the same time, King is fully aware of the diverse approaches which geomorphologists may follow, and her exposition offers instances of most of them as they are revealed in real examples.

The central purpose of the book, however, is to discuss techniques, from field surveying to the use of models. An early chapter stresses the importance of direct observation of form and character in the analysis of landscape, and of observation supplemented by the skilled use of maps and aerial photographs, by the need commonly met for a refined contouring, and by excavating and sampling. A succeeding chapter similarly stresses observation and experiment in the study of geomorphological processes—river flow and river transport, nivation and glaciation, periglacial and permafrost features, mass movement in avalanches and landslips, wind transport, and wave and tidal action. Discussion of the ways in which models may throw light on the correlation of theory with observation and experiment includes critical comment on the propriety of applying results from conceptual and scale models to processes in nature, and very full descriptions of actual models and their advantages and limitations. A chapter on cartographic and morphometric analysis as a systematization of a multitude of landform elements, and sediment analysis as a guide to erosion and transport, leads to an assessment of the value and significance of statistics in geomorphology and of the validity of geomorphological conclusions based on statistical pointers.

Parts of King's book are a little uneven in being unusually elementary or unusually specialist; and some of the examples quoted need more rigorous treatment than they are given. But to cavil at this page or that would be ungracious, when the bringing together of so much information and guidance, simply and directly put, offers nothing but good to the young student as much as to the professional geomorphologist.

T. NEVILLE GEORGE

SCIENTIFIC ENDEAVOUR AND ACHIEVEMENT

Charles Scott Sherrington

An Appraisal. By Ragnar Granit. (British Men of Science.) Pp. xii + 188 + 14 plates. (London and Edinburgh: Thomas Nelson and Sons, Ltd., 1966.) 42*s*. net.

THIS book is by an eminent Scandinavian physiologist on a great British physiologist. It was written in English and is not a translation; its smooth reading reveals Prof. Granit's command of the language. He is the Director of the Nobel Institute for Neurophysiology in Stockholm, and he twice worked in Sir Charles Sherrington's laboratory in Oxford in the late twenties. At that period Sherrington, freed from exacting commitments such as being President of the Royal Society, was able to devote much of his time to an active but not too large Honours School and to a group of graduate research workers, many of whose names are to-day familiar in neurophysiology and neurology. His work then was concerned with details of the reflex activity of the spinal cord, including the part played by the muscles and the synaptic connexions of their nerve fibres in this activity.

Sherrington (1857–1952) felt that posterity should judge a man by his work and he expressed dislike of certain long detailed biographies, hoping that nobody would attempt such an exercise on him. This wish has so far been respected. It is true that there are excellent obituaries in the journals, but further biographical writing has been tackled differently. In E. G. T. Liddell's absorbing book *The Discovery of Reflexes*, the state of nerve physiology and histology is described as it was before and during Sherrington's work for *The Integrative Action of the Nervous System*. Granit sets out to emphasize how some of the main problems of neurophysiology stem directly from what Sherrington did.

After a brief biographical and historical chapter, Granit goes on to deal with the concepts underlying Sherrington's work, and particularly with inhibition in the nervous system regarded as an active process, which is one of the main landmarks with which Sherrington's name is now associated. His work on this subject is described, as is that of his pupils and contemporaries. Then come chapters on the wider outcome of this pioneer work. First, there are the advances made possible by the development of amplifying devices; particularly by Adrian (Sherrington's joint Nobel Laureate) in recording single nerve impulses. Then comes the investigation of individual parts of the reflex arc by the use of microelectrodes. Eccles, by developing this technique, was able to penetrate single motor horn cells in the spinal cord and so get information about the activities of the synapses of an individual cell. This continued Sherrington's "pulling to pieces from the top downwards", but he also prophesied that "the elemental may not prove to be the simple" and one of the problems of to-day is, so to speak, to put the animal together again. Something of this has been taking place in Granit's own laboratory and he writes of the work on reflexes and motor control, and particularly on muscle spindles, which Sherrington had described in 1894.

The final chapter on Sherrington's writings is largely taken up with a summary of *Man on his Nature*. This is not an easy book to read, but it is lit up with passages of beauty and insight. There are also quotations from Sherrington's book of poetry *The Assaying of Brabantius*. For Sherrington, writing was always bound up with the desire to make everything "absolutely clear", and he maintained that this could be done more precisely in Latin or French than in English.

Granit's book is one to read at leisure and from which to allow something of the whole personality of Sherrington to emerge, including his capacity for the accumulation of knowledge, the power to ask the right questions in physiology and the skill to get the answers, all in an individual who was a poet, something of an artist, a lover of nature and a man who could command devotion and respect from those who came in close contact with him and worked under him. SYBIL COOPER CREED

HOW TO SUCCEED IN PAINTING

The Application of Surface Coatings

(Paint Technology Manuals, Part 4.) Pp. xiii + 345. (London: Chapman and Hall, Ltd., 1965. Published on behalf of the Oil and Colour Chemists' Association.) 35*s*. net.

The Testing of Paints

(Plant Technology Manuals, Part 5.) Pp. viii + 171. (London: Chapman and Hall, Ltd., 1965. Published on behalf of the Oil and Colour Chemists' Association.) 35*s*. net.

"'TIS a Dutch proverb, that 'paint costs nothing', such are its preserving qualities in damp climates". This quotation from Emerson serves as a heading to the foreword of Part IV of the Paint Technology Manuals published by the Oil and Colour Chemists' Association. The proverb holds only to the extent that the paint is of good quality, and that the surface has been prepared and the paint properly applied. While it is easier to succeed in painting than in business without really trying, the chances of success are enormously increased by treating the exercise in a careful, planned scientific manner.

Fortunately, this has been realized for many years in large-scale factory application of paint, and the realization is spreading to still wider circles of paint users all the time. The present two volumes, covering the whole of paint technology, should do much to aid the process of education.

Part IV continues the system of earlier volumes whereby each chapter is written by a specialist in that topic. By far the largest portion (280 pages) is concerned with various techniques for the industrial application of paint, and the application of decorative and protective coatings for constructional materials gets only 50 pages. After a chapter on equipment for industrial finishing, the material is dealt with in terms of the substrate to be coated, as is almost inevitable in view of the very specific problems found in each industry.

A severely practical outlook permeates the book, and those who expect to find reasons why things happen, or should be done, will seldom find them here; it is essentially a plain man's guide, and at that level will serve a very valuable function. But it is disappointing to find a book devoted to the application of surface coatings that completely ignores any treatment of the rheological properties of paint, and gives no background data on adhesion. Some theoretical background is given in the chapter on industrial metalware, but otherwise such material is conspicuous by its absence.

Part V, *The Testing of Paints*, differs from the other volumes in that it is essentially the work of one man, F. G. Dunkley, and the treatment is more unified. After a short chapter on test methods for production quality control, there follow chapters on tests for basic properties and tests for specific properties. Basic properties include viscosity, gloss, hardness, flexibility, durability and adhesion, while specific properties cover chemical resistance, fire retardance, electrical properties, basic paint tests and film thickness (surely this is a basic property ?).

The emphasis is on those methods of test in widespread use in Britain, but due reference is made to other tests where these have been found to be useful; an appendix collates the relevant American Society for Testing Materials, British Standard and Ministry of Defence (DEF) specifications. There is a fairly full bibliography,

but this loses much of its value by the unsystematic way in which the papers are listed. A very wide range of test methods is covered, and there will be few topics on which guidance cannot be found.

There will undoubtedly be a call, in due course, for second editions of these and other volumes in the series. When these are being considered, it would be well to consider anew the purpose they are designed to serve. When the series was conceived (and indeed during the repeated childbirths of successive volumes), the aim was to cover the requirements of the City and Guilds paint technology courses. There have been major changes in the educational pattern for paint technology since then, and the process of change is still taking place; it is likely that a rather different manner of treatment will best meet the changed circumstances. One would like to know whether the series is aimed at the technician or the technologist, for they have different needs, and whether there is sufficient scientific background material.

L. VALENTINE

ASPECTS OF TITANIUM CHEMISTRY

The Organic Chemistry of Titanium

By Raoul Feld and Peter L. Cowe. Pp. viii+213. (London: Butterworth and Co. (Publishers), Ltd., 1965.) 52*s*. 6*d*.

IT is estimated that the number of scientific publications doubles every 15 years. Quite clearly the modern chemist must grow to rely more and more on good review articles and review texts if he is to keep abreast of developments within his subject or even within his branch of the subject. This book has been planned as a comprehensive review of the chemistry of organic titanium compounds. Its value will consequently be a function of the success with which the literature has been covered and assessed critically.

The coverage of the literature is certainly impressive. The title of the volume could be more informative, since "organic titanium compound" is taken to include all titanium compounds of which carbon is a constituent, not merely those in which it is the ligand atom. Thus, apart from the obvious interest of the book to workers in organo-metallic chemistry, the chapters on alkoxides, halide complexes and low valent titanium compounds will be welcomed by inorganic chemists. It is regrettable, however, that the authors have not been more critical; much of the text reports rather than reviews the contents of the literature. To quote three examples from several, the status of *bis*(*cyclo*pentadienyl) titanium was in question when the book was written (the situation is now much clearer), yet the reader is given no indication of this fact. Also, it is likely that the concept of oxidation number is of doubtful value for compounds such as *tris*(2,2′ bipyridyl) titanium which are too readily accepted at their face value in Chapter 9. The diagram on page 7 would probably have been better omitted.

The book has three particularly strong features. The coverage of the vast patent literature is laudable. The authors have managed to filter out the more optimistic claims and report only those patents which substantiate their conclusions with at least a minimum of chemical or physical data. Since a certain proportion of the work in the field of titanium chemistry in the future may consist of more detailed examination of known compounds, the excellent tabulations of these compounds together with their physical properties are likely to place many future workers in the authors' debt. Predictably the uses of titanium compounds have been emphasized. The chapters on titanium co-polymers and Ziegler catalysts are among the strongest in the book. It was the more surprising therefore to search in vain for an assessment of titanium tetrachloride as a catalyst for the Friedel–Crafts reaction.

The book should find a place in most chemical libraries. The price is not excessive by present-day standards. Misprints are few and are confined almost exclusively to equations and formulae. The failure to report authors' initials in the references may give rise to some minor irritation, but both author and subject indexes appear to be comprehensive and accurate.

WILLIAM R. MCWHINNIE

STEROID RESEARCH

Methods in Hormone Research

Vol. 5. Steroidal Activity in Experimental Animals and Man, Part C. Edited by Ralph I. Dorfman. Pp. xiii+342. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 104*s*.

EVER since the synthesis of norethisterone (17α-ethynyl-17β-hydroxyestr-4-en-3-one), a search has been made for other useful progestational agents. Miyake and Rooks make a valuable contribution to this volume with a well presented review of those progestational steroids which have become available during the past few years. Data are provided showing the influence of nearly every substitutional site in steroids on the physiological activity of these progestational materials.

The chapter on anti-gonadotrophic steroids by Dorfman and Kincl is also a useful contribution. It deals mainly with the relationships between the structure and activity of those steroids which inhibit the biosynthesis, and sometimes the release, of gonadotrophins. The authors point out that work involving the parabiotic rat technique has certain limitations, and does not allow selective measuring of the inhibition of follicular stimulating hormone in the rat—if indeed there is any selective differentiation from luteinizing hormone to be measured. The introduction to this chapter unfortunately gives the impression that anti-gonadotrophins operate mainly at the pituitary rather than the hypothalamic level. Nevertheless, valuable data on the inhibition relationship are included. This sort of work and that of Miyake and Rooks with the progestational steroids prove that the combination of organic chemists, endocrinologists and physiologists is extremely powerful. Success in dealing with the problems of over-population will surely only be obtained by more work of this kind.

Other chapters in the book provide useful information on the effect of steroids on the central nervous system, the treatment of human malignancies with steroids, and the relative potencies of androgens. The editor is to be congratulated for supplying once again a valuable reference book. It is an excellent addition to the earlier volumes of the series.

D. EXLEY

SECONDARY METABOLISM

The Biosynthesis of Natural Products

An Introduction to Secondary Metabolism. By John D. Bu'lock. Pp. x+149. (Maidenhead: McGraw-Hill Publishing Company, Ltd., 1965.) 28*s*.

THIS book meets a need which has been increasingly felt by scientists interested in natural products. The title is somewhat misleading in that the book is not concerned with the main metabolic pathways by which amino-acids, fats, and carbohydrates are synthesized in the cell or broken down. Its subject, which has been of special interest to the organic chemist and less so to the biochemist, is the formation of a great variety of compounds such as terpenes, alkaloids, antibiotics, and a variety of other substances, chiefly by green plants or fungi. In this field of science enzyme methods have been little used, and investigations are increasingly based on the use of putative labelled precursors in whole cells or whole organisms. In this secondary metabolism, the substances concerned are

not functional constituents present in all plants, but can only be obtained from particular species of plants. Apart from this restricted taxonomic distribution, these compounds often have no obvious metabolic function but represent a fascinating challenge to the organic chemist, to the plant biochemist, to the taxonomist and to any scientists interested in the tremendous variety of chemical substances which are produced by this "secondary" metabolism.

Apart from a full account of our general knowledge in this field, the use of plant genetics in secondary metabolism is described in detail. Dr. Bu'lock also links the specific problems of secondary metabolism with general enzymology where this is appropriate. The volume represents a comprehensive and at the same time concise account of this increasingly important field. It is, however, written primarily from the standpoint of the organic chemist interested in natural products, and assumes in the reader a fairly sophisticated knowledge of reaction mechanism and modern theoretical organic chemistry. It is doubtful whether the botanist or mycologist whose chemical knowledge may have been acquired 15 or 20 years ago will find it easy to follow the detailed chemical arguments which are used. The author is, however, careful to point out the limitations of our present technique, and the fragmentary character of our knowledge, and indicates clearly where further progress may be expected.

A. NEUBERGER

STICKINESS AND CANCER

Hyaluronidase and Cancer

By Ewan Cameron. Pp. xiii + 245. (London and New York: Pergamon Press, Ltd., 1966.) 50*s*. net.

DR. CAMERON'S stimulating book presents a thesis which is stark in its simplicity. The excessive secretion of hyaluronidase is the beginning, the middle and the end of the problem of cancer.

According to Dr. Cameron, neoplastic cells arise as the result of the interaction of any carcinogenic stimulus with the protein–mucopolysaccharide ground substance, as a result of which the latter becomes increasingly viscous and impermeable. Adjacent normal cells attempt to maintain their normal environment by the increased release of hyaluronidase. "Each cell in the body is firmly embedded in a sticky viscous cement of ground substance" which is attached by hyaluronidase. Contact between the cells is reduced and this in turn permits or stimulates cell division. The preferential nutrition of tumour cells is the result of local increased stromal and capillary permeability evoked by hyaluronidase secretion. Malignant infiltration and the formation of metastases are likewise due solely to the action of the enzyme on the surrounding normal tissues and the walls of blood and lymph vessels.

Dealing first with the application of Dr. Cameron's ideas to carcinogenesis, it is very difficult to understand how his mechanism of carcinogenesis could account for the *in vitro* malignant transformation of normal cells by viruses, carcinogens, X-rays or "spontaneously". Dr. Cameron appears to be either unaware of, or to ignore, the considerable body of work published in recent years establishing the existence of tumour-specific antigens in many experimentally induced animal tumours, and the possible role of the immune response in the development of these tumours.

On the subject of the irreversible nature of the neoplastic transformation, the author considers that it is clearly a matter of cellular inheritance, but rejects the view that interaction between DNA and a carcinogen is involved, even in the initiation, with the rather cavalier phrase "there is not a shred of evidence to support its validity". Potter's deletion theory and Warburg's hypothesis are not mentioned.

When it comes to the role of cell contact in regulating cell proliferation, the author probably finds more common ground with other workers in this field. He carries the cell contact mechanism of cell proliferation much further than most, particularly when he ascribes loss of cell contact solely to the secretion of hyaluronidase. This enzyme, incidentally, has so far proved remarkably ineffective in dispersing cells in tissues. Tumour cell nutrition is dealt with without mentioning the greater capacity for glycolysis or other changes in metabolic processes which have been extensively investigated.

The release of hyaluronidase by tumour cells has been considered by many investigators to play a part in malignant invasion, but the evidence is contradictory, and Dr. Cameron's review of it does not convince this reader that hyaluronidase is the only, or even the main, enzyme system contributing to invasion. Assessing the evidence is not made easier when the role of proteolytic enzymes is dismissed in four lines.

Dr. Cameron's arguments are presented very clearly and with a coherence which is, perhaps, gained at the cost of omitting a considerable amount of established work which does not fit in with his thesis.

This book should serve a useful purpose by stimulating many investigators in the field of cancer research to re-examine carefully the basis of many of the concepts currently held. The author has made a very extensive survey of the literature pertaining to the role of hyaluronidase in cancer, which is extremely useful. G. C. EASTY

BEHAVIOUR AND NUCLEIC ACIDS

Macromolecules and Behavior

Edited by John Gaito. Pp. ix + 197. (Amsterdam: North-Holland Publishing Company; New York: Appleton-Century-Crofts, a Division of Meredith Publishing Company, 1966.) 29 guilders; 58*s*.

THIS little book has brought together some fascinating articles in a rather unusual field. They are directed in the main at the role of RNA and DNA in determining behaviour, although nearly all the experimental evidence relates to the changes in the macromolecules following on experimental procedures. Except in the last chapters, where K. H. Pribham and others present their theoretical treatment of the basis of memory, there is often a fairly tenuous connexion between the experimental data presented and rather gross behavioural patterns. Nevertheless, the book has gathered between its covers a very valuable and readable series of papers.

The chapter by Ochs on axoplasmic flow is an excellent summary of the recent work on transport of molecules of many kinds along the axoplasm. The chapter by Pevzner is a review of, among other topics, cyto-spectrophotometric methods; in the original material presented, it reveals the great extent to which such techniques have developed in the Soviet Union. Pevzner's chapter is the most provocative, as he modestly, but thoughtfully, raises the question in his brief summary as to just what the relationship is between the nucleic acids and behaviour, and just how far the biochemists can at present go in finding a meaningful answer. The difference in the time scales between the laborious and time-consuming procedures of the biochemist on the one hand, and the speed of action in nerve cells as demonstrated by the electro-physiologist, on the other, is so enormous that one rightly asks: can the two approaches ever meet?

In spite of such difficulties, this book shows that in a surprising number of instances correlation is possible, even with the attempt to show that treatment of certain types of elderly individuals with RNA leads to an improve-

ment of their general condition and, less convincingly, their memory functions.

The book is the outcome of a symposium held in 1964. The contributors to the symposium have clearly had ample time to rewrite their communications and bring them and their bibliographies up to date. While there may be a good reason for it, one name seems to be a regrettable omission from the list of contributors—Hydén of Gothenberg. His work is cited in some context or other by almost every author, and rightly; he is the pioneer in this field and should surely be regarded as the father of many of the current ideas and techniques. It would have been a pleasing gesture if this admirable little book had been dedicated to him.

J. L. MALCOLM

Human Geography

By Aimé Vincent Perpillou. Translated by the late E. D. Laborde. (Geographies for Advanced Study.) Pp. xx+522+37 plates. (London: Longmans, Green and Co., Ltd., 1966.) 50*s*. net.

THIS book could have been written only by a great scholar, for it fills its pages with example after example of man's evolution, his adaptation to environment, the techniques he has evolved to emancipate himself from that environment and in his manner of settlement. The width of Prof. Perpillou's knowledge is quite astounding. He has cast his net throughout the world in his apparently effortless quest for illustrations of man's conditions. Especially noteworthy are his examples drawn from otherwise largely inaccessible sources. In places one might wish perhaps that academic axes were not being ground quite so noticeably—for example, in his attitude to Huntington and Meitzen.

British students may be surprised by the socio-anthropological point of view of the work, though this is much in the tradition of Brunhes and de la Blache. Certainly those familiar with recent publications by British geographers on the topic will here find a totally different conception of the subject. This method is shown at its best in the sections on tropical lands where the interaction of social anthropology and more "civilized" geography is most fruitful.

One major criticism is the paucity of illustrations of a more graphic nature, especially of locational maps. The publishers and editor of the series are, however, to be congratulated on having had the initiative to invite a doyen of French geography to write specifically for English-speaking students. The author has undoubtedly set a very high standard of learning for his readers.

R. T. JACKSON

Open Tubular Columns in Gas Chromatography

By L. S. Ettre. Pp. xix+164. (New York: Plenum Press, 1965.) $4.95.

THE dramatic increase in the volume of literature devoted to gas chromatography has made the investigation of any one aspect an arduous task in terms of the effort required to abstract relevant information. Monographs produced by acknowledged experts in a given field are, in this respect, particularly welcome. This book falls into such a category. Written essentially for the practising gas chromatographer, the book is confined to practical details. When theory is introduced, the frills of mathematical derivation are limited and only those definitions and equations necessary for the proper understanding, evaluation and operation of the technique are introduced. In the simple presentation the two typographical errors which occur in equations (18) and (24) are easily recognized.

After the brief historical introduction and statement of definitions, the practical implications of the Golay equation are discussed and comparisons made with conventional packed columns together with other factors such as sample capacity. Small-bore open tubed columns (also referred to as capillary or Golay columns) have not been used as extensively as packed columns except where necessity has dictated, no doubt because of the relative ease with which the latter may be made. The author's simple account of the various techniques which may be used to coat the internal walls of the column should help to dispel any mystery associated with the process. Among other factors, surface effects are considered in the preparation of the column, both in their elimination to reduce tailing, and deliberate introduction to increase surface area and consequently stationary phase loading. The consideration of the relatively new technique of "packed capillary" columns in which some form of internal surface coating is used to obtain a relatively large surface capacity of stationary phase follows as a natural corollary.

The very small sample sizes which are necessary in order to avoid overloading of conventional open columns inevitably involve some modifications to the sample inlet system. The fourth section deals with these requirements in the gas chromatographic system and includes such important matters as stream splitting design.

After the listed bibliography, which contains 286 references, a supplement is appended, giving details of the various methods which may be used for the calculation of the "air peak" time.

E. A. WALKER

Plasma Diagnostic Techniques

Edited by Richard H. Huddlestone and Stanley L. Leonard. (Pure and Applied Physics: a Series of Monographs and Textbooks, Vol. 21.) Pp. xii+627. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) $19.50.

As pointed out in the introduction to this book, progress in experimental plasma physics depends on the development of suitable diagnostic techniques. Such development has been rapid, and it is convenient to have a single volume such as this in which most of the commonly used methods are described.

The book consists of an introduction and twelve review articles, each by an author who has specialized to some extent in the technique described. Separate chapters cover basic macroscopic measurements, magnetic probes, electric probes, spectral intensities, line broadening, optical and ultra-violet techniques, X-ray spectroscopy, far infra-red measurements, optical interferometry, microwave techniques, and particle measurements. Most of these chapters form natural subdivisions, although that on X-ray spectroscopy could have been more naturally and economically divided between the chapter on optical and ultra-violet techniques and that on spectral intensities, which contains a useful section on the various models of non-thermal plasmas, besides much else relevant to X-ray work. Each chapter is followed by a very complete bibliography. This layout suffers from the disadvantage that different methods of measuring a given parameter appear in nearly every chapter, and it would be difficult to choose the best for a given experiment without reading the entire book, despite the comprehensive index. It does, however, permit all the techniques included to be discussed systematically. There are, of course, some omissions. For instance, it is surprising that the chapter on optical and ultra-violet techniques makes no mention of the special properties of interferometers when high light-gathering power is required. Relatively small space is given to techniques based on the scattering of laser-generated radiation, but this is almost certainly a result of the rapid progress made with these methods since the volume was compiled. In general the book contains a considerable quantity of information, and should be a useful addition to the available literature.

D. D. BURGESS

MEETINGS

FERTILITY

THE annual conference of the Society for the Study of Fertility was held at Churchill College, Cambridge, during July 5–9. The meeting opened with a group of papers on the interactions between foetal and maternal tissues in early pregnancy. Oxford workers have studied the transplantation of eggs and trophoblast in the hamster and the mouse; they showed that when trophoblast tissue was transplanted into the testis some trophoblast cells reached remote areas of the host, apparently by migration along blood vessels (D. Billington). By the same technique they were able to distinguish between the trophoblastic and decidual contribution to the luteotrophic activity of the pregnant uterus (D. R. S. Kirby and T. Cowell). The enzyme and morphological changes involved in implantation in mice and rats were described in great detail (A. McLaren and C. Finn; B. L. Lobel, E. Levy and M. C. Shelesnyak) and some factors affecting early embryonic mortality in the pig were discussed (M. Phillippo).

Great interest was shown in techniques designed to facilitate the study of isolated endocrine organs and tissues; whole rat ovaries maintained in organ culture (T. Fainstat), equine granulosa cells which produced progesterone in tissue culture (C. P. Channing) and autotransplants of sheep ovaries to the neck (J. R. Goding).

The second session was devoted to a symposium on radiation effects on the reproductive system, under the chairmanship of Prof. D. Lacy. The first two papers (P. Lindop; H. M. Beaumont) described work on the radiosensitivity of oocytes and other cell types in the ovary of the mouse, an animal which has been intensively studied in this respect, and the remaining papers were concerned with irradiation effects in the male. These effects have been studied in considerable detail in the rodents, and similar work has now been extended to other species, including the goat and man (E. W. Hupp). Work with human volunteer subjects (C. A. Paulsen and H. M. Gandy) has demonstrated the extreme sensitivity of the human testis to irradiation, and has emphasized the reciprocal nature of the relationship between gonadotrophin output and germinal epithelial function. At the ultrastructural level, X-irradiation has been used in studies of organelle induction and differentiation during spermatogenesis in the grasshopper (T. N. Tahmisian).

The third session opened with a group of papers on species other than common laboratory or domestic animals. They were concerned with comparative aspects of reproduction in the chinchilla (B. J. Weir), the squirrel monkey (J. P. Bennett) and some of the larger Felidae from the collection of the Zoological Society, Regent's Park, London (R. M. F. S. Sadleir). This session also included reports on such diverse topics as the descent of the testes in the grey seal (K. M. Backhouse and H. R. Hewer), a species with a non-scrotal testis, and the influence of such environmental factors as pasture management and outbreaks of myxomatosis on reproduction in the wild rabbit (H. G. Lloyd), and the decidual changes associated with "endometrial cups" in some Equidae (J. M. King). The session concluded with three papers on the application of neurophysiological techniques to problems in the physiology of reproduction. One of these was concerned with the nature of afferent nerve fibre discharges from lactating mammary glands (A. L. R. Findlay); the others described work on the mechanism of feedback action of ovarian hormones (D. W. Lincoln; B. A. Cross and I. A. Silver).

The fourth session took the form of a symposium on the growth and function of the corpus luteum in mammals. The chairman, Dr. R. O. Greep, opened with a synopsis of earlier work on luteal function in the rat and rabbit. In the papers which followed, the emphasis lay in distinguishing between the growth of the corpus luteum and the biosynthesis and release of luteal progesterone in the guinea-pig (R. B. Heap, J. S. Perry and I. W. Rowlands), and in the interactions between the hypophysis and the uterus in controlling these processes in the sheep, sow and cow (R. Denamur, J. Martinet and R. V. Short; F. du Mesnil du Buisson; W. Hansel). Further evidence of the local effect of the uterus on the ovary was given for the sow, and a similar phenomenon was demonstrated by R. Moor and L. E. Rowson in the sheep by the surgical transfer of embryos to isolated segments of the uterus. The fact that one ovary may behave as though the animal were pregnant, while the other ovary behaves as though it were not, conflicts with the generally accepted picture of the hormonal relation between ovary and uterus, and is as yet unexplained. Work on the pituitary hormones responsible for maintaining the corpus luteum and stimulating the synthesis of luteal progesterone suggests that in the cow and the sow the main luteotrophin is LH (luteinizing hormone), whereas in the sheep it appears that the major factor is prolactin. These papers were discussed by R. Deanesly and B. T. Donovan in the light of their own work on the guinea-pig and ferret.

The fifth session opened with six papers on various aspects of reproduction in the male; it comprised work on the effect of nutrition on androgenic function in calves (T. Mann and colleagues), the preservation of human spermatozoa (M. Freund; D. Richardson), the control of fertility with drugs (B. N. Hemsworth and H. Jackson), and the effect of ageing of spermatozoa on fertilization and prenatal development (J. M. Tesh and T. D. Glover). By way of further variety, sperm dimorphism in a copepod was described by B. L. Gupta. Later papers, on reproductive function in the female, dealt with the gonadotrophin control of ovulation in several species, including human (M. E. Mitchell; H. H. Cole and R. B. Snook; M. Taymor; J. P. Bennett *et al.*).

A symposium on factors controlling the duration of pregnancy opened with a number of papers dealing with myometrial activity and the onset of parturition (B. M. Schofield; A. I. Csapo and T. Kerenyi; A. Riitta Fuchs; G. Wagner; W. R. Ward), and subsequent speakers described the influence of genotype, the immunological status of the fœtus, and a cold environment on the duration of pregnancy (M. W. H. Bishop; D. James; S. A. Barnett and M. J. Little). Attention was drawn to the distinction between animals in which the placenta produces progesterone, as in the human, and those in which it does not, as in the rabbit. It has been shown that strong uterine activity can be present without parturition, or without the maternal behaviour associated with it, in rats with progesterone implants in the hypothalamus.

A new surgical technique for the removal of the pituitary gland in the foetal lamb was described (G. C. Liggins and L. Holm), and this procedure was found to prolong pregnancy, an effect apparently mediated through the foetal adrenal gland. The chairman, Dr. A. McLaren, presented a paper on behalf of A. Psychoyos *et al.*, who have studied the relations between uterus and ovary in rats in which two sets of embryos were induced to implant asynchronously, so that the uterus was occupied by foetuses of different ages.

The final session was devoted to papers on problems in human reproduction and its control, including a morphological study of the utero-tubal junction (I. Rocker), the effect of oestrogens on the ovary (A. Netter), an analysis of fertility in married women (M. Barton), and the successful demonstration of the use of clomiphene to produce live spermatozoa in a case of Klinefelter syndrome (G. L. Foss). Work in Edinburgh has shown that chromosomal anomalies may be a major factor in unsuccessful pregnancies, and there is some evidence that the incidence increases in women over 35 years of age (M. G. Kerr).

Continued on next page

OBITUARIES

D. D. Kosambi

THE death of Professor D. D. Kosambi on June 29, 1966, at the age of fifty-nine removed a unique figure, not only from India but from all society.

Kosambi had a most unusual and comprehensive education, partly in the United States, at Harvard, where his father taught Indic studies. He acquired early an interest in mathematics, which was his main contribution to science, particularly in the field of statistics and stochastic theory. This put him in the front rank of practical mathematicians and his interest was then transferred to theoretical and nuclear physics and he worked on fundamental research at the Tata Institute in Bombay under the general direction of the late Homi Bhabha.

I am not qualified to comment on his mathematical work, especially as much of it is concerned with number theory and calculus of variation; but I had a great deal to do with him as a man of quite exceptional intelligence and charm, particularly in the work concerned with the Indian peace movement, and I learned something of his wide scope of interests. Although trained in the United States, he remained from the very beginning mainly concerned with Indian problems, including Indian economics, history and archaeology. It was a pleasure to travel with him in his own country because, for Kosambi, history was not only in the past but also in the present.

In the extremely unsatisfactory records of Indian history which he has denounced in his books, he finds the preservations of beliefs and customs in many tribes and castes. He finds, for example, not only the places of worship but also actual names, such as that of the goddess Lumbini presiding over the birth of the Buddha, which have been preserved practically unchanged for 1,500 years. I witnessed one of these survivals myself outside the National Chemical Laboratory near Poona. It was a newly established shrine with a crude figure smeared with red paint and an attendant ascetic who, for a consideration, would offer up a prayer so that the new arrival into the laboratory could be assured of a good post.

Kosambi's interests stretched further than the geography of India. I accompanied him to the National Museum of Denmark in Copenhagen and he was able to tell the curator precisely what bones he would find at the bottom of a well because the Bronze Age Danes had the same horse sacrifice as the ancient Aryans of India.

Wherever he went he was able to trace out the early trade routes still in use in the age of steam and motor cars, and often transporting the same goods, including pots made with the slow and fast wheel, that had already been made in the Indus civilization in the second millennium B.C. For this he had also to perfect his knowledge of Indian languages and poetry. His father knew the whole of the 80,000 lines of the *Mahābhārata* by heart, and I remember Kosambi taking me to the Institute in Poona where a number of aged Indian scholars and their younger disciples were occupied in providing a comparative edition of that poem from numerous manuscripts on palm leaves and other fragile bases, reckoning that they had done a good day's work when they had completed one line!

Kosambi introduced a new method into historical scholarship, essentially by application of modern mathematics. Indians were not themselves historians: they left few documents and never gave dates. One thing the Indians of all periods did leave behind, however, were hoards of coins. These carry inscriptions which list kings or date markings, but they had been in circulation before the hoard was buried and had suffered varying degrees of wear before burying. By statistical study of the weights of the coins, Kosambi was able to establish the amount of time that had elapsed while they were in circulation and so set them in order to give some idea of their respective ages. In this way he was able to date these coins, known as punch-marked coins, weighed pieces of silver of carefully standardized weight marked with various devices which connected them with a definite king, sometimes many with a single king such as the great Asoka of the Maurya dynasty. The persistence of a coin is one of the most remarkable of human characteristics. In his book, *The Culture and Civilisation of Ancient India in Historical Outline*, Kosambi shows a picture (No. 62) of a coin of a Greek king, Menander, in North India, 180–160 B.C., which he found in 1940 circulating in an open air market in Poona as an equivalent of half a rupee.

His great contributions to historical science have been his two books *Introduction to the Study of Indian History* (Popular Book Depot, Bombay, 1956) and *The Culture and Civilisation of Ancient India in Historical Outline* (Routledge and Kegan Paul, London, 1965). These provide a history of an entirely different character to anything seen before. As he himself says in his latter book:

> "But what is history? If history means only the succession of outstanding megalomaniac names and imposing battles, Indian history would be difficult to write. If, however, it is more important to know whether a given people had the plough or not than to know the name of their king, then India has a history. For this work I shall adopt the following definition: *History is the presentation in chronological order of successive changes in the means and relations of production.* This definition has the advantage that history can be written as distinct from a series of historical episodes."

In his second book, Kosambi certainly bears out his definition. He shows a picture of a two-bullock plough of the same characteristic type with vertical handle and curved yoke-pole, taken from a relief of the Bodhisattva's First Meditation in the Buddhist caves at Junnar, as in use in A.D. 200 and still in use to the present day. They are, for all practical purposes, identical.

A great deal of Kosambi's book is taken up with the description of classical Indian political science. The *Arthasāstra* is a complete and almost Machiavellian description of how the great Indian state of Magadha (300–184 B.C.) was ruled and how the institutions of the complete police state could be adapted to the tenets of the purest Buddhism. He shows that the positive task of the Mauryan State consisted of opening up the territory of the Ganges Valley, including Bengal and even Assam, by clearing the tropical forests and assimilating, through institutions of Hindic religion, the local populations.

All this may seem very far away and long ago, but to Kosambi it was all in the present as well, illuminating the current difficulties of Indian agriculture and industry.

> "When gunpowder had blown Arguna's bow and later feudalism off the map, the Indian intellectual still turned instinctively to the (Bhagavad-) Gitā to find some way of coping with patriotic needs in the new world of banks and shares, railroads, steamships, electricity, factories, and mills. . . . The Gitā is honoured oftener than read, and understood far less than it is recited. After such mixed ideas are displaced by clear-cut thinking based on a firm grasp of material reality, the work may still furnish some aesthetic pleasure for its power of expression and peculiar beauty."

J. D. BERNAL

Continued from previous page

Observations were reported on the use of an intrauterine device in the rhesus monkey by J. H. Marston *et al.*; and further papers described the effect of oral contraceptives and intrauterine contraceptive devices on endometrial blood vessels and vaginal smears (E. Grant; M. Jackson and R. Linn).

Abstracts of the papers presented at the annual meeting will be published in the October issue of the *Journal of Reproduction and Fertility*, the official organ of the Society.

J. S. PERRY
R. B. HEAP

"LIVING FOSSILS" IN WESTERN ATLANTIC PLANKTON

By DR. DAVID WALL and BARRIE DALE
Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

FOSSIL microplankton are a large group of ancient marine protista of planktonic origin that includes hystrichospheres and fossil dinoflagellates the shells of which are composed of organic rather than mineral material. They are used together with pollen and spores to date, correlate and interpret the palaeoecology of Mesozoic and Tertiary strata. Until recently, the existence of identical organisms in modern plankton was uncertain: hystrichospheres, for example, were thought to be extinct, while the youngest known fossil dinoflagellates (Miocene) were unlike living genera in critical details. Then morphological studies[1,2] of a few types of resting spores produced endogenously by common living thecate dinoflagellates quickly revealed that these spores were identical with several hystrichosphere and fossil dinoflagellate species known to occur in marine late-Tertiary and Quaternary sediments. Moreover, one such "living fossil" known as *Hystrichosphaera bentori* Rossignol (Fig. 1) was induced to excyst under laboratory conditions in single-cell culture experiments[2] to release a flagellated thecate motile stage of *Gonyaulax digitale* (Pouchet) Kofoid.

New discoveries resulting from improved extraction techniques and regular sampling of local Atlantic plankton at Woods Hole for more than 8 months have shown that many other hystrichospheres and similar dinoflagellate resting spores occur persistently in present-day oceans. More than forty types of dinoflagellate resting spores with diagnostic excystment apertures (archeopyles) have been isolated since February 1965; these include *Hystrichosphaera furcata* (Ehr.) Wetzel, *H. bulloidea* Defl. and Cooks, *H. bentori* Ross, *H. mirabilis* Ross (Fig. 2), *Nematosphaeropsis balcombiana* Defl. and Cooks (Fig. 3), *Baltisphaeridium machaerophorum* Defl. and Cooks, *B. centrocarpum* (Defl. and Cooks) Gerlach (Fig. 4), and *B. israelianum* Ross (using fossil nomenclature) together with several new species which have not been described formally, but are known to occur in the marine Pleistocene of the Caribbean Sea. Living hystrichospheres were collected from two sources, namely, the sea water and the sea floor surface layer. Those obtained directly from plankton were collected with a fine meshed (35μ) net; the remainder were isolated from humus on the sea floor or from the floors of large settling tanks where there was less turbulence. In each case selective concentration of the resting spores relative to other plankton was essential and accomplished by repeated sieving, sometimes with interposed ultrasonic vibration treatment, until only a clean 35–75μ fraction remained. An exceptionally rich sea-water sample rarely had more than thirty resting spores per litre and the most common type of hystrichosphere (*H. bentori*) seldom exceeded five cells per litre, but numerous individuals were obtained by using large volume samples of 100 l.

The resting spores of dinoflagellates recovered from local plankton at Woods Hole could be divided into four groups according to whether their excystment apertures or archeopyles were precingular, intercalary, cingular (hemispherical) or polar.

(1) Resting spores with precingular archeopyles included the "living fossil" species of *Hystrichosphaera*, *Nematosphaeropsis* and *Baltisphaeridium* already listed as common in local waters. In almost all instances the archeopyles represented the loss of a single plate-area (3″) from the dorsal surface, but rarely the opening was compound and four or five precingular plate-areas were missing, as in the cyst of *Gonyaulax polyedra* Stein[1].

The parental dinoflagellates of spores with precingular archeopyles belonged to *Gonyaulax* and *Protoceratium*. Two spores with simple archeopyles (*Hystrichosphaera bentori, H. bulloidea*) were united with their thecate stages (*Gonyaulax digitale, G. scrippsae*) in culture experiments, while seven other species of *Hystrichosphaera* have gonyaulacid parentage according to their tabulation, which was always 3–4′, 0*a*, 6″, 6*g*, 5–6‴, 1*p*, 1⁗. The spore of *Gonyaulax polyedra* was identified by its compound archeopyle and distal spinules[1] and was comparable with the common Quaternary fossil species *Baltisphaeridium machaerophorum*. Only one species of *Protoceratium*, *P. reticulatum*, is known to form spores with a precingular archeopyle[1]; these spores are similar to the Quaternary fossil *Baltisphaeridium centrocarpum* in most respects.

(2) Resting spores with intercalary archeopyles included more than twenty-five morphologically distinct categories. They were identical with fossils known to occur in Caribbean and Mediterranean Quaternary sediments in some instances and comparable with older species belonging to the fossil genera *Cleistroeisphaeridia* and *Deflandrea* in general. The archeopyles of these modern spores were basically hexagonal in shape but had variable height: width ratios (1 : 1 to 1 : 2·3) and sometimes a tendency towards asymmetry. Observations of cysts within thecae, dispersed spores and thecae released from cysts under single-cell culture experiments confirmed that the archeopyle was homologous with a dorsal intercalary plate (usually 2*a*, rarely 1*a*) and seldom compound. The position of the archeopyle was commonly subapical and dorsal, but in some instances (varieties of *P. oblongum*) the opening truncated the apex of the resting spore. In overall shape, the resting spores varied from subspherical to rhomboidal (peridinoid) to biconvex with polar compression. The majority were smooth-walled, but two types at least were spinose, including the spore of *Peridinium claudicans* Paulsen. All were deeply coloured but they varied from shades of brown to olive-green.

The parental dinoflagellates identified with these spores were species of *Peridinium* belonging to the subgenus *Orthoperidinium*, including *Peridinium oblongum* (Aurivillius) Cleve (Figs. 5 and 6), *P. claudicans* Paulsen, *P. leonis* Pavillard, *P. conicoides* Paulsen, *P. excentricum* Paulsen and *P. monospinum* Paulsen. Numerous other spores with intercalary archeopyles were found only in the dispersed condition, but their characteristic colours and often rhomboidal shapes were strongly indicative of peridinioid parentage.

(3) Two types of resting spores possessed cingular archeopyles consisting of an equatorial suture circumscribing the test apart from a small area of its ventral surface. Their natural affinities or any details of their tabulation remain unknown, but their archeopyles were basically similar to those seen in the Quaternary fossil species *Hystrichosphaeridium zoharyi* Ross.

(4) Apical excystment apertures were seen in two problematical forms. First, they occurred in certain mineralized dinoflagellate cysts (Family Calciodinellidae) which were similar to organisms first described by Gocht[3] from the Neocomian of Germany and Deflandre[4] from the Tertiary of Algeria and France. In single-cell culture experiments this circular aperture was observed to function as an excystment opening when a gymnodinioid body which quickly divided into two daughter cells was released. Secondly, empty shells of *Hexasterias* (*Polyasterias*)

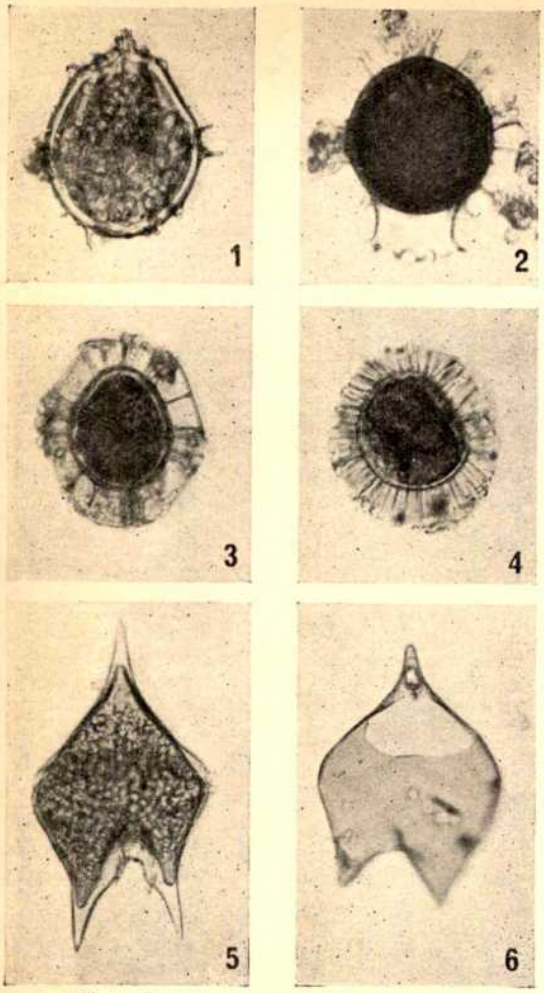


Fig. 1. *Hystrichosphaera bentori* Ross (× 500).
Fig. 2. *Hystrichosphaera mirabilis* Ross (× 500).
Fig. 3. *Nematosphaeropsis balcombiana* Defl. and Cooks (× 380).
Fig. 4. *Baltisphaeridium centrocarpum* (Defl. and Cooks) Gerlach (× 375).
Fig. 5. *Peridinium oblongum* (Aur.) Cleve (× 365). Theca enclosing a resting spore.
Fig. 6. Empty cyst of *P. oblongum* (Aur.) Cleve, showing the intercalary archeopyle (× 335).
All specimens illustrated from plankton at Woods Hole.

problematica Cleve frequently possess circular apical apertures. Dinoflagellate affinities have been suggested for this organism[5], but its parental species was not named. The striated, open hollow tubular processes make it particularly interesting as a possible surviving member of the "Tubiferum" microplankton group.

In many instances the resting spores of modern thecate dinoflagellates are morphologically distinctive (for example, *Hystrichosphaera bentori*), and identifiable in the dispersed state in which they are commonly found. Once the parental and thecate phases have been correlated by natural association or in culture experiments, the geological history of the species may be traced with the greatest precision. In general, observation of these modern resting spores supports the contention held by most micropalaeontologists that it is the most precisely defined fossil microplankton species that correspond most closely with natural groupings the members of which had mutual parents. Only a few cases are at present known where identical cysts are produced by parental dinoflagellates with different tabulations. These concern closely related neritic species or varieties of the "Oceanicum"-group of *Peridinium* (*P. claudicans*, *P. oblongum*) the present taxonomic status itself of which is open to revision.

Investigations of modern cysts clearly point to the value of the archeopyle as a criterion useful in tracing the phylogeny of dinoflagellates and in formulating diagnoses of fossil genera. They confirm by reference to more than forty different living morphological categories ("species") that the archeopyle is a fundamental characteristic of marine dinoflagellate resting spores the formation and function of which are defined precisely by genetic control. Living resting spores develop an incipient archeopylar suture before dispersion and long before their dehiscence. Fossil resting spores bear witness to the evolutionary stability of this structure since Jurassic times.

Moreover, the locus of the archeopyle is constant within narrow limits for resting spores produced by species of any modern genus according to existing information; it is precingular in *Gonyaulax* and *Protoceratium* and intercalary in *Peridinium*. Modern genera producing cysts with similar archeopyles (*Gonyaulax* and *Protoceratium*) are apparently closely related; the tabulation of *P. reticulatum* is very similar to that of *Gonyaulax*.

At present the systems of classification applied to thecate living phases and fossil resting spores are independent of each other although both rely on skeletal morphology to a large extent. Now that more comprehensive knowledge of the morphology of modern resting spores has been compiled, several recommendations based on biological studies may be presented in an attempt to ensure that the two systems of classification are compatible. A special effort should be made to determine the nature of the archeopyle before any fossil species is described. All fossil genera should be diagnosed to include mention of their archeopylar structure and species with different kinds of archeopyles should not be placed in the same fossil genus. Fossil genera the archeopyles of which originate from different series of thecal plates (precingular, intercalary) should not be placed in the same family.

The biological function and ecology of living hystrichospheres and other resting spores are of interest to the palaeontologist but are poorly understood at present. The functions of marine dinoflagellate resting spores include protection of the protoplast against conditions adverse towards the thecate stage, propagation of new plankton crops and migration of the species by spore dispersal. However, the genetic significance of this phase is unknown. The role of environmental stimuli in inducing cyst formation also is poorly understood. Resting spore formation in certain fresh-water dinoflagellates (*Glenodinium uliginosum* Schilling) has been correlated with a rapid fall in temperature prolonged over several months[7], but in marine species spore formation can occur before there is any marked decline in temperature and while the thecate stage is abundant and growth conditions appear optimal. Single-cell culture experiments have shown that temperature fluctuations can be effective in inducing excystment. The resting spore of *Gonyaulax digitale* will release its motile stage at 16°–25° C after isolation from colder water, but will not excyst under the same conditions immediately following its formation indicating that a resting period is required. Different types of marine *Peridinium* resting spores have been excysted at 6°–7° C and at 23° C, indicating that this process in different species may occur in either cold or warm water conditions. Future investigations may clarify understanding of these processes and provide ecological information concerning the seasonal and geographic distribution of hystrichospheres in the oceans.

This work was supported by a grant from the National Science Foundation and a contract with the Office of Naval Research.

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APPARENT CORRELATION BETWEEN THE LUNAR ECLIPSE BRIGHTNESS AND THE SOLAR WIND

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IN recent years there have been numerous reports of observations of luminescent brightening of the Moon assumed to be caused by bombardment of the solar corpuscular radiation[1]. Among them, unusual enhancements shown in direct photographs of the Moon taken by Kopal and Rackham[2] appear to offer the most convincing evidence for the assumed phenomenon. However, the weakness of the luminescent light compared with the sunlight reflected by the lunar surface makes observation of this phenomenon very difficult. Kopal and Rackham have found that the energy required to produce the excess of light observed in their photographs is a few orders of magnitude greater than the amount of interplanetary plasma flux so far observed from space probes[3]. The brightness of the Moon decreases by a factor of 10^3–10^5 at the time of a lunar eclipse, so that, if the interplanetary or magnetospheric plasma were to bombard the Moon in the Earth's shadow, the luminescent brightening would be readily detected during a lunar eclipse. In fact, in some of the past eclipses, the brightness of the Moon has been 15 per cent or so greater than that predicted from theoretical calculations[4]. Link[5] was the first to suggest that luminescent emission caused by enhanced radiation from the solar corona was responsible for this increase in brightness. This point prompted me to investigate, on the basis of recent results of space experiments, whether there is any correlation between the brightness of the Moon during an eclipse and the interplanetary plasma flux.

A positive-ion spectrometer flown on *Mariner* II provided Snyder, Neugebauer and Rao[6] with about 40,000 plasma spectra for the course of the entire flight to Venus. From these measurements, they found a remarkable correlation between the plasma velocity and the planetary index of geomagnetic disturbances. From the least square fit of the plots of the observed velocity, V_p, against the daily mean planetary index, ΣK_p, they derived a linear equation

$$V_p\,(\mathrm{km/sec}) = (8{\cdot}44 \pm 0{\cdot}74)\ \Sigma K_p + (330 \pm 17) \quad (1)$$

If it is assumed that the relation is correct, equation (1) can be used to determine the solar wind velocity at any time during the past 30 years for which 3 h range as well as daily mean values of K_p are available. An extrapolation of this equation yields a range of the plasma velocities from 330 km/sec for $K_p = 0$ to 940 km/sec for $K_p = 9$. Another important finding made in this experiment was that there was no strong correlation between the plasma velocity and "over-all" solar activity as determined by sunspot number and 10·7 cm radiation.

In order to minimize the various corrections necessary when combining observations of different eclipses, the data selected for the present analysis consist of a series of three-colour photometric observations (centred near λ = 4700, 5300, and 6200 Å) of eighteen eclipses between 1932 and 1957 made by Danjon, Dubois and Rougier[7]. All the measurements were taken at a point 10 min of arc inside the umbra-limb with the same "cat-eye" or double-image photometer which was specially designed for the observation of lunar eclipses by Danjon[7]. Thus, if we assume that the distribution of refracted light in the Earth's shadow is radially symmetric, these data can be combined for direct comparisons.

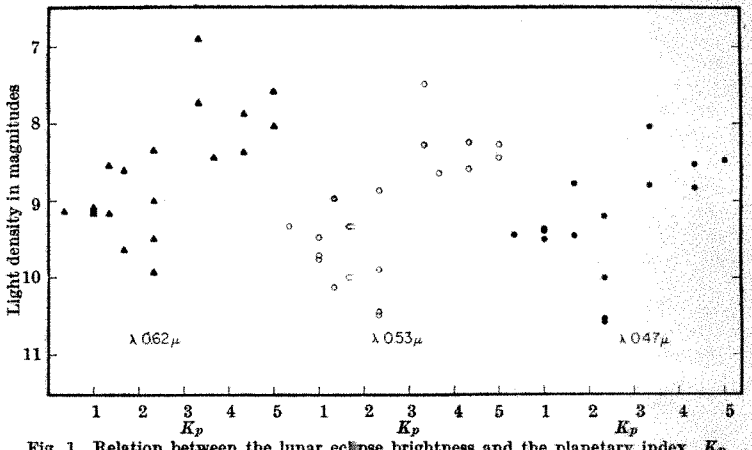


Fig. 1. Relation between the lunar eclipse brightness and the planetary index, K_p. The ordinate unit multiplied by −0·4 gives the logarithmic ratio of the brightness of an eclipsed Moon to that of the full Moon.

In Fig. 1 the observed brightness is plotted against the 3 h mean K_p at the time of each eclipse. The light density is defined by the ratio of the brightness of an eclipsed Moon to that of a full Moon, and is given in terms of the stellar magnitude scale. (The unit of stellar magnitude is equal to the logarithmic unit with base 2·512, or it corresponds to 0·4 times the logarithmic unit with base 10.) Although the range of scatters is rather large, it can be seen that there is a monotonous increase of light density with increasing K_p. Furthermore, similar plots made with 24 h mean values of K_p showing the same correlation can be seen in Fig. 1. It should be noted that theoretical calculations indicate that a point 10 min inside the umbra-limb is illuminated by light rays passing through the atmospheric region higher than about 10 km (ref. 8), which is well above the cloud region. The random fluctuations of the observed points seem to result partly from the differences in albedo of the points of observations and partly from the variations of extinction in the upper atmosphere. In view of the precision that can be expected, however, the random errors in Fig. 1 do not seem to be too serious.

Many years ago, Danjon[9] suggested that the brightness of the eclipsed Moon depended on the sunspot cycle. Using his own scheme of calibration, based mostly on the colour of an eclipsed Moon, Danjon estimated the relative brightnesses of a number of visually observed eclipses

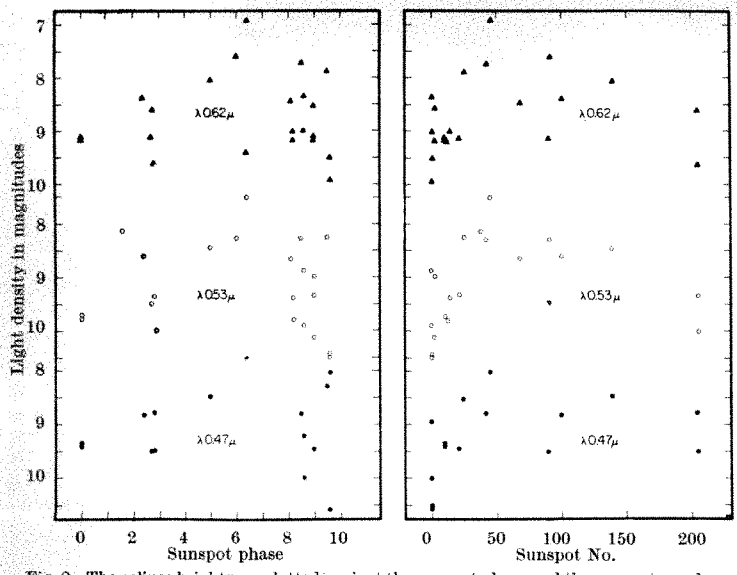


Fig. 2. The eclipse brightness plotted against the sunspot phase and the sunspot number.

between 1823 and 1920. He concluded that the brightness increases monotonously from a time of sunspot minimum until it reaches the maximum brightness just before the next sunspot minimum and then drops sharply to the minimum brightness when the sunspot activity begins a new cycle. This point can be examined for the same set of data used in the present analysis in Fig. 2, where the observed density is plotted against both the phase of sunspot cycle and the actual number of sunspots observed on the date of each eclipse. Contrary to Danjon's findings, no obvious correlation can be seen in either diagram. This appears to be consistent with the experiment by Snyder *et al.*, in which no clear relationship was detected between the plasma velocity and the sunspot number.

While it is now believed that the Earth's magnetosphere extends to the distance of the Moon's orbit, experimental knowledge of the kinetic behaviour of the plasma flow near the geomagnetic tail region is still incomplete and theoretical predictions are confusing[10]. Neglecting this uncertainty, it can tentatively be suggested that the monotonous increase in light density showing in Fig. 1 results from the fluorescent emission caused by the bombardment of interplanetary plasma. If this is so, the question of the energy balance of the assumed process must be examined quantitatively.

For convenience of calculation, the observed light density is converted to the absolute unit of radiant energy flux for 1 Å width, and is plotted against the plasma velocity derived from equation (1). All the straight lines are drawn through the points with the logarithmic gradient of 4, indicating that the emittent energy is proportional to the fourth power of the plasma velocity. Because the bombarding plasma flux is proportional to the cubic power of the velocity, we can accept the proportionality to the fourth power if we assume that either the proton density or the luminescence efficiency increases linearly with the velocity. On the other hand, if we take the former and assume that the luminescent flux is comparable with the reflected light, we obtain from the straight lines

$$\log E_\lambda = 4 \log V_p + \log N_0 + \log Q_\lambda/\Delta_\lambda + \text{constant} \quad (2)$$

where N_0 is the proton density per unit velocity, and Q_λ and Δ_λ are the efficiency coefficient and the half width of the luminescent spectrum, respectively. If we take the efficiency factor of 1·0 and assume the half-width of the luminescent spectrum to be of the order of 1000 Å, we find for the red region, N_p = 20/cm³ for V_p = 330 km/sec (K_p = 0), and N_p = 35/cm³ for V_p = 600 km/sec (K_p = 4). The blue and green regions give slightly higher densities. In reality, the luminescent emission should be a certain fraction of E_λ in equation (2), and the proton densities will be correspondingly lower. For example, if we assume the luminescence is about 10 per cent of the total emission, the corresponding proton density becomes a few per cubic centimetre which agrees with the value generally assumed for the steady flow of the solar wind.

Although this calculation is approximate, it is sufficient to show that the correlation found between the brightness of a lunar eclipse and the interplanetary K_p index can be interpreted as arising from the effect of lunar luminescence. A more detailed investigation of this interpretation, together with similar analysis for other recent observations of lunar eclipse[11], is now in progress.

I thank Prof. J. A. Van Allen and Prof. T. Obayashi for their stimulating discussions. I also thank Prof. F. Link for directing my attention to the possibility of luminescence on the brightness of lunar eclipses. This work was supported in part by two grants from the National Science Foundation.

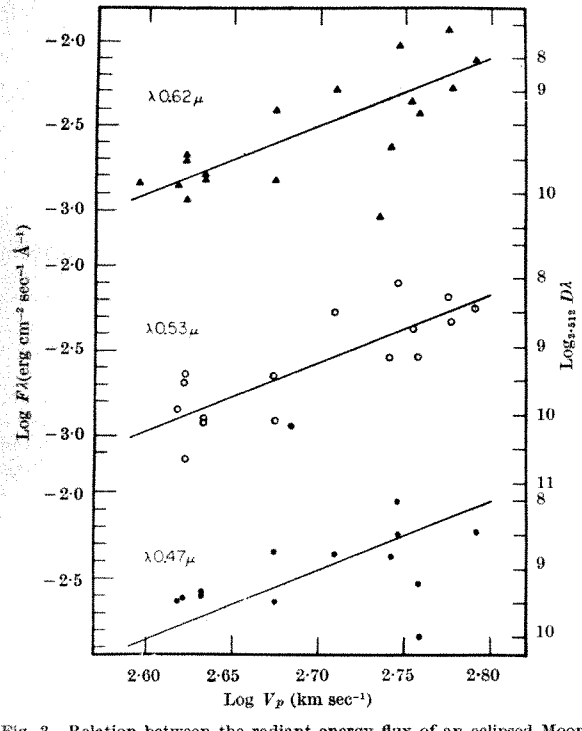


Fig. 3. Relation between the radiant energy flux of an eclipsed Moon and the interplanetary plasma velocity, V_p.

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SIMULATION OF A PERCEPTUAL PROBLEM-SOLVING SKILL

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In the physical sciences, the use of large digital computers in the development of models is now a well established technique. In psychology and the social sciences the technique, though not without its successes, has been less well accepted. One difficulty facing the behavioural scientist is that the problems in which he is interested involve a large number of variables which can rarely be systematically controlled.

A previous paper[1] reported the development of computer designed psychological test material in which the variables determining difficulty could be systematically varied and which was suitable for the study of perceptual skills and certain aspects of human problem-solving mechanisms. Because this material is being increasingly used both in the clinical field and as a research instrument[2-4] it seemed worthwhile to develop computer model-building techniques to test hypotheses about the mechanisms different subjects use in solving this type of problem. Fig. 1 shows a sample of the problems used.

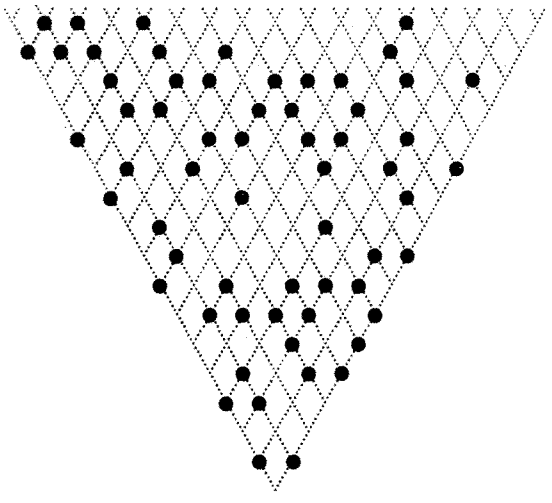


Fig. 1. An example of a difficult maze.

The display consists of a lattice background on which is superimposed a number of target dots. The subject is required to find a pathway through the lattice which obeys the following rules: it must start at the bottom vertex and end at the top row and it must lie wholly along the lattice lines; it must be unidirectional in a general upward sense; it must pass through the greatest possible number of target dots. This task may be presented in one of two forms. Either the subject may be told the maximum possible score or this information can be withheld. These two versions will be referred to as *A* and *B* respectively. Under condition *A* the subject has to discover a pathway with a specified score and is left in no doubt as to whether or not he has found a solution. Under condition *B* he has to decide himself whether further effort will produce a better solution.

A number of studies suggest that success with these problems is a function of fairly fundamental perceptual skills which correlate with intelligent adaptive behaviour. As might be expected, these skills are sensitive to brain damage[2] and the type of breakdown is a function of the site of the lesion[5]. As with many perceptual and spatial skills, there are also differences in performance related to sex, personality variables and age[4,6].

Because the format of the maze is well defined and amenable to relatively simple mathematical description, structural properties which correlate with the difficulty of individual items may be defined as numerical parameters[1]. These can be systematically varied and the effects of doing so related to the relative difficulty of different patterns for defined groups of subjects. The models described here are based on a detailed analysis of data from such a study carried out with two groups of normal subjects: 144 Royal Air Force male recruits matched with 144 female recruits for age, intelligence and education. Each subject attempted every one of a set of seventy-two mazes, each with a sixteen-rowed lattice and sixty-one target dots the arrangement of which had been specified by a computer programme. Each test set was divided into two sets of thirty-six items, one presented under condition *A* (with the solution number given) and the other under condition *B* (without the solution number). Half the subjects took version *A* followed by version *B*, the other half version *B* followed by version *A*. Practice effects were balanced by means of a randomized Latin Square design and the subjects were allowed 30 sec for each item. Thus it was possible to derive for men and women and for each condition an estimate of the difficulty of each item which was unbiased with regard to practice and fatigue effects. Further details of this experiment, henceforth referred to as Experiment *D*, have been given elsewhere[7,8]. In the present article we relate these estimates of difficulty to the successes and failures of two models in the form of computer programmes which simulate limited but contrasting aspects of the human maze solving process. This maze solving process is likely to incorporate a variety of related strategies. Of the two models described, one defines a systematic search for valid links between groups of target dots and the second a total encoding of the dot relationships.

With the present size of pattern, an exhaustive sequential search would mean that in the no-information condition subjects would have to examine 65,536 solution pathways. Such an approach would not only be time consuming but, for a human, difficult to carry out. How then do subjects go about solving the problem of linking together a maximum number of target dots? Subjectively most subjects say that they first scan the maze for areas with a high target dot density. Attempts are then made to relate these areas together. In other words, the subject makes an analysis of the groupings of target dots and the relationships between these groups in order to decide which dots can be linked to form a solution. Although ultimately defined by the lattice background, the permissibility of dot linkages might in the first instance be assessed on the basis of the angular relationships between groups.

However, some types of linkages will be more obvious than others; some dots may be perceived as linked when there is no valid path connecting them. Again some relationships, such as horizontal groupings, may appear more attractive than their problem-solving value warrants.

Grouping the target dots as runs (that is to say, into pathway segments containing target dots with no empty nodes) is a simple encoding strategy. It does not itself solve the maze but provides one possible basis for a whole

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class of strategies. For example, a subject might scan the maze, pick out a large group of dots and use this as a focal point for a pathway. Alternatively he might try to link up runs of dots working systematically from the vertex to the top (or from the top down). In this case we may visualize the subject as starting at the vertex and making a series of decisions to fork left or right based on the configuration of dots in the rows immediately ahead. If this decision is made on the basis of a more or less complete knowledge of this configuration, then the number of rows ahead that the subject will be able to scan will be very limited. Clearly most subjects will not search only from below upwards, nor if they did would their decisions be taken only on the basis of complete knowledge of a limited area. 'Look ahead' is in fact likely to be complete for the immediate neighbourhood and to spread forward on an increasingly impressionistic basis.

Such assumptions, therefore, are simplified approximations from which subjects will depart to a greater or lesser extent. The results of Experiment *D*, however, suggested that scanning from below upwards is more common than scanning from above down and that the effective 'look ahead' of some subjects is quite short. Moreover, subjects tend to adhere to, or continue to the end of, a run in preference to breaking away to a new group, even though the latter gives a better score. There is also a preference for continuing in a straight line.

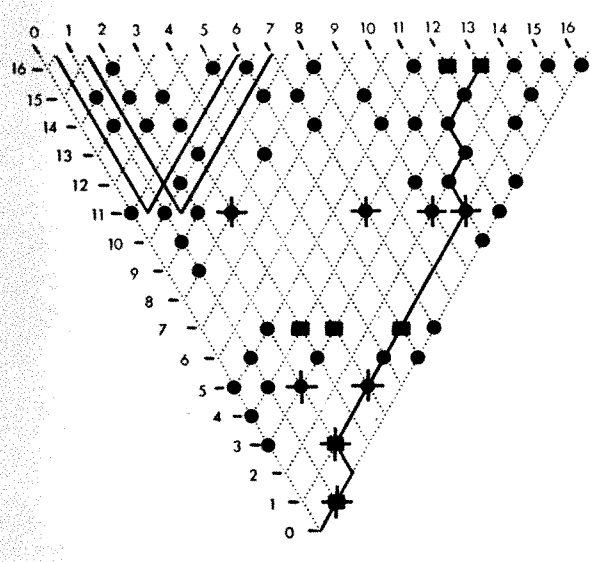


Fig. 2. This diagram illustrates (1) the first strategy, and (2) the opportunity cones used in the second strategy. (1) The target dots chosen by the strategy as base nodes are marked with a -●- and those chosen as top nodes with a box ■. The first two runs are in fact singletons (1,1), (3,2). From the second of these two base nodes three runs of three are accessible which end in three top nodes (7,3), (7,4), (7,6). All these are therefore used to generate a fresh set of base nodes. There are four of these, the two left-hand ones (11,3), (11,7) being singletons. The two right-hand ones (11,9), (11,10) belong to the same group and each generates two runs of 6. Since both can be reached only from the right of the three preceding top nodes (7,6), this is chosen from the preceding 3 runs. From here there is no preference for either of the two right-hand top nodes (16,12), (16,13) and the principle of "continuation" determines the choice of node (16,13). The final path chosen by the strategy is as marked in the diagram. (2) The two overlapping cones in the top left-hand corner define the opportunity cones based on the two apical nodes (12,1) (12,2) which in the second strategy (if the pathway had reached this point) would determine the decision taken at the node (11,1).

In the first strategy (Fig. 2) a maze solution is considered as a series of runs of dots, the direction of the pathway being from the vertex up. The end or top dot of a run will be referred to as a top node, the start or bottom as a base node. Conventionally, the vertex, which never contains a target dot, is defined as a top node; the pathway starts here and is constructed in steps. At each top node the next run of dots is chosen, which in turn terminates at the next top node of the pathway.

The procedure for choosing the next run of dots is as follows: the accessible parts of each row above a top node are scanned until a dot is found; all the accessible dots in this row are considered as possible base nodes for the next run. The next row above this is also scanned and accessible dots on this row are considered as base nodes if, and only if, they are not linked to any base node on the previous row. Clearly a base node is not always the lowest dot in a run; it is the lowest dot in a run which is accessible from a top node. Thus, from each top node except those which relate only to the top of the maze a set of base nodes can be found. The runs of dots starting on each of these base nodes are then determined, each run terminating on a next possible top node for the pathway. Of this set of next possible top nodes, the one with the maximum run of dots up to it is chosen. If there is no unique maximum, then the 'maximum' top nodes are considered in turn, and the above procedure applied to each of them. The maximum run available to each new top node is found and the next top node of the pathway is chosen on this basis. Target dots may of course act as both base and top nodes, either because they are singletons or because only the final dot in the run is accessible from the previous top node.

The general function of the strategy therefore is to start at the vertex, choose a maximum run and at the top dot of this run choose the next maximum run and so on. However, certain decisions must still be made. These affect the path taken within a group of equal runs which finish on a given top node, the path taken between a top node and the next base node, and the path when all the routes to the top are equally good. These decisions could have been selected randomly, but it seemed preferable to implement some additional hypotheses about maze-solving strategies. The first of these predicts that, other things being equal, subjects will continue in a straight line (continuation principle). If the strategy was indecisive about the very first decision, the right-hand path was chosen.

Using the patterns from Experiment *D*, this strategy was programmed and run on the University of London *Atlas* computer. Of the seventy-two items, the model failed to find a correct solution in twenty-two, and the pattern of success and failure was similar to that of the Royal Air Force subjects in that the model failed only three of the ten easiest items and passed only one of the ten most difficult. The rank correlation with success or failure was 0·38. The results are summarized in Fig. 3.

This simulation programme combines a minimal local encoding component with an elementary search procedure. Two important components are not considered at all. First, the strategy does not systematically take account of the effect of distant configurations on local decisions. Second, the strategy relates to a reduced transformation which ignores possible alternative paths through the open lattice between groupings of target dots.

The second simulation programme takes account of both these points. In this model the subject is seen as taking sequential binary decisions from the vertex to the top. At each decision point he is faced with a choice between two opportunity cones (Fig. 2); the relative attractiveness of these two cones is a function of the number of target dots each contains and the arrangement of these dots. Each target dot thus influences the decision process related to the vertex node of every opportunity cone. The problem, therefore, is to derive a function which represents for each node not the true value of its opportunity cone but the attractive force towards that node as perceived by the subject. A decision strategy can then start at the vertex of the pattern and at each bifurcation choose the most attractive node and so on up to the top. These derived values must be a function both of the pattern of the maze and of the group of people or the individual looking at the maze.

One approach makes an analogy between subjects solving the maze and current flowing in a resistance network. Subjects tend to take the path of maximum dots,

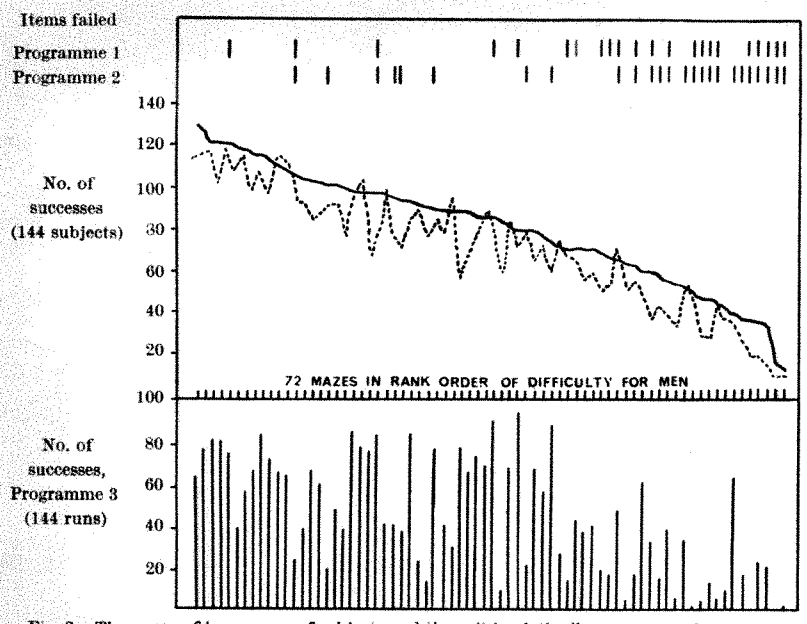


Fig. 3. The scores of two groups of subjects and three "simulation" programmes for 72 maze items. The central graph gives the number of successes for 144 male subjects (solid line) and the score for 144 female subjects (dotted line). The apparent greater variability of the female subjects reflects the fact that the items are arranged in rank order of difficulty for men. Above the graph the two rows of symbols indicate which items were failed by programmes 1 and 2. The histogram below the graph shows the number of passes achieved by programme 3 in 144 "attempts" at each item.

though not all of them succeed; current tends to take the path of least resistance, but not all of it does so. If, therefore, the maze is transformed into a resistance network in which the nodes with target dots have resistances of lower value than the nodes without target dots, and it is assumed that current can only flow along the pathways in the direction allowed to the subjects, it is possible to compute the value of the resistance from every node in the maze to the top. This value at any given node is a function of the total pattern of the cone with that node as vertex. If there are many target dots in the cone, then the value will generally be low: if these dots are arranged more in a vertical pattern than a horizontal one, and if there are dots close to the node, the value will be low again. In other words the value of the resistance from a node to the top reflects some of the perceptual components which are likely to attract a subject to that node. A similar analogy to games with strong perceptual components has been made by Shannon[9].

This model is again extremely simple and there are only two parameters to be determined. The first is the value of the two resistances. The second is a probability function determining the decision at each node. In the first instance the simplest cases were taken. The resistances were calculated on the basis that an empty node possessed unit resistance and a node with a target dot zero resistance. The decision function was always to go to the node of lowest resistance or if they were equal to the right-hand node. This programme solved forty-eight of the seventy-two items, the rank correlation with success or failure of the subjects being 0·41; the results again are presented in Fig. 3. In this form the programme gave only a single pathway. A third programme using the same resistance values was run using decision probabilities based on the arithmetical difference between the two resistance values determining each decision. These probabilities were calculated from a smoothed function derived from the decision behaviour of a subset of twelve men and twelve women in Experiment *D*. This programme was run 144 times for each maze to give a set of solutions which could be compared directly with the two sets derived from the groups of men and women. In this form and with this number of runs the programme failed to find a solution path in only two of the mazes (Fig. 3). The correlation between the performance of the programme and that of the subjects was significantly higher, being 0·60; higher correlations would almost certainly be obtained by manipulating the two parameters systematically.

One of the characteristics of the present test material which makes it particularly suitable for use with model building or simulation techniques is that there are usually a large number of different failure solutions and in general more than one correct solution. It is thus possible to assess the model in terms of the similarity of its performance to both the successes and the failures of the subjects. In the present instance we can usefully ask whether the model tended to make the same or a similar mistake on the mazes on which it failed. Because all solution paths have a common origin, a primary characteristic of an incorrect pathway is the point at which it first leaves or breaks away from a correct pathway. For the first two programmes Table 1 shows for these breakaway points the number of subjects who deviated from a correct solution at the same point as the simulation programmes and the number of subjects who at this point stayed with the correct solution pathway.

Table 1. PERCENTAGE OF SUBJECTS DEVIATING WITH STRATEGIES*

For mazes failed by:	Total (per cent)	Women *A* (per cent)	Women *B* (per cent)	Men *A* (per cent)	Men *B* (per cent)
Strategy 1 only	21·1	21·8	29·9	19·1	18·3
Strategy 2 only	22·1	18·2	23·5	19·9	22·5
Both strategies, same failure points	30·9	31·3	35·6	22·8	33·4
Both strategies, with different failure points:					
Strategy 1	21·6	19·5	26·5	18·0	22·4
Strategy 2	30·9	30·5	36·5	26·6	33·0

* The table gives the number of subjects deviating from a correct pathway at the node at which a strategy deviates, expressed as a percentage of the total number of subjects passing through the node. Under condition *A* the subject is told the solution number, under condition *B* he is not.

Finally, it is worth comparing the theoretical implications of the two types of model. In the first, specific types of behaviour are programmed to test whether or not they correspond to human maze solving skills. In the second, the relationships within the perceptual field are transformed to an analogue which allows the systematic variation of certain parameters. Although the first method is the most promising, the second can provide—and indeed has provided—useful insights into some of the mechanisms involved in this type of problem-solving behaviour.

The programmes described here were developed on the Institute of Computer Science Ferranti *Atlas* computer, and we thank Prof. R. A. Buckingham for his support and advice. We also thank Mr. Benedict Nixon for many helpful discussions. Dr. A. Elithorn and Dr. D. N. Lee were supported by a grant from the Medical Research Council, Mr. J. R. Jagoe by a grant from the Ministry of Health.

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AN ELECTROKINEMATIC THEORY OF MUSCLE CONTRACTION*

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THIS article proposes a theory of muscular contractile force development based on an electrostatic attraction of the solenoid-like structures formed by the actin and myosin filaments in striated muscles[1–5] (see Fig. 1). By assuming actin filament charge densities to be regulated by sarcoplasmic reticular calcium fluxes, we have theoretically determined that such microscopic solenoids can develop contractile forces very similar to those measured in living muscles. Our hypothesis differs from the mechano-chemical theories of H. E. Huxley and Hanson[6,7] and A. F. Huxley and Niedergerke[8,9] in that the myosin cross-bridges are not assumed to develop mechanically the contractile forces between the filaments. It differs from the electrostatic theory of Spencer and Worthington[10] in that excitation does not lead to momentary actin-site charge reversals with subsequent "jumps" of independent myosin filaments. It quite readily leads to the development of maximum tetanic force, predicts the nature of active-state tension development, and develops typical length-tension diagrams.

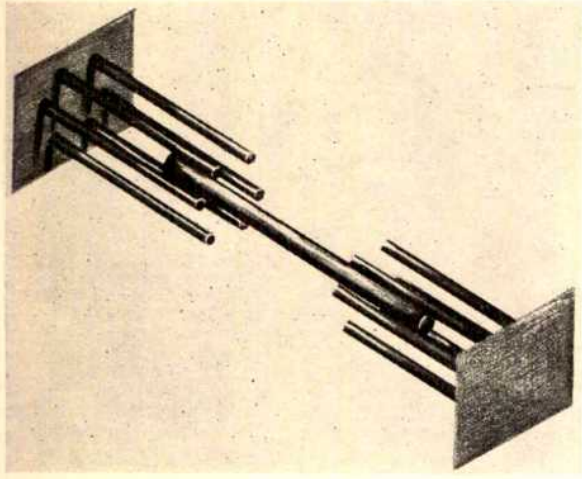


Fig. 1. The basic ordered structure formed by the actin and myosin filaments in striated muscles, a structure repeated more than 10^{14} times in each cubic centimetre of the muscle volume. Two groups of six *F*-actin filaments are shown, attached at one end to the *Z*-membranes to form a hexagonal array about a centrally located *L*-myosin filament.

The concept of charges bound to the internal filaments of both resting and active muscles is basic to this electrostatic theory. Since amino-acid analyses[11,12] and electrophoretic studies[13,14] indicate that the myosin molecule is negatively charged in nearly physiological solutions, and because the heavy meromyosin cross-bridges are known to bind firmly negatively ionized ATP[15,16], the myosin rods are considered as negative line charges in this hypothesis, as schematically depicted in Fig. 2. Although negative charges also bind to the *F*-actin filaments, primarily as ionized ADP[17–19], the recent work of Kobayasi[20] suggests that an equal number of evenly distributed positive charges bind to these filaments, which implies net charge neutrality of the actin filaments in resting muscle, also depicted in Fig. 2. Thus, no electrostatic contractile force is generated in the resting muscle owing to the net zero line charge on the actin filaments. The physical behaviour of the relaxed muscle fibre is determined solely by the viscous and elastic properties of the internal structures within the fibre[21–24].

If an electrostatic contractile force is to develop in the sarcomere during stimulation, however, the *F*-actin filaments must become positively charged. The four structures adjacent to the *Z*-membranes in Fig. 2 are assumed to play an important part here. These structures, known as the lateral cisternae of the sarcoplasmic reticulum, act as storehouses of divalent calcium cations in striated muscles[25–28]. When stimulated, they release their stored ions, which diffuse into the interfilamentary spaces, react with the filaments, and initiate the development of the contractile force[29–36]. When the excitation ceases, the cisternae actively accumulate once more those calcium ions just released[25–28,37–39]. Although the mechanism of this release and re-accumulation is not at present fully understood, it is clear that contraction and relaxation are controlled by the levels of free calcium ions in the sarcomere volume[34–36]. Concentrations of these ions greater than 10^{-7} M lead to muscular contraction; concentrations less than this cause relaxation[40–42].

Fig. 3 depicts a mechanism by which these calcium ions are postulated to cause the sarcomere to contract.

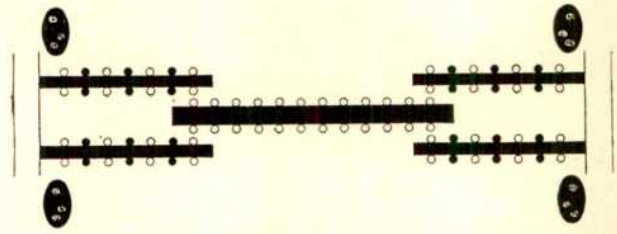


Fig. 2. Schematic diagram of sarcomere at rest, showing single *L*-myosin filament with four-*F*-actin filaments. ○, Negative charges; ●, positive charges; dotted ovals, calcium ions; solid ovals, lateral cisternae. See discussion in text.

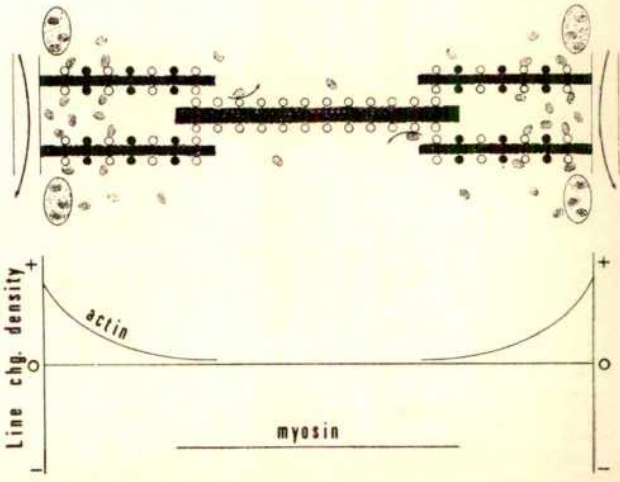


Fig. 3. Schematic diagram showing activated sarcomere. Symbols as in Fig. 2.

* Parts of this article were presented at the eighteenth Annual Conference on Engineering in Medicine and Biology, Philadelphia, Pennsylvania, on November 11, 1965.

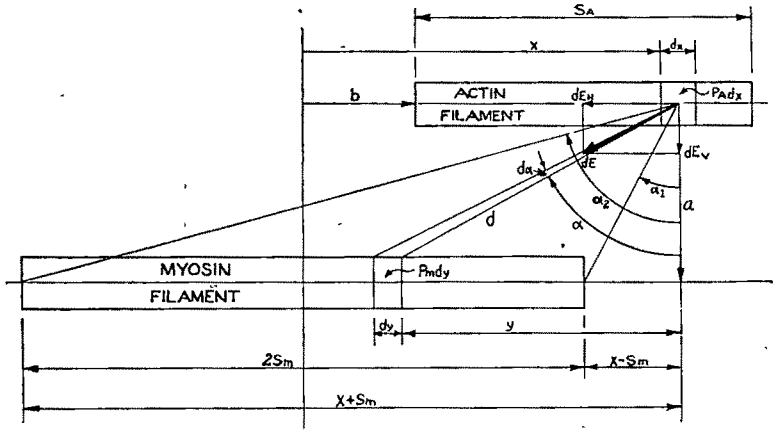


Fig. 4. Filament geometry for mathematical derivation of forces between actin and myosin rods.

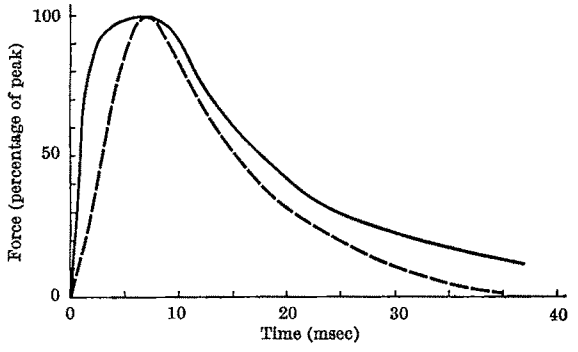


Fig. 5. Active state force development after single stimulus. ————, Experimental; — — — —, theoretical prediction by this hypothesis. Experimental curve scaled.

The muscle has just been stimulated, and the lateral cisternae have responded by releasing calcium ions into the inter-filamentary spaces*. Those ions released are assumed to react with sites on both the actin and myosin filaments in a concentration dependent fashion. Ions reacting with the actin filaments are postulated to induce a net positive charge on these filaments at their reactive sites. Because the calcium concentration is greater near the *Z*-membranes than at points distant from these structures, the actin filaments assume their greatest positive charge densities at points nearest the membranes, as depicted in the graph in Fig. 3. Reactions on the myosin filaments, which require the presence of myosin-bound ATP, are assumed to maintain the negative charge densities along these filaments as well as unlock the electrostatic bonds which form between the oppositely charged filaments during contraction. Thus, an electrostatic contractile force is developed in the sarcomere after stimulation. The positive charges on the *F*-actin filaments attract the negative charges on the *L*-myosin filaments and the muscle contracts.

When the stimulation is terminated, the sarcoplasmic reticulum actively returns those ions previously released to the lateral cisternae, which rapidly lowers the concentration of free ions in the sarcomere below the threshold value of 10^{-7} M. Because the net *F*-actin charge density is reduced to zero by this process, the muscle relaxes. Thus a complete cycle of contraction and relaxation is brought about by the release and reaccumulation of calcium ions by these reticular structures with the resulting modifications of charge densities which these ions bring about along the actin filaments.

The consideration so far represents a qualitative description of our electrokinematic theory. In order to investigate the interplay of these events in a quantitative sense, however, we must express the hypothesis in mathematical form. This derivation has been accomplished and is outlined in the following.

Assume that the myosin rod has a line density of P_m charges per metre (see Fig. 4). We desire to find the field intensity at x on the actin rod resulting from this line charge. The horizontal component of the electric field at this point is[43]

$$E_H = \frac{P_m}{4\pi\varepsilon a}\left(\cos\alpha_1 - \cos\alpha_2\right) \qquad (1)$$

where ε is the dielectric constant of the medium surrounding the rods and a is the distance between them.

Since the sarcomere volume (V) remains constant as the sarcomere length changes[44], we have

$$2\pi a^2(S_A + b) = V \qquad (2)$$

where S_A is the length of the actin filament, and b is half the width of the H zone. Expressing the cosine terms in equation (1) as functions of x and substituting the value of a found in equation (2) into the resulting equation yields:

$$E_H = \frac{P_m}{4\pi\varepsilon}\left[\frac{1}{\sqrt{(x-S_m)^2 + \frac{V}{2\pi(S_A+b)}}} - \frac{1}{\sqrt{(x+S_m)^2 + \frac{V}{2\pi(S_A+b)}}}\right] \qquad (3)$$

where S_m is half the length of the myosin filament.

If the actin filament has a positive line charge density $P_A(x,t)$, then the force tending to contract the sarcomere at any time (t) is

$$F = \int_b^{S_A+b} E_H P_A(x,t)\,dx \qquad (4)$$

where E_H is given in equation (3). (It should be noted that Debye–Hückel theory, with its many restrictive assumptions[45], is not used in this derivation. An attempt is made, however, to approximate the effects of double layer screening by reducing the filament line charge densities used in the computer solutions of equation (8).) If, however, this actin line density $P_A(x,t)$ is related to local calcium concentration $C(x,t)$ by

$$P_A(x,t) = K_1C(x,t) + K_2 \qquad (5)$$

then one must determine $C(x,t)$. This is the solution of the one-dimensional diffusion equation with reversible binding and irreversible first order reaction[46,47]. For purposes of analysis, the sarcomere is considered to be a cylinder with ends at $x = \pm(S_A + b)$. The initial and boundary conditions are then

*Although the case of *Z*-plane calcium release is analysed here, this is not a unique condition for force development in this hypothesis. Maximal isometric force is readily developed with calcium release from the *A–I* boundary, as in some types of striated muscles; however, this force is developed at a somewhat greater sarcomere length (about 2.6μ rather than 2.2μ).

$$\frac{\partial C(x,t)}{\partial x} = 0;\ x = 0, \forall\ t$$
$$C(x,t) = C_0;\ x = \pm\ (S_A + b), \forall\ t \qquad (6)$$
$$C(x,t) = f(x);\ \forall\ x,\ t = 0$$

A solution of the diffusion equation satisfying these constraints is

$$C(x,t) = C_0 \frac{\cosh x \left(\frac{K}{D}\right)^{1/2}}{\cosh (S_A + b)\left(\frac{K}{D}\right)^{1/2}} + \frac{2}{S_A + b} \cdot \Phi \qquad (7)$$

where

$$\Phi = \sum_{n=1}^{\infty} e^{-(D\beta^2 + K)t} \cos \beta x \left(\frac{(-1)^n D\beta C_0}{D\beta^2 + K} + \int_0^{S_A + b} f(x') \cos \beta x' dx' \right)$$

and

$$\beta = \frac{(2n-1)\pi}{2(S_A + b)}$$

and D and K are the diffusion and reaction rate constants, respectively. Combining equations (3), (4) (5) and (7), and rearranging, the final equation becomes

$$F = \frac{P_m}{4\pi\varepsilon} \int_b^{S_A + b} \left(K_1 C_0 \frac{\cosh x \left(\frac{K}{D}\right)^{1/2}}{\cosh (S_A + b)\left(\frac{K}{D}\right)^{1/2}} + K_2 + \frac{2K_1}{S_A + b} \cdot \Phi \right) x$$
$$x \left(\frac{1}{\sqrt{(x - S_m)^2 + \frac{V}{2\pi(S_A + b)}}} - \frac{1}{\sqrt{(x + S_m)^2 + \frac{V}{2\pi(S_A + b)}}} \right) dx \qquad (8)$$

This equation was programmed on an IBM 7090 digital computer (Stanford University Computation Center) to similate isometric experiments on striated muscle. Since calcium ions are released in pulses from the lateral cisternae in response to muscle stimuli, the solution of equation (8) was carried out in time increments equal to the pulse widths of these stimuli (2 msec). The calcium distribution in the sarcomere at the end of each time increment was used as the initial condition ($f(x)$) for the next solution increment. During these increments, the programme assigned to C_0 one of two values to generate the desired stimulation frequency $\left(\frac{1}{T2}\right)$, an "on" value of $1{\cdot}667 \times 10^{-5}\ (1 - e^{-T2/0.012})M$ or an "off" value of 10^{-8} M. (Because the lateral cisternae are not assumed to be infinite calcium sources, the "on" concentration was reduced as the frequency increased.) Other parameters used in these solutions were $D = 2 \times 10^{-8}$ cm^2/sec, $K = 15$ sec^{-1}, $\varepsilon_\pi = 40$, $S_A = 1\mu$, $S_m = 0{\cdot}825\mu$, $P_m = 4{\cdot}925 \times 10^{-10}$ coulombs/m, P_A(SAT.) $= 2{\cdot}377 \times 10^{-10}$ coulombs/m, $K_1 = 4{\cdot}4 \times 10^{-31}$ m^2 coulomb/ion, $K_2 = -2{\cdot}64 \times 10^{-11}$ coulombs/m, and $V = 3{\cdot}925 \times 10^{-21}$ m^3. The similarities between the actual experimental data and the predictions made by the computer solutions of equation (8) are the subject of the remainder of this article.

Fig. 5 depicts the time course of the active state in the muscle following a single pulse of stimulation[48-53]. The force predicted by the theoretical equation (dashed curve) is compared with the active state as determined by D. E. S. Brown by pressure potentiation studies[54] (solid curve). The active state has a rapid initial onset and a slow decline, which are properties noted in the time history of calcium concentration in the sarcomere volume.

Other features of the active state predicted by the theoretical equation but not shown here are the decline in force and the small increases in peak time and relaxation time as the muscle is stimulated at increasing sarcomere lengths[55-57].

Fig. 6 depicts the length–tension diagram of striated muscle. The muscle, initially stretched to a given length, is rapidly stimulated, and the maximum developed tension is recorded. Curves 1 and 2 are the length–tension diagrams for frog sartorius fibres as experimentally determined by Ramsey and Street[58,59] and Gordon, A. F. Huxley, and Julian[60], respectively. Curves 3 and 4 illustrate two length–tension curves which can be computed on the basis of our electrostatic hypothesis. In curve 3 the actin line density increases linearly from b to $S_A + b$. In curve 4 the line charge increases linearly from b to about $3/4\ S_A$, then remains constant (owing to saturation) over the balance of the filament. By reducing the reaction rate constant (K) in equation (8), other length–tension diagrams having greater developed forces at long sarcomere lengths, similar to those measured by Buchthal[57] and Carlsen, Knappeis, and Buchthal[61], are also predicted by this equation. The maximal isometric force of about 8×10^{-8} g/myosin filament[62,63] is readily developed in our hypothesis with filament line densities less than 5×10^{-10} coulombs/m and interfilament distances of 185–250 Å. The reduction of developed force at long sarcomere lengths arises from the increasing separation of the charge distributions as the muscle lengthens. The fall of tension at short sarcomere lengths occurs as the ends of the myosin filaments enter the regions between the Z-membranes and the charge centres on the actin filaments. Note that the form of the length–tension diagram depends on the ratio K/D in equation (8), while the twitch force depends on a weighted sum of these parameters. Since both K and D would be expected to have a temperature coefficient of the same sign, it would follow that the twitch would be affected more by changes in temperature than would be the tetanus at a given sarcomere length.

In Fig. 7 we have six curves, drawn by a Calcomp plotter in conjunction with a 7090 digital computer, which show the solutions of equation (8) as the frequency of stimulation is increased (sarcomere length, $2{\cdot}2\mu$). It should be noted that although the stimulus duration is only 2 msec, peak force development does not occur for about 7 msec. This lag in peak force development is due to the nature of the diffusion process in the sarcomere and agrees with experimental observations of the active state in striated muscle[48-53]. It should also be noted that as the interval between successive stimuli is reduced, the height of the response to the second stimulus increases; this is the active state counterpart of the force-interval curve measured in living muscle[64,65]. The curves also demonstrate a progressive fusion of the active state force

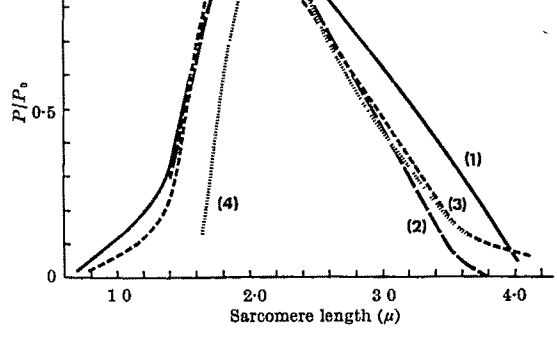


Fig. 6. Length–tension diagrams of striated muscle. Curves (1) and (2), experimental; curves (3) and (4), theoretical prediction by this hypothesis. Curve (1) scaled.

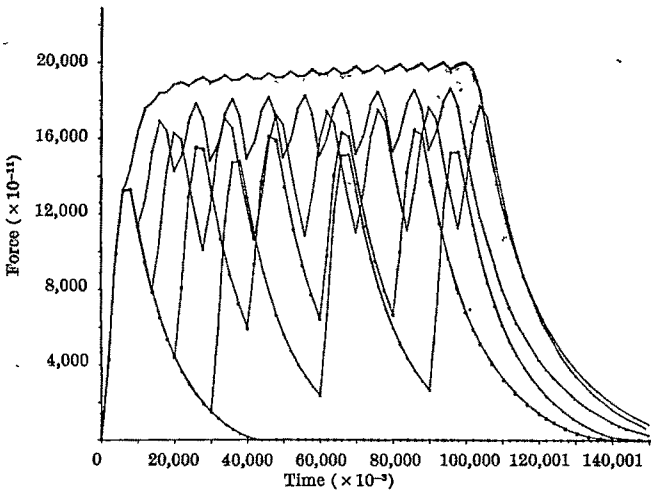


Fig. 7. Theoretical active-state forces predicted by equation (8) at increasing stimulation frequencies.

as the frequency of stimulation is increased, which is again a typical feature of muscle behaviour[57].

Thus the theoretical isometric predictions based on this hypothesis correlate well with the experimentally determined behaviour of striated muscle. These correlations, however, must be interpreted cautiously, because we are predicting the behaviour of a single force-developing element within the sarcomere and the experimental data are taken from single fibres which consist of large aggregations of these force-developing units surrounded by viscous and elastic elements. Nevertheless, for well-ordered muscles such as frog sartorius, at least, the extrapolations seem reasonable in many cases.

In summary, we have proposed a mechanism by which the force of muscular contraction could be developed in striated muscle. The mechanism does not depend on a mechanochemical interaction of the actin and myosin filaments, but rather assumes that the *F*-actin filaments, neutrally charged at rest, assume a positive line charge during stimulation, and thus attract the negatively charged *L*-myosin filaments in the manner of an electrostatic solenoid. Calcium ions, released and later accumulated again by the elements of the sarcoplasmic reticulum in response to muscle stimuli, are assumed to be responsible for these alterations in charge density on the actin filaments and thus regulate the magnitude and form of the forces developed in the sarcomere.

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ELECTROSTATIC POTENTIAL MODEL FOR THE SOLUBILITY OF THE ALKALI HALIDES IN WATER

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THE purpose of this article is to present a new relationship between the solubility of the alkali halides and the ionic radii. It was observed that a linear relationship exists between the solubility of the alkali halides and the difference of the ionic radii for any given cation family. It will be shown that this relationship is compatible with the electrostatic potential model of charged spheres.

The mechanisms involved in the solubility of the alkali halides in water has been a subject of discussion for nearly five decades. The Born equation[1] for the crystal lattice

energy is fundamental to the solubility problem and thus has provided the stimulus for numerous investigations in this field. Grimm[2] studied the dependency of the solubility both on the structure of the external electron shells and on the charges and the radii of the ions. Fajans[3] showed that the deformation of the ions of the alkali halides explained some of the variations of the physical and chemical properties of the salts, while the deformation of the ionic sheaths was found to correspond to some of the variations of the solubility of the alkali halides[4]. Balce[5] obtained a solubility law based on the electrolytic decomposition potential. In an investigation of the solubility processes of the alkali halides, Campbell[6] noted that the minima of solubilities of the salts in water corresponded to the values of nearly equal anion and cation radii. These investigations have indicated some general, but not, however, always consistent, rules.

The Born–Haber cycle describes a nearly complete analytical model for evaluating the energy changes of the solubility process of a solid in a liquid. A comprehensive thermodynamic treatment, based on the Born–Haber cycle, was described by Ladd and Lee[7]. In the Born model, the heats of solution (hydration) are considered to be exothermic if the lattice energy is greater than the energy required for solution, and endothermic if the reverse energy balance occurs[8]. The relationship between the physical properties of ionic crystals and the dimensions of ions comprising the crystals was emphasized by Pauling[9]; he did not, however, discuss the solubility of the salts.

In this article, the values of the saturated solubilities and ionic radii have been taken from previous reports. The radii of the anions and cations are denoted by R_a and R_c respectively, and are shown in Table 1. These values, taken from Goldschmidt, are discussed by Seitz[10], and he ascribes the values as being self-consistent to within about 5 per cent. The solubility values for each of the alkali halide salts were taken from Sidgwick[11]. These were recalculated in moles/100 g water and are shown in Table 2.

Table 1. IONIC RADII ACCORDING TO GOLDSCHMIDT*

	Å		Å
Fluorine	1·33	Lithium	0 78
Chlorine	1 81	Sodium	0 98
Bromine	1·96	Potassium	1 33
Iodine	2 20	Rubidium	1·49
		Caesium	1 65

* See ref. 10.

Table 2. SOLUBILITY OF THE ALKALI HALIDES IN MOLES/100 G WATER AT 25° C*

	Fluoride	Chloride	Bromide	Iodide
Lithium	0 005	1·02	1·88	1 23
Sodium	0 098	0 61	0·91	1·22
Potassium	1 65	0 46	0 55	0 87
Rubidium	1·26	0·75	0 65	0 74
Caesium	2 40	1·09	0 58	0 27

* See ref. 11.

Although not shown here, a plot of the solubilities as a function of $(R_a - R_c)$ exhibits a linear function for the cation families. However, Fig. 1, a plot of the solubility as a function of $\frac{1}{R_aR_c}(R_a - R_c)$, shows a similar relationship but a better fit. The $\frac{1}{R_aR_c}(R_a - R_c)$ values are listed in Table 3. A similar plot (not shown here) of the solubility as a function of $\frac{1}{R_cR_a}(R_c - R_a)$ did not have a linear relationship for the halogen families. Thus the linear relationship is apparently restricted to the cation families.

The four caesium salts fall on a straight line; rubidium fluoride, rubidium chloride and rubidium bromide lie in a straight line, rubidium iodide does not; potassium iodide and potassium chloride is a straight line with a given slope, while potassium bromide and potassium iodide lie on another straight line of equal but opposite slope; sodium fluoride does not lie on the straight line of sodium chloride, sodium bromide and sodium iodide; lithium fluoride, lithium chloride and lithium bromide lie on a straight line, but lithium iodide does not. For the rubidium and sodium families, a straight line was drawn through three points with a slope of $\mp\alpha$ and another straight line drawn through the fourth point with a slope of $\pm\alpha$.

The empirical solubility equation can be expressed as

$$S = \alpha\left[\frac{1}{R_aR_c}(R_a - R_c) + L_s\right] + S_0 \qquad (1)$$

where S is the saturated solubility in moles/100 g water, α the slope in moles-Å/100 g water, and S_0 a constant in moles/100 g water. The values of the constants for a good fit of equation (1) are listed in Table 4. The full significance of the constants cannot be evaluated at this time.

Table 3. $\frac{1}{R_aR_c}(R_a - R_c)$ VALUES OF THE ALKALI HALIDES (Å^{-1})

	Fluoride	Chloride	Bromide	Iodide
Lithium	0 52	0 56	0 60	0 64
Sodium	0 27	0 46	0 51	0 56
Potassium	0 00	0 26	0 32	0·39
Rubidium	−0 08	0·12	0·16	0 21
Caesium	−0 14	0 05	0 10	0 16

Table 4. EMPIRICAL CONSTANTS DETERMINED FROM THE EXPERIMENTAL DATA TO FIT EQUATIONS (1) FOR EACH CATION FAMILY

	$\left(\frac{\alpha}{\text{Moles-Å}/100\text{ g water}}\right)$	L_s (Å^{-1})	S_0 (Moles/100 g water)
Lithium	22 3	−0 517	0
Sodium	∓5 82	−0 312	−0·22
Potassium	∓4·55	−0 278	0 38
Rubidium	∓2 58	−0·165	0 61
Caesium	−7·88	−0·175	0

The two branches shown in Fig. 1 and the positive and negative slopes for the sodium, potassium, and rubidium families indicate a second-order function for equation (1). However, owing to the linearity exhibited by the branches, the positive values of $\alpha[(R_a - R_c) + L_s]$ will be con-

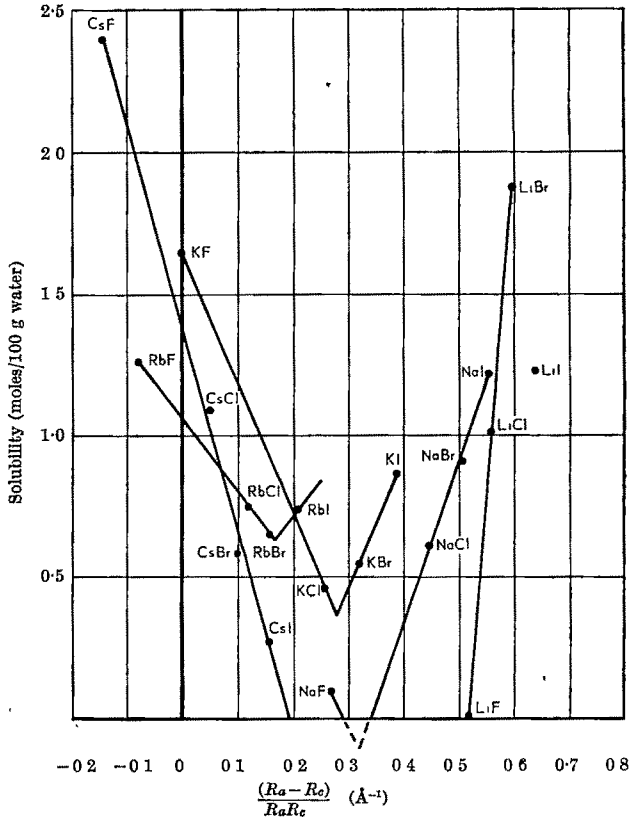


Fig. 1. A plot of the experimental solubility of the alkali halides in moles/100 g water as a function of the difference of the reciprocal of the ionic radii in Å^{-1}.

sidered only when the appropriate signs for α are taken in this discussion.

A form of equation (1) can be obtained from an approximation based on the electrostatic potential between two charged spheres. Although the water molecule has a dipole moment, it will be assumed here that during the solvation process, the water molecule behaves as either a positive or negative ion (that is, positive if a negative ion is hydrated, and negative if a positive ion is hydrated). The electrostatic potential at the shell of the cation during solvation by the water molecule is

$$V_1 = K_1 \left(-\frac{1}{R_{w-}} + \frac{1}{R_c} \right) \quad (2)$$

and for the anion solvation, the potential is

$$V_2 = K_2 \left(\frac{1}{R_{w+}} - \frac{1}{R_a} \right) \quad (3)$$

where K_1 and K_2 are constants of the medium, and R_{w+} and R_{w-} are the water molecular radii when behaving as a positive or negative "ion" respectively. Assuming $K_1 = K_2$, the energy available per molecule for solvation in water is:

$$E = V_1 + V_2 = K \left[\frac{(R_a - R_c)}{R_a R_c} + \left(\frac{1}{R_{w+}} - \frac{1}{R_{w-}} \right) \right] \quad (4)$$

The number of molecules of solute, measured in moles per given volume of water, would be proportional to E. Thus equation (1) can be implied by letting $L_s = \left(\frac{1}{R_{w+}} - \frac{1}{R_{w-}} \right)$.

The form of equation (1) is in contrast to the Born treatment, which is a function of the sum of the reciprocal of the ionic radii[8] for the solubility of the alkali halides in water.

It is noted that heat of solution does not implicitly enter equation (1). The heats of solution of the alkali halides, endothermic or exothermic, do not exhibit any consistent pattern in the $(R_a - R_c)$ relationship. The salts on the heavier halogen side, lithium chloride, lithium bromide, lithium iodide, sodium iodide and potassium iodide are exothermic, whereas the fluorine salts, potassium fluoride, rubidium fluoride and caesium fluoride, are also exothermic. For the heavier halogen salts, it is noted that the face centred crystalline structures exhibit anion–anion contact as determined by the ionic ratios $\frac{R_c}{R_a} \leqslant 0{\cdot}414$ (ref. 12). The ionic ratios of the alkali halides are listed in Table 5.

The R_c/R_a values of lithium chloride, lithium bromide, lithium iodide and sodium iodide agree with the anion–anion contact model within the error expected of the radii values[10]. It is therefore postulated that for the heavier halogen salts, the exothermic heat of solution results from the anion–anion repulsion force during the solvation process. The R_c/R_a value of potassium iodide at 0·61 does not fit the anion–anion contact model and cannot at this time be explained.

Table 5. R_c/R_a VALUES OF THE ALKALI HALIDES

	Fluoride	Chloride	Bromide	Iodide
Lithium	0 59	0·43	0 40	0 35
Sodium	0 74	0 54	0·50	0 44
Potassium	1·0	0 74	0 68	0·61
Rubidium	1·12	0 84	0 76	0 68
Caesium	1·24	0·91	0 84	0·75

The exothermic heats of solution accompanying potassium fluoride, rubidium fluoride and caesium fluoride, can be explained as follows. During the solvation process, the attraction of the water molecules to the cations is greater than that to the anions[13,14]. Thus it is assumed that for cations the radii of which are equal to or larger than the anion radii, the cation would hydrate (dislodge from the crystal lattice) before the anion. The anion–anion repulsion force would then predominate during the solvation of the cation and thus the solvation would exhibit an exothermic heat of solution. Potassium fluoride, rubidium fluoride and caesium fluoride are exothermic; $\frac{R_c}{R_a} \geqslant 1{\cdot}00$ for each and thus satisfies the conditions of the above postulate.

The endothermic heats of solution would then be explained from the converse of the anion–anion repulsion force model. The salts of which the molecules exhibit attractive forces during solvation would require energy for hydration, and thus the process would lose heat.

Two conclusions can be drawn from these observations: first, the solubility of the alkali halides in water can be expressed as a function of the differences in ionic radii based on the hard shell electrostatic potential model; second, the exothermic heats of solution result from anion–anion repulsion forces present in the crystal lattice during the solvation process and are apparently indirectly dependent on the solvation process itself. The endothermic heats of solution are explained, conversely, as arising from the attractive force present in the crystal lattice during the solvation process.

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PRESENCE OF A SERIES OF PLASTOQUINONES IN PLANTS

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CHROMATOGRAPHIC fractionation of plant lipids has revealed the presence of a family of compounds, the plastoquinones (PQ), members of which exhibit the same ultra-violet absorption characteristics, maxima at 255 and 263 mμ, although they are chromatographically distinct. Crane[1] originally described plastoquinone-9 (PQ-9 or PQ *A*) as 2,3-dimethyl-5-solanesylbenzoquinone from spinach chloroplasts and since then this source has also yielded PQ-3 (ref. 2) (with a farnesyl side chain) while PQ-4 (geranylgeranyl side chain) has been found in horse-chestnut leaves[3]. Three more plastoquinones, designated PQ *B*, PQ *C* and PQ *D* to distinguish them from PQ *A*, have been found in spinach chloroplasts[4–6]. PQ *B* was similar in chromatographic polarity to PQ *A*, whereas PQ *C* and PQ *D* were much more polar in nature and were shown to have a hydroxyl group by infra-red

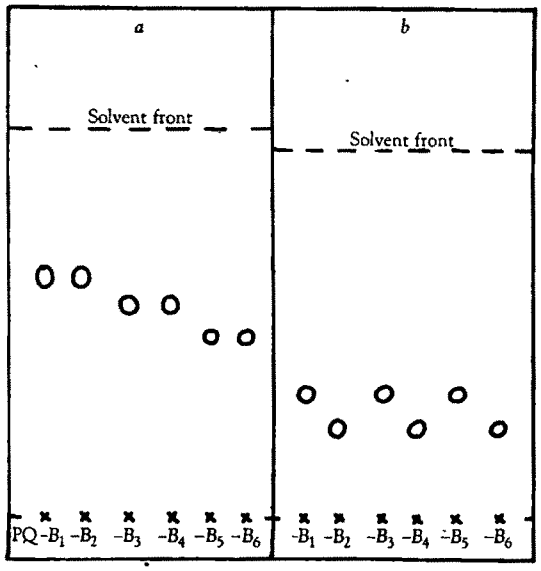


Fig. 1. Adsorption (*a*) and partition (*b*) thin layer chromatograms of purified "PQ *B*" type quinones from *Ficus*. About 10 μg of each sample was applied. Solvent: adsorption, 12 per cent di-iso-propyl ether in light petroleum. Partition, 2·5 per cent water in acetone. Stained with Emmerie-Engel reagent after reduction with sodium borohydride.

spectroscopy[7]. Samples of PQ *B*, PQ *C* and PQ *D* have been examined by mass spectrometry[8] and PQ *B* was reported to have two hydrogens less than PQ *A*, while PQ *C* and PQ *D* had an oxygen atom more than PQ *A*. Thus it was suggested[8] that PQ *C* (or PQ *D*) could be a hydroxy PQ *A* or a hydrated PQ *B*.

In the present examination of plant lipids a column fraction of similar polarity to PQ *B* has been obtained which on adsorption and partition thin layer chromatography could be resolved into six distinct components each with the 255, 263 mμ spectrum. It is proposed to call these compounds PQ B_1, PQ B_2, PQ B_3, PQ B_4, PQ B_5 and PQ B_6; identification of one or all of these compounds with PQ *B* as previously described awaits further study. It has also been shown by thin layer chromatography that there are at least six distinct compounds (each with the 255, 263 mμ chromophore) eluted from a column in the PQ *C*–PQ *D* region. The assignment of trivial names for these quinones is difficult because we cannot identify them with Crane's PQ *C* or PQ *D* specifically. On thin layer chromatography the six quinones are split roughly into two groups of three which may correspond broadly with PQ *C* and PQ *D*. As PQ *C* was the first "polar" plastoquinone to be described[4] we will call the six "polar" plastoquinones described here PQ C_1, PQ C_2, PQ C_3, PQ C_4, PQ C_5 and PQ C_6 for relative simplicity. While it is possible that there is some relationship between the two groups "PQ *B*" and "PQ *C*" we do not wish to imply any connexion between PQ B_1 and PQ C_1, etc.

Leaf tissues were obtained from the University Botanical Gardens, Ness, a few hours before use. Elder (*Sambucus nigra*) was examined by one of us (W. T. G.) in some earlier work on "PQ *B*" and so values for "PQ *C*" were not obtained for this tissue. Again the "PQ *B*" fraction from *Gossypium* contained a congener which intensively absorbed ultra-violet light and which was difficult to remove; this tissue was eliminated from the "PQ *B*" survey. The leaves were extracted by homogenizing in acetone and diethyl ether/light petroleum (40°–60° C) was added to the filtered extract and the acetone was washed out with water. The extracts were chromatographed on acid-washed alumina (Woelm) deactivated to Brockmann Grade 3 and six fractions were collected, 0·25 per cent diethyl ether/light petroleum (E/P), 1 per cent E/P, 3 per cent E/P, 8 per cent E/P, 20 per cent E/P and ether. PQ *A* was present in the 1 per cent E/P fraction, "PQ *B*" was in the 3 per cent E/P fraction while 20 per cent E/P eluted the "PQ *C*–PQ *D*" material. All three of these fractions exhibited ultra-violet absorption at 255, 263 mμ. Plastoquinone *A* was estimated by reduction with sodium borohydride using a $\Delta\varepsilon$ mol. of 18,700. Chlorophyll was determined by the method of Arnon[9].

The 3 per cent E/P fractions, which contained "PQ *B*" and α-tocopherol as the major ultra-violet-absorbing components, were chromatographed quantitatively on preparative thin layers (500μ) of silica-gel *G* impregnated with "Rhodamine 6 *G*" and developed in chromatotanks with 12 per cent di-iso-propyl ether/light petroleum. Under ultra-violet light[10] three distinct bands were seen migrating in front of α-tocopherol on the developed chromatograms and these were eluted separately and rechromatographed on paraffin-impregnated silica-gel *G* layers (250μ) developing with 97·5 per cent aqueous acetone in the saturation chamber of Davies[11]. The developed plates were sprayed with 0·001 per cent fluorescein in ethanol and examined under ultra-violet light. In this system each of the three bands from the adsorption plates separated into two fractions (see Fig. 1) and these were eluted.

The samples were chromatographed on columns of alumina (3 g), light petroleum eluting the paraffin and 6 per cent E/P bringing off the quinones. The quinone content of each fraction so obtained was estimated by reduction with sodium borohydride in ethanol (assuming $\Delta\varepsilon$ mol. at 255 mμ of 18,700 for all PQs). All six quinones possessed the typical PQ spectrum, $\lambda_{max.}$ 255 mμ, inflexion 263 mμ and gave the same isosbestic points at 232 and 276 mμ on reduction.

A small sample of the 20 per cent E/P fraction (0·3–0·5 mg) was subjected to two-dimensional thin layer chromatography with 15 per cent ether/benzene as solvent in both dimensions. The developed chromatogram was sprayed with borohydride solution, acid and then Emmerie–Engel reagent[12] and the quinones were thus detected as pink spots. Seven quinones were detected in a diagonal line across the plate (see Fig. 2). There was one relatively polar spot and two groups of three. Two portions of each sample were chromatographed and the developed chromatograms were sprayed with 0·001 per cent fluorescein and the quinones seen in ultra-violet light as dark areas on a fluorescent background. The silica-gel corresponding to each spot was scraped off

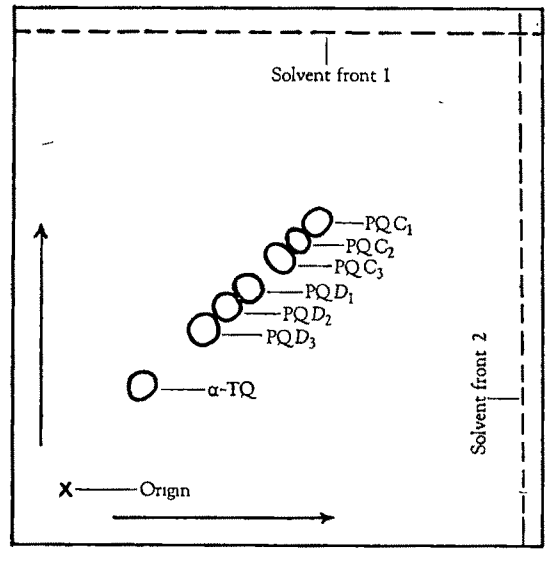


Fig. 2. Two-dimensional thin layer chromatogram of polar plastoquinone fraction from *Gossypium*. Solvent: 15 per cent di-iso-propyl ether/benzene in both dimensions. A sample of the 20 per cent ether/light petroleum fraction from column chromatography of *Gossypium* lipids was treated to the two-dimensional thin layer chromatography and was stained with Emmerie-Engel reagent after reduction with sodium borohydride.

Table 1. PLASTOQUINONES *A* AND *B** AND CHLOROPHYLL† CONTENT OF LEAVES

	Chlorophyll	PQ *A*	PQ B_1	PQ B_2	PQ B_3	PQ B_4	PQ B_5	PQ B_6
Ficus elasticus	0·69	145	11·1	13·1	16·7	12·9	5·2	11·2
Passiflora xallardii	2·24	108	4·9	3·9	3·4	4·9	1·9	2·9
Fuchsia arborescens	1·75	36·0	3·2	4·2	3·6	1·5	0·6	2·0
Stephania japonica	2·83	76·9	2·1	3·4	1·2	2·3	0·1	1·1
Ixora coccinea	1·60	14·6	2·5	2·5	2·3	1·7	0·6	0·9
Urtica dioica	1·57	82·3	2·3	3·0	2·6	1·9	0·7	1·2
Pinus nigra	1·06	29·4	6·2	3·9	4·5	7·3	2·2	3·9
Sambucus nigra	1·26	160	11·2	21·2	9·4	11·2	4·6	4·8

* Quinone values expressed as μmoles × 10^3/g fresh weight.
† Chlorophyll values expressed as mg/g fresh weight.

Table 2. PLASTOQUINONE *C**, α-TOCOPHEROL QUINONE* AND CHLOROPHYLL† CONTENT OF LEAVES

	Chlorophyll	PQ *A*	PQ C_1	PQ C_2	PQ C_3	PQ C_4	PQ C_5	PQ C_6	α-TQ
Ficus elasticus	0·69	145	16·6	24·7	13·3	11·3	13·7	14·0	34·8
Passiflora xallardii	2·24	108	35·1	38·3	26·0	15·3	28·6	20·5	14·8
Fuchsia arborescens	1·75	36·0	2·2	3·0	1·7	0·2	5·2	1·9	8·0
Stephania japonica	2·83	76·9	8·4	10·2	8·9	4·2	6·3	5·5	22·9
Ixora coccinea	1·60 %	14·6	6·1	8·1	6·1	3·0	4·9	4·3	13·3
Urtica dioica	1·57	82·3	1·4	1·4	1·7	1·2	2·5	1·9	11·9
Pinus nigra	1·06	29·4	5·8	10·6	17·2	‡	‡	‡	8·4
Gossypium herbaceum	2·57	136	13·9	17·7	15·8	15·2	42·2	13·3	23·4

* Quinone values expressed as μmoles × 10^3/g fresh weight.
† Chlorophyll values expressed as mg/g fresh weight.
‡ Unidentified lipid interfered with borohydride reduction.

(material from the two plates was bulked) and extracted twice with ether. The absorption spectrum of each fraction in ethanol was examined and the most polar spot showed maxima at 262 and 269 mμ which is indicative of α-tocopherol quinone. All the other six spots gave spectra with maxima at 255 mμ and inflexions at 263 mμ. The plastoquinones and α-tocopherol quinone were determined by borohydride reduction using the Δεmol. figures of 18,700 at 255 mμ for PQs and 17,840 at 262 mμ for α-tocopherol quinone.

Table 1 shows the content of "plastoquinones *B*" in the various leaf samples. The total "PQ *B*" level was in general much lower than that of PQ *A* and varied from 0·0036 μmole/mg chlorophyll in *Stephania* to 0·102 μmole/mg in *Ficus*. *Pinus* was exceptional in that the total "PQ *B*" (28·0 μmole/g fresh weight) was of the same order as PQ *A* concentration (29·4 μmole/g fresh weight). The relative amounts of each "PQ *B*" type quinone varied between tissues; however, the PQ B_1 plus PQ B_2 fraction (eluted together from adsorption thin layer chromatogram) accounted for the highest proportion while the PQ B_5 fraction always occurred at the lowest level.

All the 20 per cent fractions gave similar two-dimensional thin layer chromatograms showing the same seven spots. The quinones were (in order of increasing polarity) PQ C_1, PQ C_2, PQ C_3, PQ C_4, PQ C_5, PQ C_6 and α-tocopherol quinone. Table 2 shows the content of the quinones in the leaf samples. The ratio of PQ *A* to "PQ *C*" varied; for example, in *Passiflora* and *Ixora* there was more "PQ *C*" than PQ *A* and in *Gossypium* the levels were about the same. In *Urtica* and *Fuchsia*, however, there was very much more PQ *A* than "PQ *C*". α-Tocopherol quinone was found to be present at levels generally much lower than those of PQ *A* and lower than the total "PQ *C*". In general PQ C_2 was in the highest amount in the "PQ *C*" group and there were relatively large amounts of PQ C_5 in some cases. There was usually much more "PQ *C*" in a plant than "PQ *B*". All the plastoquinones in the "*C* group" gave isosbestic points at 231 and 307 mμ on reduction of the quinone to quinol with borohydride and these positions are similar to those recorded for PQ *C* and PQ *D* by Threlfall, Griffiths and Goodwin[7]. These isosbestic points are slightly different from those given by the "PQ *B*" components, and the reason may be that, because of the methods used, the "PQ *B*" materials were purified to a greater extent.

It might be argued that this multiplicity of "PQ *B*" and "PQ *C*" fractions is merely a reflexion of the instability of the materials and that many of the PQs reported here are artefacts. However, the various chromatograms obtained could always be reproduced and there was no indication of changes in pattern; furthermore, the presence of six quinones in each group cannot be without some significance of familiarity.

One of us (J. C. W.) is the recipient of a Science Research Council research studentship. Part of the equipment used in this work was provided by a grant from the Science Research Council. We thank Prof. R. A. Morton for his help and interest in this work.

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STEREOCHEMICAL CODE OF AMINO-ACID RESIDUES: THE MOLECULAR CONFORMATION OF GRAMICIDINE *S*

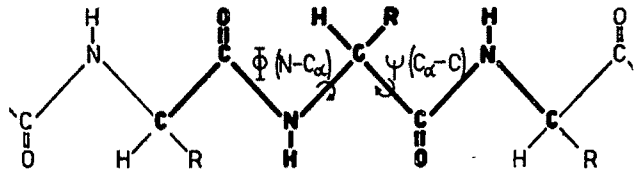
By PROF. A. M. LIQUORI, P. DE SANTIS, A. L. KOVACS and L. MAZZARELLA

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THE possible conformations of helical polypeptide chain with amino-acid residues in equivalent positions are restricted by their van der Waals interactions[1–3]. The limited number of permissible conformations includes those established experimentally by X-ray analysis.

We have published contour maps showing the conformational free energy of helical poly-L-alanine and poly-L-proline as a function of the angles of rotation about the bonds adjacent to the α-carbon atoms. Figs. 1 and 2 are the same maps redrawn according to the new con-

vention[4]. The maps show that only five helical configurations are sterically permissible ($R\alpha,\beta,\gamma,\delta$, $L\alpha$). Amino-acids with bulky side-chains, such as valine, threonine or isoleucine, which are repeated indefinitely, can distort any of these helices, but especially the α-helix[2,3]. Accordingly, only a restricted number of conformations are permissible for a polypeptide chain made up of non-identical residues. The chain, which would not necessarily consist of conformationally equivalent residues, would have a tertiary structure defined by the sequence of conformational states of its component residues. We have calculated the potential energy of the following unit:



This does not correspond to an amino-acid residue but to that part of the chain the conformation of which is determined by two angles of rotation $\psi_{C_\alpha-C}$ and Φ_{N-C_α} adjacent to the α-carbon atom. The energy map obtained in this way makes it possible to select the most stable conformation of an L-amino-acid residue in a polypeptide chain independent of its nearest neighbours in the sequence. Such a calculation shows that the only conformational states permissible for any one "unit" are those which we shall call $R_{a,b,c,d}$, La, shown in Fig. 3, which generate by repetition the helical conformations $R\alpha,\beta,\gamma,\delta$, $L\alpha$ permissible for homopolymers of L-amino-acids. However, the minima of the energy map calculated without the condition of conformational equivalence are broader because the co-operative interactions which stabilize a helix are absent. Nevertheless, this procedure

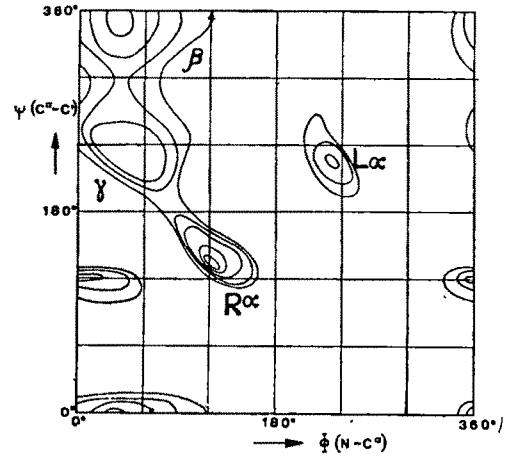


Fig. 1. Two-dimensional plot of the van der Waals conformational potential energy of a chain of poly-L-alanine as a function of the angles of rotation ψ (Cα−C′) and Φ(N−Cα) about the non-rigid skeleton bonds of amino-acid residues.

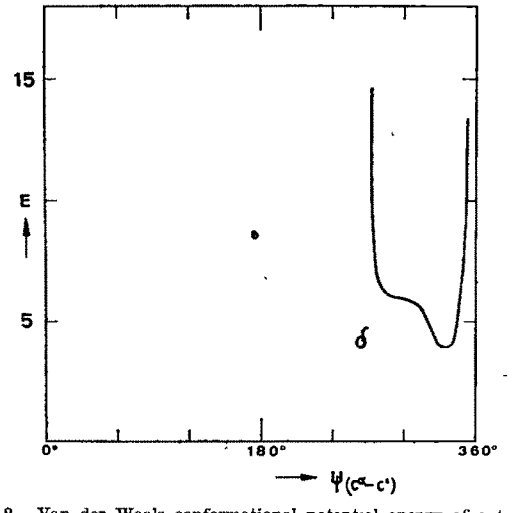


Fig. 2. Van der Waals conformational potential energy of a trans-poly-L-proline chain as a function of the angles of rotation ψ (Cα−C′) about the non-rigid skeleton bond.

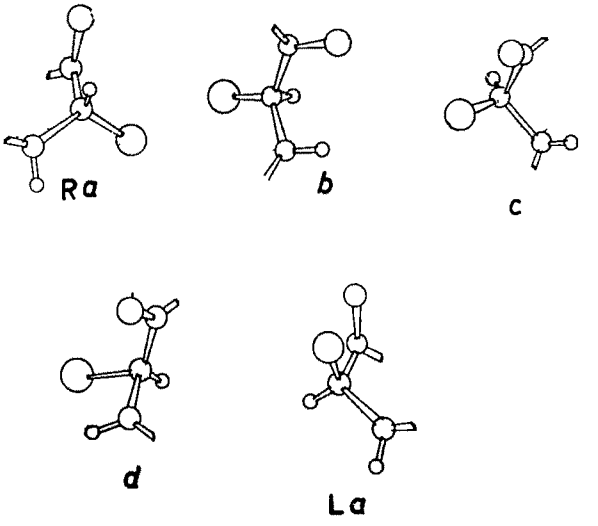


Fig. 3. Allowed conformations of L-amino-acid residues in a polypeptide chain. The peptide bonds are broken.

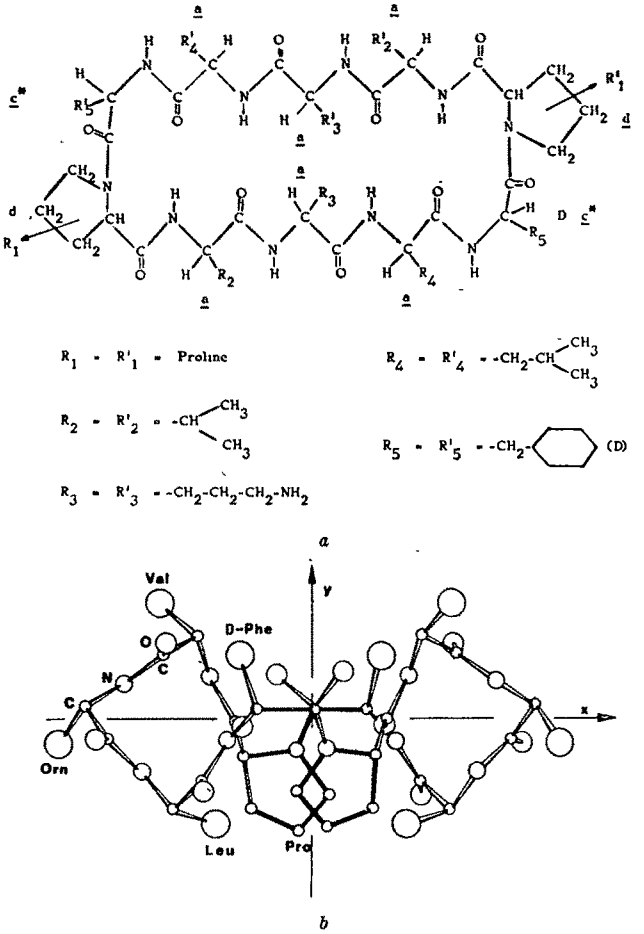


Fig. 4. *a*, Primary structure of gramicidine *S*. *b*, Model of structure of gramicidine *S*. The *y* axis is parallel to the dyad axis.

Table 1

		x(Å)	y(Å)	z(Å)
	N	−2·7	1·1	−1·5
	Cα	−3·0	2·2	−0·6
Valine	C′	−4·0	1·7	0·4
	O	−3·8	1·9	1·7
	Cβ	−4·0	3·0	−1·5
	N	−5·0	0·9	0·1
	Cα	−6·0	0·3	0·9
Ornithine	C′	−5·3	−1·0	0·19
	O	−5·6	−1·0	0·31
	Cβ	−6·6	−0·7	0·0
	N	−4·4	−1·5	1·5
	Cα	−3·6	−2·4	2·3
Leucine	C′	−2·9	−1·6	3·3
	O	−2·9	−2·0	4·5
	Cβ	−2·5	−2·7	1·3
	N	−2·2	−0·6	2·9
	Cα	−1·4	0·3	3·8
D-Phenylalanine	C′	−0·1	0·3	3·3
	O	0·8	1·2	3·6
	Cβ	−1·9	1·5	3·5
	N	0·4	−0·8	2·7
	Cα	1·7	−1·0	2·1
	C′	2·0	0·0	1·0
Proline	O	1·7	−0·2	−0·2
	Cβ	1·6	−2·3	1·4
	Cγ	0·6	−3·1	1·5
	Cδ	−0·5	−2·2	2·4

may enable the most stable conformations to be identified and a tertiary structure to be predicted on a rational basis from the primary sequence. Such a method was tested for the cylic decapeptide gramicidine *S*.

The ten residues are identical in pairs (Fig. 4*a*). We determined the sequence of permissible conformational states that can be assigned to the amino-acid residues in order to satisfy as closely as possible the condition of ring closure. Such states are indicated in Fig. 4*a*. It should be noted that for D-Phe we have chosen a state *c** which is enantiomorphous of state *c* permissible for L-amino-acid residues. Starting with this model and adjusting it so as to close the ring, the structure shown in Fig. 4*b* was obtained. This consists of two antiparallel single turns of right-handed α-helix bridged by proline and phenylalanine. Each helical section contains one hydrogen bond between the first and fourth amino-acid residues. A dyad axis perpendicular to the direction of the helix relates one half of the molecule with the other. The atomic co-ordinates with respect to the axial system of Fig. 4*b* are given in Table 1. The dimensions of the molecule agree with those given by Schmidt *et al.*[5] on the basis of a preliminary X-ray analysis.

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MICROPULSATION MEASUREMENTS DURING THE PUGET SOUND EARTHQUAKE OF APRIL 29, 1965

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On April 29, 1965, a severe earthquake occurred in the Puget Sound area which was later reported to be about magnitude seven on the Richter scale. Algermissen and Harding[1] have recently published the location and magnitude of the earthquake as determined by an analysis of seismic records from an extensive array of seismometers and from damage surveys in the area. These authors have also given a description of the geological setting and history of seismicity in this area.

The purpose of this article is to report an interesting observation made by a pair of three-component micropulsation detectors located near the epicentre. Normally this equipment is used to measure rapid changes in the Earth's magnetic field induced in a stationary induction loop. If the magnetic field is uniform and unchanging, an electromotive force (E.M.F.) can also be induced in the coils if they undergo a rotation, but not if they undergo a simple translation. The three loops are shown schematically in Fig. 1, and their expected response to the compressional or *P* wave and the distortional or *S* wave is indicated. The *P* wave arrives first and induces a rocking motion in the vertically mounted coil ($\dot{H}_z$), but not in the two coils ($\dot{H}_x$ and $\dot{H}_y$) lying flat on the Earth's surface. As the slower *S* wave passes, the Earth's surface tilts and induces rotation in all three coils.

The two stations are located near Tulalip, Washington (48·077° N., 122·192° W.), and Cougar Mountain, Washington (47·531° N., 122·125° W.). At Cougar Mountain, two telluric current probes (E_x and E_y) were also functioning, and their response was similar to that of $\dot{H}_x$ and $\dot{H}_y$ at the same station. The signals from the three magnetic coils at each station and from the two telluric current probes at Cougar Mountain were amplified, telemetered to Seattle, and recorded on a pair of slow-speed (15 cm/h) magnetic tape recorders. Timing marks derived from the local power lines were also recorded hourly. The data were later recovered and analysed by playing the tapes back at high speed on a recording oscillograph. A reproduction of these data is given in Fig. 2.

The E.M.F.s induced in the moving coils were sufficiently large to cause saturation in the amplifier chain, so that details of the Earth movement cannot be derived from these data. However, it can be seen that the $\dot{H}_z$ coils responded to both the *P* and *S* waves, whereas the $\dot{H}_x$

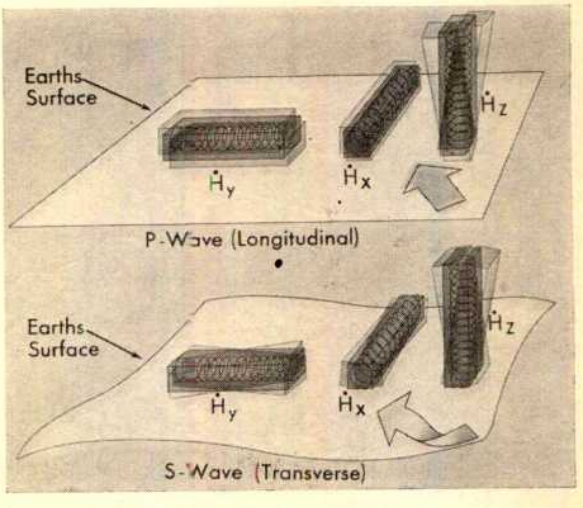


Fig. 1. Schematic representation of the three induction loops and their response to *P* and *S* waves.

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and $\dot{H}_y$ coils responded only to the S waves. It is also apparent that the signals arrived first at Cougar Mountain. The separation of the P and S waves is particularly clear in the Tulalip records, and the delay time for the arrival of the S wave after the P wave is about 11·25 sec. Because the three magnetic components at Tulalip were recorded on the same magnetic tape as one telluric current component (E_x) at Cougar Mountain, a reasonably accurate measurement of the relative arrival times of the wave at the two stations was also possible. As can be seen in Fig. 2, the S wave arrived at Cougar Mountain just as the P wave arrived at Tulalip.

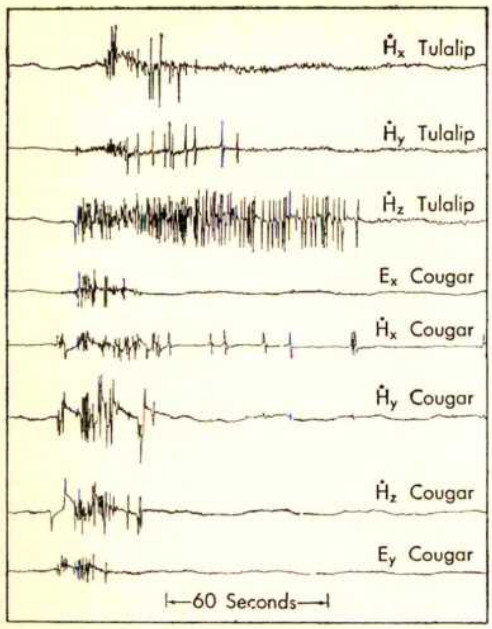


Fig. 2. Recordings of the magnetic field and earth-current detectors at Tulalip and Cougar Mountain at the time of the Puget Sound earthquake, April 29, 1965.

For the purposes of this article, it will be assumed that the velocity of the P wave, v_p, is 6 km/sec, and that the velocity of the S wave, v_s, may be represented[2] by

$$v_s = \frac{v_p}{\sqrt{3}} \qquad (1)$$

Using these velocities and the times already mentioned, it is possible to deduce from equation (2)

$$D_T = 6T = (6/\sqrt{3})(T + 11{\cdot}25) \qquad (2)$$

that the distance of Tulalip from the epicentre, D_T, was about 92 km, and the P wave arrived there at $T = 15{\cdot}4$ sec after the earthquake. Because this is the time for arrival of the S wave at Cougar Mountain, it is possible to determine the separation distance, D_C, of that station from the epicentre. Hence

$$D_C = (6/\sqrt{3})(15{\cdot}4) \simeq 53 \text{ km} \qquad (3)$$

The separation distances of the two stations from the epicentre (122·3° W., 47·4° N., 59 km depth) published by Algermissen and Harding[1] can also be computed. These distances are 96 km and 62 km for Tulalip and Cougar Mountain, respectively, and are shown in Table 1.

Considering how difficult it is to determine the exact time of onset of a P or S wave, and the assumptions that

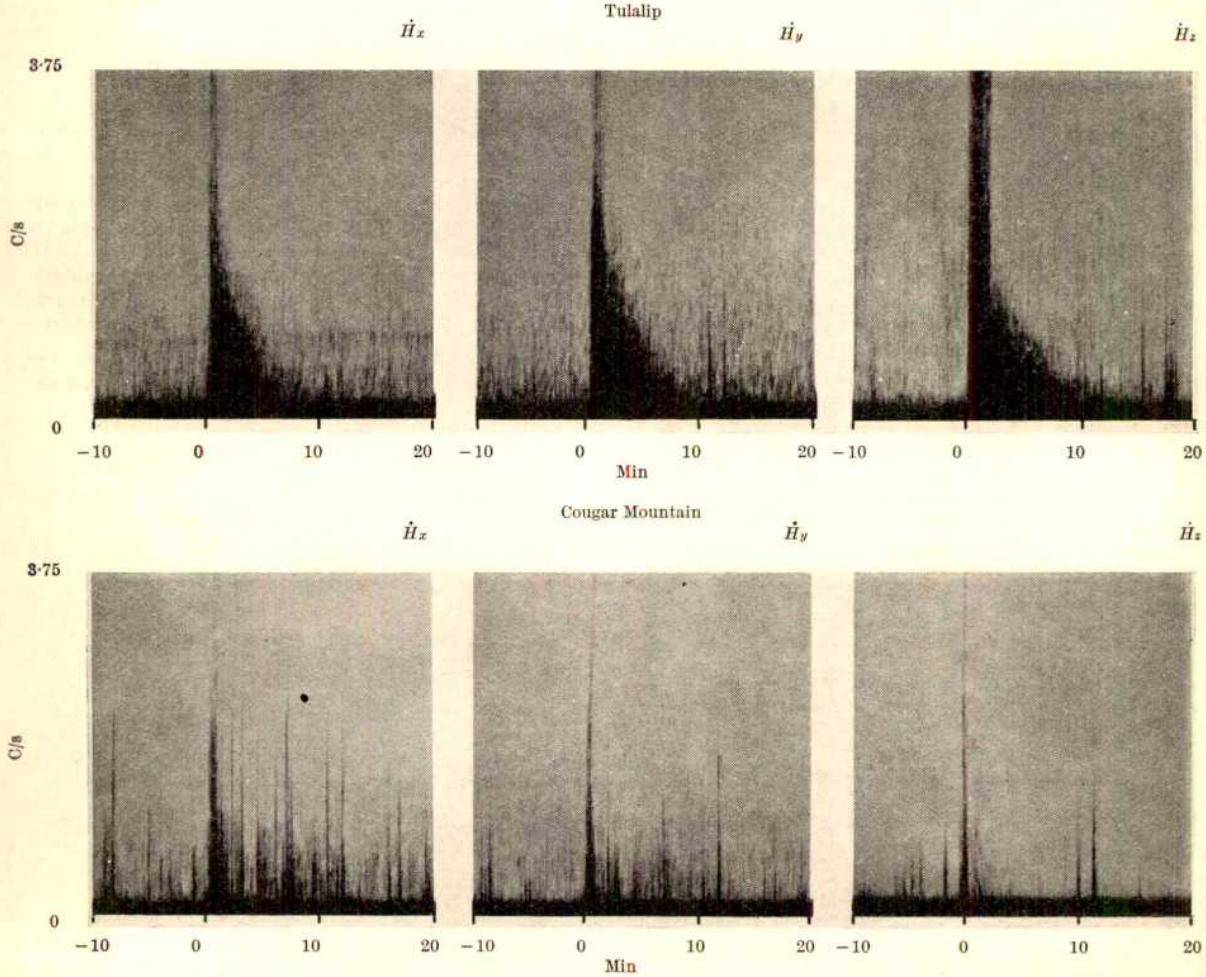


Fig. 3. Spectral analysis of the earth motion at the two stations during the Puget Sound earthquake, April 29, 1965.

Table 1. SEPARATION OF THE STATION FROM THE EPICENTRE

Station	Location	Epicentral separation according to magnetic records	Epicentral separation (Algermissen and Harding (ref. 1))	Residual distance
Cougar Mountain	47·531° N 122·125° W	53 km	62 km	9 km
Tulalip	48·077° N 122·192° W	92 km	96 km	4 km

were made concerning the velocities of the P and S waves, the residuals are certainly within the limits of error of this experiment.

Spectral analysis of Earth movement. The magnetic tapes were also played back on a 'Sonagran' spectrum analyser to determine the frequency content of the Earth movement as a function of time after the earthquake. These data are presented in Fig. 3. It can be seen that the motion was considerably smaller and terminated much more rapidly at Cougar Mountain than at Tulalip. Tulalip is located on a sedimentary area, whereas the Cougar Mountain station is situated on top of an extensive coal deposit with considerable surface outcroppings.

Magnetic precursors. In recent years several observations have been reported of magnetic changes before earthquakes. These seem to fall into two main classes: first, very slow changes which are detected as an increase in the total magnetic flux density at the surface of the Earth over a period of several years before the earthquake[3]; and second, very rapid disturbances of large magnitude in the hours just preceding the seismic disturbance[4]. Breiner[5] has reported actual measurements of this type for very small earthquakes in Nevada in 1962.

The general explanation for these phenomena is usually given as a change in magnetic permeability of the piezomagnetic rock resulting from changes in stress before the earthquake. It is clear that the pattern of magnetic disturbance before an earthquake will be difficult to predict because of the lack of knowledge of the magnetostrictive properties of the underlying rock, and because of the complicated behaviour of the stress distribution before and after faulting.

Because the micropulsation equipment described in this article responds to a time rate of change of the magnetic flux density, it is incapable of measuring any long-term variation. Stacey and Westcott[6] have recently discussed the limit of observability of the seismomagnetic effect due to local variations in geomagnetic disturbances. Our two stations are unevenly spaced near the epicentre, and for this reason we are in a very favourable position to detect any rapid variation that may have taken place and discriminate effectively against natural micropulsation activity.

Rather large natural micropulsation activity was evident at both stations after the magnetic storm of April 17, 1965, but the period just preceding the earthquake was exceptionally quiet. It was not possible to detect any magnetic perturbation which could be considered to have arisen from a seismomagnetic effect before the earthquake. Of particular interest is the fact that no magnetic disturbance was detected at the instant of the earthquake. If any magnetic disturbance was present, it was buried in the system noise. In all models based on the response of magnetostrictive rock to changing stress, and in the measurements of Breiner[5], a large magnetic signal would be generated at the instant the stress was relieved by the Earth movement. The fact that no effect was noted at the exact time of the earthquake makes it very unlikely that any appreciable change in the local magnetism had occurred before the earthquake either in a very slow build-up or in an abrupt fashion.

Because the noise level of the amplifier chain corresponds approximately to 2 milligammas/sec, we may set this as an upper limit to the time rate of change of magnetic flux density at the detectors at the instant of the earthquake. Assuming also that the magnetic impulse caused by the relaxation of piezomagnetic rock would be generated in a period less than a couple of seconds, it can be inferred that the upper limit on the maximum change of the Earth's magnetic field at the time of the earthquake was 10^{-2} gamma, and that the upper limit on the dipole moment of the stressed region was $2{\cdot}5 \times 10^{13}$ C.G.S. E.M.U. This is smaller than the value derived by Breiner[5] for a microseism in Nevada.

Thus in summary, the E.M.F.s induced in micropulsation coils have been analysed to compute an epicentre and to look for possible magnetic signals generated by the Puget Sound earthquake of April 29, 1965. The epicentre calculated by this method agrees with that published by Algermissen and Harding[1] within the limits of experimental error. The search for magnetic precursors yielded negative results. The upper limit to the change of dipole moment of the stressed region has been determined to be $2{\cdot}5 \times 10^{13}$ C.G.S. E.M.U. The fact that the focus was so deep may explain the lack of any appreciable effect. The results of this experiment indicate that, at least in this area, the possibility of using the piezomagnetic effect as a useful pre-indication of earthquake activity is rather remote.

We thank F. G. Marshall of the Boeing Company for many useful discussions and comments.

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TIME OF CHIASMA FORMATION IN RELATION TO THE TIME OF DEOXYRIBONUCLEIC ACID SYNTHESIS

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IN recent years interest in hypotheses to explain genetic recombination, or chiasma formation, has increased. This is in part due to the increasing volume of abnormal crossing-over data which high resolution microbial genetic systems have been providing and in part due to the fact that studies on chromosome chemistry and ultrastructure are beginning to converge and provide hope that an understanding of recombination phenomena in molecular terms may not be too distant. The purely mechanical torsion hypothesis[1] is no longer satisfactory, for it is unable to account for the molecular precision with which crossing-over occurs, and until recently the most widely accepted hypothesis, particularly to explain microbial recombination results, was the copy-choice hypothesis[2,3]. Greatly modified from the form in which it was originally suggested by Belling[4], this entails a synthetic error at the time that new chromatids are being synthesized. The theoretical and practical limitations

of this hypothesis are considerable when applied to semi-conservative DNA replication and to chromosome behaviour in "higher" organisms, however[5], and many modifications have been suggested to circumvent some of these difficulties. Doubt was first thrown on the tenability of the copy-choice hypothesis when it was discovered that recombination in virus *lambda* of *Escherichia coli* involved "chromosome" breakage and could apparently take place in the absence of DNA replication[6,7]. Though proof was not presented that recombination could not also accompany replication, particularly in other systems, faith in copy-choice was certainly shaken. More recent work, including the observations reported below, collectively show that the copy-choice hypothesis must now be discarded.

In the present experiments, which were designed to test the relation between the time of chiasma formation and the time of DNA synthesis, use was made of the fact that an environmental change, in the form of high-temperature treatment, will modify chiasma formation in locusts in a predictable fashion. Minutes before beginning such a treatment, cells undergoing DNA synthesis were labelled with radioactive thymidine. Labelled stages were identified autoradiographically and the time taken for labelled cells to reach diplotene and metaphase I compared with the time taken for chiasma frequency to be modified at these stages of meiosis.

The experimental material used was the desert locust, *Schistocerca gregaria*, for my earlier studies have established both the normal chiasma properties of this species at male meiosis[8] and the manner in which these are modified when the chiasma frequency is greatly reduced by high temperature treatment at a constant 40° C[9,10]. Three different labelling experiments were carried out, two involving heat treatments and one involving control temperature (30° C) alone. These may best be described separately.

In the first experiment twelve young adult male locusts were injected with tritiated thymidine (6-$T(n)$ specific activity 24·8 c./mmole). This was provided in an isotonic saline to reduce any effects of osmotic shock. Each locust received 0·02–0·025 ml. (about 10 μc.) abdominally, without anaesthesia. The locusts were then placed in an incubator maintained at 40° C and fed twice daily on fresh rye seedlings and bran. They received no light except during feeding times. One individual was taken on the third and ninth days of heat treatment, two on the fourth, fifth, sixth, seventh and eighth days. The testes were removed by vivisection, cleaned of fat body and fixed in acetic-alcohol (1 : 3). Autoradiographs were made from Feulgen-stained squash preparations of part of the testes using 'Kodax *AR*10' stripping film. The exposure time was 50 days. The remainder of the testis material was refrigerated and, once satisfactory autoradiographs had been obtained, chiasma frequencies were scored in propionic-orcein squash preparations made from this material. Chiasma frequencies were scored at diplotene when high and at metaphase I when very low (see refs. 9 and 10 for reasons). Twenty cells from each individual were scored and standard deviations and means calculated.

A constant temperature of 40° C greatly reduces chiasma frequency in this species and can lead to complete failure of bivalent formation. There is an initial time lag of several days before the expression of this effect, however, the most drastic reduction beginning at 6 days[9]. In the present experiment, using locusts bred in this Department, from material kindly supplied 2 years ago by the Anti-Locust Research Centre, London, the pattern of response differed slightly from that reported earlier: slightly greater sensitivity was noted. The great heterogeneity of response of different individuals after the initial lag of several days[9] suggests a strong genetic element in the sensitivity to heat treatment and the slight differences in response noted probably reflect some genetic differences between the stocks used.

The individual sampled on the third day of treatment was found to possess a low mean chiasma frequency, but this was just within the normal control mean range (Fig. 1). In some cells, however, a measure of distal localization was found (Fig. 2) which indicated that the heat-treatment effect was just beginning to be shown. This was borne out on days 4 and 5, where both individuals sampled on each of these two days showed reductions in chiasma frequency, with some accompanying localization, which placed them well below the control mean range (Fig. 1; 3–7). Univalents were first found on day 5 (Fig. 5). As before, the most drastic initial reduction was found on day 6 (Fig. 1; 8–10). On subsequent days all individuals showed varying degrees of univalence.

On days 4 and 5, when appreciable reductions in chiasma frequency were found at diplotene and metaphase I, the autoradiographs showed that the latest labelled cells, which were involved in premeiotic DNA synthesis at the time that the treatment was begun, were still at zygotene. This first wave of labelled cells did not reach diplotene-metaphase I until the sixth day (Fig. 11), coinciding with the rapid drop in chiasma frequency previously established for this day. Although no label was found beyond zygotene on the fifth day, on the sixth day label was found over both chromatids at all stages of meiosis from pachytene—anaphase II in both of the individuals sampled. This shows that the period of premeiotic DNA synthesis in this species is no longer than 1 day at 40° C and also that leptotene and zygotene are of 5 days duration at 40° while all of the remaining meiotic stages from late pachytene to anaphase II are completed within 1 day at 40° C. No label at metaphase I or II was found on days 7 or 8, but another wave of labelled cells was found at these stages in the one individual taken on the ninth day. As only half the chromosomes were labelled in this individual at metaphase I, this must, on a semi-conservative model of chromosome replication, have been the third wave of labelled cells to pass into meiosis, corresponding to those cells which were undergoing DNA replication two spermatogonial mitoses before meiosis. The second wave of labelled cells must have passed through meiosis between the seventh and eighth days and been missed (Fig. 1). This would make the time of meiosis 6 days and the time of spermatogonial mitosis 1·5 days at 40° C.

More important than these considerations of meiotic timing is the observation that the reduction in chiasma frequency begins some 2 days before the labelled cells reach diplotene-metaphase I. This shows quite clearly that the events which are affected by the heat treatment and which lead to a reduction in chiasma frequency (which may coincide with the time of chiasma formation

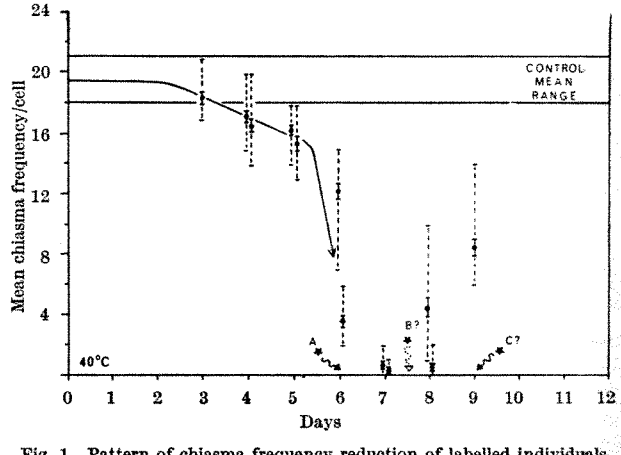
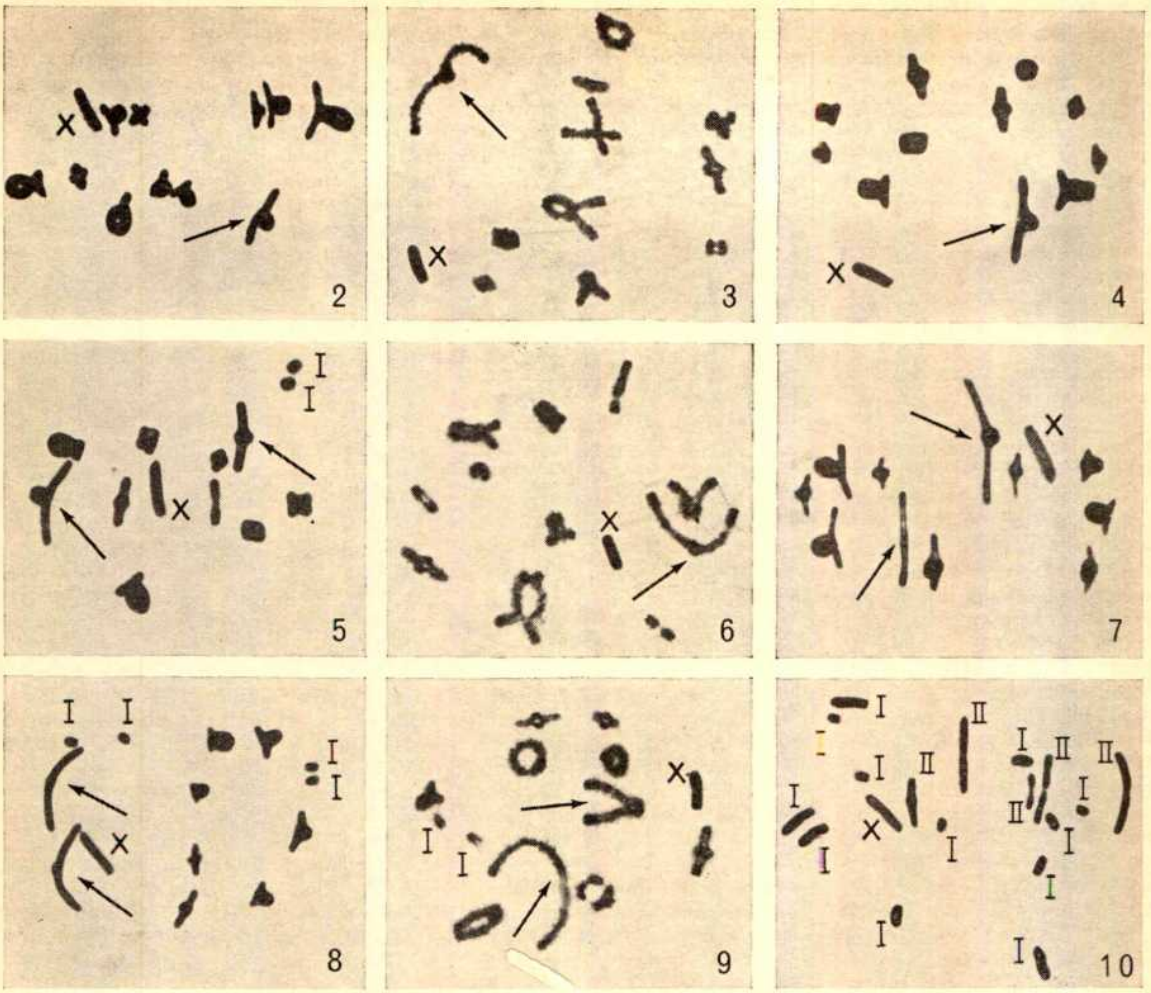


Fig. 1. Pattern of chiasma frequency reduction of labelled individuals of *S. gregaria* achieved at a constant 40° C. Means, standard deviations and (dotted) cell ranges are shown for each individual sampled. The first wave of labelled cells to reach metaphase I did so in both individuals on day 6 (*A*). The third wave arrived on day 9 (*C*). The second must have reached this stage between days 7 and 8 (*B*).



Figs. 2–10. Chiasma frequency reduction with accompanying localization observed at diplotene and metaphase I after 3–6 days at a constant 40° C. Fig. 2: 3 days heat treatment: atypical distal localization beginning. Figs. 3–4: 4 days heat treatment: slight chiasma frequency reduction with distal chiasma localization. Figs. 5–7: 5 days heat treatment: greater reduction with univalent formation beginning. Figs. 8–10: 6 days heat treatment: reduction, localization and univalence all well marked. (Figs. 2, 4, 5, 7, 8, 10: metaphase I, Figs. 3, 6, 9: diplotene.) (All × *c.* 900.)

or, more likely, precede it) do not occur at the time of premeiotic DNA synthesis but take place at least 2–3 days (at 40° C) after this period of DNA synthesis is complete. This places the time of chiasma formation in zygotene or early pachytene nuclei.

The second experiment carried out was the timing of meiosis at the "control" temperature of 30° C. Ten male locusts were injected with about 10 μc. of tritiated thymidine in saline, as before, and maintained in an incubator at a constant temperature of 30° C. One individual was taken each day on days 3, 8, 9, 10, 11, 12, 13, 14, 15, 16. On autoradiographs prepared as before and exposed for 40 days it was found that labelled cells reached diplotene—metaphase I on days 13 and 14. This shows that the premeiotic DNA synthetic period and the duration of the pachytene—anaphase II stages are both approximately 2 days at 30° C compared with 1 day at 40° C, while the total duration of meiosis is 14 days at 30° C, compared with 6 days at 40° C. This gives a Q_{10} for this temperature range of 2·3.

It was known from earlier experiments[11] that a heat-treatment of 2 days at 40° C could irreparably modify the chiasma properties of those cells which were at the sensitive stage of meiosis when the heat-treatment was applied and that if returned to a temperature of 30° C a pulse of cells showing reduced chiasma frequencies, with univalent formation, would pass through meiosis. Because

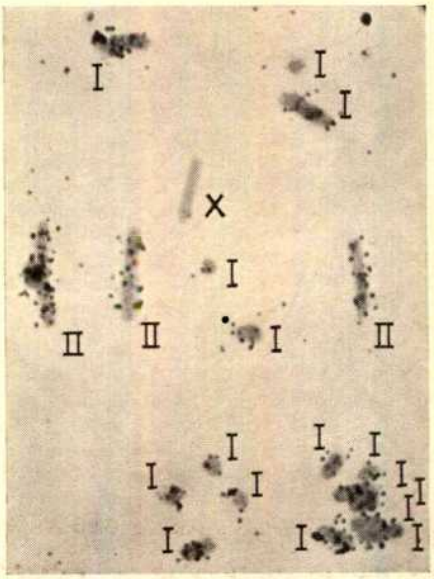


Fig. 11. Autoradiograph from one of the two individuals sampled on day 6 showing the arrival of tritiated thymidine-labelled chromosomes at metaphase I. 3 bivalents and 16 univalents are present in this cell. The late replicating Z chromosome is unlabelled. (× 1,800.)

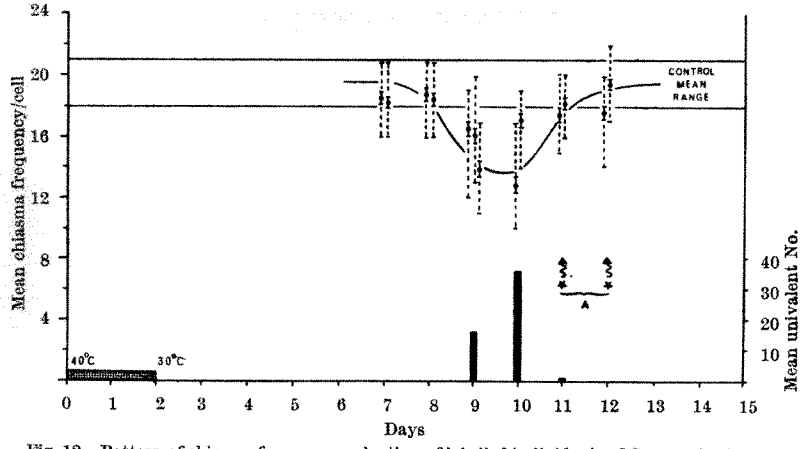


Fig. 12. Pattern of chiasma frequency reduction of labelled individuals of *S. gregaria* observed at 30° C after 2 days heat treatment at 40° C. Means, standard deviations and cell ranges again provided for each individual. One of the two individuals sampled on days 11 and 12 showed labelling in both chromatids at metaphase I and II (*A*). These cells were engaged in premeiotic DNA synthesis at the beginning of the heat treatment.

of the much slower rate of development at 30° C, however, these affected cells do not reach diplotene—metaphase I until a week or so after returning the locusts to this lower temperature. It was considered that this slower developmental rate found at 30° C might lead to a clearer separation of the time of premeiotic DNA synthesis from the time at which the high temperature treatment begins to affect chiasma formation. Accordingly, in the third labelling experiment, fourteen male locusts were injected, as before, with about 10 μc. of tritiated thymidine in saline and heat-treated at 40° C for 2 days. They were then transferred to an incubator maintained at 30° C and two locusts were dissected on days 7, 8, 10, 11 and 12; three were taken on day 9. One of the fourteen injected died. When autoradiographs were successfully developed (exposure time 60 days) chiasma frequencies were once again scored in twenty cells from the remainder of each testis, in propionic-orcein squash preparations, and standard deviations and means calculated. The results are shown in Fig. 12.

On days 7 and 8 both individuals sampled possessed low mean chiasma frequencies, but these were well within the normal control range. On the ninth and tenth days, however, marked reduction in chiasma frequency was evident, with accompanying univalent formation along with the tendency for the localization of those chiasmata which do form to the chromosome ends. On days 11 and 12, recovery was well under way, though it was by no means complete. It was found that the autoradiographic results confirmed and extended those obtained before. When chiasma formation was greatly reduced on days 9 and 10, the most advanced label was confined to zygotene nuclei. Labelled cells did not reach diplotene—metaphase I until days 11 and 12, when one of the two individuals taken on each of these two days showed labelling in all chromatids at metaphases I and II. The fact that both individuals on these days did not have identical labelling properties suggests that the wave of labelled cells passes through these later stages of meiosis within one day after this combined 40–30° C treatment, with a mean of about 11·5 days, and might be missed if only one individual is sampled per day.

The observation that the labelled cells do not reach metaphase I until days 11 and 12 shows that not only is the time of chiasma formation, or the time of some possibly synaptic stage preceding chiasma formation which is sensitive to heat treatment, not coincident with the time of premeiotic DNA synthesis, but it is sufficiently separated from this for a considerable heat-treatment effect to be manifest and recovery to be well advanced before the labelled cells pass through mid-meiosis. Clearly any hypothesis to explain chiasma formation, such as the copy-choice hypothesis, which involves the formation of chiasmata at the time of premeiotic DNA replication can no longer be entertained. Hypotheses which require chiasma formation to occur before the time of DNA synthesis[12] are even further from the truth. These findings are in agreement with the conclusions drawn by Lawrence, based on the effects of radiation on chiasma frequency[13,14] and recombination[15], that these take place after DNA synthesis is complete, during the more classical times of zygotene or pachytene.

While this work was in progress, two independent sets of observations of a comparable type, using genetic recombination rather than chiasma frequency, were published. In one[16], a 1 day heat-treatment of 35° C was given to *Drosophila melanogaster* females and the effect of this heat shock on recombination frequency determined on subsequent days at 25° C. As described above in *Schistocerca*, a wave of affected cells passed through meiosis giving a maximum effect in eggs laid on the ninth day. The effect itself differed in being an increase in crossing over adjacent to the centromere of chromosome II, though this may well reflect an overall decrease, with the tendency for proximal localization. The time taken for tritiated thymidine-labelled oocytes to mature was determined in autoradiographs of histological sections of ovaries. Because the mean time of oocyte maturation within the ovary was also 9 days, the authors came to the conclusion that recombination does occur at the same time as DNA synthesis. They did not determine the mean time of oviposition of labelled eggs, however, which is more relevant. To explain the discrepancy with our results on locusts, this need only be longer than the time of egg maturation within the ovary by 1 or 2 days. Alternatively, the time of the prophase (synaptic ?) stage sensitive to the heat treatment may, in this material, be separated from the time of DNA synthesis by an interval no greater than 1 day. In this case a 1 day sampling programme would lack the resolution necessary to separate these stages. In the second report of this type[17], recombination was measured in the liverwort *Sphaerocarpus donnellii*, subjected to a heat treatment of 35° C during the daytime. It was concluded that an effect of the heat treatment could be obtained after the fifteenth day post-fertilization, although DNA synthesis, as estimated by radiation-induced mutation studies, is completed by this time. These findings are thus in direct agreement with those obtained in *Schistocerca*.

One final observation which provides valuable support for the conclusions drawn above is that of Westergaard[18] using the Ascomycete fungus *Neotiella rutilans*. Here nuclear fusion of two haploid nuclei takes place immediately before meiosis in the ascus. Using Feulgen microspectrophotometry he has concluded that, in this species, DNA doubling appears to occur before nuclear fusion. In this instance, recombination between dissimilar genotypes could hardly occur at the time of DNA synthesis if, at this time, the two haploid nuclei have not yet fused.

These findings in *Schistocerca*, *Sphaerocarpus* and *Neotiella*, three completely different life forms, are sufficiently clear for us now to be able to say with certainty that chiasma formation and genetic recombination do not occur at the time of premeiotic DNA synthesis or earlier. In *Schistocerca*, chiasma formation must take place at least several days after premeiotic DNA synthesis is complete, during the classical time of zygotene or early pachytene and true breakage and reunion must be involved. Whether hybrid DNA formation[5] is also involved in this

exchange process will, perhaps, be one of the next things to attempt to decide.

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STRUCTURE DEVELOPMENT IN AGGREGENS

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UNDER appropriate conditions, established strains of cultured mammalian cells aggregate[1] and with continued cultivation such aggregates grow in size and eventually fragment. The cycle is repeated as the individual fragments grow in size and fragment[2]. In a growing culture, cells which adhere to each other in a group taking the form of a relatively organized mass are referred to as "aggregens". In our initial studies the development of aggregens from three strains, *HEp*$_2$, *WISH*, and *L* strain was determined[2]. The aggregens of these strains varied both in external appearance and in internal organization. It was found, however, that in all cases there was a relatively thin peripheral layer of healthy cells and active multiplication occurred in this region, while the interior portion of the aggregens seemed to consist of deteriorating or dead cells. The cells in the outer layer did not appear to exhibit any definite structural features. As the growth of cells in the form of aggregens provided one of the requisites for structural differentiation, however, we thought that conditions might be found which would permit such differentiation to occur.

We have now been able to form aggregens from fourteen additional established strains of mammalian cells (Table 1). We used the same methods for aggregating cells[3] and culturing aggregens[2] as in our previous experiments. We observed that some of the strains formed structures and this article reports the details of our observations.

Table 1. CHARACTERISTICS OF AGGREGENS OF VARIOUS CELL STRAINS

Amorphous	Structured
Chang liver*	Chang conjunctiva*
HeLa-Gey†	Girardi's heart-*H**
HEp$_2$†	Girardi's heart*
*L*929-*DC*‡	HeLa-S_3†
Monkey heart*	Monkey kidney-*MK*$_2$*
Monkey kidney-*MK*$_1$*	Pig kidney*
Monkey kidney-*MKG**	
Mouse lymph node‡	
Mox 12-6‡	
Swine lymph node*	
Wish amnion*	

Cell strain: *Normal epithelial; † malignant epithelial; ‡ normal fibroblastic.

The development of structures was first noted in aggregens formed from cell strain *MK*$_2$. This strain has been studied more extensively than the others. Isolated from normal monkey kidney in 1955, it has been maintained in continuous cultivation since then[4]. In sections of aggregens of this strain (Fig. 1) transverse longitudinal sections of tubular structures were noted in the healthy outer portion. In addition to the presence of these it was noted that all the remaining cells in this region of the aggregen were arranged in various patterns and were not randomly orientated as they are in aggregens with amorphous layers[2]. Whereas the cells of the other strains required a number of days to aggregate, the cells of the *MK*$_2$ strain aggregated quite rapidly and complete aggregation of all the cells occurred within 24 h. When aggregation was carried out on the roller drum in a peptone medium containing β and γ globulin serum fractions, a number of aggregates formed in the tube. This medium was removed at the end of 24 h and a medium consisting of 10 per cent horse serum in Eagle's mixture was added. Shortly after, the smaller aggregates joined together to form a single large aggregate. When the initial aggregation procedure was carried out in the medium containing serum, a single large aggregate formed within 24 h.

In order to determine the time sequence for the development of structures in *MK*$_2$ aggregens, a large number of aggregates were made. They were fixed, sectioned, and stained immediately after aggregation had been observed. This process was repeated at daily intervals during the differentiation of the aggregates. The youngest aggregates did not exhibit any tubular structures but, although the arrangement of cells was essentially amorphous, there appeared to be grouping of cells within the aggregate. Structures did not develop in the aggregens until a certain size had been achieved, and degenerating and dead cells were observed to be present in the interior of the aggregen. With continued growth of the aggregen, the structures of the outer healthy layer were no longer observed and had an amorphous appearance as also did the cells in the outer layer of daughter aggregens that developed following the fragmentation of the parent aggregen. Aggregens apparently grow by the multiplication of cells in this healthy layer at the periphery, and when the outer layer reaches a certain size the cells on its inner border become ill and die probably as a result of the lack of nutrients available to them[2]. It would appear, therefore, that after the period in the growth of the aggregen during which structures develop, the next generation of cells are incapable of forming differentiated structures. Moreover, it would also seem that when the structures "arrive" at the inner border of the outer layer they are obliterated by the necrosis that occurs here.

Clone cultures were developed from the *MK*$_2$ cell strain using Puck's method[5] and aggregates were made with cells from the clone cultures. Structures similar to those noted in aggregens of the original *MK*$_2$ strain were observed to develop in aggregens of these clone cultures. Furthermore, the sequence of structure development was similar to that in both parent and daughter aggregens.

The structures observed in aggregens of other strains of cells were mainly in the form of vesicles (Fig. 2). The aggregens formed from one of the HeLa sublines (S_3) (Fig. 3) appeared to contain large numbers of adjacent

vesicles which resembled the organoid type of structure in tumours produced *in vivo* by this strain[6]. However, aggregens of another subline of HeLa (Gey) were amorphous. This variation, dependent on the subline of HeLa used, contrasted with the situation observed in HeLa and L strains; for here the aggregens produced from numerous clone cultures of both strains manifested no structural differences from those observed previously[2]. On the basis of their cellular organization, the aggregens that have been studied thus far can be divided into two general groups, "amorphous" and "structured". A distribution of the cell strains in these groups is given in Table 1. The types of organization which we describe here developed in our standard medium (10 per cent horse serum in Eagle's mixture). It is possible that, under different environmental conditions, or by the addition of different substances to the growth medium, differences in the organization of the cells of respective aggregens might have been observed. In preliminary experiments along these lines some differences in organization have been noted.

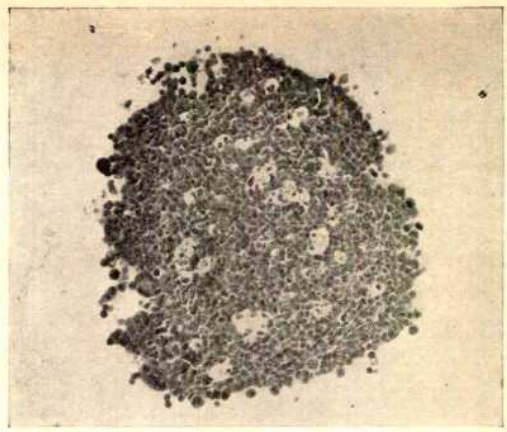
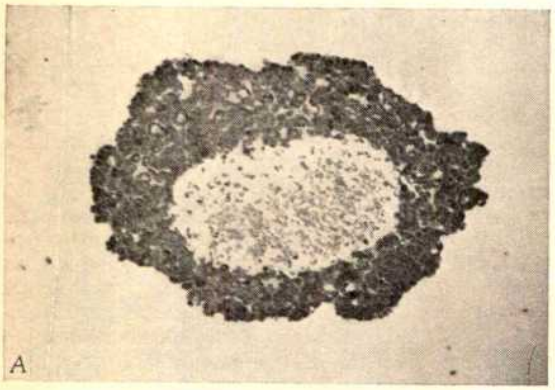
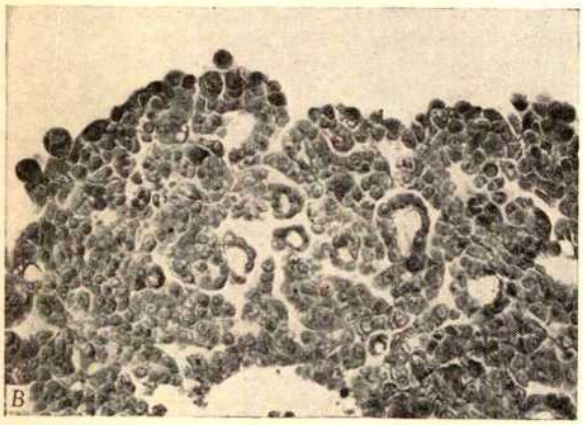


Fig. 2. Cross-section of aggregen of Girardi's heart strain (× 72).





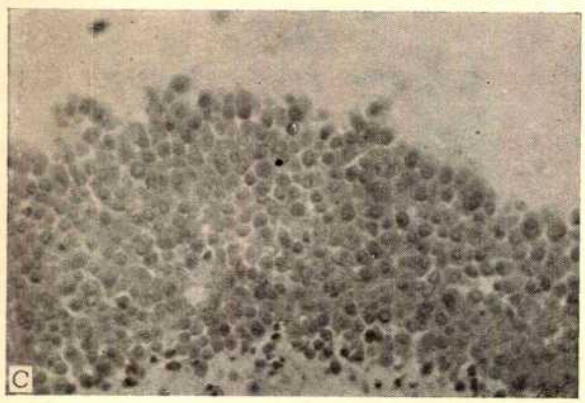


Fig. 1. Aggregens of MK_2 strain. *A*, Cross-section of structured aggregen (×,42). *B*, Segment of outer layer of *A* (× 150). *C*, Segment of outer layer of older aggregen without structures (× 180).

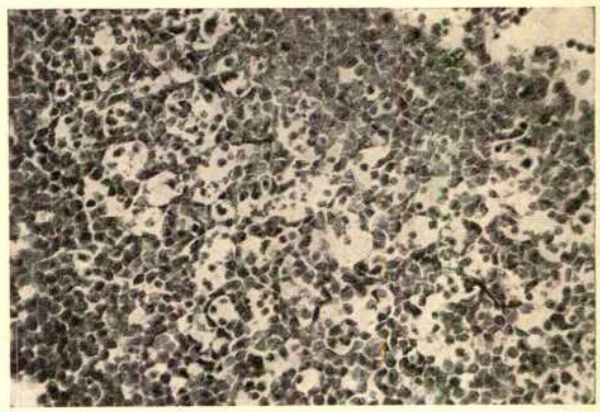


Fig. 3. Cross-section of outer layer of aggregen of HeLa S_3 strain (× 120).

Discussion. It was previously observed that, although cultured mammalian cells can aggregate, it takes from a few days to a week for this to occur[1,3]. With cells from chick embryos up to about 10 days of age, aggregation occurs relatively rapidly and is complete within 24 h. The ability of the cells to aggregate decreases as the embryo grows older, or if the embryonic cells are maintained as a monolayer[7]. Although they have been grown in culture for more than 10 years, cells of the MK_2 strain aggregate in the same manner as do cells taken from young embryos; that is, in a serum containing medium they form a single large aggregate within 24 h. In view of the fact that they can form definite structures, it would seem likely that the cells of this particular strain of cultured cells are endowed with some of the unique properties of cells present in young embryos.

The development of structures in aggregens of this strain appears to occur at a particular time in their cycle of growth as aggregens. Their potential for producing the structures is not apparently expressed until the cells have interacted for a certain time. The cessation of structure development with increasing age of the aggregens suggests that continued association of cells in the tissue-like mass finally results in the repression of the ability to form these structures. The sequence of events can be likened to embryonic development in which a particular type of differentiation occurs at a given period in the growth of the embryo and then ceases.

It is evident that the production of structures does not result from the interaction and sorting out of different types of cells in the original culture because structure

development occurred in clone cultures derived from the parent MK_2 strain. Although it cannot be said that the ability to form structures still resides in every cell of the original strain, clearly the potential to form these structures is present. The structures observed in MK_2 aggregens often exhibited a definite tubular shape; however, there was great variety both in the size and conformation of the tubules. During cytodifferentiation of the embryo the development of specific structures is assisted by the structures already present. These provide support and may also act as guides to groups of differentiating cells. It may therefore be possible to produce more definite and regular development of structures if aggregens are produced from mixtures of the MK_2 strain and another strain of cells, with the latter strain providing some form of support or matrix for the MK_2 cells. The observation that the organization in the aggregens produced with a tumour cell strain, HeLa S_3, is similar to structures observed in these tumours grown *in vivo* lends support to the previous suggestion that the properties of these cells in aggregens may resemble those of natural tumours[2]. No variations in the structure of the aggregens derived from clones of the HEp_2 and L strains of cells were observed. The differences which we noted in structure development using different strains of HeLa cells may reflect a greater variability among cells in cultures of the latter strain than in those of the other two. Without any doubt, the ability to form structures is associated with a surface property of cells. Yamada[8] has observed that HeLa cells form different types of clones; for example, tight clones and loosely packed clones[8]. The various properties of the surface of cells, as manifested by the type of clones that form, may be related to their ability to develop structures when grown as aggregens.

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PRECIPITATION OF TOBACCO MOSAIC VIRUS BY MACROMOLECULES: A METHOD FOR ESTIMATING MOLECULAR VOLUMES

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TOBACCO mosaic virus (TMV) diffuses through agar gels[1] but not through gelatine gels[2]. To investigate the cause of this unexpected anomaly the behaviour of TMV in solutions of gelatine and agar was investigated. It was established that the virus is precipitated by solutions of gelatine and agar above specific threshold concentrations. The addition of TMV to solutions containing the threshold concentration, or more, of gelatine or agar leads to the immediate formation of a visible precipitate. No visible reaction is detected below the threshold concentration.

The present article is concerned with the use of this type of precipitation reaction between TMV and macromolecules as a simple and rapid means of estimating the volume displaced by the macromolecules.

The mechanism by which TMV is precipitated is thought to be one of incompatibility and mutual spatial exclusion between the virus and macromolecules, that is, another manifestation of the phenomenon described in its various aspects by Albertsson[3], Ogston and Phelps[4], Laurent and Ogston[5] and Laurent[6]. When a large enough quantity of the macromolecular material has been added to confine the virus in a volume sufficiently small for the distance between the central long axes of the particles to be of the order of 200–250 Å, the particles aggregate and form needle-like crystals[7]. Taking the TMV particle to be a rod with a length of 3,000 Å and diameter of 150 Å, and a molecular weight of 40 million (ref. 8), it can be calculated that 1 mg of TMV contains $1{\cdot}5 \times 10^{13}$ particles and that these occupy 0·009567 ml. when the interparticle spacing is 250 Å.

Because of the tendency of TMV particles to distribute themselves so as to occupy all the available space as uniformly as possible[7], it is reasonable to postulate that in a mixture of TMV and a macromolecular species all the space not occupied by the macromolecules must be occupied by TMV, and vice versa. Thus when a macromolecular species is added step by step to a suspension of TMV, the virus particles are thought to occupy a progressively smaller fraction of the volume of the solution, with a progressively smaller distance between particles, until at the threshold concentration the TMV particles are less than 250 Å apart and they aggregate. This threshold concentration of macromolecules is thus the concentration required just to fill or to occupy all the remaining space in the solution. From an estimate of this remaining volume, obtained by subtracting the volume occupied by the TMV from the total volume, it is possible to calculate the volume occupied by a single macromolecule by using the molecular weight of the macromolecules and Avogadro's number ($6{\cdot}023 \times 10^{23}$ molecules per gram molecular weight) (ref. 9). This in turn can be used to obtain the dimensions of an equivalent sphere.

The validity of this hypothesis and its applicability as a general method for estimating molecular volumes were tested by observing the precipitation of TMV by a range of compounds, the molecular dimensions of some of which have already been investigated by other methods.

The following macromolecular materials were used: agar ('Ionagar No. 2', Oxoid), bovine serum albumin (Armour), casein (light, white, soluble, British Drug Houses), dextran 2,000 (Pharmacia), dextran grades *A* and *C* (British Drug Houses), gelatine (fine leaf *E*, Société d'Exploitation des Produits Chimiques Coignet), hyaluronic acid (ox synovial, kindly provided by Prof. A. G. Ogston), and *Rhizobium meliloti* strain *F* polysaccharide[10].

The procedure used for the precipitation was simple. Where sufficient material was available, samples were dissolved in a phosphate buffer[11] and various dilutions made. With gelatine the reaction was carried out with 1 ml. volumes in small tubes, and with all the other materials in 3 ml. volumes in 1 cm cuvettes. Small samples of TMV were added to the solutions and thoroughly mixed. The gelatine experiments were carried out with the addition of 0·03 ml. containing 0·63 mg of TMV, and the experiments with other materials with the addition of 0·04 ml. containing 2·4 mg of TMV. The

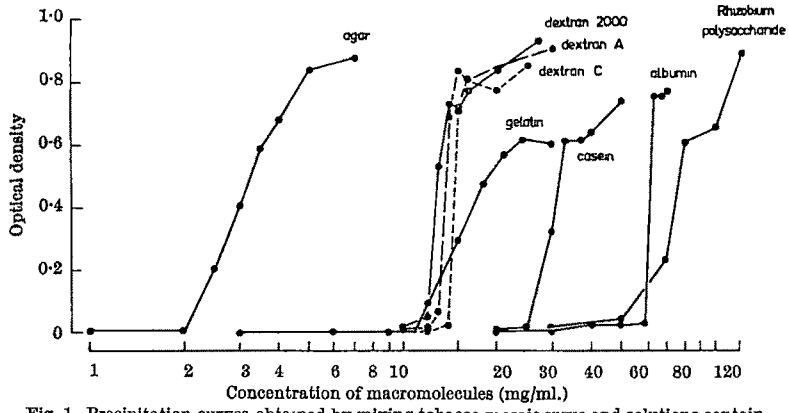


Fig. 1. Precipitation curves obtained by mixing tobacco mosaic virus and solutions containing various concentrations of macromolecular materials. Optical density measurements were made 30 min after mixing.

mixtures were allowed to stand for 30 min at room temperature, except agar, where incubation at 45° C was used to prevent the formation of a gel. The optical density of the mixtures was determined at 750 mμ. It was found necessary to make this measurement without agitation of the tubes or cuvettes in order to avoid temporary changes in optical density. When the turbid suspensions were shaken the optical density rapidly decreased by about 20 per cent but returned to its former value within a few minutes when left stationary. For this reason the mixtures were always made directly in the vessels in which the optical density readings were to be made.

In order to minimize the weight of material required when using bovine serum albumin, a direct titration procedure was utilized instead of the series of dilutions. Small volumes of a concentrated albumin solution (150 mg/ml.) were added to 1·5 ml. of TMV suspension in a cuvette. After each addition the solution was mixed and allowed to stand for 15 min before the optical density was determined and more albumin added. When the mixture was turbid the end-point was sharply defined.

The results obtained by these procedures are shown in Fig. 1. The shapes of the curves for all the macromolecules were the same: below a certain threshold concentration, characteristic for the material, there was no precipitation, but above the threshold concentration the optical density of the mixture rapidly increased with increased concentration until a maximum density was reached.

Examination of the precipitates by light microscopy showed needle like crystals of the type described by Cohen[12], except in the case of hyaluronic acid where tactoids[7] were observed. A precipitation curve was not obtained with hyaluronic acid because this material was not available in a sufficiently concentrated form for dilutions to be made. The threshold concentration for hyaluronic acid was taken to be 4·4 mg/ml. because this was the only concentration at which turbidity was obtained when TMV was added, and this was the highest concentration of hyaluronic acid available.

The following points (all obtained from unpublished data) are considered to support the view that the mechanism of precipitation is one of mutual incompatibility between the virus and added macromolecules, which results in compression of the virus into smaller fractions of the solution until phase separation in the form of crystallization or the formation of tactoids occurs. (1) Electron micrographs of the precipitated material show closely packed arrays of parallel rods, indicating that the precipitates contain only TMV. (2) The amount of precipitation is directly proportional to the amount of virus present in the mixture and is independent of the macromolecular material provided sufficient is present to exceed the threshold concentration. (3) When a sufficient amount of macromolecules has been added to achieve maximum turbidity and the mixtures are centrifuged at low speed (11,150*g* for 15 min) no virus remains in the supernatant liquid. Thus all the virus is aggregated and none is left in the volume of the mixture occupied by the macromolecules. (4) The threshold concentrations found here for dextrans (12·5–14·5 mg/ml., depending on molecular weight) are similar to the concentration (14 mg/ml.) at which the sedimentation behaviour of dextrans was found by Ogston and Woods[13] to show a change. These authors interpreted this as resulting from the fact that the dextran molecules are no longer separate and discrete, that is, this is the minimum concentration of dextran required for the molecules just to fill all the available space.

Table 1. MOLECULAR VOLUMES AND RADII OF EQUIVALENT SPHERES CALCULATED FROM MACROMOLECULAR THRESHOLD CONCENTRATIONS REQUIRED TO PRECIPITATE TOBACCO MOSAIC VIRUS

Macromolecular substance	Molecular weight	Threshold concentration (mg/ml.)	Concentration of molecules at threshold (No./ml.)	Unit volume per molecule (Å³)	Radius of equivalent sphere (Å)	Molecular shape and dimensions reported *in lit.*
(*a*) Results obtained in reactions with 0·80 mg TMV/ml. Volume occupied by TMV at threshold: 0·00765 ml./ml.						
Agar	160,000	2·04	$7·69 \times 10^{15}$	129×10^6	313	Not available
Albumin	69,000	63·5	554×10^{15}	$1·79 \times 10^6$	75	Almost spherical, major axis 144–150 Å (ref. 14)
Casein	16,300	25	924×10^{15}	$1·07 \times 10^6$	64	Rod, length 150 Å, radius 8 Å (ref. 15)
Dextran 2,000	2,000,000	12·5	$3·76 \times 10^{15}$	264×10^6	398	Not available
Dextran *A*	200,000	13·5	$40·7 \times 10^{15}$	$24·4 \times 10^6$	180	Not available
	~275,000		$29·6 \times 10^{15}$	$33·5 \times 10^6$	200	
Dextran *C*	60,000	14·5	145×10^{15}	$6·84 \times 10^6$	118	Not available
	~90,000		$97·0 \times 10^{15}$	$10·2 \times 10^6$	135	
Rhizobium polysaccharide	8,000	57·5	$4,328 \times 10^{15}$	$0·229 \times 10^6$	38	Not available
(*b*) Result obtained in reaction with 0·63 mg TMV/ml. Volume occupied by TMV at threshold: 0·00603 ml./ml.						
Gelatine	90,000	11	$73·4 \times 10^{15}$	$13·5 \times 10^6$	148	Spherical random coil (ϱ_σ = 165–175 Å) (ref. 16)
(*c*) Result obtained in reaction with 1·6 mg TMV/ml. Volume occupied by TMV at threshold: 0·0153 ml./ml.						
Hyaluronic acid	13,000,000	4·4	$0·204 \times 10^{15}$	$4,825 \times 10^6$	1,048	Almost spherical random coil (ϱ_σ = 2,300 Å)

The results obtained for the molecular volumes, calculated from the threshold values shown in Fig. 1, are presented in Table 1. It will be seen that the molecular dimensions, expressed arbitrarily as radii of equivalent spheres, are of the same order as the published data where these are available.

Some of the results require comment. Little seems to be known of the molecular dimensions of agar. The value found here applies only to agar solutions. When turbid agar–TMV mixtures are allowed to gel the turbidity disappears within 15–30 min, showing that the TMV particles are no longer confined to a small volume. Agar molecules in the gel state thus appear to occupy less volume than when in solution.

The result for albumin (diameter of equivalent sphere 150 Å) is close to the dimension of the major axis of the prolate ellipsoid (150 Å) reported by Oncley, Scatchard and Brown[17] and the later prismatic molecule with dimensions 144 × 45 × 22 Å (ref. 14). However, other recent estimates of the albumin molecule by gel filtration in dextran[18] and diffusion through agar[19] give values of

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BRITISH ASSOCIATION SUPPLEMENT—NOTTINGHAM, 1966

WHICH WAY TO CHANGE?

AN organization which goes back to the Great Reform Bill, which served as a learned society of the most vigorous kind through most of the nineteenth century, and which has retained a good deal of its original homespun independence, cannot fail to command respect and even affection. This is one of the reasons why it is good to see, with another revolution of the calendar, that the British Association for the Advancement of Science is still alive, that its programme is packed with the names of interesting people, and that the crowds will flock to Nottingham for the meeting in spite of the change in the timing of the summer public holiday in Britain. So much is common ground.

These days, however, each recurrence of the annual meeting of the association is also a time for asking what purpose is really served by the meeting and, for that matter, by the association as a whole. One difficulty is that the annual meeting has been overtaken by events, and particularly by the increasing speed with which news of discovery spreads within the scientific profession and beyond by means which are faster than annual gatherings can hope to be. Another is that science as a whole has become less accessible to the interested layman, although this is too frequently exaggerated. The immediate result, however, is that the British Association, in the annual meeting and in its less obvious work, has become less and less concerned with the advancement of science as such, and more and more of a popularizer, propagandist and proselytizer. Nobody complains at this change, which is at once desirable and inevitable.

Why then the talk of crisis? The annual report for the financial year to March 31 is a gloomy document. The association has been dipping into its meagre reserves, so that its Victorian sponsors must be turning in their graves. Revenues have risen buoyantly in the past decade, but costs have outstripped them. One result is that, in spite of the stringent economies, there is likely also to be a deficit in the year ahead. Another is that the association has taken to complaining, somewhat more querulously than may everywhere seem justifiable, that the Department of Education and Science should pay a larger share towards the educational work now being undertaken. Nevertheless, even though this is not a good time for fund raisers in Britain, the association and those outside who wish it well should not refrain from hoping for the comparatively little luck that could banish anxiety for a year or two to come.

In the long run, however, survival will require more than mere luck, which is why it is good to learn that the council of the association is now looking carefully at its motives for continuing to exist. Everybody will wish the council well. It is to be hoped that it will be as courageous and as imaginative as were the old people who first recognized the vacant place in British life for a scientific society which could turn its energies to evangelism and tub-thumping when circumstances required. There are two sorts of remedies that the council should be looking for.

In the first place, the annual meeting should be given a much firmer impact on the outside world. At present, of course, great crowds of people derive great pleasure from it, and will continue to do so in the years ahead. But the council is also right to recognize that the annual meeting is the focal point for the rest of its work throughout the year. As such, it deserves to be more exciting and more stimulating.

This year, at Nottingham, the association has taken a tiny step in this direction by arranging three symposia, outside the usual framework of the meeting, concerned with separate issues of public importance. The promise of the kind of discussion which might help to resolve public doubt has not, unfortunately, materialized. All but one of the speakers advertised for the symposium on supersonic aircraft, for example, belong to organizations committed to their development. Only Sir William Hildred, from the International Air Transport Authority, is in a position to be sceptical about their value without being actively disloyal to his employer. There is no sign on the programme of the man from the Noise Abatement Society, or of the taxpayer who may well consider that

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This year's meeting of the British Association for the Advancement of Science is the fourth to have been held in Nottingham. The pages which follow include extracts from the Presidential Address in 1966 and some historical material, compiled by the editorial staff of *Nature*, which may help readers to appreciate how the British Association has changed over the years.

a nation which finds it hard to make ends meet should now squander £200 million, and a large part of its tiny skilled labour force as well, on such a risky venture.

If the British Association really wishes to make a mark on issues like these—and there is every reason why it should—then it must take a leaf out of the old books, and appoint committees to take up these important causes, see that intelligent reports on them are fully discussed in public, and then commit to them such prestige as it can command. There is a host of issues to be taken up. Such a course would often bring the association up against official doctrines, but it used not to be afraid of that. Perhaps the greatest of its present troubles is that it has been overtaken by gentility.

The money troubles are subsidiary but dependent. They would vanish overnight if the association abandoned its lecture services and some of its other novel undertakings. So why not then abandon them? This may seem a cruel question when there is undoubted evidence of sound achievement in the recent past, but it should not be dismissed lightly. Everything depends on what kind of organization the association wishes to become. If it sees itself as providing a continuing educational service of a special kind, handling large sums of public money and similar sums from industry and charity as well, then there will be a whole sequence of general treasurers going grey before their time. There should be some way of organizing these educational activities so that they finance themselves. If the lectures, for example, are as much appreciated as the association says, then local education authorities should be easily persuaded to pay more towards them. If, on the other hand, 3*s*. 9*d*. is more than the market is prepared to pay for providing a child with a single lecture, it is better to learn that now and not later. With the alternative methods of communicating science to young people, the association should not be surprised that the Department of Education and Science should be reluctant to increase its grant.

All this suggests that the association would be very much happier, and more fully in control of its own affairs, if it came to think of itself as what it used to be—an initiator of new projects, a catalyst of new ideas and a defender of those which are old and cherished. It should be ready to show other organizations how good works may be accomplished, but chary of undertaking too much on its own account. Most of all, it should beware of too much dependence on government departments. The arrangement with the Ministry of Technology by which the association has undertaken to encourage an interest in technical matters among young people is particularly suspect, for it is hard to know precisely how it will be able to earn its keep. And in the long run, of course, the objective should be to find some way of bringing forward from within itself a flow of new and vigorous ideas. There is every reason to believe that such activity would be at least as welcome now as it used to be. It would also be fun, which is not a negligible consideration.

INFORMATION

How best to manage scientific information? Section X of the British Association has had the wit this year to arrange a discussion on this important problem. More than that, it has had the good luck to recruit as speakers people who live with the problem every day —the Director of the National Lending Library, the Head of the Information Division at the National Science Foundation, and a representative from Aslib. Sir James Cook, the Vice-Chancellor of the University of Exeter, is devoting his presidential address to the same cause. No doubt the room in which the section will be meeting will echo for days with the sound of that evocative phrase "the information explosion". But what really is the problem?

In the first place, there are mechanical issues which have thrust themselves on public attention. The development of devices which simplify the reproduction of printed text has opened up the possibility of unfamiliar means of circulating information. Some people suggest that the most familiar means of communication between people—the printed page—may instead be replaced by an electrical connexion to some central bank of computers, and there is even talk of transferring to the machines not merely the task of storing information, but also the more subtle tasks of digestion and criticism. The first thing to be recognized is that the obvious advantages of speed and comprehensiveness which possibilities like this can offer are accompanied by certain risks. In particular, there is a danger that the machines, which are necessarily better at storing facts than ideas, may oversimplify the traffic in scientific information, and may neglect that function of the scientific literature which is to stimulate as well as to inform. There is also a real danger that some schemes intended to make the circulation of the scientific literature more efficient would, in reality, help to put it out of business. Those who make machines are as aware of these risks as the potential users, but that is not enough!

How to handle the scientific information is, however, comparatively easily decided. There remains the nasty suspicion that all the improvements of efficiency which the machines now seem to offer may merely enable a much greater volume of scientific information to saturate and overwhelm potential users. So many working scientists complain of the problems of assimilating information, and of distinguishing archival material from that which is intended to stimulate further work, that the nature of the scientific literature probably requires careful study. In other words, there is a need to attend to the quality of the literature as well as to its handling. At the very least, there is a need that it should become more pithy, more intelligible, and more effective as a means of intellectual communication within the growing profession of science. As things stand, there are plenty of suggestions for managing the computing machinery more effectively; improving the quality of the literature is unfortunately more difficult.

LAND AND HUMAN POPULATION

The following are extracts from the Presidential Address delivered on August 31, 1966, at the Nottingham Meeting of the British Association, by Sir Joseph Hutchinson, Drapers' Professor of Agriculture, University of Cambridge.

THE characteristic of our age is the dominance we have achieved over our environment. We can organize ourselves so that, according to Burton and Wibberley[1], "The six English conurbations of London, the West Midlands, south-east Lancashire, west Yorkshire, Merseyside and Tyneside, contain 17 million people at an average density of 8,050 persons per square mile", and we can establish a camp at the South Pole where men can live in reasonable comfort for months at a time, and maintain contact with the rest of the world. We can feed ourselves better than ever before. There are black spots in the food situation, but the chance of a man dying of starvation anywhere in the world is less now than it ever has been hitherto. We have even acquired the skill to escape from this Earth and to begin the exploration of space.

There is therefore a wealth of scientific achievement, but I believe it is important to temper any account of our successes by drawing to your attention what I believe to be our most serious failure. We can, with our present knowledge, feed, clothe and equip a larger human population than ever before, but a large and increasing share of our productive effort is absorbed in providing for further population increase and, despite our successful conquest of practically the whole of the plant and animal kingdom, we have not yet begun seriously to undertake the control of our own species.

Biologically, this is not a difficult undertaking. Even without modern knowledge and techniques, there have been periods in the past when our numbers in Britain were static, or even declined. The relation during the past millennium between population increase and prosperity has been discussed by van Bath[2], and he has drawn attention to the manifold interactions between the size of harvest and opportunities for industrial employment on the one hand, and nutritional level, age at marriage, and the incidence of epidemics on the other. Van Bath was concerned with a period ending in A.D. 1850, but we need to go no further back than the 1930's, within the experience of many of us here, to note a time when the birth rate was inadequate for replacement. So population increase is not inevitable.

Our difficulties in population control are social and emotional. In Britain we have not yet really accepted that population pressure is an immediate concern of our own, and not just a vague menace in distant parts of Asia. We forget that, in the United Kingdom, there are 570 of us to the square mile, whereas in India there are only about 385 (ref. 3). Western Europe is one of the most densely populated areas on Earth. Whether this amounts to over-population is, of course, another matter. I believe that Britain is in fact over-populated now, and will inevitably be more heavily over-populated in the future, whatever we may decide to do about it. Over-population means, in simple terms, too many people for the resources in land that are available to them. Recently there has been an important step forward in the definition and estimation of over-population. William Allan in his very valuable book *The African Husbandman*[4] has developed the concept of "Critical Population Density", the maximum population density that can be supported by a given agricultural system without progressive deterioration of the land. Allan was concerned with subsistence farming in a purely agricultural community, but the concept can be usefully applied much more widely than this. It is a characteristic of our biological environment that we can for a limited period exploit it in excess of its real productive capacity, and only after a lapse of time is it apparent that we have initiated in it a progressive decline. We have spent working capital as well as income, and when the capital has been depleted the income falls. This is easy to see in African shifting cultivation, when a reduction in the period of the recuperative fallow leads to degenerative changes in the natural vegetative cover, accelerated erosion of the soil, and declining yields in the cropping period. That the degeneration of the environment is a real problem, and may, and indeed does, lead to an almost irreversible decline in productivity, is evident from the present state of the Mediterranean lands after six or so millennia of overcutting, overgrazing, and overcultivation, and from the present state of heaths and moors in Britain for which we have the evidence of pollen diagrams that they once carried a forest cover[5]. One is reminded of the forester's bitter comment on so much of the agricultural development of Latin America—"Cut down wealth to sow poverty!".

This concept of "Critical Population Density" is relevant in all human communities. It is in the natural course of events that a developing community should service its development first by bringing into use its unused resources. There comes a time, however, when all the readily available resources have been brought in. At this point of time, the population density has reached the critical level, and the choices before the community lie between the over-exploitation of resources to the long-term detriment of the system, the planning of new systems of resource use that are both more conservative of resources and more productive of real income, and population control.

The history of population growth and the agricultural exploitation of the land in Britain is one of alternation between the first two of these choices, culminating in the position we have reached in the last decade, of an abundance of farm produce, even though the growth of our population continues unchecked. Our present food supply position might lead us to the complacent conclusion that we have now learnt how to match with increasing supplies our ever-increasing needs. The historical evidence is against such a conclusion, and current assessments of our need for, and use of, our limited land indicate that our present abundance may not be long continued.

Developing countries have in common a heavy dependence on agriculture, and agricultural systems that are still in the main exploitive. They differ substantially among themselves in the extent to which there is pressure of population on land, and in the nature and the degree of sophistication of the relationship between town and country. In all of them, the great need is for the initiation of agricultural revolutions of the kind through which Britain has passed. Having so recently been ourselves

through the fertilizer and mechanization revolution, we have been disposed uncritically to advocate fertilizers and machines for other countries. This was not the way that we emerged from exploitive to improving agriculture, and we have good evidence that these agents of our latest revolution are most successful where land is held on modern systems of tenure, and where the art of husbandry has already assimilated the practices of fertility conservation.

In India we know that the improvement of fertility is possible. We can see it in the highly fertile fields in the immediate vicinity of the villages. We know also that yield increases are obtainable from fertilizers. The agents of agricultural improvement work as they do in Europe. But the change in habits and customs necessary to establish the systematic conservation and distribution of the elements of fertility has hardly begun, and social inertia is such that land reform is extremely slow. Agriculture is static, and we are more in need of incentives than of knowledge, if change is to come about.

When European agriculture stagnated, some stimulus from outside the industry was necessary before a new phase of improvement began. It was the food shortages of the war-time blockade that set going the application of industry to agriculture in recent years. There is now a stimulus to Indian agriculture that could be used to initiate agricultural change if there is the imagination and determination to see it through. India is engaged on a great extension of water conservation and irrigation, often in multipurpose schemes involving power generation and industrial development. Here are opportunities to get agriculture moving, by undertaking land reform, transferring some surplus population from the land to industry, and providing the education and advice on the new farming practices necessary to ensure the conservation of the elements of fertility. In India the sequence of improvement must start with the mitigation of climatic risk, but then it should follow the pattern of this country —land reform, fertility conservation, and then fertility improvement. I only lived four years in India. I left with an unfulfilled ambition—to see whether, using both fertility conservation techniques and fertilizers, one could achieve in twenty years the level of fertility built up over 2,000 years on the village perimeter. I believe we know enough to set about such an enterprise, and, since that would mean multiplying productivity by two or three, the impact of such an achievement on India's food problems would be very large indeed.

African problems are of a different kind. There are vast areas where the rainfall is good and reasonably reliable, and the population small. But over most of them the soils are very ancient, and have been leached to the poverty line. Even in Uganda, where the climate is good and the soils better than the African average, fertility levels are not such as to sustain a high level of production. As land is abundant, it has been impossible to maintain growth in agricultural production over half a century, and in particular there has been a great increase in production for export. Little has been achieved in improving the productivity of the land, but the need for greater productivity has not been apparent while production could readily be increased by bringing more land into cultivation. Pressure on the land is still not great, but pressure on the market for export crops is increasing. It is quite clear that the increase in national income to which Uganda aspires cannot be financed by increases in the exports of cotton and coffee. But unless agriculture can contribute substantially, the increase in national income will be slow. Here also we can learn from western European experience. Buganda, the central area of Uganda, gained through the mailo system of freehold in land, the advantages Britain gained from the enclosure movement. The next stage in British agricultural progress stemmed from the example of high farming in the Netherlands, where the stimulus of town demand led to the development of intensive farming. In Uganda there is substantial urban development that has already had a great impact on the farming up to 20 miles or more from the town boundaries. Milk is produced in quantity and retailed in the towns by men who take it in on bicycles. The staple foodstuffs are taken into the town markets on bicycles and lorries. Traditional cash crops are of little importance near the towns, but the food crops are fully integrated into the cash economy. This is the new growing point of Uganda agriculture, as food and industrial crops provided the growing point of the agriculture of the Netherlands. What is now needed is the drive and imagination to initiate a new system of farming where livestock products and food crops have a ready sale, and where the conservation of fertility that can be practised with mixed farming can be developed, and supplemented by utilization of town wastes and fertilizers. Uganda agriculture could traverse in two decades the trail opened up over two centuries by the agriculture of western Europe.

It will be apparent that I advocate for both India and Africa a pattern of agriculture that many advanced practitioners of British agriculture are giving up. The conservation of fertility means the conservation and distribution of bulky organic manures, and this is an expensive matter where labour costs are high. But we are considering developing economies where labour costs are low, and where fertilizers have to be bought with the proceeds of the sale of low-value agricultural produce. A poor country, hoisting itself up on its own resources, must use first the local materials and the home labour force. This is the way we started on the improvement of our own land and, even if it could be demonstrated that the whole process could be successfully carried out with inorganic fertilizers, it would still remain true that only by making the most of local materials, and using local labour, has a poor country any hope of escape from exploitive agriculture. Only the rich can afford to squander organic manures. But this is not all. Western European mixed farming revolutionized the nourishment of our people as well as the nutrition of our soils, and its establishment in developing countries would be the surest way of ensuring a high dietary standard for the human population.

Let me be quite clear. In advocating the sequence of change and improvement through which western Europe has achieved high agricultural productivity, I am not suggesting that the products of industry should be eschewed until the practices of the Norfolk improvers have been applied. The successive revolutions through which we have passed can—and should—be telescoped. I do not believe that their order can be reversed.

Let us now look to the future. The lands that remain untilled are agriculturally unpromising, and in any case are likely to be wanted to meet the needs of growing local populations. The security of Britain's food supply depends on the continued improvement of the productivity of the lands, here and overseas, that we now farm. That there is a real prospect of this continued improvement, we can be assured. Exploitive agriculture has given way to conservation and improvement in all the advanced communities of the temperate parts of the world. But other needs and other problems face us, with our limited land resources and our large and increasing population. Of these, the most important are water supply and effluent disposal, and recreation. Britain is well watered. Our rivers run the year round, and we have great underground aquifers on which we have been accustomed to draw. But a population of 50 millions, living as we do, and running vast industries that use prodigious quantities of water, is now drawing on our supplies at a rate that will soon exceed summer availability. Moreover, we all too easily overlook the fact that our rivers are not only the source of most of our domestic and industrial water. They are the only possible recipients of our domestic and industrial effluent, with all the pollution that that entails. Here we must distinguish between organic and industrial pollution. Given a summer flow adequate to dilute sewage effluent, organic pollution

is dissipated by water life, and rivers contaminated with sewage become fresh and sweet by natural means. Industrial pollution is another matter. We have learnt a lesson with insecticides. Persistent poisons turn up persistently in unexpected places, and they are cumulative. The most persistent of all water contaminants are industrial wastes, containing indestructible matter such as metal salts. Water so polluted must run to the sea. We are getting pollution conscious, and control of effluent discharges is much better than it was, but we still have much to learn, and we still need to develop the kind of public conscience that will make the proper disposal of effluents a matter of civilized behaviour rather than of law enforcement. Indeed we still have to get our own thinking straight on our handling of persistent materials.

Water supply and effluent disposal must be planned together. Of water we have enough, but it is going to cost us a great deal to keep it pure, and to ensure that we have enough all the year round. Deforestation and drainage have increased the amplitude of seasonal fluctuations. Agricultural demand for irrigation is increasing where supplies are most limited, and at the time of year when they are at their lowest. We must store our winter surplus, and there are only two methods of storage, in surface reservoirs and in the aquifers from which we pump a part of our supplies. Surface reservoirs submerge land, much of it good agricultural land, some of it with vegetation of great natural and scientific interest, and some of it of great recreational value. But storage we must have, and we must site it where it will do least damage. Storage in estuaries may help, but the maintenance of flow to keep our rivers from degenerating into open sewers will alone require substantial up-river storage. Recharge of aquifers we have only recently begun to take seriously, but we have some limited experience and we urgently need more extensive experimentation.

Recreation and amenity also make demands on our limited land. The countryman has recreation and amenity on his doorstep, and his holidays are in consequence much less important in his life than are those of the townsman. Most of us live in towns now, very many of us in vast conurbations from which escape is expensive and time-consuming. If we have a semi-rural home, it is at the expense of our family life, since one member of the family is out of the house from dawn to dusk, five days a week. So we get the great summer exodus to the country and the sea, the growing demand for rural cottages as weekend retreats for those who can afford two homes, and the great increase in fishing, sailing, mountaineering and—remarkably!—caving. This is the relief we seek from the stress of urban life, and in our consideration of the use of our land, we must take it increasingly into account. Indeed, already our resources near the great cities are under strain.

Away from the industrial regions there are great areas suited to recreational use, though they are less accessible to those who need them most. Wales, and large parts of Scotland, could profitably provide recreation for many more than now enjoy them. Here is an opportunity in land use, and with wise management it should be possible to make a working adjustment between the interests of the naturalist, the farmer, and the holiday-maker. These are areas that are agriculturally of low productivity, and it might be argued that they should be surrendered to the tourist industry. This would be a mistake. Holiday-makers go to the country, but they do not go to empty country. They want both services and companionship, and the size of population necessary to maintain a high amenity value is greater than that needed to service the tourist industry. Thus the need, and the opportunity, to maintain vigorous local communities as centres to which holiday-makers can go. Farming, forestry, and tourism should be together in Britain's hill country.

To these competing needs for land must be added the need for land for urban growth and industrial expansion. And this emphasizes the basic problem with which we are faced. To provide the food, the water, and the recreational areas we need for our present urban population will take all our limited resources in land. Yet inexorably our population grows, our cities grow, and the pressure on our land increases. In all our thinking, population growth is accepted as inevitable, and indeed it is, in the sense that any action to control it will only be effective after a substantial lapse of time. We are now familiar with the 'population explosion', and with the predictions of hunger or intolerable overcrowding in times beyond the limit of the lives of most of us. But very few of us have accepted the population problem as one which we should face and solve. We may reasonably hope that our resources in land will be sufficient, if wisely managed, to meet the needs of our expanding population for our lifetime. But, even on the most optimistic forecast, that leaves us barely time to meet what we can foresee of the needs of the twenty-first century. We find the population problems of our own race difficult for the same reason. We have the knowledge and the technology for control, but we lack the vision to plan and initiate a programme that we know will take several human generations to bring to fruition.

This is a matter of our hopes and ambitions for our own children. The problems of young people in an urban environment, and the stress and disorientation they suffer, are apparent already. No doubt there is still much we can do to mitigate the strains of urban life, and to maximize its advantages. But do we really believe that a population twice the present size—as it may well be a century hence—can live and work and enjoy adequate leisure and recreation within the confines of this island? And if we do not, now is the time to set about ensuring that our numbers are stabilized.

We need first a target figure. I would like to set 40 millions, though I believe that, short of catastrophe, it would take two centuries to achieve it. Perhaps more important than a target figure, we need a social philosophy that will embrace the concepts and consequences of a stable population. This will come easily to a biologist, who is accustomed to thinking of growth in terms of a sigmoid curve, with a slow start, a grand period, and a levelling off to an asymptote. The ecological concept of the climax involves a stable population, within which individuals grow and mature and die, while the community maintains its vigour and stability.

The heart of the whole matter is, of course, the birth rate. The fall in the birth rate to less than replacement rate in the 1930s and during the 1939–45 war shows how sensitive our community is to constraints on our multiplication such as economic depression and the hazards of war. We may be encouraged to believe, therefore, that if we really appreciated the threat to our children's well-being posed by unrestricted increase in their numbers, we might take advantage of the techniques of control that are available to us. Surely we can learn to take account of the fact that large families are detrimental to the interests of our children later in their lives, and to accept as normal a family size of one or two, or occasionally three, children. For, make no mistake, Britain already carries a population as great as the environment can support without degeneration, and it will call for all the knowledge and skill we can command to prevent irreparable damage before we achieve a stable population even if we set about stabilization without delay.

This is the great remaining challenge of our time. We have mastered the physical world and the world of biology. We ourselves remain untamed. Our greatest need is to master the threat of our own numbers.

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THE BRITISH ASSOCIATION IN NOTTINGHAM — A CENTURY AGO

EXACTLY a century ago the British Association for the Advancement of Science met, for the thirty-fifth time, at Nottingham. Its influence in Britain was then at its height. Close on 2,000 people were members, and the membership was sufficiently permanent for its names to be printed at the end of each annual report. Most of the sections which now exist then also held their separate meetings, although the sections individually and the general assembly of the British Association as a whole made a practice of passing resolutions on matters that seemed to them to be of public importance. The Nottingham meeting was serious and concerned with a number of administrative matters such as the association's spending on scientific research. It was also, however, a cheerful meeting. As always the new president delivered his address at 8 o'clock, there was a soirée the following evening, and evening discourses on the Friday and Monday—the second of them by Joseph Hooker on insular flora. After another soirée on the Tuesday the meeting broke up with a discussion on the afternoon of Wednesday, August 29, at which the proceedings of the general committee were reported to the general meeting as a whole, and the whole body of the membership was given an account of how the committee had decided to spend its resources. At that time the association was reasonably prosperous. From a few years after its formation it had begun making small grants of money to those engaged on scientific research. In most years something of the order of £1,000 was spent on these activities and, after the thin time which the association apparently experienced in the late 1840s, the funds available were sufficient for it to allocate £1,750 13*s.* 4*d.* to scientific research at its meeting in Nottingham in 1866. The present treasurer will no doubt envy the state of affairs which allowed the association to lay out these funds from its income derived almost exclusively from subscriptions —dividends were then a mere £250 a year.

The variety of the general committee's interests is quite apparent from the report of its proceedings. In the first place, it was concerned with the affairs of the Kew Observatory and indeed spent £600 on supporting the work there. During the year the Observatory calibrated a dip circle for a Russian naval officer and did similar work for the Indian Survey. The Observatory was also engaged in the instrumentation of the survey being carried out of Zanzibar, and calibrated instruments for the Admiralty as well. Self-recording magnetographs were in fashion and Kew played a leading part in instructing workers from what is now called the Commonwealth, as well as from Russia and the United States, in the operation of these devices. Mr. Whipple, described as the Magnetical Assistant, is commended for his diligence. The Observatory was also deeply engaged in gravity surveys in India, and a Mr. Loewy at the Observatory was charged with special responsibility for seeing whether "Captain Kater's pendulum is still in a state to justify its adoption as an instrument to give correct determination of the lengths of the second's pendulum".

Sunspots were recorded. The Government was given advice about the setting up of a meteorological department, and Kew volunteered to be the first station. The Observatory asked the council of the British Association to consider favourably a request that a young clerk should be employed to tabulate magnetic and meteorological recordings at the Observatory.

The British Association was also preoccupied with what was called its Lunar Committee—a body of men among whom Sir John Herschel and Mr. J. N. Lockyer (who later founded this journal) were prominent. In 1866 they were given £120 to spend, and one of the products of their work is to be found in a report of the meeting at Nottingham as an attempt to construct a map of the Moon 100 in. in diameter. In retrospect the report of the committee's work is striking for the detail and care with which it tackled the problem of estimating the shapes and sizes of objects visible on the surface of the Moon. There is also a detailed discussion of the problem of deciding on reference points for lunar mapping and, in the drafts of the map which were available at Nottingham, attempts were made to indicate that some parts of the Moon were "higher" or "lower" than others. In its description of the crater Hipparchus, the committee was able to say: "The north boundary differs in a very marked degree from the S.W., inasmuch as the land on the S.W. rises to a considerable elevation, while on the N.N.E. it is depressed. The north boundary consists of cliffs which do not appear to rise much, if at all, above the surface of Hipparchus. It is this feature which occasions the evanescent character of Hipparchus, so that in about forty-eight hours after the terminator has passed it, this boundary is no longer discernible." The committee's careful listing of craters and other features on the Moon's surface was compiled from drawings and photographs by professional and amateur observatories throughout the country and abroad. The committee had no illusions about the difficulties of this work, and emphasized at several points in its report at Nottingham that "the map is not intended to be a perfect or complete lunar map, but only a guide to observers in obtaining data from the complete map".

The Committee on Electrical Standards was reappointed in 1866 and was to do valuable work throughout much of the nineteenth century. The Nottingham meeting was also told of the work done by a committee on "Uniformity of Weights and Measures" which boasted in its report that "during the year steps of great importance have been taken to promote the adoption of one common decimal system of weights and measures both at home and abroad". A conference had been held in Frankfurt on this subject. The committee complained that this conference had adopted a curious proposal that the metric system should be linked to the English system by defining a foot as three decimetres, but it was heartened by the way in which the United States Congress had in the previous year introduced bills making the use of the metric system lawful in the United States. The committee promised to press the cause of decimalization at the then impending exhibition in Paris in 1867, recording with

satisfaction that interest in decimalization in India was high but that administrators in India were divided about the virtues of different systems. At home the picture was less cheerful. "The only legislative measure recently passed is one for transferring to the Board of Trade the Department of Weights and Measures previously connected with the Office of the Comptroller of the Exchequer". The committee complained that there was no provision for the acquisition of standard metric weights and measures. "The law on the subject is in a very anomalous state." It recorded that a deputation had called on the President of the Board of Trade and that the noises of goodwill which he made were of no value because "the session was too far advanced and nothing has been done". The committee wanted to see education "among the young" on the metric system, a change of heart among the pharmacists, the adoption of the metric system by the railways, and a continuing effort by itself to promote the cause of decimalization.

As it happens, the cause of astronomical observations in the southern hemisphere was as much an issue in 1866 as it is a century later. The observation of meteors was also being encouraged by the British Association, and the committee responsible for this reported at Nottingham details of several visible meteors going back to an observation in 1768 on Cook's voyage around the world, but mostly collected by devoted amateurs and members of the British Association in the previous decade.

Perhaps the most adventurous of the British Association's projects was the work of the Balloon Committee, including "Colonel Sykes, Mr. Airy, Lord Wrottesley, Sir David Brewster, Sir J. Herschel, Bart., Dr. Robinson, Dr. Fairbairn, Dr. Tyndall, Dr. W. A. Miller, and Mr. Glaischer". During the year the committee had sponsored three flights by balloon with the object of collecting physical and meteorological information. One ascent took place from Woolwich Arsenal in October 1965. Mr. James Glaisher took off after the Sun had gone down, chiefly because the committee was anxious to have information about the temperature lapse in the lower atmosphere at night. Mr. Glaisher was surprised to find that the temperature did not lapse at all but actually increased with height up to 1,900 ft. He was also surprised to find that the humidity decreased with height. Mr. Glaisher made an attempt to measure ozone with slips of paper containing chemical reagents. He had trouble with his Davy lamp, which was the only way of reading his instruments. He was overcome with the brilliance of the sight of London from the balloon and felt it "impossible to convey any adequate idea of the brilliant effect of London viewed from an elevation of 1,300 ft. on a clear night when the air is free of mist". In the event the balloon crossed London by the Marble Arch and the Harrow Road and came down at Highmoor in Oxfordshire after dawn. One of Mr. Glaisher's conclusions was that it would be valuable to carry telescopes on balloons to high altitudes so that an observation might be made of the Milky Way and other stars.

Two other ascents that year seemed to be less successful. In December 1865 the temperature lapse was marked, but the balloon was buffeted much by the winds, and a lamp was put out by accident at 2,400 ft. (by which time the temperature was down to freezing) so that the journey was abandoned. Not until April 1866 was another ascent possible, and this time the journey started from Windsor and finished in Sussex. The committee of the association agreed to continue with the balloon flights and allocated a further £50 to the work of the committee—a modest amount, it would appear, in all the circumstances.

The less spectacular parts of the association's work also have a curiously up-to-date appearance. There was, for example, a committee concerned with the transmission of sound under water, whose formation had been prompted by experiments carried out in 1826 in the Lake of Geneva, and whose motive was to find some way of communicating between two ships "in company at sea, or between a ship and the coast during foggy weather". Another committee was exploring Palestine; the archaeology of Kent's Hole in Devon was supported with £100; and there were projects to excavate fossil elephants from Malta, fossil crustacea from wherever they could be found, fauna from the Kilkenny coalfields, and similar palaeontological work in Iceland, Ireland and Scotland. Finally, the association was exercised by the problem of the performance of steam ships and the need to make ships' plates impenetrable to bullets and other projectiles. Much of the work was carried out at Shoeburyness where shots were fired at ships armoured with various kinds of plating. One of the conclusions was a suggestion as to how wooden ships might be most effectively armoured with steel and iron. The committee was able to make suggestions about the tactics to be followed by ships so as to minimize the danger that their armour would prove ineffectual. It also commented on the relative advantages of the American and English systems of artillery; the Americans were apparently inclined to use heavy shot at low velocity in the hope of shattering the armour, while the British Navy looked for penetration of the armour with the intention of killing the crew, damaging the machinery and sinking the vessel. By 1866 the committee was not in a position to take a firm line about the advantages or disadvantages of the two systems.

Compared with contemporary meetings of the British Association, the attention paid a century ago to matters like these may seem remarkable. In practice the general committee of the British Association did even more to sponsor research by drawing attention to problems which needed solution, and, in this spirit, it encouraged an investigation of methods of solving simultaneous equations, the study of the chemistry of cast iron, and the difficult matter of how best (if at all) to present scientific evidence in courts of law.

As was the custom in the nineteenth century, the address by the president, Mr. William Robert Grove, Q.C., M.A., F.R.S., was an attempt to survey the then state of science in Britain. He began his address, however, with a declaration about the importance of public understanding of science. "It is sad to see the number of so-called educated men who, travelling by railway, voyaging by steamboat, consulting the almanac for the time of sunrise or full-moon, have not the most elementary knowledge of a steam-engine, a barometer, or a quadrant; and who will listen with a half-confessed faith to the most idle predictions as to weather or cometic influences, while they are in a state of crass ignorance as to the cause of the trade-winds or the form of a comet's path. May we hope that the slight infiltration of scientific studies, now happily commenced, will extend till it occupies its fair space in the education of the young, and that those who may be able learnedly to discourse on the Eolic digamma will not be ashamed of knowing the principles on which the action of an air-pump, an electrical machine, or a telescope depends, and will not, as Bacon complained of his contemporaries, despise such knowledge as something mean and mechanical."

In the second half of the nineteenth century the members of the British Association were also concerned to understand what astronomy was doing. The orbits of the double stars were easy enough to understand, but how could nebulae be accounted for in terms of gravitation as known on the surface of the Earth? Meteorites were also a puzzle, especially because they seemed to radiate from particular points in the sky. It was remarkable to the then president that the number of asteroids had increased so that there were a total of eighty-eight planets, and the notion that the space between Mars and Jupiter might be filled with planets varying in size from Jupiter to a cannon ball was already current.

But why should the Earth be so dense? On the assumption that meteorites are a kind of primeval rock, and the

suggestion—due to a French geologist—that rocks become more like meteorites the further their source beneath the surface of the Earth, it was still a puzzle to know why the average specific gravity of the Earth should be between five and six and why that of olivine was a mere 3·3. The explanation suggested by the president was that the oxidation of rocks was confined to regions near the surface of the Earth and that the deeper rocks were more dense because they were free from oxygen.

Mr. Grove was also interested by the measurement of the spectrum of a star which appeared suddenly on May 12, 1866, "equal in brilliancy to a star of the second magnitude". Its brightness diminished in a few days and its spectrum was sufficiently unusual, with bright emission lines superimposed on a continuous spectrum, to suggest that something new was happening. As to the surface of the Moon, the appearance of the craters "is not unlike that seen on the surface of some metals such as bismuth, when cooling from fusion and just previous to solidifying". May it not therefore be that the Moon is really substantially metallic with an oxide coating too thin to be seen? This at least was the suggestion, and one of the reasons why the British Association was anxious to keep its lunar committee at work, but the president admitted that the problem "needs more examination and study before any positive opinion can be pronounced". No doubt the frustration of not knowing what the Moon was made of helped the president to complain, as he did with some bitterness, that instruments ought to be improved.

With Joule a member, and actively engaged on much of the association's work, it is not surprising that the presidential address should have taken up the equivalence of heat and mechanical energy. The president himself had been impressed with an experiment demonstrating this in an unfamiliar way. "A train of multiplying wheels ended with a small metallic wheel which, when the train was put in motion, revolved with extreme rapidity against the periphery of the next wheel, a wooden one. In the metallic wheel was placed a small piece of phosphorus, and as long as the wheels revolved, the phosphorus remained unchanged, but the moment the last wheel was stopped by moving a small lever attached to it, the phosphorus burst into flame. My object was to show that while motion of the mass continued, heat was not generated, but that when this was arrested, the force continuing to operate, the motion of the mass became heat in the particles. The experiment differed from that of Rumford's cannon-boring and Davy's friction of ice in showing that there was no heat while the motion was unresisted, but that the heat was in some way dependent on the motion being impeded or arrested. We have now become so accustomed to this view that whenever we find motion resisted we look to heat, electricity, or some other force as the necessary and inevitable result." But where does heat energy come from? The president suggested that there must be a sharp distinction between influences such as heat and influences such as magnetism, which must be "cosmical and not merely terrestrial". There is no connexion to be found between sunspots and magnetism. And as to the heat of the Sun itself, the theory that this might be caused by the impact of meteorites was not convincing because it was hard to believe there could be enough meteorites.

The president was also perplexed by what is known as Albers's Paradox, pointing out that it was hard to imagine what could be happening to the radiation from the Sun and smaller stars. "If the universe have no limit and it is difficult to conceive one, heat and light should be everywhere uniform; and yet more is given off than is received by each cosmical body, for otherwise night would be as light and as warm as day. What becomes of the enormous force thus apparently non-recurrent in the same form? Does it return as palpable motion? Does it move or contribute to move suns and planets? and can it be conceived as a force similar to that which Newton speculated on as universally repulsive and capable of being substituted for universal attraction? We are in no position at present to answer such questions as these; but I know of no problem in celestial dynamics more deeply interesting than this, and we may be no further removed from its solution than the predecessors of Newton were from the simple dynamical relation of matter to matter which that potent intellect detected and demonstrated."

Thanks to his understanding of molecular physics, the president was able to be entirely confident that the heat delivered up by burning coal really comes from the Sun much earlier, at the time of its formation. He pointed out, however, that coal fields would become exhausted and that it was impossible to predict what changes would then be necessary if energy were to be used for practical purposes. "The waste of fuel in ordinary operations is enormous, and can be much economized by already known processes. It is true that we are, at present, far from seeing a practical mode of replacing that granary of force the coal-fields; but we may with confidence rely on invention being in this case, as in others, born of necessity, when the necessity arises.

"I will not further pursue this subject; at a time when science and civilization cannot prevent large tracts of country being irrigated by human blood in order to gratify the ambition of a few restless men, it seems an over-refined sensibility to occupy ourselves with providing means for our descendants in the tenth generation to warm their dwellings or propel their locomotives."

But there was electricity just round the corner. The president pointed out that "Mr. Wilde and Mr. Holz" had found that it was possible to increase "electrical power" by coupling electrical coils and electro-magnets together. "There seems hardly a limit to the extent to which mechanical may be converted into electrical force." There were also experiments with machines which "Mr. Holz" had been experimenting with.

The tone of the president's address in 1866 was optimistic. "As phlogiston and similar creations of the mind have passed away, so with hypothetic fluids, imponderable matters, specific ethers, and other inventions of entities made to vary according to the requirements of the theorist, I believe the day is approaching when these will be dispensed with, and when the two fundamental conceptions of matter and motion will be found sufficient to explain physical phenomena." He was, of course, before his time.

The aftermath of the great argument about evolution, which took place at Oxford in 1862, was still perplexing the association. The president in 1866 set out to summarize the arguments for and against. His task was made easier by the controversy then raging in France between Pasteur and Pouchet on spontaneous generation, although Mr. Grove gave victory to Pasteur. He was particularly impressed with Pasteur's argument that the assertion of spontaneous generation had become possible only for more and more "lower forms of life" as experimental techniques had become better. But there were difficulties about talking about the origin of species without a definition of what a species really consists of. "The more gaps between species are filled up by the discovery of intermediate varieties, the stronger becomes the argument for transmutation and the weaker that for successive creations." Indeed, the president then was a firm believer in continuity. He confessed that he leant to the view that the successive changes in organic beings do not take place by sudden leaps and said that "if the facts are stronger in favour of one theory than another it would be an affectation of impartiality to make the balance appear equipoised". The prejudices of education were against new views, he said. The acid test was whether a new theory would "remove more difficulties, require fewer assumptions and present more consistency of observed facts than that which it seeks to supersede".

NOTTINGHAM BETWEEN THE WARS

The third annual meeting to be held in Nottingham convened on September 1, 1937. The programme and the proceedings were similar in form to those of the nineteenth century, yet the problems of the twentieth century, practical as well as intellectual, were also plainly evident.

THE meeting of the British Association at Nottingham in 1937 was the third to have been held there, and was by all accounts successful and stimulating. The association was at that time deeply engaged in a number of issues of public importance, ranging from the importance of adult education in science to the planning of land use in Britain. The proceedings were enlivened by the appearance of H. G. Wells as president of the Educational Section, and by his eloquent plea for a sensitive appreciation of the character of education and its role in the modern world (see page 1061, this supplement). This was also the year in which George VI agreed to become patron of the British Association, and in which the then president, Sir Edward Poulton, undertook to represent the British Association at the Coronation on May 12. The balance sheet was in a reasonably healthy condition, and if there were warning signs of the difficulties which at present cause the treasurer such anguish, there was no cause to be unduly worried by them.

The Nottingham meeting was inaugurated at the Albert Hall, Derby Road, on September 1, 1937. The president spoke on the *History of Evolutionary Thought, as recorded at Meetings of the British Association*, and concerned himself with a review of the doctrine of evolution from its foundation through the mid-nineteenth century to the development of cytogenetics in this century. The meetings of the sections were held at what was then known as the University College of Nottingham. There were evening discourses on the Friday and Monday, the first by Dr. R. E. Slade on *Grass and the National Food Supply* and by Professor (later Sir) James Gray on the *Mentality of Fishes*.

Financially, the mid-thirties were a watershed for the British Association, for it was then that the association began to find it difficult to finance its scientific activities out of the income from membership subscriptions and investment revenue. The total budget for the year worked out at something less than £5,000, while investments produced a cheerful £1,500 and membership subscriptions somewhat more than £2,000 even in a year that was only moderately well attended. What with the sale of publications and occasional large donations, the association could cover its modest outgoings comfortably, and yet spare some hundreds of pounds each year for the support of scientific research. In addition there was a substantial expenditure on small grants for research from various special funds administered by the council of the British Association, so that the total expenditure on research projects in 1937 amounted to £720.

Even so, Mr. P. G. H. Boswell, the general treasurer, was worried. He pointed out that the association had not found it possible to meet its target of an annual allocation of £500 to a contingency fund, and that the accumulated surplus over five years was merely £1,940. He was also concerned that the association's support of research projects of various kinds had increasingly tended to become support for long-established committees. Like all charitable foundations, the association wished to regard itself as an initiator of new work. "The true function of the association, in making grants to research committees, is the initiation of particular pieces of research rather than their quasi-permanent endowment." But wishes are one thing and realities another; alternative sponsors for work begun were apparently as hard to come by in the thirties as they are now.

In the thirties, the various sections of the association were still passing resolutions on various matters of scientific or public importance. Frequently the council of the association, which alone was empowered to act on the basis of sectional resolutions, was also required to decide what should be done about them, and the report of the meeting in 1937 includes records that the council had found it impossible to act on a resolution passed the previous year at Blackpool, by the Chemistry Section, and which declared that the "extent to which chemistry is applied for beneficient purposes in connection with the industry of the British nation and the health of its citizens is enormously greater than the scope of its employment for purposes of warfare". As a result, the resolution went on, "chemists as a body view with grave concern the increasing use of science for destructive ends".

At Nottingham itself, the sections of the association were earnestly productive of resolutions. The mathematicians and physicists, for example, were sad to note, "on the occasion of a visit to the grave of George Green, the world-famous mathematician of Nottingham", that "deterioration is taking place in the tombstone, and would respectfully suggest that representations should be made to the proper authorities to take such steps as seem fit to maintain in good condition the resting place of this renowned man". Green, who worked for a time as a miller but later graduated from Cambridge as a mathematician, was, of course, the man who did much to put into mathematical language the work of Faraday and others on electro-magnetism.

The zoologists at Nottingham were anxious that Lord Rothschild's house at Tring should continue to be used as a centre for research and as a museum, even though it had passed into the hands of the British Museum. The geographers made a gentle protest at the decision by the published. The engineers wanted the council of the British Association to use its influence with the engineering institutions so as to create better channels for the exchange and dissemination of information about engineering research and development. The anthropologists wanted anthropology made compulsory in the studies of probationer civil servants in India and Burma. Zoologists and others asked for some public investigation of the ways in which wild animals—pests and others—were dealt with in British agriculture; one of the questions at issue was whether the gin trap was humane or otherwise.

In the late thirties the research committees were more numerous than the sections of the association. One of them—the committee on seismological investigations—remains alive, and in 1966 includes at least one member who was active in the late thirties. But the Mathematics and Physics Section was also engaged on the calculation of mathematical tables, and, in collaboration with the chemists, on the determination of thermal conductivities of rocks by making direct measurements in deep mines

and boreholes. Through the activities of the seismological committee, the British Association had become the sponsor of much detailed work, and, by its support, the University Observatory at Oxford was able to contribute to the work of the Seismological Survey. The six Milne–Shaw seismographs belonging to the association were scattered around the world at Oxford, Edinburgh, and in Australia and South Africa. It was largely because of the association's support for seismology that the Oxford Observatory was able to equip itself with a large brass globe some 18 in. in diameter and conveniently ruled and drilled with holes so as to assist in fixing the epicentres of earthquakes.

The Committee on Mathematical Tables was busy on Bessel Functions, and about to start work on the tabulation of Airey Integrals. During the year the committee made arrangements for its mechanical computing machine to be housed at the Galton Laboratory at University College, London, under the eye of Professor R. A. Fisher, and plans were in hand to employ a full-time operator (out of a budget of £200). At the same time, the Committee on the Measurement of Thermal Conductivities in Rocks completed plans for drilling a borehole 100 ft. deep, at a cost of £40, having convinced itself that thermal conductivity could be inferred without much inaccuracy from the measurements of temperature which could be made at various depths.

The geologists were particularly active. The committee responsible for collecting photographs of particular geological interest was able to add 154 items to its collection of nearly 9,000 photographs. A project was under way to ensure that full advantage should be taken of quarry working in the oolite at Stow-on-the-Wold to collect what evidence there might be of fossil reptiles, and a great many specimens came to light during the year. The quarries on which this interest was lavished had previously yielded up some splendid dinosaur remains.

The excavations at Kent's Cavern near Torquay, in which a committee of the association was first engaged a century ago, were still in progress. During the year a pit was dug in the floor of the cavern, and from it was excavated a substantial quantity of mammalian remains but only a few flints to indicate human presence. In Derbyshire, the cave known as the Whalley Rock Shelter had been found to be a primitive workshop site, with a yield of some hundreds of pieces of stone artefacts. Yet another committee was hard at work on the excavation of ancient mining sites in Wales and Northern Ireland, and was able to date with some accuracy the succession of improved techniques at a refuse dump on the Great Orme (Llandudno), and in particular to demonstrate that the Romans had worked there.

The principal work of the research committees, at least so far as the meeting at Nottingham was concerned, was in education. The geologists had been particularly concerned to make a study of the ways in which the teaching of geology in the schools could be improved, and this will no doubt be considered a curious premonition of the discussions to be held at Nottingham this year on the teaching of geology. In 1937, as now, the geologists felt neglected by the school curriculum, but at Nottingham they were more interested in showing how geology could be introduced into the school curriculum than with making the general case that geology should not be neglected. They were apparently encouraged by the way in which the school examinations in the thirties were being reorganized in such a way as to make a more general study of science a commonplace in grammar schools, and they argued that geology was a particularly suitable subject for study in those schools sited near what were called "natural geological laboratories". The committee also considered that the industrial demand for geologists would grow rapidly (though it did not cite exploration for petroleum as one of the causes of growing demand) with the result that there would be a dearth of suitably trained people "unless something is done to stimulate the entry of students into geological courses".

The committee argued that it was particularly important to include some teaching of geology early in the school career of most schoolchildren because "only a small proportion of those who enter for the various school examinations pass on to university work. Most of them take up posts in industry and commerce at once. Of these, a considerable proportion who have no further academic training obtain posts where they are sooner or later concerned with mining, building, engineering or other problems in which even a small acquaintance with geological matters would be of real assistance. In ordinary life, too, opportunities for using scientific knowledge are continually increasing in number. The applications of geology are certainly not fewer or less important than those of other sciences. Unless some work is introduced into school curricula, not only will there be a serious restriction in the numbers who will seek to take up geology as a profession, but many who may be later concerned with the applications of geology will hardly know of the existence of a science which has close contacts with their work".

The other educational theme to dominate the proceedings at Nottingham in 1937 was that of adult education in science. This had been a continuing concern of the association since the early thirties. By 1937 the committee responsible for the work had been able to collect a mass of information about the extent of adult education in Britain. As a result, it felt confident enough to present to the meeting at Nottingham a detailed report showing in great detail the place of science in adult education as it was and as it should be.

This cause was close to the association's heart. The Committee on *Science in Adult Education* produced for the Nottingham meeting a detailed analysis of the then current practice, which showed that only some seven or eight per cent of the various adult education courses—run by evening institutes, the Workers' Educational Association and by extra-mural departments of universities—were devoted to scientific subjects. Scientific studies were most neglected in the south-east of England and London, where they took up only one or two per cent of the time of the evening institutes. In the hope of making the spread of education in science for adults easier, the committee drew up detailed syllabuses for studies in astronomy, anthropology, biology and the like. This activity chimed in well with the presidential address to the section on Education Science in Nottingham that year.

For the rest, the programme for 1937 has a curiously familiar appearance. There was a symposium on the industrial use of X-rays led by Sir William Bragg, a paper on rate processes by Henry Eyring, and talk about the best way of observing eclipses and of introducing algebra into the school mathematics curriculum. The chemists were told about chemotherapy and—by W. T. Astbury—about the X-ray analysis of protein structures. Between them the geologists made a thorough survey of the Midlands, and the zoologists ranged from sex ratios in wild animals to the technique of recording bird song. There was some economics of an academic character, a paper on the possible use of electrical vibrations in television, a paper on *Palaeolithic Man in Nottinghamshire* and an anthropological study of the painted headboards on horse-drawn carts. The physiologists, much moved by what Wells had said about the need for a liberal education in science, made a concerted plea for the inclusion of physiology in the education of all young people.

By all ordinary tests, the meeting was a success. Altogether there were 2,027 people in attendance at various times, roughly half of them for the duration of the meeting. There were four or five hundred casual visitors and persons described in the reports as "foreigners".

H. G. WELLS ON EDUCATION*

In 1937 Mr. H. G. Wells was the president of the Section of the British Association on Educational Science. The substance of his address to the session, delivered on Thursday September 2, 1937, is reproduced here because it faithfully if brilliantly reflects the flavour of the British Association in the thirties, because of the historical importance of Wells's ideas on education, and also as a way of recalling that this year is the centenary of Wells's birth.

SECTION L of the British Association is of necessity one of the least specialized of all sections. Its interests spread far beyond professional limitations. It is a section where anyone who is, so to speak, a *citizen at large* may hope to play a part that is not altogether an impertinent intrusion. And it is in the character of a citizen at large that I have accepted the very great honour that you have offered me in making me the president of this section. I have no other claim whatever on your attention. Since the remote days when as a needy adventurer I taught as non-resident master in a private school, invigilated at London University examinations, raided the diploma examinations of the College of Preceptors for the money prizes offered, and, in the most commercial spirit, crammed candidates for the science examinations of the university, I have spent very few hours indeed in educational institutions. Most of those were spent in the capacity of an enquiring and keenly interested parent at Oundle School. I doubt if there is any member of this section who has not had five times as much teaching experience as I have, and who is not competent to instruct me on all questions of method and educational organization and machinery. So I will run no risks by embarking on questions of that sort. But, on the other hand, if I know very little of educational methods and machinery I have had a certain amount of special experience in what those methods produce and what that machine turns out. I have been keenly interested for a number of years, and particularly since the war, in public thought and public reactions, in what people know and think and what they are ready to believe. What they know and think and what they are ready to believe impresses me as remarkably poor stuff. A general ignorance—even in respectable quarters—of some of the most elementary realities of the political and social life of the world is, I believe, mainly accountable for much of the discomfort and menace of our times. The uninstructed public intelligence of our community is feeble and convulsive. It is still a herd intelligence. It tyrannizes here and yields to tyranny there. What is called elementary education throughout the world does not in fact educate, because it does not properly inform. I realized this very acutely during the latter stages of the war and it has been plain in my mind ever since. It led to my taking an active part in the production of various outlines and summaries of contemporary knowledge. Necessarily they had the defects and limitations of a private adventure, but in making them I learnt a great deal about—what shall I say ?—the contents of the minds our schools are turning out as taught.

And so now I am proposing to concentrate the attention of this Section for this meeting on the question of what is *taught as fact*, that is to say on the *informative side of educational work*. For this year I suggest we give the questions of drill, skills, art, music, the teaching of languages, mathematics and other symbols, physical training and development, a rest, and that we concentrate on the inquiry: *What are we telling young people directly about the world in which they are to live* ?

*Reproduced by permission of Professor G. P. Wells.

Now when I set myself to review the field of inquiry I have thus defined, I found it was necessary to take a number of very practical preliminary issues into account. As educators we are going to ask what is the subject-matter of a general education ? What do we want known ? And how do we want it known ? What is the essential framework of knowledge that should be established in the normal citizen of our modern community ? What is the irreducible minimum of knowledge for a responsible human being to-day ?

I say irreducible minimum—and I do so, because I know at least enough of school work to know the grim significance of the school time-table and of the leaving school age. Under contemporary conditions our only prospect of securing a mental accord throughout the community is by laying a common foundation of knowledge and ideas in the school years. No one believes to-day, as our grandparents—perhaps for most of you it would be better to say great-grandparents—believed, that education had an end somewhere about adolescence. Young people then left school or college under the imputation that no one could teach them any more. There has been a quiet but complete revolution in people's ideas in this respect and now it is recognized almost universally that people in a modern community must be learners to the end of their days.

Nevertheless it is true that what I may call the main beams and girders of the mental framework must be laid down, soundly or unsoundly, before the close of adolescence. We live under conditions where it seems we are still only able to afford for the majority of our young people, freedom from economic exploitation, teachers even of the cheapest sort and some educational equipment, up to the age of 14 or 15, and we have to fit our projects to that. And even if we were free to carry on with unlimited time and unrestrained teaching resources, it would still be in those opening years that the framework of the mind would have to be made. We have got to see therefore that whatever we propose as this irreducible minimum of knowledge must be imparted between infancy and, at most, the fifteenth or sixteenth year.

And next let us turn to another relentlessly inelastic packing-case and that is the school time-table. How many hours in the week have we got for this job in hand ? The maximum school hours we have available are something round about thirty, but out of this we have to take time for what I may call the non-informative teaching, the native and foreign language teaching, teaching to read, teaching to write clearly, basic mathematical work, drawing, various forms of manual training, music and so forth. A certain amount of information may be mixed in with these subjects but not very much. They are not what I mean by informative subjects. By the time we are through with these non-informative subjects, I doubt if at the most generous estimate we can apportion more than six hours a week to essentially informative work. Then let us, still erring on the side of generosity, assume that there are 40 weeks of schooling in the year. That gives us a maximum of 240 hours in the year. And if we take ten

years of schooling as an average human being's preparation for life and if we disregard the ravages made on our school time by measles, chicken-pox, whooping-cough, coronations and occasions of public rejoicing, we are given 2,400 hours as all that we can hope for as our time allowance for building up a coherent picture of the world, the essential foundation of knowledge and ideas, in the minds of our people.

Our question becomes therefore: 'What should people know—whatever else they don't know? Whatever else we may leave over—for leisure-time reading, for being picked up or studied afterwards—what is the irreducible minimum that we ought to teach as clearly, strongly and conclusively as we know how?'

We begin telling true stories of the past and of other lands. We open out the child's mind to a realization that the sort of life it is living is not the only life that has been lived and that human life in the past has been different from what it is to-day and on the whole that it has been progressive. We shall have to teach a little about law and robbers, kings and conquests, but I see no need at this stage to afflict the growing mind with dates and dynastic particulars. I hope the time is not far distant when children even of eight or nine will be freed from the persuasion that history is a magic recital beginning 'William the Conqueror, 1066'. Concurrently, we ought to make the weather and the mud pie our introduction to what Huxley christened long ago as Elementary Physiography. We ought to build up simple and clear ideas from natural experience.

We start a study of the states of matter with the boiling, evaporation, freezing and so on of water and go on to elementary physics and chemistry. Local topography can form the basis of geography. We shall have to let our learner into the secret that the world is a globe—and for a time I think that has to be a bit of dogmatic teaching. It is not so easy as many people suppose to prove that the world is spherical and that proof may very well be left to make an exercise in logic later on in the education. Then comes biology. Education, I rejoice to see, is rapidly becoming more natural, more biological. Most young children are ready to learn a great deal more than most teachers can give them about animals. I think we might easily turn the bear, the wolf, the tiger and the ape from holy terrors and nightmare material into sympathetic creatures, if we brought some realization of how these creatures live, what their real excitements are, how they are sometimes timid, into the teaching. I don't think that descriptive botany is very suitable for young children. Flowers and leaves and berries are bright and attractive, a factor in aesthetic education, but I doubt if, in itself, vegetation can hold the attention of the young. But directly we begin to deal with plants as hiding-places, homes and food for birds and beasts, the little boy or girl lights up and learns. And with this natural elementary zoology and botany we should begin elementary physiology. How plants and animals live, and what health means for them.

There I think you have stuff enough for all the three or four hundred hours we can afford for the foundation stage of knowledge. Outside this substantial teaching of school hours the child will be reading and indulging in imaginative play—and making that clear distinction children do learn to make between truth and fantasy—about fairyland, magic carpets and seven league boots, and all the rest of it. So far as my convictions go I think that the less young children have either in or out of school of what has hitherto figured as *history*, the better. I do not see either the charm or the educational benefit of making an important subject of the criminal history of royalty, the murder of the Princes in the Tower, the wives of Henry the Eighth, the families of Edward and James I, the mistresses of Charles II, Sweet Nell of Old Drury, and all the rest of it. I suggest that the sooner we get all that unpleasant stuff out of schools, and the sooner that we forget the border bickerings of England, France, Scotland, Ireland and Wales, Bannockburn, Flodden, Crecy and Agincourt, the nearer our world will be to a sane outlook on life. In this survey of what a common citizen should know I am doing my best to elbow the scandals and revenges which once passed as English history into an obscure corner or out of the picture altogether.

But I am not proposing to eliminate history from education—far from it. You will see how large a proportion of our treasure of 2,400 hours I am proposing to give to history. This next section represents about 800 to 1,000 pre-adolescent hours. It is the school-boy–school-girl stage. And here the history is planned to bring home to the new generation the reality that the world is now one community. I believe that the crazy combative patriotism that plainly threatens to destroy civilization to-day is very largely begotten by the schoolmaster and the schoolmistress in their history lessons. They take the growing mind at a naturally barbaric phase and they inflame and fix its barbarism. I think we underrate the formative effect of this perpetual reiteration of how *we* won, how *our* Empire grew and how relatively splendid *we* have been in every department of life. We are blinded by habit and custom to the way it infects these growing minds with the chronic and nearly incurable disease of national egotism. Equally mischievous is the furtive anti-patriotism of the leftish teacher. I suggest that we take on our history from the simple descriptive anthropology of the elementary stage to the story of the early civilizations.

We are dealing here with material that was not even available for the school-masters and mistresses who taught our fathers. It did not exist. But now we have the most lovely stuff to hand, far more exciting and far more valuable than the quarrels of Henry II and a Becket or the peculiar unpleasantness of King James or King John. Archaeologists have been piecing together a record of the growth of the primary of civilizations and the developing roles of priest, king, farmer, warrior, the succession of stone and copper and iron, the appearance of horse and road and shipping in the expansions of those primordial communities. It is a far finer story to tell a boy or girl and there is no reason why it should not be told. Swinging down on these early civilizations came first the Semitic-speaking peoples and then the Aryan-speakers. Persian, Macedonian, Roman followed one another, Christendom inherited from Rome and Islam from Persia, and the world began to assume the shapes we know to-day.

Look at our time-table and what we have to teach. If we give history four-tenths of all the time we have for imparting knowledge, that still gives us at most something a little short of 400 hours altogether. Even if we think it desirable to perplex another generation with the myths of the Creation, the Flood, the Chosen People and so forth, we haven't got the time for it—any more than we have the time for the really quite unedifying records of all the Kings and Queens of England and their claims on this and that. No reason why much of that stuff should not be picked up in private reading—by those who like that sort of thing. But so far as the school time-table goes we are faced with a plain alternative. One thing or the other. Great history or hole-and-corner history? The story of mankind or the narrow, self-righteous, blinkered stories of the British and the Jews?

There is a lot more we have to put into the heads of our young people over and above history. It is the main subject of instruction but, even so, it is not even half of the informative work that ought to be got through in this school stage. We have to consider the collateral subject of geography and a general survey of the world. We may have a little mapmaking here, but I take it what is needed most are reasonably precise ideas of the various types of country and the distinctive floras and faunas of the main regions of the world. We do not want our budding citizens

to chant lists of capes and rivers, but we do want them to have a real picture in their minds of the Amazon forests, the pampas, the various phases in the course of the Nile, the landscape of Labrador and so on, and also we want something like a realization of the sort of human life that is led in these regions. We have enormous resources now in cheap photography, in films and so forth, that even our fathers never dreamt of—to make all this vivid and real. New methods are needed to handle these new instruments, but they need not be overwhelmingly costly.

Geography and history run into each other in this respect and, on the other hand, geography reaches over to biology. Here again our schools lag some fifty years behind contemporary knowledge. The past half-century has written a fascinating history of the succession of living things in time and made plain all sorts of processes in the prosperity, decline, extinction and replacement of species. We can sketch the wonderful and inspiring story of life now from its beginning.

And finally, we have the science of inanimate matter. In a world of machinery, optical instruments, electricity, radio and so forth we want to lay a sound foundation of pure physics and chemistry on the most modern lines—for everyone. Some of this work will no doubt overlap the mathematical teaching. And finally, to meet awakening curiosity and take the morbidity out of it, we have to tell our young people, and especially our young townspeople, about the working of their bodies, about reproduction and about the chief diseases, enfeeblements and accidents that lie in wait for them in the world.

That, I think, completes my summary of all the information we can hope to give in the lower school stage. And as I make it I am acutely aware of your unspoken comment. *With such teachers as we have* ! Well, I think that it is a better rule of life, *first* to make sure of what you want and then set about getting it, rather than to consider what you can easily, safely and meanly get, and then set about reconciling yourself to it. I admit we cannot have a modern education without a modernized type of teacher. Everything I am saying now implies a demand for more and better teachers—with better equipment. And these teachers will have to be kept *fresh*. It is stipulated in most leases that we should paint our houses outside every three years and inside every seven years, but nobody ever thinks of doing up a school teacher. There are teachers at work in this country who haven't been painted inside for fifty years. They must be damp and rotten. Two-thirds of the teaching profession now is in urgent need of being either reconditioned or superannuated. In this advancing world the reconditioning of both the medical and the scholastic practitioner is becoming a very urgent problem indeed, but it is not one that I can deal with here.

And there is another matter also closely allied to this question of the rejuvenation of teachers, at which I can only glance now, and that is the bringing of school books up to date. In this informative section of school work there is hardly a subject in which knowledge is not being vigorously revised and added to. Our school work does not follow up contemporary digesting. Still less do our school libraries. They are ten, fifteen years out of date with much of their information.

Then our new citizens have to understand something of the broad elements in our modern social structure. They should be given an account of the present phase of communication and trade, of production and invention, and above all they need whatever plain knowledge is available about the conventions of property and money. On these conventions human property stands, and the efficiency of their working is entirely dependent on the general state of mind throughout the world. We know now that what used to be called the inexorable laws of political economy and the laws of monetary science are really no more than rash generalizations about human behaviour, supported by a maximum of pompous verbiage and a minimum of scientific observation. Most of our young people come on to adult life, to employment, business and the rest of it, blankly ignorant even of the way in which money has changed slavery and serfdom into wages and employment and how its fluctuations in value make the industrial windmills spin or flag. They are not even warned of the significance of such words as inflation or deflation, and the wage earners are the helpless prey at every turn towards prosperity of the savings-snatching financier. Any plausible monetary charlatan can secure their ignorant votes. They know no better. They cannot help themselves. Yet the subject of property and money—together they make one subject because money is only the fluid form of property—is scarcely touched on in any stage in the education of any class in our community. They know nothing about it; they are as innocent as young lambs and born like them for shearing.

Thus I budget, so to speak, for our 2,400 hours of informative teaching. We have brought our young people to the upper form, the upper standard. Most of them are now going into employment or special training and so taking on a role in the collective life. But there remain some very essential things which cannot be brought into school teaching, not through any want of time, but because of the immaturity of the growing mind. If we are to build a real modern civilization we must go on with definite informative instruction into and even beyond adolescence. Children and young people are likely to be less numerous proportionally in the years ahead of us in all the more civilized populations, and we cannot afford to consume them in premature employment after the fashion of the preceding centuries.

Social organization is equally a matter for definite information. 'We are all socialists nowadays.' Everybody has been repeating that after the late Lord Rosebery for years and years.

A certain elementary training in statistical method is becoming as necessary for anyone living in this world of to-day as reading and writing. I am asking for this much contemporary history as the crowning phase, the graduation phase of our knowledge-giving. After that much foundation, the informative side of education may well be left to look after itself.

I put [this] before you as a statement of my convictions. I do not know how it will impress you and I will not anticipate your criticisms. It may seem impossibly bold and Utopian. But we are living in a world in which a battleship costs £8 million, in which we can raise an extra £400 million for armaments with only a slight Stock Exchange qualm, and which has seen the Zeppelin, the radio, the bombing aeroplane come absolutely out of nothing since 1900. And our schools are drooling along much as they were drooling along 37 years ago.

There is only one thing I would say in conclusion. Please do me the justice to remember that this is a project for Knowledge Organization only and solely. It is not an entire scheme of education. It is only a part and a limited part of education—the factual side of education. There are 168 hours in the week and I am dealing with the use of rather less than six during the school year of less than 40 weeks—for 10 years. It is no good saying, as though it were an objection to my paper or to me, that I neglect or repudiate spiritual, emotional and aesthetic values. They are not disregarded, but they have no place at all in this particular educational scheme. I have said nothing about music, dancing, drawing exercise and so on and so forth. Not because I would exclude them from education but because they do not fall into the limits of my subject. You no more want these lovely and elementary things mixed up with a conspectus of knowledge than you want playfulness in an ordnance map or whimsicality on a clock face. But the spiritual, emotional, aesthetic lives our children are likely to lead will hardly be worth living, unless they are sustained by such a clear, full and sufficient knowledge as I have ventured to put before you here.

CRISIS AND CHALLENGE

The annual report for 1965–66 talks of financial crisis and intellectual challenge. Where will the money come from to support the new activities which have been started? How will the annual meeting be changed so as to make a greater impact on the membership and the rest of the country?

THE dominant part of the annual report of the British Association for 1965–66, which will by now have been presented to the General Committee at Nottingham, is the report of the General Treasurer, Mr. I. H. Cox. According to what he says, the British Association faces a "grave crisis" in its affairs. In the year past it has been drawing on its reserves, and is likely to have to do so again in the year ahead. Though income is not actually falling, the costs of the projects on which the association has set its heart are rising steadily, with the result that the hunt for economies, which has perplexed the association for the past decade, must continue.

The financial position is all too simple. The British Association no longer has a comfortable cushion of investments on which to fall back. Ironically, the market value of the securities which it owns is hardly different (at £41,000) from what it was thirty years ago. The income from investments is less than 3·5 per cent of the total budget (in 1965–66) of £66,000. Moreover, membership subscriptions are no longer sufficient to cover a substantial part of the cost of keeping going, even though the subscription has recently been raised to four guineas (£4·2) and even though the generosity of local industries and institutions is usually sufficient to cover the cost of the annual meeting. (At Nottingham, the overhead costs will be borne by the ratepayers of that city.) The result is that the association has now to support itself increasingly by means of grants and donations from foundations, industries and public bodies. Making sure that grants continue is necessarily a continuing cause for anxiety. Trying to arrange that they should be increased must often seem a superhuman undertaking.

The immediate outlook is this. Income in the year to the end of March 1966 was £60,000, or roughly £5,000 less than in the previous year. Although the membership increased, and the association sold more copies of its publication *The Advancement of Science*, the past year was also one in which a number of covenants made seven years ago by industrial companies came to an end. (Even so, the association still received £22,000, or a third of its total income, from this source.) But as income has fallen, opportunities to spend money have increased. The result is that the General Treasurer expects that the association will be in deficit in the current year as well, even though it will begin to enjoy a grant from the Ministry of Technology intended to allow the association to embark on a programme for persuading young people to take an interest in technology. It is no wonder that the officials of the association feel curiously frustrated by circumstances outside their own control. This is how the treasurer puts it:

"As to the future, I regret my inability to be more reassuring than my predecessor in his last report. For any inter-meeting activities to take place (and this is an area in which the association's work has developed fast) advance financial provision must be made, and the council has felt that it could not but approve a budget for 1966–67 which again anticipates a deficit—£5,000—even after substantial economies in staff and a further drastic reduction in subventions to branches and area committees which permits no material development. As a continuing policy, however, budgeting for deficit is clearly suicidal and, as I write, the closest reassessment of the association's deployment of its slender funds is being made and new sources of income are being energetically sought. In the course of this I greatly hope that new financial policies may emerge in time for me to report upon them to the General Committee when it meets in Nottingham in August.

"Let there be no doubt, however, that for the present the association faces a grave crisis in its affairs. This is all the more ironic and regrettable in view of the recent reorientation of its activities more specifically to meet the needs of the times by creating a better understanding of the commercial and intellectual life of the nation, and by offering a forum where different disciplines can be brought to bear on matters of national moment, and broadening the horizon of young people so that those with the aptitude for careers in science and technology may discover themselves, and be discovered, before it is too late. Of the pressing need for these things, none can have serious doubt; that they lie squarely within the role and competence of the British Association is also generally accepted; what appears to be wrongly assumed, however, is that we can somehow continue to perform our national function without adequate support from public funds. Business, industry and charitable foundations are already exhibiting their faith. The further sum we sought—and still seek—from this other source to afford a stable central structure from which all else stems (£12,500 a year and pound for pound up to another £25,000) seems a pitifully small sum to ask and be refused."

The last passage is a reference to the way in which the association has been trying to persuade the Department of Education and Science—so far without success—to increase the annual payment of £12,500 towards the general cost of the association's work. Elsewhere in his report the Treasurer says that it is "deeply disappointing" to have to report that this request had been declined in November 1965. It is, no doubt, a measure of the association's despair about the financial outlook that it can risk being as openly critical about a government department on which it will have to continue to rely for a measure of financial support, at least in the foreseeable future.

There is, of course, no doubt that the character of the association has changed enormously in recent years. The form of the annual meeting may have remained more or less unchanged in the past century, but its flavour is now very different from what it used to be. The association's direct support of research and innovation has died away to a trickle—though the Seismological Committee continues, and although the geologists are working on the improvement of the curriculum and the anthropologists are studying burial sites and ancient field systems.

There has, however, been a rapid growth of the association's work in schools and with the public. The annual report makes no bones about the importance which is now attached to these activities.

"For the last decade the association has done wonders, within the limits imposed by its scant financial resources, in providing throughout the year and all over the country a massive and successful service of lectures and meetings for schools and other audiences, at which the pattern of

the annual meeting is translated into a less formal and often most exciting style."

The annual report goes on to draw attention to its work with visual aids and in various other studies intended to help with education and educational experiment.

The lecture service is now the central component of the association's contemporary claim on public attention—and financial support. How does it work? The headquarters of the British Association, the two branches (the Southern Branch centred on Southampton and the West Midlands Branch at Birmingham) and the nineteen area committees which now exist all undertake to provide speakers on scientific subjects for audiences in schools and those provided by adult organizations. During 1965–66 a total of 1,719 lectures was provided, four-fifths of them by the area organizations. In the annual report now current, the association claims that the total audience during the year was 128,000, including nearly 40,000 adults. This is marginally less than the corresponding figure for 1964–65.

The total cost of providing the lectures seems to have been about £24,000, of which some £7,000 came from local sources—chiefly local education authorities approached by area committees of the British Association. This works out at 3*s*. 9*d*. for each member of the audience, although the net cost to the association was only three-quarters of this. The annual report says that during 1964–65 the area committees were asked to restrict their spending on lectures and other similar activities, and that a still further reduction has been called for in the current financial year. The extent to which this will hamper activities will no doubt be determined by the success with which individual area committees are able to raise extra funds from their local authorities.

The association also has encouraging news to report about its work with young people. For some years it has been organizing "Science Fairs" at which young people from schools exhibit the results of project work carried out either at school or in their spare time. The area committees of the British Association take the initiative in organizing these events, though some of the work and all of the money are provided by the *Sunday Times*. (Dr. T. A. Margerison of the *Sunday Times* has played a particularly valuable part in the development of this work.) In the past few months the science fairs have been turned into a remarkably successful series of television programmes, thus setting at rest the anxieties on the part of the council of the British Association as these are expressed in the annual report. Another new venture is the organization of what is called a young scientists' association based on Southampton, and there is also a scheme for replicating a meeting for young people at which visits to factories and other industrial establishments play a prominent part.

The association also conducts a number of programmes supported by ear-marked grants, chiefly from charitable foundations, which run to something like £5,000 a year. One of these is a project to maintain a continuing interest in the development of school curricula in mathematics. Another is intended to make it easier for teacher training colleges to keep in touch with scientific developments, usually by receiving a distinguished lecturer on some special topic, but sometimes by making visits to scientific or industrial establishments. Then there is a scheme for encouraging youth clubs to carry out scientific investigations, frequently in the form of ecological investigations spread over a considerable interval of time. The association also maintains a visual aids committee, with a full-time executive officer who arranges programmes for the annual meeting of the association and who keeps liaison with film makers and film users, in industry and education, in the hope of increasing the mutual benefit of the interested parties.

Though the annual meeting may now seem less important than it used to, the association quite properly says, in the current annual report, that this meeting is a "focal point" for the rest of its work. If the meeting is now less of a means of communication between professional scientists than it was a century ago, it retains the pattern the original purpose of which was to provide an annual review of developments in the whole of science in the course of the preceding year. Joint discussions involving members and speakers from different sections have always been a feature of the British Association, and just as there is now a committee intended to find ways of exploring common ground between botanists and zoologists in cytology and genetics, so in the past there have been similar committees attempting, with varying degrees of success, to raise for discussion topics lying across the conventional boundaries between different branches of science.

In the past year or so the officers and officials of the association have been looking for ways of giving this annual occasion more bite, and more impact on public affairs. In this spirit, there has appeared on the annual programme an increasing number of public discussions, involving members of different sections of the association, on issues of some topical importance. In recent years there have been symposia on topics such as the provision of food for the impoverished half of the world and the supply of fuel and other sources of power. This year, at Nottingham, there are to be discussions of three topical issues—supersonic aircraft, drugs and their safety, and the problem of meeting the steadily increasing demand for fresh water from countries of all kinds, advanced and otherwise. As things have turned out, the public discussions of these questions have tended to become rehearsals of well known points of view. This was particularly true of the symposium at Cambridge in 1965 at which the distinguished heads of the nationalized industries concerned with the production of fuel outlined the well known policies of their organizations. The trouble, of course, is that an open meeting of the British Association is not an ideal setting for critics of official fuel policy to have their say. It would seem only fair to recall that in 1937 there was a discussion as vigorous as any likely to take place this year at Nottingham on the issue of how best to use the land of Britain. The British Association as such had earlier taken sides on this question by sending a resolution to the Government asking that a statutory body should be established to undertake what was called town and country planning, but there were several of the members who did not take kindly to the word *planning*.

None of these considerations is directly relevant to the anxieties which beset the Treasurer. There may even be some comfort for him in knowing that the day is gone when the ability of the association to spend money is determined by the size of the attendance at the annual meeting; that, too, could be embarrassing, at least in years when bad weather or competing attractions kept people away from the annual meeting. In any case, there is no longer a city in the United Kingdom that could accommodate so many people at the same time that their membership fees would keep the association going from one year to the next. Yet the meeting remains the focal point, and its success will inevitably determine how easily the association as a whole can win the attention of those who must in future keep it supplied with funds.

There is every reason to believe that the council of the association is aware of the problems which confront it. The annual report, which is remarkably frank for documents of this kind, says that "Institutions no less than individuals must, if they are to survive, react quickly and effectively to changes in the world around them. It is therefore satisfying to report that the council has, during the year, engaged in continuing discussions aimed at redefining both the association's purposes in the modern world and at clarifying the ways in which it can most effectively implement those purposes".

LEEDS, 1967

Sir Willis Jackson

SIR WILLIS JACKSON, who will be President of the British Association in 1966–67, and who will deliver the Presidential Address at Leeds in 1967, has been an elder statesman for as long as most people can remember, but he is a mere 62 years old. He was born in Burnley and educated there and at the University of Manchester. His first job was as a lecturer in electrical engineering in the then College of Technology at Manchester, from where he moved to Oxford in the early thirties. He went into industry, with Metropolitan Vickers Electrical Co., for a brief spell before the Second World War and then became professor of what was called electrotechnics at the University of Manchester and professor of electrical engineering at the Imperial College of Science and Technology. His public reputation was enormously enhanced by his spell as Director of Research and Education at Associated Electrical Industries (Manchester), Ltd., in the fifties. There Sir Willis was responsible for building up an efficient and imaginative scheme for the training of people at all levels, especially young apprentices. The success of the training scheme was such that it became a model followed widely by other similarly placed companies in British industry. Sir Willis left industry to become head of the Department of Electrical Engineering at Imperial College in 1961.



For the past decade Sir Willis has also been extremely active as a member of committees concerned with public policy towards science and technology. He joined the Advisory Council on Scientific Policy in 1961 and has remained the chairman of the sub-committee responsible for scientific manpower since he took over from Sir Solly Zuckerman in 1963. The sub-committee has recently been preparing an up-to-date survey of the use made of scientific manpower in Britain which is due to be published in September 1966. The chances are that this document will have an important bearing on the discussions now going on about the spending of public money on science on one hand and technology on the other.

The election of Sir Willis Jackson as President for 1967 will do much to gratify the wish of the British Association to be more intimately concerned with technology.

DUNDEE, 1968

Dame Kathleen Lonsdale

SCIENTISTS throughout the United Kingdom, whether suffragettes or not, will be delighted to learn that Professor Dame Kathleen Lonsdale has been elected President of the British Association for the Advancement of Science for 1968. Professor Lonsdale was one of the first two women to be elected to the Royal Society of London; she now becomes the first woman to hold the presidency of the British Association—an election long overdue.

A pioneer in the field of X-ray crystallography, Professor Lonsdale worked with Sir William Bragg's original team in the early 1920s. Since then her work has encroached on the structure of the benzene ring, magnetic anisotropy of crystals and molecules, X-ray scattering in diamonds and ice, and diamond/graphite transitions; more recently it has been applied to medical fields in which she is investigating bladder stones and other calculi.

Throughout her views on various aspects of life, science and politics there runs a strong ethical thread. Professor Lonsdale is known as a Quaker and a pacifist. She is forthright in her views, and her refusal to register for wartime duties led to a prison sentence in 1943.



She is known to hold strong views as to the moral obligations of the scientists. As citizens she believes they ought to be vigilant to see that the applications of science which ease their own conditions of life do not lead to unnecessary danger for other people. She has also said that they have a duty towards the general public and towards the statesmen and politicians to make sure that they are well informed—here perhaps lies the reason for her constant support of the activities of the British Association over many years.

Professor Lonsdale is an inspiration to all who work with her in the Department of Chemistry at University College, London, or come into contact with her through her extensive committee work with the International Union of Crystallography, the Royal Institution, and various prison and borstal institutions.

Married to Dr. T. J. Lonsdale, a scientist now retired but previously at the Road Research Laboratory, Professor Lonsdale has two daughters, one son, six grandsons and two granddaughters.

Continued from page 1050

72 and 74 Å, respectively, for the diameter of the equivalent sphere.

The result for casein illustrates one difficulty of the proposed present method for estimating molecular dimensions associated with molecules far removed from spherical shape. The κ-casein molecule has been reported to be a rod 150 Å long and 16 Å in diameter[15]. Such a molecule can be calculated to have a radius of gyration of 43 Å and an equivalent sphere radius of 19 Å. The present result by TMV precipitation indicates that the domain occupied by casein molecules is larger than either of these dimensions.

The molecular weight of dextran fractions had little effect on the threshold concentration, which indicates that a given weight of dextran occupies a volume fraction that is independent of the size of the molecules. This coincides with the observation by Laurent[6] that the effect of various dextrans on the solubility of albumin was the same for equal weights of all dextrans, regardless of the molecular weight of the dextran used. The close agreement with the data of Ogston and Woods[13], already referred to, is valuable support for the validity of the hypothesis underlying the technique.

The result obtained here for gelatine solutions also applies to gelatine gels. Unlike that with agar, the turbidity of gelatine–TMV mixtures is not affected by the sol-gel transformation, which suggests that gelatine molecules occupy the same volume in sols and gels. This is similar to the situation with dextran, where no essential difference was found in the exclusion phenomenon in gels or solutions[18].

The molecular radius found here for hyaluronic acid is less than half the value of the radius of gyration obtained by light scattering (Ogston, personal communication). It is possible that the present result is low because the space-excluding efficiency of a molecule probably decreases as the diameter of the excluding molecule increases, relative to a fixed size of excluded molecule[4]. Some compression of the hyaluronic acid molecules may be necessary to exclude the TMV particles from the interstices between the hyaluronic acid molecules.

The results shown in Table 1 suggest that, provided some caution is taken in interpreting the data, the TMV precipitation method can be used as a rapid and simple technique to obtain some idea of molecular dimensions. In its simplest form it is not necessary to measure the optical density of the turbidity achieved by adding TMV to the macromolecules. Such measurements were made here in order to establish the nature of the precipitation curves. All that is required is the detection of the 'end-point' or threshold concentration by visual observation of the lowest concentration of macromolecule required to give a visible turbidity. This will permit the technique to be applied to small volumes of sample.

I thank Prof. A. G. Ogston for valuable discussions on the mechanism of the phenomenon.

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POSSIBLE LOCALIZATION OF THE GENES FOR THE KIDD BLOOD GROUP ON AN AUTOSOME INVOLVED IN A RECIPROCAL TRANSLOCATION

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This article describes a family in which several members show a chromosomal abnormality. It has been possible with the aid of meiotic studies to interpret this as a reciprocal translocation involving two autosomes. Studies of nineteen autosomal marker genes suggest the possible location of the genes determining the Kidd blood group system on one of the chromosomes involved in the translocation.

The family was selected for chromosomal study because a young couple (II 5 and 6 in Fig. 1) had produced a stillborn, heavily malformed daughter and had a history of one previous, spontaneous abortion. The chromosomes of the abnormal child were not examined. The mother was apparently normal, but the father showed a very slight abnormality in that the short arm of one chromo-

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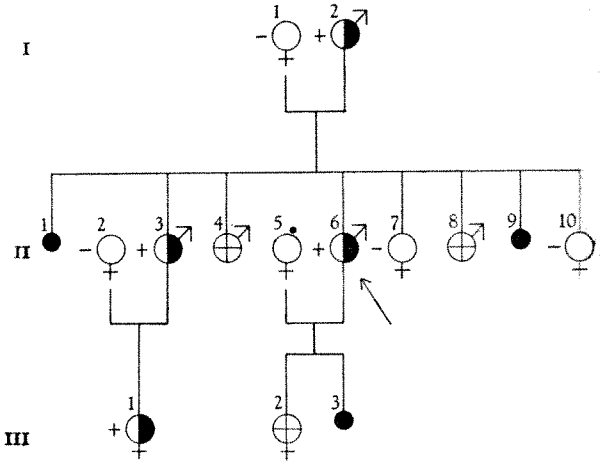


Fig. 1. Pedigree of family Z. Semifilled symbols indicate translocation carriers, empty ones chromosomally normal individuals, crossed ones individuals not studied, and small filled circles spontaneous abortions not cytogenetically analysed. The results of the Kidd blood group testing are indicated to the left of the symbols by + = Jk_a + and − = Jk_a −.

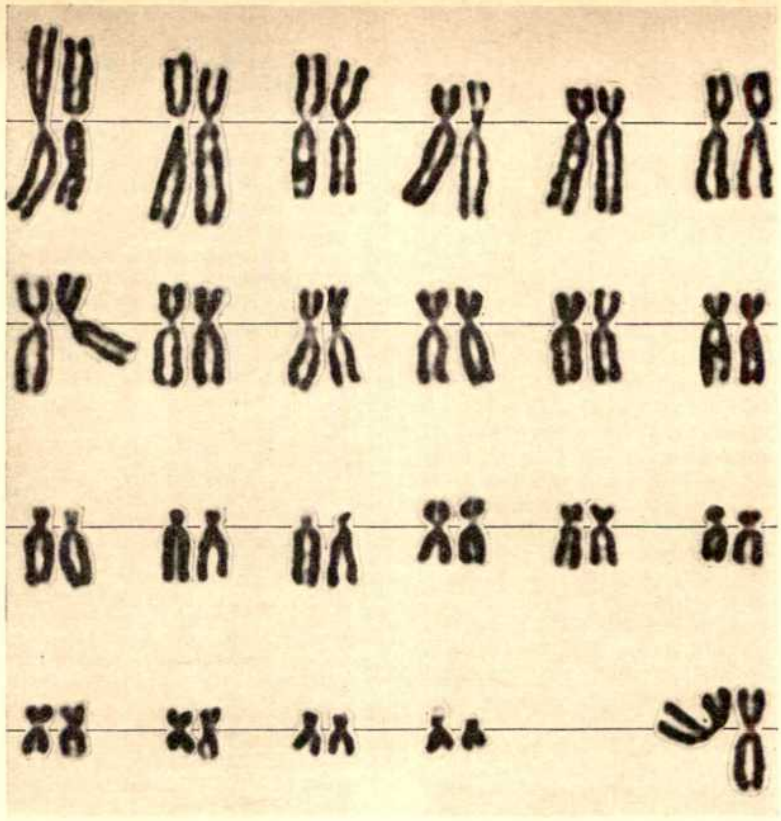


Fig. 2. Karyotype obtained from a mitotic blood cell from the niece of the propositus. Note the short, short arm on the right hand chromosome of pair No. 2.

some No. 2 was slightly shorter than usual (Fig. 2). The abnormality was very slight and in some cells it was scarcely detectable, probably because of variation in contraction of the chromosomes. A similar abnormality was detected in the grandfather, uncle and cousin, while the grandmother and two aunts showed no detectable abnormality. All these individuals, carriers of the translocation and normals, were apparently healthy.

A testicular biopsy was obtained from II 6 and it showed a normal histological picture. Altogether forty-nine cells in diakinesis or first metaphase were analysed. All cells demonstrated an apparently normal XY bivalent and one characteristic quadrivalent (Fig. 3). Twenty autosomal bivalents were found in eighteen cells, and nineteen in five cells. In the remaining twenty-six cells the exact number of bivalents could not be determined with certainty. A chain of four chromosomes was never observed, nor cells with twenty-three bivalents. Judging from the shape and size of the quadrivalent, chromosome No. 2 and one chromosome in the group 6–12 seemed to be involved in the translocation. All individuals with an abnormal karyotype could thus be considered as translocation carriers.

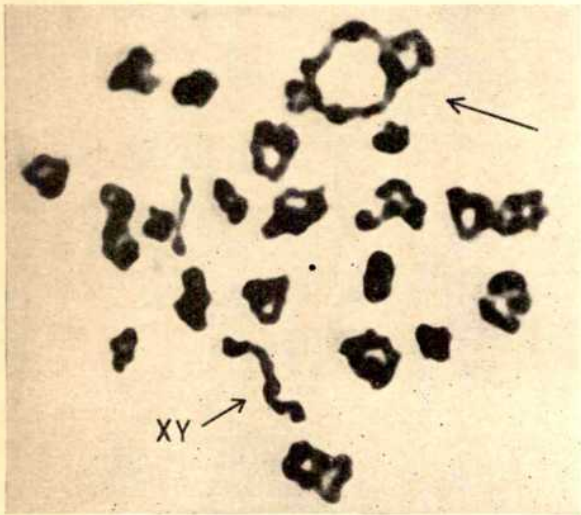


Fig. 3. First meiotic metaphase in a testicular cell from the propositus, twenty-one bivalents and one quadrivalent. The XY bivalent and the quadrivalent formed by the chromosomes involved in the translocation and their homologues are indicated by arrows.

Nineteen autosomal marker genes were investigated to determine their linkage relationships with the chromosomal abnormality. Seven systems gave information, and of these *Rh*, *MNSs*, *Hp*, *Gm* and *Gc* showed definite evidence of crossing-over with the marker chromosomes. The only one to give any suggestion of linkage was the Kidd (Jk) blood group system. The results are shown in Fig. 1. The probability of finding such a family, assuming complete linkage due to the suppression of crossing-over, is 16 : 1 in favour of linkage. Without this simplifying assumption the probability of linkage of some degree is about 7 : 1. It must be remembered, however, that the initial odds of finding linkage in these circumstances is dependent on the length of the two chromosomes involved in the translocation in relation to the total chromosomal length. If this is taken to be about 1/10, then the final odds for linkage lie between 3 : 2 in favour and 7 : 10 against. While one cannot be more precise at this stage, this family does provide a suggestion that the genes determining the Kidd red cell antigens may be located either on chromosome No. 2 or on one of the chromosomes in the group 6–12.

These investigations were supported by grants from the Swedish Cancer Society and Medical Research Council, BIOI EURATOM, the University of Pavia, and the National Heart Institute, National Institutes of Health, Bethesda, Maryland.

LETTERS TO THE EDITOR

ASTRONOMY

Neutrino Energy Losses from Vibrating Neutron Stars

IT has been suggested by Cameron[1] that the spectra of discrete X-ray sources in the sky may arise from two sources, each attributable to the presence of neutron stars: a thermal component emitted from the photosphere of the star and a non-thermal synchrotron or bremsstrahlung component emitted by electrons accelerated in a vibrating magnetosphere. It is assumed that the latter process derives its energy from stored gravitational energy in the form of radial oscillations with characteristic fundamental frequencies in the 10^3 c/s range.

Calculations by Bahcall and Wolf[2,3] indicate that neutrino cooling of non-oscillating neutron stars by way of the reactions $(n,n) \rightarrow (n,p,e \text{ or } \mu,\bar{\nu})$, $(n,\pi^-) \rightarrow (n,e \text{ or } \mu,\bar{\nu})$ and their inverses can reduce surface temperatures below observable limits in a matter of months. Tsuruta and Cameron[4] have pointed out, however, that this view is overly pessimistic because at neutron star densities ($\sim 10^{13}$–10^{16} g/cm^3) the pion-neutron interaction is predominantly repulsive which makes it unlikely that pions will be present at all. On this basis, cooling times are extended to times of the order of 10^5 years.

In this communication I shall examine Cameron's other conjecture in order to determine whether significant amounts of vibrational energy can be stored for long times. The temperature effect will be excluded by considering only zero temperature neutron stars.

If the star is in hydrostatic equilibrium the major component of its composition will be neutrons, with added traces of protons, electrons and negative muons. The abundances of these particles, in the non-interacting Fermi gas model, are easily determined by the condition imposed on their Fermi energies (including rest mass), namely,

$$E_F(n) = E_F(p) + E_F(e) = E_F(p) + E_F(\mu^-) \quad (1)$$

plus charge neutrality. In this model no pions appear until densities reach $\sim 10^{17}$ g/cm^3 (ref. 5). For this case there are no neutrino losses from neutron-neutron scatterings because only zero energy neutrinos can be emitted. If, however, the star is set into vibration so that portions of the interior are subject to sufficiently rapid density fluctuations, then equation (1) is no longer satisfied and neutrinos can be lost from the system.

Bahcall and Wolf[2,3], Finzi[6], and Ellis[7] have computed matrix elements and phase space integrals for the $(n,n) \rightarrow (n,p,e \text{ or } \mu,\bar{\nu})$ reactions in the non-zero temperature case for neutron stars in hydrostatic equilibrium. Their results are in remarkable agreement. It appears that this process will provide the dominant neutrino energy loss mechanism from vibrating neutron stars. I have followed Bahcall and Wolf's course and have used their matrix elements while adapting their phase space integral calculation to the case where equation (1) is not satisfied. We obtain, for the neutrino luminosity L_ν

$$L_\nu \approx 0{\cdot}74 \times 10^7 (\rho_N/\rho)^{\frac{1}{3}}[|\Delta_e|^8 + |\Delta_\mu|^8 p_F^2(\mu)/p_F^2(e)] \text{ ergs/g sec} \quad (2)$$

where

$$\Delta_e = E_F(n) - E_F(p) - E_F(e) \text{ MeV} \quad (3a)$$

$$\Delta_\mu = E_F(n) - E_F(p) - E_F(\mu) \text{ MeV} \quad (3b)$$

$p_F(e)$ and $p_F(\mu)$ are the electron and muon Fermi momenta, and ρ_N is nuclear density equal to $3{\cdot}7 \times 10^{14}$ g/cm^3. L_ν is a function of time because the Fermi energies and momenta change as the density changes during vibration.

A somewhat similar calculation has been performed by Finzi[8], but at only one density, so that no realistic model was used. Finzi suggested that some unknown mechanism transfers vibrational energy to the surroundings of a neutron star with a half-life of $\simeq 55$ days, thus accounting for the exponential tails of Type I supernova light curves. However, more recently Morrison and Sartori[9] have proposed a detailed theory which can account quantitatively for the shape of these light curves and the details of the observed spectra. This theory depends only on the flash of light at maximum luminosity and is thus not related to the nature of a supernova remnant.

To incorporate these results into a realistic neutron star model we have used the results of Tsuruta[5,10] based on equations of state derived from two nuclear potential calculations of Levinger and Simmons[11]. The two potentials, V_β and V_γ, yield models the density profiles of which are sufficiently different so as to represent a considerable spread in possibilities. The star is assumed to oscillate in a sinusoidal, homologous fashion such that each element of mass experiences a radial displacement proportional to its equilibrium radial distance from the stellar centre with all elements moving in phase. (Prof. A. G. W. Cameron informs me that these assumptions may be significantly in error.) The fundamental frequency is obtained from the variational principle of Misner and Zapolsky[12]. On this basis, an approximate expression for the total vibrational energy is

$$E_{\text{VIB}} \approx 2\pi\omega^2 \xi^2 \int_0^R r^4 \rho(r) dr \quad (4)$$

where ω is the circular frequency and $\xi = \delta r/r$ is the ratio of the maximum radial displacement δr of a mass element during expansion (or compression) to the equilibrium position. By the homology assumption ξ is a constant for all elements. R is the total radius.

For a given model, the rate of decrease of E_{VIB} is given by

$$\dot{E}_{\text{VIB}} = -4\pi \int_0^R L'_\nu(r) \rho(r) r^2 dr \quad (5)$$

$L'\nu(r)$ is the neutrino luminosity of equation (2) integrated over as many cycles as occur in 1 sec.

Six models have been calculated assuming an initial value of $\xi = 0{\cdot}05$. Table 1 lists the relevant parameters for each.

Table 1. NEUTRON STAR MODELS

Potential	Mass (in $M_\odot$)	Radius (in km)	E_{VIB}/ξ^2 (ergs)	ω (radians/sec)
V_β	0·34	11·7	$9{\cdot}6 \times 10^{51}$	$1{\cdot}0 \times 10^4$
V_β	0·62	7·0	$3{\cdot}3 \times 10^{52}$	$1{\cdot}96 \times 10^4$
V_β	0·89	5·7	$4{\cdot}4 \times 10^{52}$	$2{\cdot}09 \times 10^4$
V_γ	0·33	14·1	$2{\cdot}3 \times 10^{52}$	$1{\cdot}23 \times 10^4$
V_γ	1·07	12·3	$1{\cdot}7 \times 10^{53}$	$1{\cdot}67 \times 10^4$
V_γ	1·80	11·2	$1{\cdot}4 \times 10^{53}$	$1{\cdot}23 \times 10^4$

The time variation of E_{VIB} is shown in Fig. 1 for all models. It can be shown that

$$E_{\text{VIB}} \approx [E_{\text{VIB}}^{-3}(t=0) + \text{constant} \times t]^{-1/3} \quad (6)$$

where t is the elapsed time. After an elapsed time equal to the life of the Crab Nebula ($\sim 3 \times 10^{10}$ sec), vibrational

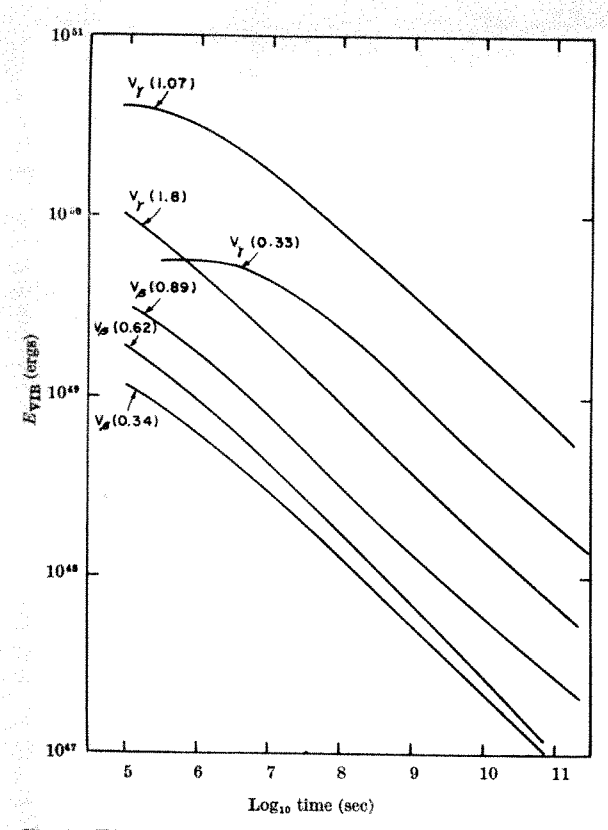


Fig. 1. Vibrational energy versus elapsed time for six neutron star models.

energies are still in the range $\gtrsim 10^{47}$ ergs for V_β models and $\gtrsim 10^{48}$ ergs for V_γ. Without knowing how efficiently vibrational energy can be dissipated into the surrounding medium, it is difficult to interpret these figures. However, the energies are probably large enough to account for the rate of electromagnetic radiation from the Crab Nebula which is $\sim 10^{37}$ ergs/sec based on figures given by Gould[13]. If this rate has been relatively constant for the last 1,000 years, then the total loss has been $\sim 3 \times 10^{47}$ ergs, which is comparable with, or less than, the vibrational energy which can still be stored at the present time. I have also calculated the decrease in vibrational energies for non-zero initial temperatures and find that the above conclusions still hold for initial temperatures as high as 10^9 °K (ref. 14).

I thank Prof. A. G. W. Cameron who suggested and guided this work, and Dr. S. Tsuruta who was prompt in making additional neutron star models and frequency calculations at my request.

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Frequency Splitting of Solar Radio Bursts

As part of a new experimental and theoretical programme of solar radio research in Tasmania, a number of dynamic spectrographs are being built to obtain information of the amplitude, polarization and direction of arrival of radio bursts with high time and frequency resolution. The first two instruments, which have been in operation since early 1965, have the following specifications: (*a*) frequency range 8–230 Mc/s, bandwidth 200 kc/s sec^{-1}, scan rate 200 sec^{-1}; (*b*) frequency range 24–28 Mc/s, bandwidth 50 kc/s sec^{-1}, scan rate 200 sec^{-1}. Each uses a fixed log periodic antenna directed obliquely into a horizontal reflecting screen. In receiver (*a*), the first oscillator frequency is swept with a varactor over the range 440–662 Mc/s and there are three intermediate frequency amplifiers at 670 Mc/s, 50 Mc/s and 10 Mc/s respectively. Records of dynamic spectra are made on 35 mm film moving at 18 in./h for 8 h each day and intermittently with a film speed of 6 in./min during solar bursts. The operation of the faster cameras is initiated by the bursts.

Apart from a few bursts, mainly type III, nothing of significance was recorded until the major series of radio outbursts which began on March 16, 1966, and continued until March 29. This event was characterized by a single spot group which, when observations began at 2200 U.T. on March 16, was situated at approximately 45° north latitude and 60° east of the meridian. Large numbers of type I and III bursts and drift pairs and some U-bursts were recorded.

In addition, many narrow bandwidth bursts, apparently of a type not so far reported, were observed in the frequency range 20–50 Mc/s. On the wide range records (spectrograph *a*), they appeared as sharp lines of almost constant frequency and lasting for 1–2 sec (Fig. 1). They generally occurred in groups of about ten individual bursts distributed in frequency from 20 Mc/s to 50 Mc/s, with the higher frequency components beginning slightly earlier. The frequency-time slope of the leading edges was in the range -4 Mc/s to -10 Mc/s, that is in the same range as for type III bursts. The rate of occurrence of the burst groups was approximately 10/h during March 17 and 18. With the higher frequency resolution of the 24–28 Mc/s spectrograph, the individual bursts were observed to be made up of two components separated slightly in frequency (Figs. 2*a*, *b* and *c*). The mean frequency interval was 0·12 Mc/s and its range of values 0·05–0·25 Mc/s. The centre frequency of each component normally decreased with time at the mean rate 0·1 Mc/s and the mean duration was 1·2 sec.

A possible explanation of these split pairs is the excitation of electromagnetic waves near the ordinary and extraordinary mode critical frequencies. The small frequency separation, about $fH/2$, would then imply that the source is located in a region of low magnetic field intensity ($\sim$ 0·12 oersted). The generation of two magneto-ionic modes of opposite polarizations in the solar corona has already been examined by Zeleznyakov[1] in the theory of plasma Cerenkov radiation, and it is interesting that he found the extraordinary wave will in general not be observable because of high attenuation unless the magnetic field intensity is very small. With a field intensity of 0·12 oersted, the extraordinary wave might still be seen but could be expected to be much weaker than the ordinary wave which would correspond to the lower frequency component of the split pair. However, examination of the records shows in general that the higher frequency component is the stronger. With this mechanism, the two components would have opposite polarization.

An alternative process is Doppler shifted cyclotron radiation from electrons[2,3]. It has been shown by Fung and Yip[4,5] that in the solar corona, electrons moving in the magnetic field of a sunspot may generate cyclotron radiation which will be observable mainly in the ordinary

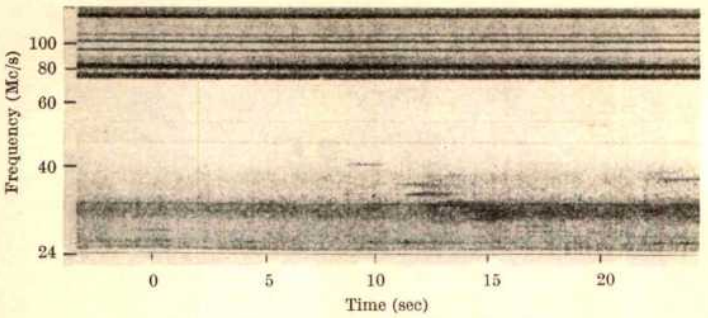
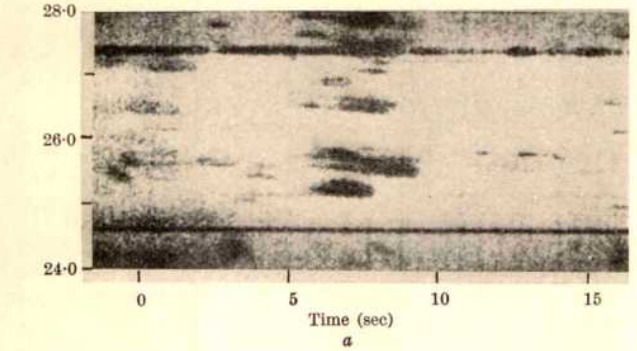
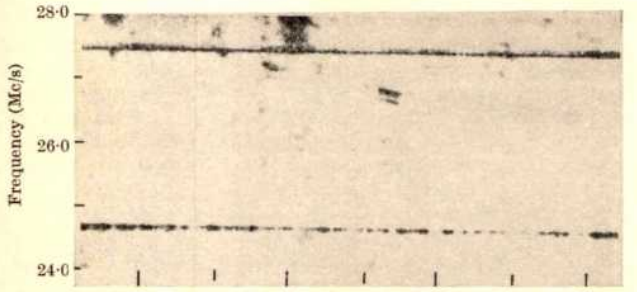


Fig. 1. Wide range record showing split pair group.





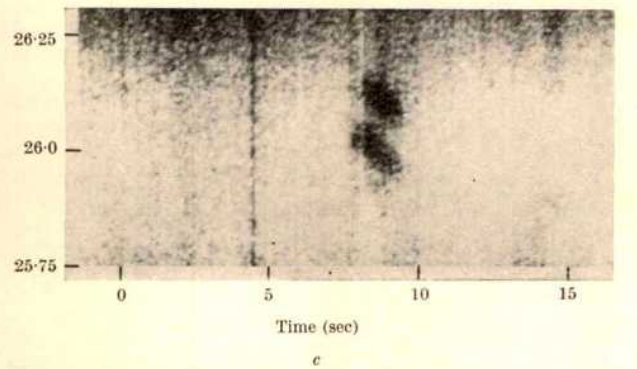


Fig. 2. *a*, 24–28 Mc/s record showing frequency splitting of bursts within a group. *b*, Individual pair. Note the greater intensity of the higher frequency component. *c*, Enlarged record showing frequency-time slope.

mode in the vicinity of the second harmonic of the cyclotron frequency. The frequency will depend also on the direction of emission, and two waves of differing frequencies corresponding to forward and backward emission normally will be observable. The velocity of the guiding centre would need to be only about 0·01 *c* to produce the small frequency splitting observed. If the mean direction of motion is outwards through the corona, then this velocity is consistent with the observed rate of change of the centre frequency. The polarization of both components would be ordinary in this event.

Polarization observations may thus be expected to throw more light on the origin of the split pairs, and in association with directional measurements may produce new information about the dynamics of particles and the magnetic fields in the solar corona.

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PLANETARY SCIENCE

Anti-matter Content of the Tunguska Meteor

Cowan *et al.*[1] have advanced the hypothesis that the 1908 Tunguska meteor explosion resulted from the anti-matter content of the metecrite. Although their analysis indicates a probable upper limit of 1/7 for the anti-matter content of the Tunguska meteor, this result depends to a large extent on the validity of certain assumptions used in the calculations.

The present analysis should be considered an extension of the former work and assumes as its starting point the previously determined four charged pions produced per nucleon pair annihilated. Whereas Cowan *et al.* assumed that only negatively charged pions are absorbed by atmospheric nuclei (and thus instrumental in producing carbon-14), the cross-section data of Marshak[2] indicate that the mean free path for nuclear absorption is essentially the same for both positively and negatively charged pions. This is reasonable as the pion-nucleon interaction arises mainly from the nuclear rather than electrostatic forces. If the charged pion mean free path σ for nuclear absorption is taken as 80 g cm^{-2} in air[3], it is possible to compare the fraction of pions which decay with the fraction which are absorbed provided the pion mean life is known. Horwitz *et al.*[4] report an average total energy E_T of 380 MeV per charged prong in the $p\bar{p}$ annihilation. The charged pions then have an average kinetic energy E_K of 240 MeV (the rest mass energy E_0 is 140 MeV) and a small relativistic correction to the mean life τ_m of the pion is necessary. A conservative estimate of the increased pion mean life τ' can be made by assuming an essentially constant speed equivalent to E_k = 240 MeV. Since $E_T = \gamma E_0$, $\tau'_m = \gamma\tau_m$ ($\gamma = [1-\beta^2]^{-\frac{1}{2}}$, then τ'_m = (380/140) (2·54 × 10^{-8} sec) = 6·9 × 10^{-8} sec.

If n_0 represents the total number of charged pions produced initially by annihilation reactions, then the number of pions n remaining at some later time t would be $n = n_0 \exp - \left(1/\tau'_m + \frac{\bar{\rho}\bar{v}}{\sigma}\right)t$, where v and ρ represent respectively the average pion speed and atmospheric density along the flight path. Since $\bar{\rho}\bar{v}/\sigma$ is effectively the probability of absorption per unit time, then by letting $\lambda_a = \bar{\rho}\bar{v}/\sigma$ and $\lambda'_m = 1/\tau'_m$, the above expression may be written $n = n_0 \exp - (\lambda_a + \lambda'_m)t$. Since in this form the expression for n is similar to that for branched radio-

active decay, branching ratios for decay and absorption may be defined as $R_d = \frac{\lambda'_m}{\lambda'_m + \lambda_a}$ and $R_a = \frac{\lambda_a}{\lambda'_m + \lambda_a}$, respectively. For an explosion at a height of 5 km, $\bar{\rho} = 7{\cdot}3 \times 10^{-4}$ g-cm^{-3}, and if $\bar{v} = 0{\cdot}9\ c$ ($v = 0{\cdot}93\ c$ for $E_k = 240$ MeV), then $R_d = 0{\cdot}983$ and $R_a = 0{\cdot}017$.

Whereas the previous analysis assumed that 50 per cent of the charged pions were absorbed thus producing a 7 per cent increase in radiocarbon activity, the calculations already given indicate only 1·7 per cent are absorbed thus yielding only a 0·24 per cent increase in radiocarbon activity. Although this value may only be correct to within a factor of two owing to over-all uncertainties in the initial assumptions, it is questionable whether a 0·24 per cent increase in radiocarbon activity would be detectable. Cowan *et al.* report an increase equivalent to about 1 per cent in some tree rings around 1909, but Suess[5] finds no change whatsoever in another sample. These results are consistent with the hypothesis that the Tunguska meteor was entirely anti-matter in content.

A closer look at the mechanisms which initiate thermonuclear and anti-matter explosions is now in order. Of the 200 MeV available as a result of uranium-235 fission about 85 per cent is converted immediately into kinetic energy of very short range fission fragments (less than 3 cm in air at sea level), thus liberating a tremendous amount of energy within a relatively small volume. The formation of a fireball from a fission-fusion explosion arises primarily from this phenomenon.

In contrast to this approximately 65 per cent[4] of the energy available in a $p\bar{p}$ annihilation goes into charged pions and the remainder into neutral pions (assuming the kinetic energy is the same as that of the charged pions). The charged pions which are absorbed ($\approx$ 2 per cent of the total) by atmospheric nuclei result in neutron and proton emission from excited nuclei. Because the neutron mean free path in air is much too large to permit much absorption close to the explosion centre, only proton interactions will contribute any significant amount of energy to the growth of the fireball. Thus, of the charged pions which are lost through the process of nuclear absorption, less than 1 per cent are effective in contributing to the thermal yield of the explosion.

In evaluating the contribution of the charged pions which decay (greater than 98 per cent of the total generated) to the thermal yield it is well to note that the decay products are muons and neutrinos ($\pi^+ \rightarrow \mu^+ + \nu$). The neutrinos, of course, contribute essentially no energy whatsoever to the thermal yield. The muon contribution depends on the average energy at which the pions decay. Using pion range-energy curves[3] a value of $-dE/dx = 2 \times 10^{-3}$ MeV/cm is deduced for rate of energy loss of 200 MeV pions in air at sea level. More than 98 per cent of the pions decay within six half-lives, and using the above datum, calculations show that the pions suffer little energy loss during this interval (less than 15 MeV on the average). By calculating the maximum and minimum muon energies (in the laboratory system) resulting from pion decay, the average muon kinetic energy is taken as about 160 MeV. Owing to the extremely large absorption mean free path in air, nearly all the muons will decay before being absorbed. Because the maximum kinetic energy of the electron from muon decay is 52·3 MeV in the muon reference frame, a certain portion of these decay electrons will possess enough energy in the laboratory system to initiate electronic showers and with lower energy decay electrons will aid in the development of a fireball.

As mentioned earlier, more than 30 per cent of the $p\bar{p}$ annihilation energy is channelled into neutral pions. Owing to the extremely short mean life ($\approx 10^{-15}$ sec) these pions disappear rapidly yielding mostly high energy quanta (99 per cent) and a smaller proportion (1 per cent) of electrons and positrons. The absorption mean free path[6] of 50–100 MeV quanta in air is about 67 g cm^{-2}, implying that most of this energy will be absorbed at large distances, thus adding relatively little to the thermal yield of the explosion. Thus, the over-all contribution to fireball growth from neutral pion decay electrons and positrons may be about the same as for those from muon decay.

Although qualitative in some respects, these calculations permit a comparison to be made of the energy distribution between an ordinary nuclear explosion and an anti-matter explosion. In an air burst[7] of a fission-fusion device roughly 50 per cent of the energy is dissipated in blast and shock effects, 35 per cent of the total yield appears as thermal radiation, 5 per cent as initial nuclear radiation, and 10 per cent as residual radioactivity. These percentages are of course dependent on altitude. The significant point for this type of explosion is that a major proportion of the particles emitted in the fission-fusion reactions dissipate their energy within a short distance, thus producing conditions favourable for the formation of a fireball.

In contrast the greater percentage of primary particles produced in an anti-matter explosion rapidly decay, and the secondary emissions will in general be absorbed over a relatively large volume. Qualitatively it might be expected that within a certain critical volume near the explosion centre the secondary and to a lesser extent the primary emissions would produce conditions favourable to the development of a fireball. Under these conditions it would not be surprising if the percentage of thermal yield from an anti-matter explosion was considerably less than that resulting from an ordinary nuclear explosion.

The observations of an eye-witness to the Tunguska meteor explosion are significant at this point. As Cowan *et al.* point out, the fireball did not last long. S. B. Semenov, an observer, just managed to lower his eyes as the explosion occurred, and when he looked again the fireball had disappeared. The account of P. O. Kosolopov in the same connexion has similar implications. This relatively short duration fireball (presumably only several seconds) is difficult to reconcile with the 33 sec fireball[7] to be expected from an ordinary 30 megaton thermonuclear explosion (the calculated equivalent yield of the Tunguska burst). It definitely appears that the relatively short duration fireball is reasonable evidence against the Tunguska explosion being thermonuclear in nature. A Monte Carlo calculation would be necessary to evaluate properly all the parameters involved, but it certainly seems possible that an anti-matter explosion of this yield would have a fireball of only a few seconds owing to the vastly different nuclear interactions inherent in the process itself. Finally, very recent studies indicate the possibility that certain shock haloes in biotite are of anti-matter origin[8].

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Strontium : Calcium Ratio in Tourmalines from South-west England

GOLDSCHMIDT has pointed out[1] that during the cooling of an igneous magma the earliest formed calcium minerals would be poor in strontium relative to calcium while later calcium minerals would show a progressively higher

proportion of strontium to calcium. In his opinion this would be so because the calcium ions would be taken into the crystal lattice in preference to strontium, because they were more abundant and because they had a smaller ionic radius and would form a stronger bond. Other workers[2,3] have shown that the ratio of strontium : calcium in plagioclases does increase as calcium decreases with fractionation in certain igneous rock series.

Samples of tourmaline have been separated from some of the various rock types that make up the granitic masses of south-west England and their strontium and calcium content determined by emission spectrographic analysis[4]. When the ratio of strontium : calcium of each tourmaline sample is plotted against calcium content several features of interest emerge. In Fig. 1 tourmalines taken from granites, aplites and pegmatites and some of those from quartz-tourmaline rocks form a series in which both the ratio of strontium : calcium and the absolute amount of strontium increases as calcium decreases. If the granites, aplites and pegmatites are considered to make up a fractionation series then the variation in the ratio of strontium : calcium with fractionation shown by others to be present in plagioclases may also be said to exist in tourmalines from the granitic rocks of south-west England. In addition, on the basis of the low calcium content and the high ratio of strontium : calcium in their constituent tourmaline, certain quartz-tourmaline rocks can be considered to be the product of extreme fractionation.

A second generation of tourmaline, usually occurring as blue-green acicular crystals which sometimes form stellate aggregates and grow around corroded nuclei of an earlier generation of tourmaline, has long been recognized in the granites of south-west England[5] and may be attributed to late-stage hydrothermal activity. Perhaps the most striking feature of Fig. 1 is the way in which the samples (*H*), selected as of undoubted hydrothermal origin on the basis of their mode of occurrence, may be seen to be quite separate from all the other samples. The ratio of strontium : calcium for the hydrothermal samples is as much as ten times greater than that for other tourmaline samples containing similar amounts of calcium.

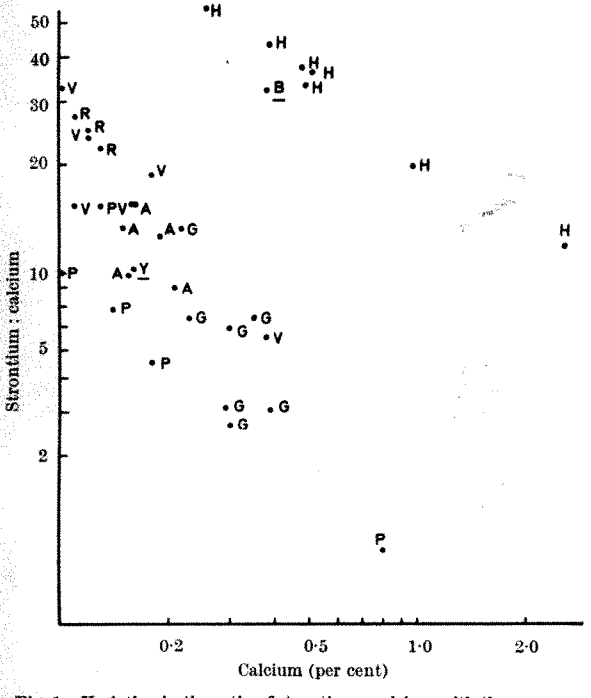


Fig. 1. Variation in the ratio of strontium : calcium with the percentage of calcium in tourmalines from south-west England. (Percentage of strontium × 1,000)/percentage of calcium is plotted against the percentage of calcium. Origin of pure tourmaline samples: *G*, granite; *A*, aplite; *P*, pegmatite; *R*, quartz-tourmaline rock; *V*, quartz-tourmaline veins; *H*, hydrothermal tourmaline; *B*, blue rim; *Y*, yellow central zone tourmaline from Castle an Dinas quarry, near Penzance.

The ratio of strontium : calcium may be used as an aid in establishing whether or not a given tourmaline sample is of hydrothermal origin. Tourmaline from a zone of reddened granite in Castle an Dinas quarry near Penzance, on examination in thin-section, was found to consist of anhedral yellow grains each with an outer zone of blue tourmaline. Samples of both the yellow and the blue tourmaline were obtained by careful hand-picking of the pure mineral fragments and analysed separately. The yellow central zone (*Y*) contains 0·16 per cent calcium and 16 p.p.m. strontium and falls into the same region in Fig. 1 as the samples from aplites. On the other hand the blue outer zone (*B*) contains 0·38 per cent calcium and 122 p.p.m. strontium and falls into the same region as the hydrothermal samples, which strongly suggests that the growth of the blue rims was of hydrothermal origin. Certain other tourmaline samples from quartz-tourmaline veins and from some quartz-tourmaline rocks were often found to have a patchy yellow and blue coloration in thin-section, and if their ratios of strontium : calcium were plotted on Fig. 1 they would fall between the fractionation series and the hydrothermal tourmalines. These samples are interpreted as having undergone some degree of hydrothermal alteration.

The ratio of strontium : calcium in tourmaline may, therefore, be used not only as an indicator of fractionation and to distinguish hydrothermal tourmaline from other varieties of tourmaline but also to detect tourmaline that has suffered some degree of subsequent hydrothermal alteration.

I thank Prof. F. W. Cope and Dr. C. S. Exley for encouragement and guidance in this work which was carried out in the Department of Geology, University of Keele.

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ENGINEERING

Wind Forces on Groups of Structures

A RECENT letter by Gunn and Malik[1] raises the possibility that the collapse of the Central Electricity Generating Board's cooling towers at Ferrybridge in 1965 might have been caused by the unusual aerodynamic loads present on this type of configuration.

Very little is known about the flow around groups of bodies, and a preliminary investigation has just been completed in this laboratory of the pressures on one cylinder in a group of cylinders as shown in Fig. 1. The cylinders, 2 in. in diameter and 28 in. high, were mounted on the floor of the 5·5 ft. by 4 ft. working section of a low speed wind tunnel and tested at a Reynolds number based on the diameter of the cylinder of 15×10^5. The pressure distribution on one of the cylinders in the rear row was measured for various angles of inclination of the group to the flow direction. The pressure tappings were placed around the cylinder in a plane 9 in. below the top.

One interesting feature of these results is that over a small angle of inclination α the force F on the cylinder in the rear row changes by almost 100 per cent. Fig. 2 shows these results, and it can be seen that if the main stream is inclined to the group at an angle of 26° then a change of $\pm$ 2° in the wind direction can produce a

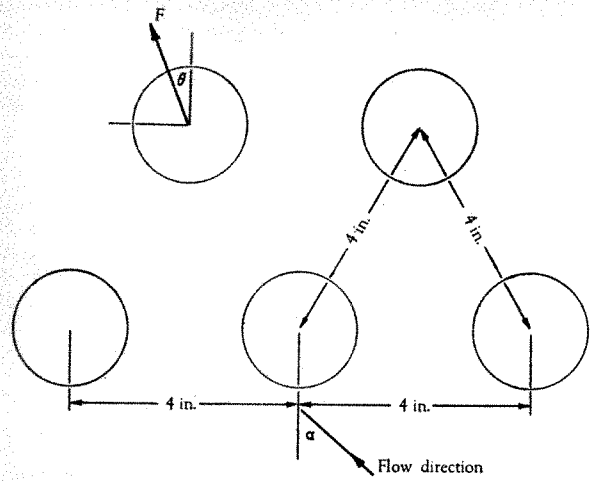


Fig. 1. Experimental configuration. Flow velocity u, density ϱ, and cylinder diameter $d = 2$ in.

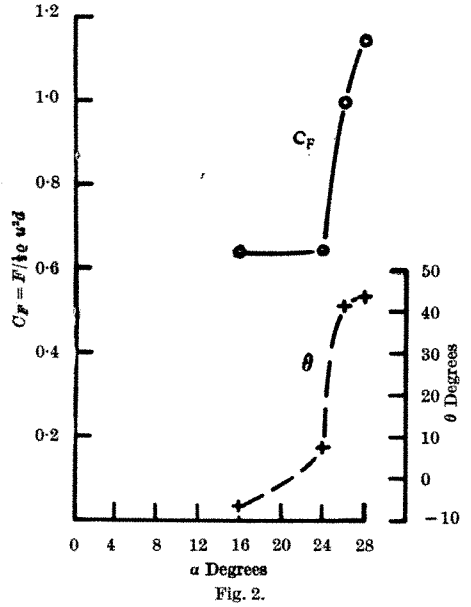


Fig. 2.

change in the non-dimensional force coefficient C_F of about 0·5. Also shown on Fig. 2 is the variation of the direction of this force with the angle α.

This result may be relevant to the collapse of the cooling towers, because it is quite conceivable that if the mean wind direction were at this critical inclination to the group and the direction of the wind were oscillating by a few degrees about this mean direction then this would produce a large fluctuating force on the towers. The frequency of the fluctuating force would be determined by the frequency of the wind oscillation and not by the frequency of oscillation of the wake of the cylinder.

The tests mentioned here were, of course, at a Reynolds number much lower than those of the actual towers, but it is thought that the flow producing these results is not very sensitive to Reynolds number.

A similar result was found earlier by Mitchell, also in this laboratory, for the flow around a group of rectangular blocks (to be published).

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The Central Electricity Generating Board now considers the collapse of the cooling towers to be due to structural weakness.—Editor, *Nature*.

Flow of Gas at Low Pressure

WHEN the diameter of a round tube is very much larger than the mean free path (m.f.p.) of the molecules of a gas flowing through it the volumetric flow F of the gas in the axial z direction is given by the expression

$$F = \frac{\pi a^4}{8\mu} \frac{dp}{dz}$$

where a, μ and p are the tube radius, and the gas viscosity and pressure, respectively.

When the width to thickness ratio of a rectantular tube as well as the thickness to m.f.p. ratio are very large, the flow of gas dF per unit width dw of the tube is likewise given by the expression

$$\frac{dF}{dw} = \frac{b^3}{12\mu} \frac{dp}{dz}$$

where b designates the inner thickness of the tube.

Conversely, at low pressures when the m.f.p. is very much larger than the dimensions of a uniform tube of circumference 0 and cross-section area A extending in the z direction, and under the assumption that the accommodation coefficient is unity, that is, that molecules bounce off the walls in a Maxwellian distribution, the mass flow of gas is generally given by expressions equivalent to (1) (ref. 1)

$$Q = \frac{4}{3} \bar{c} \frac{A^2}{0} \frac{d\varrho}{dz} \qquad (1)$$

where $\bar{c}$ and ϱ designate the average speed of the molecules and the gas density respectively. It is immediately apparent that equation (1) cannot have a general application. For example, did the general cross-section consist of two parallel connected round tubes of different radii a_1 and a_2, the inequality

$$a_1^3 + a_2^3 \neq \frac{(a_1^2 + a_2^2)^2}{a_1 + a_2}$$

would demonstrate the inapplicability of (1).

When the tube is round, equation (1) can be written

$$\frac{Q}{A} = q = \frac{2}{3} \bar{c}\, a \frac{d\varrho}{dz} \qquad (2)$$

where q designates the average mass flow per unit cross-section area of the tube. This is the case in which (1) is correct, as the following calculation will verify. The method used consists of first calculating the mean free path, $\bar{e}$, of the low-pressure gas molecules in a round tube, and, second, the mean square $\overline{e_z^2}$ of the free paths projections in the axial z direction. The axial molecular diffusivity, D_z, is then given by

$$D_z = \frac{1}{2} \frac{\overline{e_z^2}}{e} \bar{c}$$

because the mean squared random travel of a molecule in the z direction in a time t is given by $2D_z t$, and the average mass flow per unit area is given by

$$q = D_z \frac{d\varrho}{dz} \qquad (3)$$

$\bar{e}$ is obtained immediately from the observation that, since the rate of molecular collisions with the tube wall is $\frac{1}{4} \bar{c}\, \nu$ per unit area, where ν designates the number of molecules per unit volume, for a single molecule in a tube of radius a, length l and wall area $2\pi a l$ we should have

$$\nu = \frac{1}{\pi a^2 l} \text{ and } \frac{\bar{c}}{\bar{e}} = \frac{1}{4} \bar{c}\, \nu \,.\, 2\pi a l = \frac{1}{2} \frac{\bar{c}}{a}$$

whence

$$\bar{e} = 2a$$

In order to calculate $\overline{e_z^2}$, a polar system of co-ordinates is placed on an element of the inner wall, the elevation δ

of any direction being measured from the axial z direction, and the azimuth φ from an inner directed normal to the tube at the point considered. $\overline{e_z^2}$ is calculated from the general formula

$$\overline{e_z^2} = \frac{\int z^2 \, d\Omega}{\int d\Omega}$$

where z designates the z projection of any path originating on the wall element and where $d\Omega$ designates any properly weighted elementary solid angle from the wall element considered. Any unweighted solid angle is given by $\sin\delta \, d\delta \, d\varphi$ and the weight $\sin\delta\cos\varphi$ must be applied to correct for the foreshortening of the elementary area for slanted directions. z is given by

$$z = \frac{2a \cos\varphi \cos\delta}{\sin\delta}$$

We obtain for $\overline{e_z^2}$

$$\overline{e_z^2} = \frac{\int_0^{\pi} d\delta \int_{-\pi/2}^{\frac{\pi}{2}} \frac{4a^2 \cos^2\varphi \cos^2\delta}{\sin^2\delta} \sin^2\delta \cos\varphi \, d\varphi}{\int_0^{\pi} d\delta \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} \sin^2\delta \cos\varphi \, d\varphi}$$

$$= \frac{4a^2 \cdot \frac{\pi}{2} \cdot \frac{4}{3}}{\frac{\pi}{2} \cdot 2} = \frac{8}{3} a^2$$

whence for D_z

$$D_z = \frac{1}{2} \frac{\frac{8}{3} a^2}{2a} \bar{c} = \frac{2}{3} a\bar{c} \qquad (4)$$

and substitution of this value for D_z in equation (3) yields equation (2).

It is illustrative to note that the average net mass flow given by equation (3) is not uniform throughout the tube. Thus, for example, we may calculate the flow through an element of unit area of the tube cross-section near the tube wall by noting that, for a uniform density gradient $d\rho/dz$, the rate of mass flow from a small area at the height z above the cross-section element considered is given by

$$\frac{\frac{1}{4}\left(\rho_0 + z \frac{d\rho}{dz}\right) \bar{c} \, d\Omega}{\int d\Omega}$$

where $d\Omega$ designates the properly weighted solid angle through which that small area is seen from the element of the tube cross-section at which the system of polar coordinates is centred, and where ρ_0 designates the gas density at that small element. The proper solid angle weight for this calculation is $\cos\delta$, which measures the foreshortening of the cross-section element for directions slanted with respect to the normal to that element, and we obtain for the z $(d\rho/dz)$ term

$$q_{lw} = \frac{\frac{1}{4}\bar{c}\frac{d\rho}{dz} \int_0^{\frac{\pi}{2}} d\delta \int_{-\frac{\pi}{2}}^{\frac{\pi}{2}} 2a \frac{\cos\varphi\cos\delta}{\sin\delta} \sin\delta\cos\delta \, d\varphi}{\int_0^{\frac{\pi}{2}} d\delta \int_{-\pi}^{\pi} \sin\delta\cos\delta \, d\varphi}$$

$$= \frac{2a}{4} \bar{c} \frac{d\rho}{dz} \frac{\frac{\pi}{4} \cdot 2}{\frac{1}{2} \cdot 2\pi} = \frac{a\bar{c}}{4} \frac{d\rho}{dz}$$

where it must be noted that the integral with respect to $d\varphi$ in the numerator extends over only half the domain of the denominator because the proximity of the wall ensures that from one half the solid angle viewed by the cross-section element the rate of molecular flow is determined by ρ_0.

q_{lw} represents, therefore, the excess of mass flow downward which results from the greater gas density above the cross-section. Likewise, the calculation of the mass flow upward would reveal a deficiency of gas flow of the same magnitude due to the lower density of the gas below the area considered, so that the total mass flow per unit area near the wall would be

$$q_w = 2q_{lw} = \frac{1}{2} a\bar{c} \frac{d\rho}{dz} \qquad (5)$$

or somewhat less than the average mass flow given by equation (4).

A similar calculation for the excess mass flow from above through an element of the cross-section in the centre of the tube would give

$$q_{lc} = \frac{1}{4} \bar{c} \frac{d\rho}{dz} \frac{\int z \, d\Omega}{\int d\Omega}$$

$$= \frac{1}{4} \bar{c} \frac{d\rho}{dz} \frac{\int_0^{\frac{\pi}{2}} d\delta \int_0^{2\pi} a \frac{\cos\delta}{\sin\delta} \sin\delta\cos\delta \, d\varphi}{\int_0^{\frac{\pi}{2}} d\delta \int_0^{2\pi} \sin\delta\cos\delta \, d\varphi}$$

$$= \frac{1}{4} a \bar{c} \frac{d\rho}{dz} \frac{\frac{\pi}{4} \cdot 2\pi}{\frac{1}{2} \cdot 2\pi} = \frac{\pi}{8} a \bar{c} \frac{d\rho}{dz}$$

and adding a like contribution resulting from the deficiency in the mass flow from below

$$q_c = 2q_{lc} = \frac{\pi}{4} a \bar{c} \frac{d\rho}{dz} \qquad (6)$$

This mass flow is greater than the average given by equation (4) which is thus seen to be intermediary between a higher flow in the tube centre and a lower flow at the tube side.

If we now consider a rectangular tube of inner width and thickness B and t, respectively, the classical formula (1) gives for the mass flow per unit area

$$q = \frac{Q}{Bt} = \frac{4}{3} \frac{\bar{c}}{Bt} \frac{B^2 t^2}{2(B+t)} \frac{d\rho}{dz} \cong \frac{2}{3} t \bar{c} \frac{d\rho}{dz} \quad , B \gg t \qquad (7)$$

When we attempt to verify equations (7) by calculating the diffusivity of molecules in the z direction, we obtain readily for $\bar{e}$, using an argument similar to the one used for the case of a round tube:

$$\bar{e} = 2t$$

On the other hand, when we calculate $\overline{e_z^2}$ we have for the elementary weighted solid angle:

$$d\Omega = \sin^2\delta \cos\varphi \, d\delta \, d\varphi$$

and for the projection z of any path:

$$z = t \frac{\cos\delta}{\cos\varphi \sin\delta}$$

and it is clear that the integral $\int z^2 d\Omega$ taken over the entire solid angle viewed from a wall element is infinite.

It can easily be verified that an infinity is also obtained when the mass flow through an element of the tube cross-section is calculated as was done for a round tube.

This result illustrates the care with which some of the subtleties of kinetic theory must be tackled and suggests that the study of gas flow at low pressure through any but round tubes may require a computer approach and a presentation of the data through multiple entry tables.

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THE SOLID STATE

Rehbinder Effect in Lubricated Metal Cutting

THE mode of action by which lubricants reduce cutting forces during machining operations has been the subject of many conflicting hypotheses[1–5]. Shaw *et al.*[6] stimulated new interest in this field with the observation that carbon tetrachloride caused a reduction in cutting forces of some 10–15 per cent if applied to the workpiece surface and allowed to evaporate immediately before cutting. This phenomenon has been the subject of a recent investigation in which both $^{14}CCl_4$ and $C^{36}Cl_4$ were used to study the location and distribution of the tagged species in copper chips cut at slow speed.

The fluid was applied to the workpiece surface ahead of the advancing shear zone (Fig. 1) in sufficiently small amounts to have evaporated visibly before deformation of the surface occurred. Nevertheless, a reduction in cutting force of some 10 per cent was achieved. By taking direct activity counts on the resultant chip it was determined that chemical reaction had occurred predominantly on the rough surface of the chip remote from the chip/tool contact under these conditions. Lubrication of the chip/tool interface had not occurred. By preparing autoradiographic records from chip taper sections and also by directly counting the activity on chip surfaces progressively subjected to acid dissolution, the distribution of activity in the body of the chip was determined. There was, however, no evidence for the existence of a concentration gradient of tagged material across the chip section, which would have indicated diffusion of the applied fluid into the chip itself.

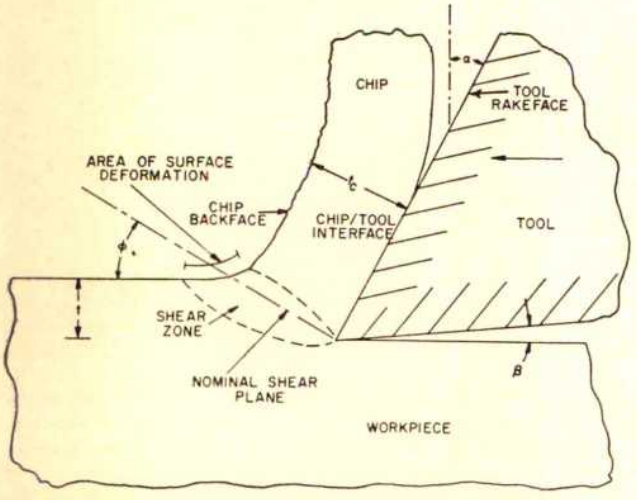


Fig. 1. Diagram showing chip formation and giving nomenclature. ϕ, Shear angle; α, rake angle; β, flank clearance angle; t, undeformed chip thickness; t_c, chip thickness.

The evidence obtained suggested that carbon tetrachloride changes the mechanical properties of the deforming metal by a purely surface chemical reaction. Some confirmation that the shear zone was sensitive to change in condition of the deforming surface area was, therefore, sought. Electropolishing the deforming area during a cut produced the same changes in cutting parameters that had previously been obtained by the reaction of carbon tetrachloride in the same area. Fig. 2 shows a dynamometer trace and longitudinal chip section for a cut—lubricated throughout with electrolyte (phosphoric acid–ethanol–water mixture)—during which the deforming surface was from time to time electropolished. The reduction of cutting force and increase in shear angle which result from what is again a purely surface effect are clearly demonstrated.

The effect of adsorbed media on the rate of strain hardening of metal single crystals was first demonstrated by Rehbinder[7], and attributed to diffusion of physically adsorbed molecules into microcracks thought to be present in the surface. It was suggested that this diffusion process caused microcracks to propagate, so weakening the metal. More recently Kramer[8] showed that, during tensile tests with single crystals of aluminium and copper which were immersed in stearic acid solutions, chemical reaction and localized solution of the test specimen accompanied a reduction in the rate of strain hardening of the metal. Removal of the surface by electropolishing the specimens during the tensile test produced similar changes in the rate of strain-hardening[9], and it was argued that the function of the surface active media used by Kramer, and earlier by Rehbinder, was to produce localized surface removal in regions of dislocation pile-up below the metal surface. Because the surface represents a

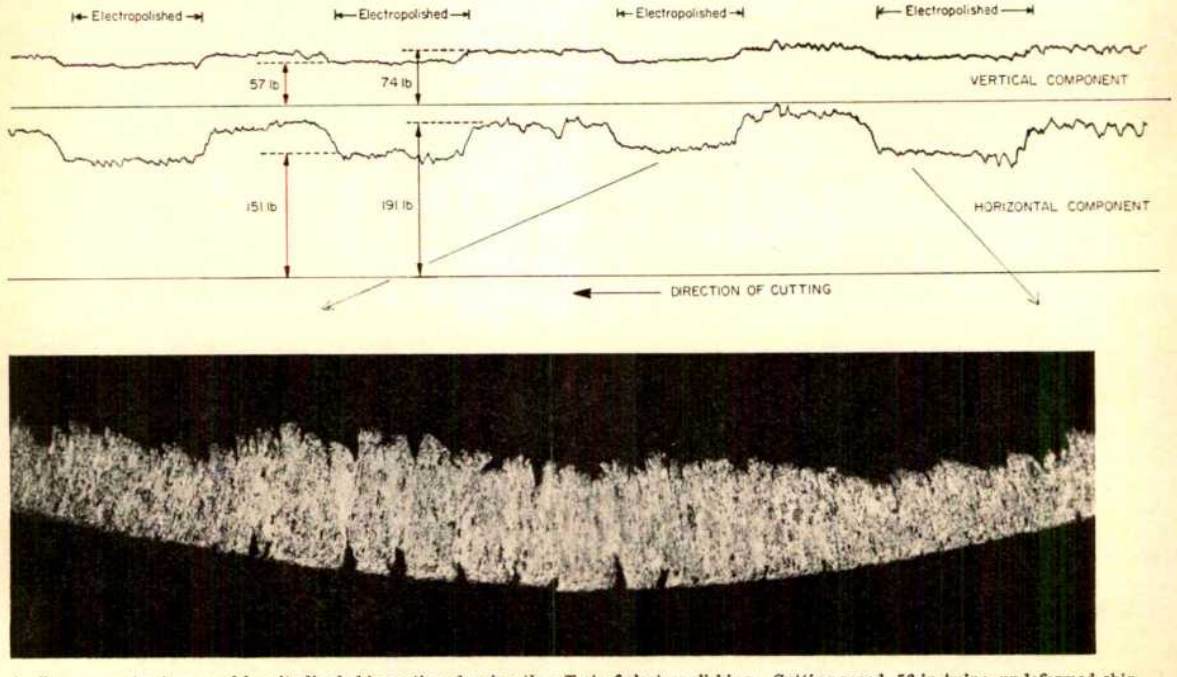


Fig. 2. Dynamometer trace and longitudinal chip section showing the effect of electropolishing. Cutting speed, 56 in./min; undeformed chip thickness, 0·004 in.; rake angle, 15°; metal, 99·3 per cent copper.

partial barrier to the egress of dislocations, removal of the surface would lower this barrier and accelerate the flow of dislocations from the crystal; in this way the rate of strain hardening of the metal undergoing deformation would be reduced.

In this investigation, because at the most only a few monolayers of carbon tetrachloride can be present on the surface at the time of deformation, solution of the reaction product is ruled out. The surface-mechanical interaction cannot be the result of localized surface removal. The emphasis has therefore been transferred from surface removal to the chemical reaction itself. It is proposed that the barrier to dislocation flow out of the metal results from the resistance to fracture of the interatomic bonds linking the atoms which constitute the surface. Passage of a dislocation out of the metal to form a slip step involves the breaking of many strong bonds and leaves the atoms forming the slip step in a high energy condition with an excess of electron charge. The presence on the crystal surface of electronegative species, which could by reaction during slip step formation take up the excess charge, must reduce the resistance of the intermetallic bonds to fracture and facilitate slip step formation. On this basis the reaction of carbon tetrachloride will accelerate dislocation flow from the metal and reduce the rate of strain hardening in the shear zone. It can be shown that a reduction of the strain hardening rate will result in the higher shear angle and reduce cutting force; these are, in fact, observed.

It appears that the surface-mechanical interaction of fluids such as carbon tetrachloride which occurs during the cutting process is basically the same phenomenon as that observed originally by Rehbinder during tensile tests with metal single crystals, and can therefore be classified as a Rehbinder effect. Further evidence has shown, however, that this Rehbinder effect does not occur in metal cutting when the whole of the cutting zone is flooded with lubricant and suggests, paradoxically, that Rehbinder, who used flood lubrication in his metal-cutting studies, could not have observed the effect in metal cutting which bears his name.

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Fatigue Crack Propagation in Metals

A PARAMETER often used for correlating the propagation rate of fatigue cracks is the stress intensity factor K. This follows the work of Irwin[1], who showed that the elastic stresses at a point near the root of a crack always have the form

$$\sigma = \frac{K}{\sqrt{2r}} f(\theta) \quad (f(\theta) \leqslant 1)$$

where r and θ are polar co-ordinates, with the origin at the crack tip, of the point. The expression is only valid if $r \ll a$ (the crack half length).

This report provides K values for crack propagation rates of 10^{-5} and 10^{-4} in. per cycle obtained from fatigue experiments on various metals—the object being to try to correlate the K values for fixed crack propagation rates with the tensile properties of the metals. Most of the experiments were made on test pieces of $\frac{1}{2}$ in. × 1 in. cross-section tested in bending, the K values being calculated from the measured bending moments using the boundary collocation calculations of Gross and Srawley[2]. Table 1 gives the K values and includes some values obtained from tension experiments on panels 3 in. wide. (Experiments on specimens cut from the same sheet of *D.T.D.*5070 aluminium alloy gave very good agreement when tested in either tension or in bending.) The loading in the fatigue experiments was of the form $1{\cdot}1\ P \pm P$ (stress at the crack tip always tensile) and the K values were calculated from the alternating load (P) rather than the peak load ($2{\cdot}1\ P$) which is sometimes used. Table 1 also gives the tensile properties of the metals and values of K/E, where E is Young's modulus. It will be seen that the K values for the two propagation rates do not correlate with the tensile properties of the materials but that the values of K/E are reasonably constant. When log K/E is plotted against log da/dn a straight line of slope 3·6 is obtained and over the range investigated ($da/dn = 10^{-6} - 10^{-4}$ in./cycle) a reasonable approximation to the fatigue crack propagation rate for all the metals is given by the equation

$$\frac{da}{dn} = 3{\cdot}43 \times 10^{7} \left(\frac{K}{E}\right)^{3{\cdot}6} \qquad \begin{pmatrix} K \text{ in lb./in.}^2 \times \sqrt{\text{in.}} \\ E \text{ in lb./in.}^2 \end{pmatrix}$$

This suggests that the main factor governing the propagation rate of fatigue cracks is the elastic strain concentration at the tip of the crack and not the flow properties of the metals as might be expected. It should be noted that the experimental values were obtained from tests made in laboratory air so that it is possible that in some cases the low values of K/E resulted from the rate of crack propagation being accelerated by corrosion fatigue.

Another point to be considered is the effect of frequency on fatigue crack propagation. It has been shown by Schijve, Broek and de Rijk[3] and by Bradshaw and Wheeler[4] that for aluminium alloys a change of frequency of 100 to 1 produces a change in the propagation rate of fatigue cracks of about 1 to 2. This is probably a corrosion fatigue effect because for tests on aluminium alloy *in vacuo*[4] the same change in frequency produced a much smaller change in the rate of crack propagation. It seems, therefore, that in the absence of corrosion the propagation rate of fatigue cracks is not influenced to any great extent by the time during which the stress is applied.

From fractographic studies of fatigued surfaces it has been shown by Forsyth and Ryder[5] and by Schijve[6] that at high propagation rates ($> 10^{-3}$ in./cycle) the crack advances every stress cycle, and recent work by Hertzberg[7] indicates that this relation holds for crack propagation rates as low as 10^{-6} in./cycle. It seems therefore that once the cracking process has started it repeats itself every cycle.

The implications of these considerations are that for the propagation of fatigue cracks a metal can be regarded as a continuum in which the rate at which the crack advances is governed by the geometry of the system and the distribution of elastic strain near the crack tip. It is well known that a plastic zone forms ahead of the crack during fatigue cycling before failure occurs, so plastic flow would have to be taken into account in any mathematical treatment of the problem.

A possible qualitative mechanism by which the crack grows will now be suggested. Once a crack has formed, probably by a dislocation mechanism, the highly strained material at the end of the crack flows to form a plastic zone in front of the crack. Eventually, owing to restraint by the surrounding material a condition of triaxial tension is reached in a small region at the crack tip. In this region the shear stress is less than that required to cause flow and a large elastic tensile strain can occur. The material fails in tension and the crack advances until the strain is relieved by plastic flow in the surrounding material. Reverse flow occurs in the plastic zone as the stress is removed and during the next cycle the condition of triaxial tension again occurs, the material fails and the crack advances. It seems probable that this process can

Table 1. FATIGUE CRACK PROPAGATION EXPERIMENTS ON VARIOUS METALS

Test method	Magnesium alloy *L*128 Bending	Aluminium alloys *L*65 Bending	2024/T3 Bending	*D.T.D.* 5070 Bending and tension	Naval brass *B.S.S.* 251 Bending	Titanium alloy 811 Tension	Ferritic steel *E.N.* 24 Bending	Austenitic stainless steel *E.N.* 58 *B* Bending
Material thickness (in.)	0·5	0·5	0·16	0·14	0·5	0·0625	0·5	0·5
				K, lb./in.^{3} × $\sqrt{}$in.				
$da/dn = 10^{-5}$ in./cycle	2,200	4,200	3,500	4,300	4,300	5,600	11,400	9,000
$da/dn = 10^{-4}$ in./cycle	3,500	7,600	6,000	7,700	7,400	9,600	23,000	17,000
				$K/E \times 10^{4}\sqrt{}$in.				
$da/dn = 10^{-5}$ in./cycle	3·4	4·0	3·5	4·1	3·8	3·1	3·8	3·0
$da/dn = 10^{-4}$ in./cycle	5·4	7·2	6·0	7·4	6·5	5·3	7·6	5·7
				Tensile properties of metals lb./in.^{2}				
Limit of proportionality	9,000	40,000	—	—	10,500	45,000	—	—
0·1 per cent proof stress	19,000	60,000	54,000	51,000	29,000	148,000	—	—
0·2 per cent proof stress	21,000	64,000	55,000	58,000	30,000	150,000	117,000	51,500
Ultimate tensile stress	30,500	70,000	70,000	65,000	57,500	160,000	145,000	96,000
Young's modulus. $E \times 10^{-6}$	6·5	10·5	10·0	10·5	11·5	18	30	30
Elongation (per cent)	4	11	16	6·5	42	13	16	45

occur only if the thickness of the material is appreciably greater than the plastic zone size, a condition which always applied in the experiments reported here.

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A System of Nomenclature for the Fibrous Calcium Silicates, and a Study of Xonotlite Polytypes

FIBROUS calcium silicates occur as polytypes because each $(Si_3O_9)_\infty$ or $(Si_6O_{17})_\infty$ chain can occupy alternative positions differing by $b/2$ relative to sheets of calcium atoms[1–7]. In an attempt to limit the number of "trivial" names, the International Mineralogical Association's Commission on Mineral Names[8] has recommended the use of suffixes such as wollastonite-Tc and -2M, but cases with several variable parameters were not adequately covered. This communication describes a comprehensive system of nomenclature, which uses suffixes derived from the reciprocal lattice. This system could be extended to cover polytypes in other inorganic phases.

Diffraction patterns from polytypes of any species of calcium silicate have certain reflexions in common, which indicate a "pseudo-cell" corresponding to the repeat unit for the calcium and most of the oxygen atoms. Weaker, often streaky reflexions are also present, associated with the positions of the silicate chains. A smaller reciprocal cell in accord with the weak reflexions can be defined on the same axes as the reciprocal pseudo-cell, as in Fig. 1. A suffix $LUVW$ is then allocated to define the polytype, so that Ua^* = pseudo-a^*, Vb^* = pseudo-b^* (V is almost invariably 2), and Wc^* = pseudo-c^*; U, V and W are also the ratios between the true and pseudo-repeat distances along the pseudo-cell axes in the real lattice. The prefix L represents the Bravais lattice type (P, C, A, F or I), and indicates translational relationships between adjacent pseudo-cells. The usual conventions for selecting the true unit cell are ignored, as they would obscure the relations between polytypes, but a more conventional unit cell may be selected later if required. On the proposed system, wollastonite-Tc and -2M would be renamed wollastonite-C221 and -P121, respectively, and the two known polytypes of foshagite, $Ca_4Si_3O_9(OH)_2$ (ref. 7), would bear the suffixes A122 and F222.

Xonotlite, $Ca_6Si_6O_{17}(OH)_2$, is a typical fibrous calcium silicate, comprising triple rows of calcium atoms, double silicate chains, and hydroxyl ions[2,3]. Consideration of the structure suggests the possibility of four regular polytypes, xonotlite-P121, -C221, -A122 and -F222, and two others, -P∞21 and -A∞22, with complete disorder within (001) planes. Reciprocal lattices for these polytypes are shown in Fig. 1. Earlier I used electron diffraction to identify xonotlite-A122 in a specimen from San Francisco[9]. A specimen stage for the electron microscope permitting tilt through angles up to 55° about any selected axis has recently been used to investigate polytypes in samples from four other localities, in Newfoundland, Puerto Rico, Pakistan and Aberdeenshire, Scotland. Six synthetic specimens have also been examined; they were prepared hydrothermally at temperatures of 165, 185, 200° C (ref. 10), and 350° C (Dr. K. Speakman), for times between 25 and 120 h. The Aberdeenshire specimen comprised A122, F222, A∞22 and P∞21 polytypes, and intergrowths with values of 1 and 2, or 2 and 4, for W. All the other natural crystals were A122 or A∞22 types. Synthetic specimens prepared at 165° and 185° C were all A∞22, but the higher preparation temperatures favoured intergrowths of A∞22 with P121 or P∞21. Reflexions with $W = 1$ were stronger than for $W = 2$ in the 350° C specimen.

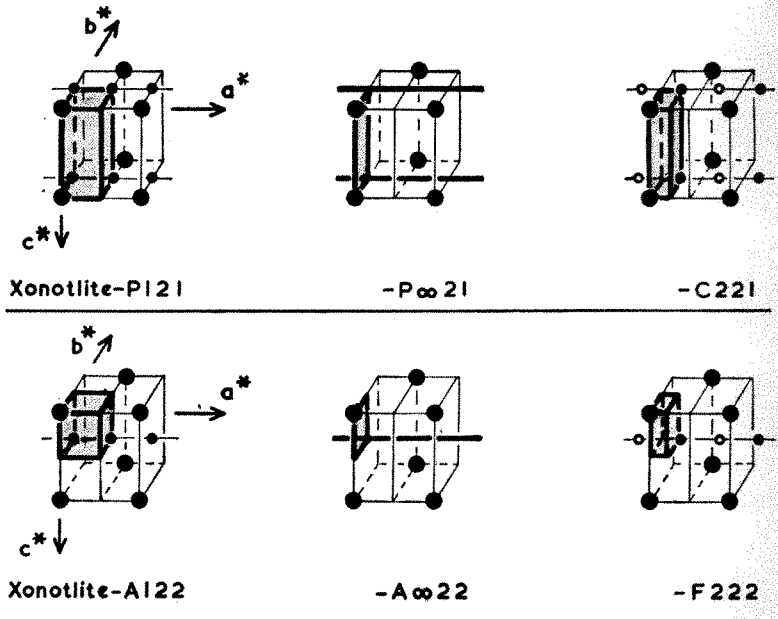


Fig. 1. Reciprocal lattices for polytypes of xonotlite, with their symbols on the proposed system of nomenclature. For the polytypes in the upper half, c is not doubled, that is, $W = 1$, while for those in the lower half, c is doubled, and $W = 2$. Reciprocal pseudo-cells are outlined in thin lines; the true reciprocal cells defined on the same axes are outlined in thick lines and shaded. For polytypes C221 and F222, small closed and open circles represent reflexions for the twinned forms.

Completely ordered polytypes were observed in natural crystals only for $W = 2$, and in synthetic crystals only for $W = 1$. The temperatures of formation of the natural specimens are not known, but the Aberdeenshire specimen, which contained P∞21 polytype, occurred in a metamorphic region, with considerable evidence of high-temperature products. The results therefore suggest the possibility of a low-temperature xonotlite with $W = 2$, and a high-temperature form with $W = 1$. Consideration of the central row of calcium atoms, in each triple row parallel to b, shows that each calcium has one hydroxyl ion co-ordinated where $W = 2$ while two and no hydroxyls alternate where $W = 1$. Taylor and I have shown[7] that similar rows of calcium atoms in foshagite have equal co-ordination of hydroxyl ions, possibly because of forces owing to the relative polarizations of Ca—OH and Ca—OSi bonds. The present investigation suggests that such forces may be dominant at lower, but not at higher, temperatures of formation for xonotlite. In order to test this hypothesis, samples of xonotlite will be prepared with very long reaction times at various temperatures, and data on the natural localities will be examined for evidence of formation temperatures.

I thank Prof. H. F. W. Taylor, Dr. A. Aitken, Dr. K. Speakman, and Dr. H. G. Macpherson for the specimens of natural and synthetic xonotlite, and for their interest in this work.

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PHYSICS

Piezoelectric Effect in Poly-γ-methyl-L-glutamate

THIS communication presents some experimental evidence illustrating the piezoelectric effect in certain synthetic polypeptides.

It has been recently established that a number of high polymeric substances show a piezoelectric effect[1,2]. The substances so far investigated have been naturally occurring polymers such as cellulose, keratin, silk fibroin and collagen. Actual specimens used in the study have been taken from wood[3-5], wool[6], silk[7], bone[8-10] and tendon[11].

The effect can be accounted for by taking into consideration the crystal symmetry and unidirectional orientation of crystallites of such polymers. It seems likely that the origin of piezoelectricity in cellulose and protein results from the abundance of hydrogen bonds in their crystal structure. Until now, the piezoelectric effect in synthetic polypeptides—in which the hydrogen bond plays an essential part in determining its molecular configuration and crystal structure—has not been investigated.

Thin films of poly-γ-methyl-L-glutamate were kindly provided by the Ajinomoto Chemical Co., Tokyo. The film was stretched to twice its original length, and this resulted in a thickness of about 0·025 mm. The electrodes, each with a diameter of 10 mm, were fixed on either side of circular films of 20 mm diameter by painting them with silver paste. One end of the film was clamped to a small metal bar connected to the centre of an electromagnetic vibrator and the other to a frame which could be adjusted so as to give an appropriate tension to the film. Through two thin, flexible silver foils attached to the electrodes, the electric signal was led to a differential amplifier and then to a synchroscope.

If a longitudinal vibration was applied to the film by the electromagnetic transducer, an electrical polarization with the same frequency as that of the mechanical vibration was produced on the faces of the film and could be presented on the vertical axis of the synchroscope. If a voltage proportional to the coil current in the electromagnetic transducer was applied to the y axis of the synchroscope, a Lissajous figure, showing the relationship between stress and polarization of the specimen, was observed. During measurement, the circular film was rotated to various angles to the oriented direction so that the stress could be applied in different directions.

The apparent piezoelectric modulus d, which is the coefficient connecting the electrical polarization and mechanical stress, is plotted in Fig. 1 against the angle θ between the direction of stress and the orientation axis. The points are the average of experimental results for two films. The curve is well represented by a formula $d = d_{max} \sin 2\theta$, as would be expected for such unidirectionally oriented substances. The absolute value of the piezoelectric modulus d_{14} for these films of poly-γ-methyl-L-glutamate is $2{\cdot}8 \times 10^{-8}$ C.G.S.E.S.U., which is about half the d_{11} coefficient of quartz crystal.

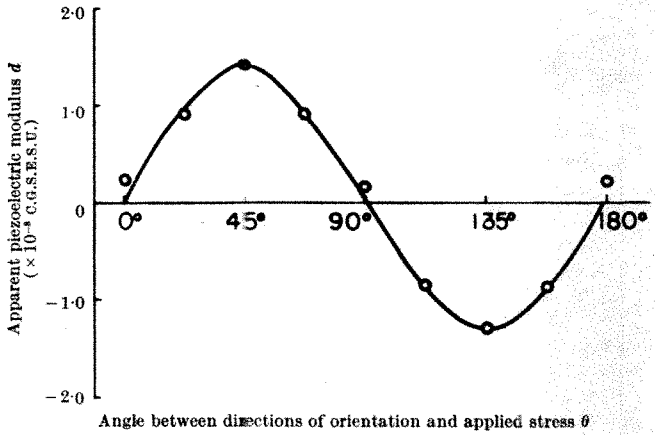


Fig. 1.

It is significant that the value of piezoelectric modulus is fairly large. This substance does not absorb water because methylation of the acidic group occurs. The molecular conformation is the α-helix form. The degree of polymerization is 3,200. The degree of crystallinity is unknown, but would be higher than 30 ~ 40 per cent, as roughly estimated by X-ray diffraction patterns. It is believed that many synthetic polypeptides other than poly-γ-methyl-L-glutamate will also show a similar piezoelectric effect and that such studies might provide fundamental clues toward understanding the piezoelectric effect observed in a number of biological substances such as hair, bone and tendon.

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Potentials of Metallic Powder Formation on the Mercury Dropping Electrode

THE formation of electrolytic metallic powders on solid electrodes at a certain potential in the region of the limiting current was first observed with copper, gold and cadmium[1,2]. However, the conditions of electrodeposition on the solid electrodes make it impossible to determine precisely the corresponding potentials[3] and to construct a series of potentials of metallic powder formation.

Using the phenomenon of metallic dendrite formation on the dropping mercury electrode[4], it is possible to determine the potential of the powder formation with a relatively high precision (±25 mV) by microscopic observation. In the case of cadmium, for example, the powder begins to form at a potential more negative than −0·9 V (Fig. 1). At the same time the concentration of the metal ion must be greater than a certain minimum value (0·0125 M for Cd^{2+}). In this way we determined the series of potentials of formation (ε_p) of various metallic powders on the mercury dropping electrode (Fig. 2). Fig. 2 shows that these potentials are independent of the concentration and that the minimum value is in general about 0·01 M. Experimentally it was found that the formation of powders is favoured by a certain value of the rate of growth of the surface of the drop. In the case of cadmium, in a range of concentration of 0·1–0·4 M, the metallic dendrites are formed when a fast capillary (t_1 = 3 sec) is used, while in the range of 0·025–0·05 M it is necessary to use a slow capillary (t_1 = 75 sec). The presence of gelatine favours the dendrite formation by diminishing the threshold of the concentration. The experimental observation shows also that the dendrites are not in close contact with the mercury; immediately after direct contact with mercury is established, the particles are rapidly dissolved in the mass of the drop and often (in the case of the cadmium, for example) an intense hydrogen discharge is observed on the non-dissolved dendrite portions.

These experimental results are in good agreement with the quantum mechanical mechanism suggested for the formation of electrolytic metallic powder[1,3] and make possible a more complete explanation of the phenomenon.

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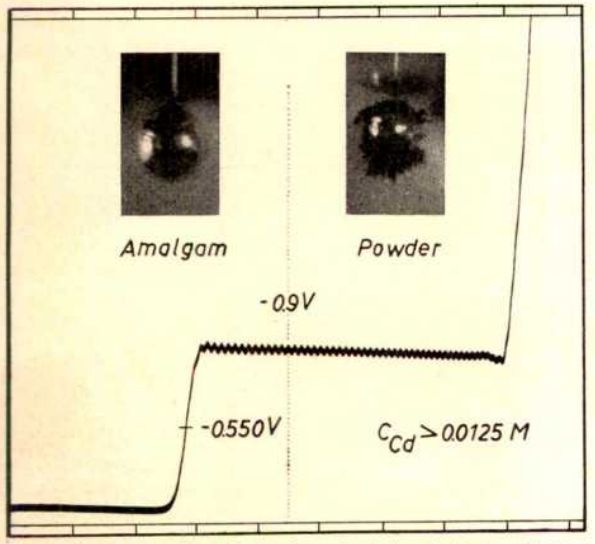


Fig. 1. Amalgam and powder regions of cadmium (cadmium chloride) discharge on dropping mercury electrode in 2 M potassium nitrate and 0·05 per cent gelatine solution. Beginning at 0 V, −200 mV/absc.

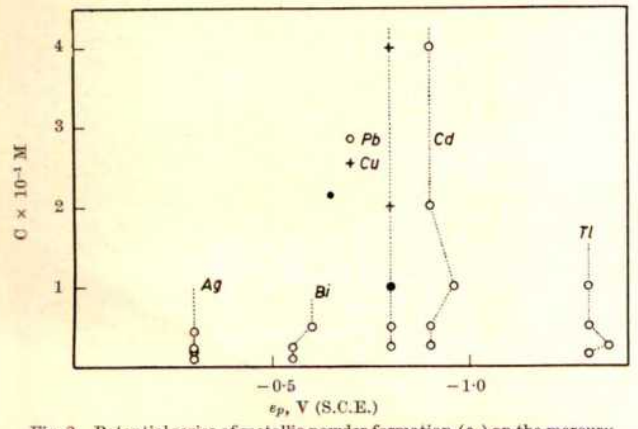


Fig. 2. Potential series of metallic powder formation (ε_p) on the mercury dropping electrode as a function of metal concentration. Silver nitrate in 3 M sulphuric acid, bismuth nitrate in 4 M hydrochloric acid and lead nitrate, cupric chloride, cadmium chloride and thallium nitrate in 2 M potassium nitrate base solution, 0·05 per cent gelatine.

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Nucleation of the Solid Phase by Cavitation in an Undercooled Liquid which expands on Freezing

HICKLING[1] has proposed that nucleation by cavitation in an undercooled liquid which expands on freezing is caused by adiabatic compression into a region where a high pressure phase is stable: the high pressure phase nucleates and later transforms to the stable low pressure phase. Originally, his arguments were intended to explain only the results on water, but were later extended to include other materials which expand on freezing[2], although no such observations were available.

In recent experiments we have found that gallium, which expands on freezing, behaves like water, benzene, nickel, etc., in that the solid phase can be nucleated

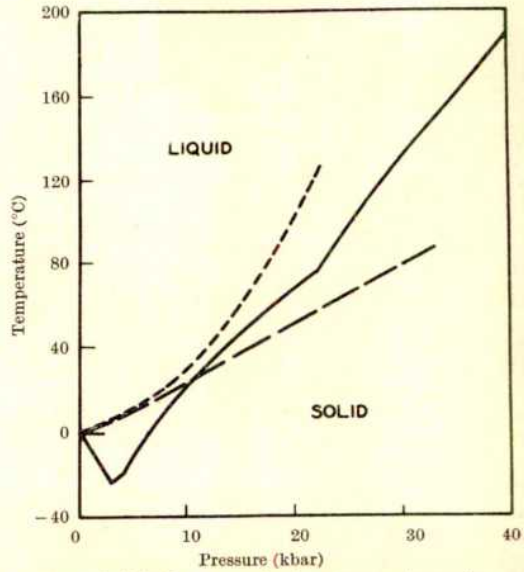


Fig. 1. Lines of adiabatic compression in water superimposed on a plot of the equilibrium melting line of ice. — —, Reversible adiabatic compression. - - -, Shock-wave adiabatic compression.

in the undercooled liquid by cavitation. A 2 c.c. sample of gallium contained in a sealed glass tube could be undercooled 25° (to 5°C) but nucleated in an ultrasound field with a few degrees undercooling. We believe that this is the first observation of such nucleation in a material other than water which expands on freezing.

In calculating the path taken during the collapse of a cavity, Hickling has considered only the temperature rise which results from reversible adiabatic compression. The collapse of a cavity is not reversible in the thermodynamic sense. The conditions should more closely approximate those in a shock front, as specified by the Rankine-Hugoniot[3] relationships. Using these equations we have calculated the irreversible part of the heating under such conditions[4]. This gives the temperature rise as indicated by the dotted line in Fig. 1. It is evident that the irreversible heating is sufficient so that the high pressure solid phase will not nucleate, because the liquid phase is stable over the entire path. The mechanism suggested by Hickling cannot, therefore, be operative in water. The nucleation must occur while the system is locally under tension, as has been suggested by Hunt and Jackson[4].

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Effect of Precipitation on Transmission through the Atmosphere at 10 Microns

THE development of high power maser sources at 10·6 microns[1] has made possible an investigation of the characteristics of propagation through the atmosphere at this wavelength. Our investigations were conducted over the same 2·6 km path in Holmdel, New Jersey, which was used at shorter wavelengths by T. S. Chu and D. C. Hogg[2].

A continuous gas-flow 10·6μ oscillator, provided by T. J. Bridges[3] and operated to produce 0·5 W, was used as the source in our experiments. Observations were made continuously during most of the period from December 1965 to the end of May 1966. The 7 mm diameter output beam of the maser was directly transmitted during most of the work, although a 6 in. diameter Cassegrainian telescope was also used. The receiver was a thermopile placed at the focus of a 6 in. mirror. Mechanical chopping of the transmitted beam at 10 c/s and synchronous detection yielded a measuring range between 50 and 70 dB depending on the configuration used.

Fogs. During the past North American winter on ten occasions—each lasting several hours—we observed attenuation, due to fog, larger than our measuring range. On one such occasion we observed a fade of 50 dB which lasted a few minutes and which we were able to correlate with the movement into the beam of a patch of fog which intercepted less than half the 2·6 km path when the attenuation was maximum.

Snow. Because theoretical predictions of snow attenuation at different wavelengths have not so far been possible, it was of particular interest to observe the wavelength dependence of this effect. On six occasions during the past winter, appreciable snowfalls occurred and in each case there were periods, typically several hours long, during which the attenuation exceeded 50 dB. On one such occasion, simultaneous measurements were made by T. S. Chu at 0·63μ and 3·5μ. A comparison of the data indicates that the attenuation in decibels at 10μ was about 50 per cent greater than at the shorter wavelengths.

Rain. Most of the appreciable rainfalls during this period were accompanied by light fogs which produced the dominant attenuation and, therefore, obscured the effects resulting from the raindrops. On three occasions, however, rainfalls essentially free of fog were recorded. The attenuations observed were all between 8 and 10 dB/mile/in./h precipitation rate. (The rates measured ranged up to 2 in./h.) This result can be compared with the theoretical prediction of 11·5 (in the same units) by Chu[4]. Because this theory does not take into account the fact that some of the forward scattered signal is collected by the receiver, we consider the agreement between theory and experiment to be quite good.

We thank T. J. Bridges and T. S. Chu for their co-operation in this experiment.

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Variable Focal Length Lenses using Materials with Intensity Dependent Refractive Indices

RECENT theory[1–3] and experiments[4–7] have demonstrated the existence of an intensity dependent non-linear component in the refractive index of some materials. This non-linearity in the refractive index has been observed to rotate an elliptically polarized optical maser beam[4,5] and to lead to self trapping[6,7]. This communication considers the two step nature of focusing which leads to the conclusion that a sample tank filled with one of these non-linear materials can be made to behave like a lens. It will be shown that it is possible for beam trapping to occur within the non-linear medium but for the beam to come to a focus outside the medium tank (see Fig. 1).

Consider, for example, a diffraction limited beam of the form

$$E^2 = \frac{8\pi^2 a^2 \eta_0}{c\lambda^2 R^2} P_t \left\{\frac{2J_1(ka\beta)}{ka\beta}\right\}^2 \qquad (1)$$

where a is the radius of the aperture, λ is the free space wavelength of optical maser, η_0 the refractive index, c the velocity of light, R the distance from aperture, P_t the total beam power, $J_1(ka\beta)$ the Bessel function, and $\beta = \sin\theta$. If the non-linear refractive index is of the form

$$\eta_1 = \eta_0 + \eta_2 E^2 \qquad (2)$$

then the non-linear portion becomes

$$\eta_2 E^2 = \frac{8\pi^2 a^2 \eta_0 \eta_2}{c\lambda^2 R^2} P_t \left\{\frac{2J_1(ka\beta)}{ka\beta}\right\}^2 \qquad (3)$$

From equations (2) and (3) it is obvious that the refractive index is not uniform across the beam owing to the power distribution in the beam. This means that the centre of the beam will be slowed more than the edges as shown in Fig. 1. The net result of this non-uniform phase delay across the beam is identical to what occurs in a lens as a result of the increased path length in the centre of the lens. If the same considerations are used here as in the case of thick lenses it is possible to determine an effective focal length for our non-linear system.

When the phase delay between the outer edge and the centre of the beam is computed for passage of the beam a distance ΔR in the tank, the effective difference in path

length between the centre and the extremal rays is shown to be

$$\Delta l = \frac{8\pi^2 a^2 \eta_2}{c\lambda^2} P_t \left(\frac{\Delta R}{R_0 (R_0 - \Delta R)} \right) \quad (4)$$

Substituting for Δl using the Sagitta equation

$$\Delta l = \frac{a^2}{2R_0}$$

the distance in the sample tank at which self-trapping occurs (that is, the beam is rendered parallel) is seen to be

$$\Delta R = (1{\cdot}64)\eta_0 a^2 \left[\lambda + \frac{16\pi^2 \eta_2}{c\lambda} P_t \right]^{-1} \approx \frac{0{\cdot}103\, \eta_0\, c\lambda\, a^2}{\pi^2 \eta_2 P_t} \quad (5)$$

Thus equation (5) gives the minimum path distance necessary to render the diverging diffraction limited beam parallel. If the path length in the tank is further increased, the beam will necessarily converge and focus outside the tank. As the tank length is increased the focal point moves toward the tank-air interface and for a distance in the medium beyond ΔR of R_f given by

$$\delta R = R_f = \frac{a^2 \eta_0}{3{\cdot}8} \sqrt{\frac{c\eta_0^2}{\eta_2 P_t}} = a^2 K \quad (6)$$

(which is very nearly what Kelley[3] obtained) the beam will focus in the tank. Thus it is seen that it is possible for the beam to focus outside the tank for a tank length l between

$$\Delta R < l < \Delta R + R_f \quad (7)$$

With these ideas in mind it is now possible to determine the relationship between the focal length of the non-linear system and the tank length l. Because

$$\delta R = l - \Delta R \quad (8)$$

it is obvious that the radius of the beam at the air-tank interface is given by the relationship

$$\rho^2 = \frac{a^2 K - \delta R}{K} \quad (9)$$

Furthermore, the path difference between the extremal and the central ray of the beam is

$$\Delta l = \frac{4{\cdot}9\pi^2 \eta_0 \eta_2}{\lambda c} P_t \left\{ 1{\cdot}64 \frac{a^2}{\lambda \delta R} + 1 \right\}^{-1} \quad (10)$$

Using the Sagitta equation again, the focal length is found to be given by the relationship

$$f' = \frac{\rho^2}{2\Delta l} \quad (11)$$

This must, however, be modified because there is a change of index of refraction at the tank-air interface. The focal length of the system thus becomes

$$f = \frac{f'}{\eta_0} = \frac{a^2 K - \delta R}{2\eta_0 K \Delta l} \approx \frac{a^2 K - l}{2\eta_0 K \Delta l} \quad (12)$$

the approximation being valid as $\Delta R \approx 0{\cdot}05\, R_f$.

A theoretical focal distance has been calculated for a 10 cm length tank l filled with carbon disulphide, an input beam power P_t of 100 kW and an aperture diameter of 0·5 mm. From ref. 5, η_2 is found to be $3{\cdot}1 \times 10^{-11}$ E.S.U. at a λ of 6943 Å. From equation (12), the focal length f is thus found to be 18·5 cm.

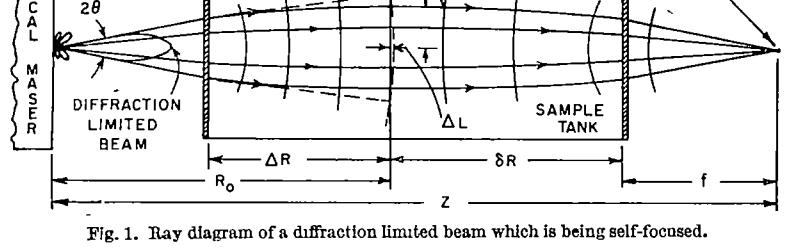


Fig. 1. Ray diagram of a diffraction limited beam which is being self-focused.

It should be pointed out at this time that in this crude analysis only the centre and extreme rays have been considered in arriving at the focal distance of equation (12). For a complete analysis all intermediate rays should be considered, which would yield the spherical aberration of the system. It will turn out that the spherical aberration is dependent on the beam intensity profile and would be different for a Gaussian distribution than for an aperture limited distribution. Thus it is that this lens in general has a tendency for spherical aberrations; however, it should be possible to select a beam profile such that no aberration occurs. It should also be noted that the focal point of equation (12) is dependent on both power and frequency owing to the dependence of f on P_t, λ and $\eta_2(\omega)$.

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CHEMISTRY

New Evidence for the Fine Structure of Porous Carbons

Most speculations about the detailed pore structure of carbons have been unsupported by direct experimental evidence. The classification in terms of micropores, transitional pores and macropores was developed mainly by Dubinin *et al.*[1] on the basis of adsorption and mercury penetration investigations. These methods, however, give no information about the detailed pore geometry and only limited information about the statistical character of the structure. Molecular sieve investigations[2] have led other workers to the conclusion that the pores are often slot-like. Wolff[3] and Mizushima[4] have proposed more specific models in which active carbons are pictured as aggregates of graphite-like plates which impose a slot-like character on the spaces between them.

We have obtained evidence, based on a combination of adsorption and electron microscopic investigations, which enables us to propose a more complete structure for carbons produced by the decomposition of powdered or pelletted polyvinylidene chloride (PVDC): the same model is thought to be applicable to carbons formed by the decomposition of hexaiodobenzene, coconut shell and anthracite coal.

When the initial stages of the decomposition of PVDC occur below the softening point of the polymer (~180° C) the morphology of the original polymer sets the pattern for the structure of the carbon. Once about 10 per cent of the decomposition has taken place at the lower temperature, the nature of the final carbon is insensitive to the conditions under which the reaction is completed. In the present work, carbons ("low-temperature" chars) were prepared by carrying out about 50 per cent of the decomposition below 180° C, and completing decomposition

(100 ± 0·2 per cent) by raising the temperature to 750° C.

Adsorption of benzene at 25° C by low temperature PVDC chars is accompanied by a hysteresis loop extending from the saturation vapour pressure down to a relative pressure of about 0·25 (Fig. 1*a*, *d*). When samples of such chars are finely ground (to pass 400 B.S.S. sieve size) the powder retains its high adsorptive capacity, but the hysteresis loop is largely and sometimes completely eliminated (Fig. 1*a*, *d*): similar results (though not always leading to complete elimination of hysteresis) have been obtained with carbon prepared from hexaiodobenzene decomposed below its melting point (Fig. 1*b*), with coconut shell charcoals (Fig. 1*c*), and with anthracite coal charcoals. Soft coal charcoals, however, still retain a hysteresis loop after grinding.

Electron microscopic examination of finely ground low temperature PVDC chars shows that they consist very largely of thin platelets (200–500 Å thick) of carbon the porosity of which is too fine to be observed. These observations are consistent with the view that the carbon platelets have been formed from polymer crystallites. A detailed investigation of ultrasonically dispersed samples of the PVDC powder from which our carbons have been prepared shows that they are highly crystalline. Although a variety of habits have been observed, typical crystals are of the order of 100 Å thick and occur as sheets, or more often as ribbons (possibly formed by rupture of the sheets in preparing the samples for electron microscope investigation) 2–3μ wide and 30μ or more long. The carbon particles formed by pyrolysis are usually thicker than the single polymer crystals: multi-layer crystals in the original polymer, which are broken down by ultrasonic dispersion, are probably bonded together during the cross-linking stage of the decomposition.

The major part of the adsorptive capacity of the final carbon is associated with the very fine and extensive micropore structure of the pseudomorphs of the original polymer crystals. These pores are formed by the loss of hydrogen chloride from the crystals: on the basis of the folded chain picture of polymer crystals[5], one would expect

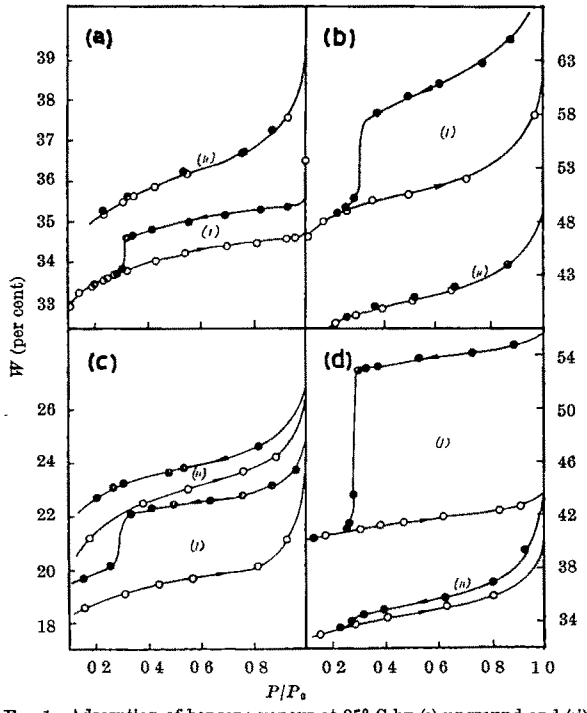


Fig. 1. Adsorption of benzene vapour at 25° C by (i) unground and (ii) finely ground samples of. *a*, carbon from decomposition of PVDC powder (sample *B*9-1077 from Dow Chemical Company) pelletted at 75,000 lb./in²; *b*, carbon from hexaiodobenzene decomposed below its melting point; *c*, activated coconut shell charcoal; *d*, carbon from decomposition of PVDC powder (sample *EP*-961-10-9 from Dow Chemical Company) unpelletted ○, Adsorption; ●, desorption.

these pores, generally, to be oriented normally to the crystal surface. They are of molecular dimensions, and consideration of the stereochemistry of the cross-linking reactions which are needed to complete the decomposition makes it plausible that they should have a slot-like character: this structure occurs within the platelets. The stacking together of these porous crystal pseudomorphs forms a second structure of voids with which the capillary condensation hysteresis is associated. The elimination of hysteresis by breaking down the void structure by grinding is essentially the converse of the experiments of Carman and Raal[6], who were able to create void structures and so to induce hysteresis of this kind by pelletting fine non-porous silica and carbon black powders. The similarity in the response to grinding of hexaiodobenzene carbons, coconut shell charcoal, and anthracite charcoals suggests that these materials have a structure similar to that already described: work to confirm this is in hand. In each case the nature of the starting material and its observed behaviour during carbonization make it probable that the basic crystal structure is retained in the carbonization process, although the random nature of cross-linking reactions destroys the regular internal structure so that the carbons show no clearly distinguishable X-ray patterns.

Carbons formed by the decomposition of PVDC above its softening point, of hexaiodobenzene above its melting point, or of PVDC in solution, exhibit small or vanishing hysteresis loops in benzene adsorption and electron micrographs show them to have a much more uniform dispersed structure apparently formed by the decomposition of amorphous material. The PVDC chars prepared by Wynne-Jones *et al.*[7] from the glassy polymer obtained by exposing liquid vinylidene chloride to sunlight show no hysteresis and presumably also have a similar structure.

These views are supported by further work on PVDC polymers the softening point of which has been raised by ultra-violet irradiation or by flash photolysis; and on PVDC/PVC copolymers with lower softening points. They are also consistent with the results of an investigation of the kinetics of the decomposition of these polymers.

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Losses due to Adsorption during Filtration of Aqueous Solutions of Polycyclic Aromatic Hydrocarbons

Although it has been pointed out that some adsorption of a solute may occur during filtrations[1] there is apparently no general awareness of the possible magnitude of such losses with dilute solutions of certain solutes. Loss by adsorption of polycyclic, aromatic hydrocarbons during filtration[2] or dialysis[3] has been mentioned briefly, and such adsorption has been shown[4] to be the cause of a major discrepancy in some recent attempts to determine the solubilities of such hydrocarbons in aqueous solutions of deoxyribonucleic acid (DNA)[3,5–7]. Stauff and Reske[8] found filtration to be unsuitable for the removal of benzo[*a*]pyrene in determinations of solubility in aqueous protein solutions; they attributed the observed 20–50 per cent error to mechanical denaturation of the protein

by the glass frit during filtration, but it is quite possible that adsorption of the hydrocarbon was also a contributing factor. Except for a brief investigation of the amount of protein adsorbed during the filtration of aqueous solutions through 'Millipore' filters[9], however, there has been no detailed report on the extent of losses due to adsorption.

In the course of some preliminary experiments on solubilization undertaken 3 years ago, I was able to trace the erratic results obtained for the solubilities of pyrene and benzo[*a*]pyrene in aqueous solutions of polyvinylpyrrolidone (PVP) or DNA to adsorption of the hydrocarbons by the filter media.

To evaluate the extent of this adsorption, experiments were carried out on solutions of phenanthrene in distilled water, because phenanthrene is sufficiently soluble in water to permit changes in concentration to be observed spectrophotometrically by means of the strong absorption band at 251 mμ. To reduce errors arising from the background absorption resulting from ultra-violet absorbing materials leached from the filters and from light-scattering by suspended particles, the base-line technique[10,11] was used for determinations of concentration. It is especially important to use this method in solubilization experiments if the solvent is a solution of a polymeric material. The solute often appears to bring about changes in the light-scattering and sedimentation properties of the polymer, with the result that the background absorption of the reference blank and the experimental solution are no longer exactly comparable. (Application of the base-line technique to the spectral curves shown in Fig. 3 of ref. 7, using readings at 385, 395 and 405 mμ, indicates that the concentrations of benzo[*a*]pyrene actually remain quite constant at about 1×10^{-6} M during centrifugation. The spectral changes shown in this figure appear to be the result of the more rapid sedimentation of the DNA in the hydrocarbon-containing solution than in the blank[3]. I have also observed a similar effect in PVP solutions of these hydrocarbons.) All spectrophotometric measurements were made on a double-beam recording spectrophotometer using matched, fused quartz absorption cells with the appropriate solvent or solution as a reference. The results obtained are shown in Table 1.

The solutions used were filtered solutions which showed no particles of undissolved phenanthrene when examined visually in ultra-violet light[12]. A membrane filter (solvent-resistant; pore size 0·45μ) was the material usually used for this preliminary filtration. No essential difference in the amount of phenanthrene adsorbed was observed when suspensions were used instead of pre-filtered solutions. Comparable results were also obtained when aqueous solutions of naphthalene, pyrene or anthracene were filtered, and the relative losses were greater with the less soluble hydrocarbons. For example, when an aqueous solution of pyrene was filtered through a sintered glass filter (fine frit) the concentration of pyrene in the first 50-ml. portion of filtrate was 44 per cent of the original concentration. Similarly, no anthracene could be detected spectrophotometrically in the final 20 ml. portion of filtrate after filtration of 270 ml. of an aqueous suspension of anthracene through 'Polypore *AM*-7'. No adsorption of hydrocarbon was observed from solutions in aqueous ethanol (20 or 50 per cent) or in other organic solvents. When solutions of pyrene, benzo[*a*]pyrene, benz[*a*]anthracene, dibenz[*a*,*h*]anthracene, or similar hydrocarbons in aqueous solutions of PVP or DNA were filtered there was a marked loss of hydrocarbon in the filtrate.

For several hydrocarbons, particularly with the membrane filters, some of the adsorbed hydrocarbon could be washed out of the filter with a small amount of water after the filter had been allowed to dry. Occasionally, supersaturated solutions were obtained in this way. Most of the adsorbed hydrocarbons could be washed out of alcohol-resistant filters with ethanol.

Such polymeric materials as PVP and DNA were also adsorbed from aqueous solution, but the filters were quickly saturated by these materials and, after the first 20–50 ml. of filtrate had been collected, further changes in concentration of the filtrate were not usually observed. In contrast to this rapid saturation, and the saturation of membrane filters by protein[9], the filters are not readily saturated by the hydrocarbons. Even after filtration of sufficient solution to give apparent saturation, refiltration through the same filter often produced a further reduction of the concentration of hydrocarbon in the filtrate.

From Table 1, it may be seen that the amount of hydrocarbon removed from solution by adsorption during filtration depends to some extent on the composition of the filter, but that the pore size of the filter also has a very marked effect, indicating the influence of changes in surface area as well as in the rate of filtration. The pronounced increase in the amount of hydrocarbon removed from solution when two filters are used instead of one (see the data obtained with 'Polypore 27*A*') is also a result of increased surface area and decreased rate of flow. The effect of changes in the rate of filtration alone may be seen particularly well in the data for 'Polypore *AM*-1' and 'Polypore *GM*-6', where a decrease in the rate of filtration resulted in a sharp decrease in the concentration of phenanthrene in the filtrate.

Munck[13] used silicone-treated glassware to reduce the adsorption of steroids from aqueous solutions; but, in the present work, similar treatment of fritted-glass filters with a silicone preparation did not produce any clear-cut effect on the amount of phenanthrene adsorbed during filtration. The reduced affinity of the treated surface for

Table 1. CHANGES IN CONCENTRATIONS OF PHENANTHRENE IN FILTRATE DURING FILTRATION OF SOLUTIONS OF PHENANTHRENE IN DISTILLED WATER

Filter medium	Pore size (μ)	Original conc. (M × 10)	Filtrate fractions* First Vol. (ml.)	First Conc. (%)†	Second Vol. (ml.)	Second Conc. (%)†	Third Vol. (ml.)	Third Conc. (%)†	Fourth Vol. (ml.)	Fourth Conc. (%)†
Glass, coarse frit	40–60	6 5	15	84	15	96	60	99		
Glass, fine frit	4–5 5	6 9	15*s*	64	15*s*	84	60*s*	96		
Paper, 'Whatman No. 1'		5 3	15	56	15	74	60	87	60	93
Paper, 'Whatman No. 2'		4·5	15	24	15	50	60	74	60	79
Membrane filter-holder‡ (sintered stainless steel)		6 8	15	54	15	87	60	96		
'Millipore *HA*'§	0 45	7 3‖	140	0	50	2	50	7	80*s*¶	42
'Millipore *OH*'§ solvent-resistant	1 5	6·9	15	1	50	14	100	26	365	41
'Polypore *AM*-1'‡	5	6·1‖	50*s***	41	50*s*††	31	50*s***	64	540*s*	79
'Polypore *AM*-3'‡	2	3·5	25	0	50	0	50	0	50*s*	17
'Polypore *AM*-5'‡	0 65	6 4	50*s*	0	50*s*	2	50*s*	6	50*s*	14
'Polypore *AM*-7'‡	0·3	7·8‖	75*s*	0	75*s*	0	75*s*	0	340*s*	0
'Polypore *GM*-6'‡ gravimetric	0 45	5 9	50*s*	28	50*s*	53	50	32	50*s*	66
'Polypore 27*A*'‡ solvent-resistant	0 45	7·5‖	15	2	50	70	50	91		
'Polypore 27*A*' two membranes superimposed	0 45	7·9‖	15	0	60	0	75	1	75	24

* Allowed to filter by gravity except where designated 's' (suction).
† Expressed as percentage of the original concentration.
‡ From Gelman Instrument Co.; 2 in. diameter (1962 designations).
§ From Millipore Filter Corp.; 47 mm diameter.
‖ A suspension, not necessarily saturated.
¶ After filtration of three 50 ml portions.
** Filtered rapidly.
†† Filtered more slowly by reducing the suction

the hydrocarbon appears to be counteracted by the effect of the reduction in the rate of filtration brought about by the hydrophobic surface of the filter.

In addition to the errors introduced by this adsorption during filtration, particles of hydrocarbon (seen as bright fluorescent specks when the filtrates are viewed in ultraviolet light) occasionally pass through paper or sintered-glass filters. The centrifugation procedure of Kofahl and Lucas[14] was found to give fairly reproducible results, even with viscous solutions of highly polymerized DNA. Two centrifugations, with 'Pyrex'-wool plugs centrifuged down on to the residue, were sufficient to ensure removal of suspended hydrocarbon. Comparison of the spectra of the resulting solutions and of the original suspensions, using the base-line technique, showed the change in concentration of the dissolved hydrocarbons to be less than 10 per cent during this procedure. By this method, considerably less variation is observed than when filtration is used to remove undissolved hydrocarbon. The loss may arise from adsorption on the centrifuge tubes and on the glass-wool plugs.

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X-ray Fluorescence Analysis of Heavy Elements in a Light Matrix

WHEN solids or liquids are irradiated with X-rays the elements in the substance emit secondary fluorescent radiation. The intensity of the fluorescent radiation from an element in a mixture is usually not proportional to the concentration of that element. The reason for this is that the heavy element exhibits an X-ray absorption markedly different from the matrix. Thus a plot of the intensity of the emitted wavelength (L line) against concentration of the heavier element in the mixture gives a curve which, as the concentration of the element increases, becomes parallel to the concentration axis (Fig. 1L).

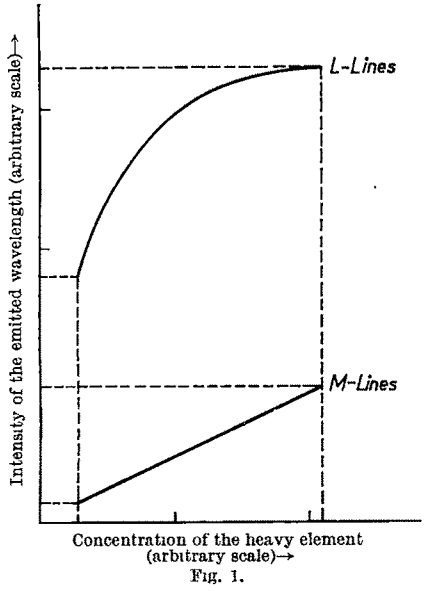


Fig. 1.

Addink, Kraay and Witmer[1] have shown that by adding quantities of strongly absorbing material, a linear relationship can be effected between the intensity and concentration; however, special considerations are required when the results so obtained are interpreted. The same effect can be accomplished by making intensity measurements at a longer wavelength when the absorption in the heavy element and matrix is more or less equal (Fig. 1M). Under these conditions, the analysis resembles the measurement of the concentration of a light element in a light matrix. Thus, for example, when the concentration of lead in silicates is determined by this technique it is the intensity of the Pb$M\alpha_{1,2}$ line which is measured instead of the usual Pb$L\alpha_1$, and when the concentration of bismuth in sodium carbonate is determined it is the Bi$M\alpha_{1,2}$ line and not the Bi$L\alpha_1$.

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Extension of the Periodic Table

IN the course of an investigation concerning the behaviour of orbital objects around a nucleus, in which the properties of various simple theoretical models were studied to determine under what conditions the exchange of radiation and/or particles obeying the "inverse square law" could account for other interaction laws (not necessarily only at the nuclear or atomic level), the following series was found:

$$\frac{n_1}{1^2} + \frac{n_2}{2^2} + \frac{n_3}{3^2} + \ldots\ldots = 2k \quad (k = 1, 2, 3, \text{etc.})$$

It arose in more than one model, and is of little significance to the more ambitious main investigation. For example, the factor "2" in the right-hand side is to be expected whenever certain conditions of symmetry are postulated: and the left-hand side occurs at once when considering the "classical" potential energy of "n_r" electrons in the "r^{th}-th Bohr orbit[1]; and also in other models (for example, in which the radius of the "r"-th orbit is proportional to "r" and "inverse square law" attractions are summed)

Table 1

k	n_1	n_2	n_3	n_4	
1	2	—	—	—	
2	2	$1\,2.2^2$	—	—	
3	2	$2.2.2^3$	—	—	etc.
4	2	$2.2\,2^2$	$1\,2.3^3$	—	
5	2	$2.2\,2^2$	$2.2\,3^2$	—	
6	2	$2\,2.2^2$	$2.2.3^2$	$1\,2\,4^2$	
		etc			

From the infinite number of solutions of this equation, however, the regular pattern of solutions given in Table 1 is highly suggestive.

In the original investigation, the "n_1", "n_2", etc., referred to the number of orbital objects in successively remote orbits from the nucleus, and the regular pattern of solutions already given tallies precisely with the gross electron structures of the noble gases[2] as shown in Table 2.

Table 2

Gas	k	First period	Second period	Third period	Fourth period	Atomic No., that is $n_1+n_2+n_3+n_4+$ etc.
Helium	1	2	—	—	—	2
Neon	2	2	8	—	—	10
Argon	3	2	16	—	—	18
Krypton	4	2	16	18	—	36
Xenon	5	2	16	36	—	54
Radon	6	2	16	36	32	86

Continuing the pattern, one can predict the gross electron structures of all the noble gases yet to be discovered. For example, the next heavier one will have an electronic structure, corresponding to $k = 7$, of:

$$2 : 2.2.2^2 : 2.2.3^2 : 2.2.4^2,$$

that is $2 + 16 + 36 + 64$ = atomic number 118;

and the next after that, corresponding to $k = 8$, will be:

$$2 : 2.2.2^2 : 2.2.3^2 : 2.2.4^2 : 1.2.5^2,$$

that is $2 + 16 + 36 + 64 + 50$ = atomic number 168.

By noting how the intervals between the noble gases are occupied in the existing Periodic Table[3], a prediction can be made in some detail of the properties of all elements up to atomic number 118, the next noble gas; and a less specific, but more dramatic, prediction of the properties of even heavier elements can be made, as follows:

The regular pattern of solutions requires, of its very nature, that the intervals between successive noble gases go in pairs of equal numbers of elements; thus, from helium to neon is eight elements, as is the interval from neon to argon; from argon to krypton is eighteen elements, as is the interval from krypton to xenon; and so on.

With each successive pair of intervals, more and more elements have to be accommodated, so while there is a one-to-one correspondence between the elements in one interval and those in the "paired" interval, there has to be a drastic change when the next interval occurs. Thus, the eight elements from helium to neon match the eight elements from neon to argon; but the interval from argon to krypton copes with its larger number of elements by dispersing them fully into *A*- and *B*- sub-groups, and by introducing a transition group of three elements (iron, cobalt and nickel). The following interval from krypton to xenon matches this plan exactly, element-for-element. However, the next interval again, from xenon to radon, has so many elements that it must adopt an even more drastic plan, and in addition to using every available place in the Periodic Table it must lump together fifteen elements in a single place (the lanthanides, atomic numbers 57–71). On this scheme, the interval from radon to the next (undiscovered) noble gas should match the interval xenon to radon exactly, element for element, so that the fifteen elements from actinium (atomic number 89) to lawrencium (atomic number 103) should occupy only one place in the Periodic Table (refs. 4, 5 and 6 illustrate early discussion along these lines), and elements 108, 109 and 110 should be transition-type elements corresponding to osmium, iridium, and platinum, and so on.

There is reason to believe that extremely heavy atoms are most unstable[7,8] so the interval from atomic number 118 to 168 may never be reached. But if it is, because this starts another pair of intervals one can predict that a dramatic device to fit the elements into the interval will necessarily appear.

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Fundamental Stretching Vibration in Liquid Water

WE have recently investigated the fundamental stretching vibration of liquid water in the infra-red region 4,000–2,000 cm^{-1} using the thin cell technique developed by Thompson[1]. The spectra, recorded under high-resolution conditions on a Perkin–Elmer 125 spectrometer, are shown in Fig. 1. It is apparent that the absorption in the 3,450 cm^{-1} region is complex and composed of three bands with frequencies of ~3,625 cm^{-1}, 3,410 cm^{-1} and 3,250 cm^{-1}. The band profile is almost identical to that observed in the Raman effect (see, for example, Schultz and Hornig[2]).

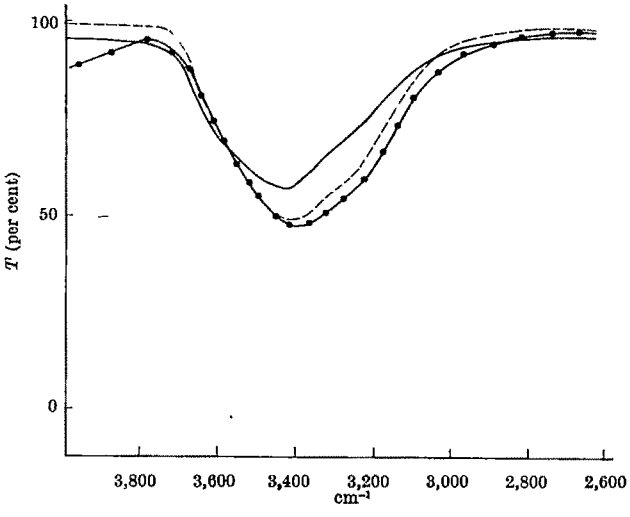


Fig. 1. Effect of temperature on the OH stretching absorption of water. – – – –, *c.* 35° C; ———, *c* 60° C; —●—, *c.* 5° C.

The most interesting observation, however, is the effect of temperature on the band system. As the sample is cooled from ambient temperature (~35° C when in the sample compartment of the spectrometer) to about 5° C, the intensity of the low-frequency band 3,250 cm^{-1} rises while that of the 3,625 cm^{-1} band falls. When the temperature is raised above ambient to about 60° C the 3,625 cm^{-1} band increases while the 3,250 cm^{-1} band decreases in intensity. Schultz and Hornig's[2] Raman spectra show exactly similar behaviour.

We now believe that the simplest explanation for these infra-red and Raman observations is in terms of three different types of water molecules with differing degrees of hydrogen bonding, as postulated by Buijs and Choppin[3] from their investigations of the combination band $\nu_1 + \nu_2 + \nu_3$ at ~8,300 cm^{-1}.

In the past we have advocated[4] a different explanation for the observed infra-red and Raman bands of liquid water using, like Schultz and Hornig, the idea of Fermi resonance to explain the Raman bands at 3,625 cm^{-1} and 3,250 cm^{-1} as a combination and overtone band respectively. At that time the available infra-red spectra indicated that our assignment was reasonable. In view of the infra-red data presented here, however, we believe that an explanation in terms of three types of water molecules is preferable to the earlier one which made use of the idea of Fermi resonance between bands separated by the order of 200 cm^{-1}. The assignment of the infra-red and Raman bands at 35° C is probably as given in Table 1.

The frequencies given in Table 1 for both infra-red and Raman results are taken directly from the recorded spectra. The differences between these three fundamental frequencies are consistent with the differences found by Buijs and Choppin[3] in the 8,300 cm^{-1} region in the infra-red.

Table 1

Raman	Infra-red	
3,620	3,625 cm^{-1}	Vibration of non-bonded water molecules
3,415	3,410 cm^{-1}	Vibration of water molecules with one proton involved in hydrogen bonding
3,250	3,250 cm^{-1}	Vibration of water molecules with both protons involved in hydrogen bonding

We are continuing our investigations on the temperature variation of the infra-red and Raman bands of liquid water and hope to enlarge on the use of spectroscopic data to define the properties of bulk water.

We thank Dr. C. Smart for advice and encouragement.

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Photolytic Degradation of Textile Fibres in the Far Ultra-violet

Although it is generally recognized that the action of far ultra-violet radiation on textile fibres is photolytic in character, investigations of the influence of oxygen and water vapour on the complex reactions involved have been mainly confined to cotton cellulose. The temperature of the system and the parameter of degradation are other factors which profoundly influence the nature of the results[1]. In the experiments described here we have used as parameter the loss in tensile strength of the textile yarn which was exposed to the radiations from a low pressure mercury–vapour lamp emitting mainly at 2537 Å. The yarn was contained in the inner compartment of a brass cell fitted with a quartz window. The cell was maintained at a constant temperature by circulating a suitable heating (or cooling) medium through the outer compartment. During the experiment a stream of gas brought to the required temperature was passed through the cell. For the exposures (Fig. 1) in dry oxygen

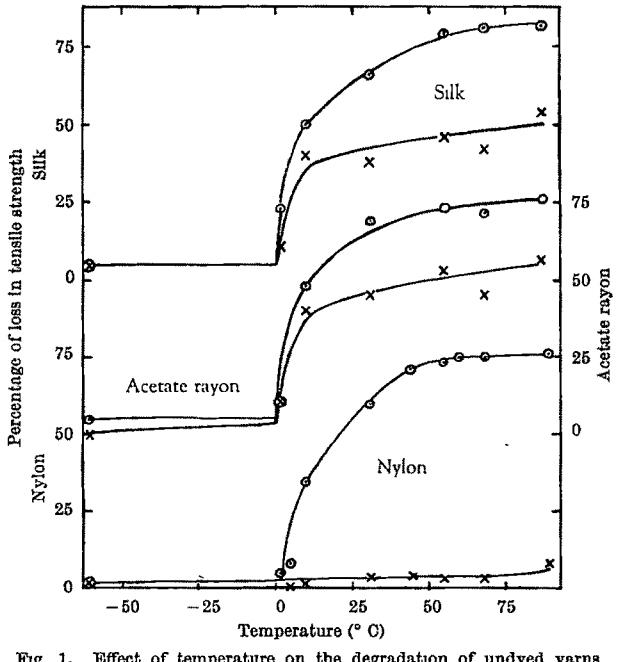


Fig. 1. Effect of temperature on the degradation of undyed yarns exposed to low pressure mercury-vapour lamp in dry oxygen (○) and dry nitrogen (×) for 20 h.

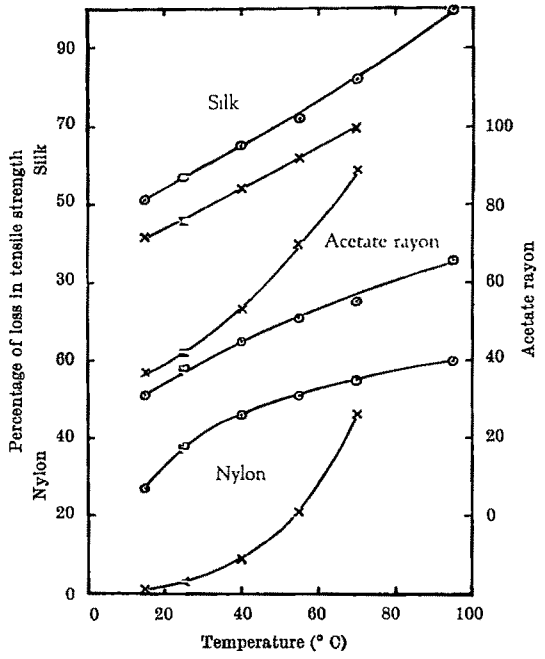


Fig. 2. Effect of temperature on the degradation of undyed yarns exposed to low pressure mercury-vapour lamp in air at 0 per cent relative humidity (○) and 100 per cent relative humidity (×) for 24 h.

and nitrogen involving temperatures down to −60° C the quartz face of the brass cell formed part of the wall of an airtight lamp chamber. At the bottom of the chamber was a tray of phosphorus pentoxide to absorb moisture and prevent the deposition of ice on the cold quartz plate. In addition a stream of dry nitrogen was passed continuously through the chamber. For the experiments (Fig. 2) with dry and moist air in which the lowest temperature was 15° C, this procedure was unnecessary and the cell was irradiated in a large well ventilated cupboard. Where dry gases were used they were dried with phosphorus pentoxide. In experiments performed in air at 100 per cent relative humidity, air was bubbled through a sintered glass filter immersed in distilled water which was maintained at the same temperature as the cell.

The effect of temperature on the degradation of some textile fibres exposed in dry oxygen and dry nitrogen to 2537 Å radiation is shown in Fig. 1. The fibres comprised scoured undyed yarns (free from delustrant or other additive) of nylon (hexamethylene diamine-adipic acid, Du Pont de Nemours, 4-fold, 45 den. 13 fil.), cellulose secondary acetate rayon (Courtaulds, 250 den. 48 fil.), and silk (degummed Japanese silk, 190 den.). With all three yarns the degradation in both gases is of negligible proportions at temperatures below about 0° C, but rises rapidly as the temperature is increased above this value. These results are in some contrast to those obtained with cellulose fibres. Results for undyed cotton yarn using fluidity in cuprammonium as the parameter of degradation have been reported[1] at temperatures down to 8° C. Further results at −60° C have shown that the change in fluidity (and also in reducing power) is of the same order as that at 5° C in the corresponding atmosphere. Moreover, with undyed cotton yarn (and also with scoured undyed viscose rayon) the loss in tensile strength in dry nitrogen and dry oxygen seems to be of the same order in the two gases and is only slightly affected by temperature over the range from −60° C to +90° C. As the temperature of the system falls to 0° C, it does not show the marked decrease in strength loss that is evident in Fig. 1 with silk, nylon and acetate rayon.

Nylon is particularly sensitive to the presence of oxygen in the irradiation system, and it would seem likely that in the absence of oxygen there is extensive recombination

of the free radicals formed on photolysis of the polyamide. Even in the presence of oxygen recombination would seem to be favoured at low temperatures. The reactions involved are undoubtedly complex as is shown by results which have been obtained with transparent films of nylon and other polymers[2], and may involve cross-linking of the polymer molecules[3].

The effect of temperature on the degradation of yarns of undyed silk, nylon and cellulose acetate rayon on irradiation in air at 0 and 100 per cent relative humidity is illustrated in Fig. 2. Except with acetate rayon the strength loss of the yarn is inhibited by water vapour. This effect is particularly evident with nylon, but as with acetate rayon, the strength loss increases substantially and at a much greater rate than in dry air, as the temperature is increased. The effect of water vapour on the degradation of cotton yarn by 2537 Å radiation has been shown[1] to depend on the parameter of degradation; the loss in strength being greater in the absence of water vapour, and the reverse being the case when the chain length of the cellulose (as measured by fluidity in cuprammonium) is considered. This evidence seems to suggest that in the presence of air and water vapour the polymeric free radicals formed on photolysis undergo secondary reactions involving not only gaseous oxygen but also water itself. It is possible that in certain circumstances hydrogen can be abstracted from a water molecule by the polymeric free radical. The resultant hydroxyl radicals would be expected to have a greater oxidative effect on cellulose than on polyamide fibres.

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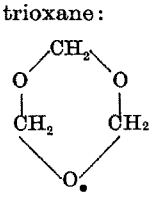
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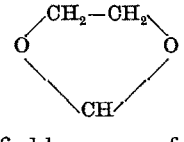
Formation of Cyclic Molecules by Macro-radical Reactions

MACRO-RADICALS formed by thermal degradation of polymers are known to participate in depolymerization[1] with subsequent detachment of a volatile compound. We have found another type of macro-radical reaction, namely, the formation of cyclic molecules.

A radical $\sim OCH_2—OCH_2—O\dot{C}H_2$ is formed by thermal degradation of polyformaldehyde. As a result of chain conformation by addition of a free-valency carbon atom to oxygen or to another carbon atom this radical may form cyclic atoms of trioxane:



or of dioxalane:



These were identified by means of chromatography and mass-spectrometry. Sometimes cycles with many links are also formed; this was demonstrated by mass-spectrometry.

Similar macro-radical reactions were observed during an investigation of the thermal degradation of polyvinylchloride. Here hydrochloric acid is the primary reaction product. Its detachment results in the formation of a macro-radical with a system of conjugated bonds:

$$\sim CH = CH - CH = CH - CH - \dot{C}H$$

In closing the cycle this radical is capable of detaching a benzene molecule. Benzene formed as a secondary product was identified by chromatography or mass-spectrometry.

So far as we know, no such cyclization of radicals in the degradation of polymers has previously been reported, though similar reactions of light radicals are known[2].

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BIOCHEMISTRY

Effect of Temperature on s-Ribonucleic Acid Aggregation induced by Magnesium Ions

RECENTLY, the aggregation of *s*RNA from *Escherichia coli* has been reported[1]. At neutral *p*H and at 25° C the aggregation process is dependent on nucleic acid and magnesium ion concentration. In 0·02 M magnesium, the aggregates dissociate into monomer units at low nucleic acid concentrations. This report shows that a modest increase in temperature (from 25° C to 45° C) partially dissociates the aggregates and that the decrease in molecular weight is reflected in the viscosity thermal profile.

E. coli *s*RNA was obtained from General Biochemicals, Chagrin Falls, Ohio, and freed of protein by repeated mechanical shaking with phenol. The aqueous supernatant was then chilled and the *s*RNA precipitated with two volumes of an ethanol–potassium chloride (0·3 M) solution. This was repeated twice. The final precipitate was dissolved in 0·01 M ethylenediamine tetraacetic acid (EDTA) and dialysed overnight against 0·01 M EDTA at 4° C. The solution was then treated twice with bentonite—50 mg/25 ml. of solution[2]. EDTA was then removed by dialysis against frequent changes of doubly distilled water at 4° over a period of 72 h. The aqueous *s*RNA was finally lyophilized.

The calibration, thermal control, use and calculation procedures with the Price–Phoenix light scattering photometer have already been described[1]. The instrument was modified in one respect; a narrow slit nosepiece was mounted in front of the photometer and the instrument refocused by a reflectance method. Viscosity measurements were made with a Ubbelohde viscometer with a solvent flow time of about 90 sec (25° C) or with a conventional viscometer with a 0·8 mm bore having a flow time of about 300 sec (25° C). Temperature regulation in viscometry was achieved as previously described[1]. For either viscometry or light scattering measurements, dust was removed by 'Millipore' filtration. *s*RNA concentration was estimated spectrophotometrically[3].

A low ionic strength medium used in these and previous investigations[1] was chosen on the basis of the observations of Lipsett *et al.*[4]. These investigations clearly show a competition between moderate concentrations of neutral salt and magnesium ions for binding sites on polyribonucleotides and suggest that an investigation of the effects of magnesium ions on nucleic acids should be made in a low ionic strength medium.

Fig. 1 shows the effect of increasing temperature on the molecular weight of *s*RNA. In neutral salt, the apparent

molecular weight (about 25,000) is only slightly decreased as the temperature is raised to 45° C, and essentially quantitative reversibility is obtained when the solution temperature is brought back to 25° C. In solutions which are 0·02 molar in Mg^{2+} and 0·01 molar in potassium acetate, the molecular weight of the aggregated form of *s*RNA at 25° C is roughly equal to that of a dimer. Heating to 45° C results in a drop to about 37,000. The process is about 95 per cent reversible, as is indicated by the checked circles. Heating to temperatures much above 45° C for longer periods of time in the presence of magnesium results in extensive aggregation[1].

Fig. 2 shows the intrinsic viscosity in the presence of magnesium ions at 25°, 35° and 45° C. At 25° C, the variation in η_{sp}/C with concentration clearly reflects the dissociation process reported earlier[1]. At 35° and 45° C the dependence of η_{sp}/C on concentration is less than that at 25° C. This is probably a consequence of the decrease in degree of aggregation at each of these temperatures (Fig. 1) although possible conformational changes cannot be ruled out. Linear extrapolation of the 25° C data above 3 mg/ml. leads to a value of $0{\cdot}074_5$ dl./g for the aggregate form, in good agreement with the value of 0·076 dl./g reported earlier[1]. In view of the scatter of the data below 3 mg/ml. at 25°, 35°, and 45° C, apparent intercepts at zero concentration were averaged giving 0·041 ± 10 per cent dl./g for the monomeric form of *s*RNA in magnesium ions.

These results show that the aggregation of *s*RNA induced by magnesium ions is, within a limited temperature range, thermolabile. In this respect it resembles the low temperature aggregation reported by Henley *et al.*[5]. The nature of the aggregation is unknown. Whether it results from magnesium ion phosphate links or base–base interaction or both is at present conjectural.

In solutions which are 0·3 molar in potassium chloride and 0·01 M molar in potassium acetate, the intrinsic viscosity at 25° C of *E. coli* *s*RNA monomer is 0·059 dl./g (ref. 6). Clearly, in a low ionic strength magnesium ion containing solvent the effective hydrodynamic volume of

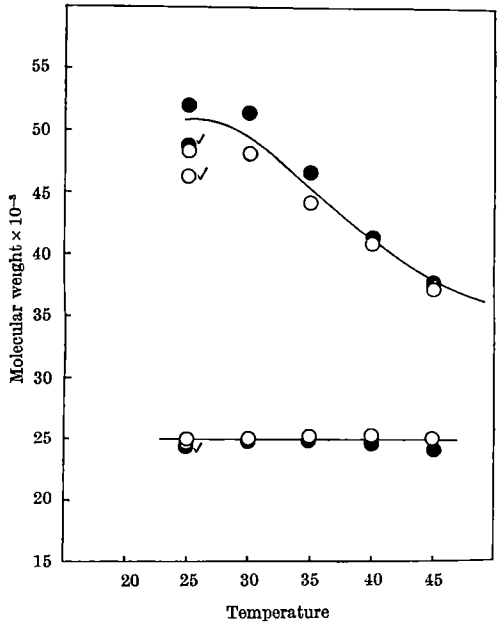


Fig. 1. The effect of temperature on the molecular weight of *s*RNA. ○, *s*RNA XI, ●, *s*RNA XII. Solvent systems: upper curve, 0·02 molar in magnesium acetate and 0·01 molar in potassium acetate, *p*H 7·0. *s*RNA XI, 4·25 mg/ml.; *s*RNA XII, 3·2 mg/ml. Lower curve, 0·3 molar in potassium chloride and 0·01 molar in potassium acetate. *s*RNA XI, 3·8 mg/ml.; *s*RNA XII, 2·95 mg/ml. Reversal from 45° to 25° C indicated by checked circles. Light scattering artefacts arising from thermal effects on the cell housing block and cell were corrected by measuring the turbidity of a 1 per cent solution of bovine serum albumin as a function of temperature. All solutions were kept 5 min at temperature before results were recorded.

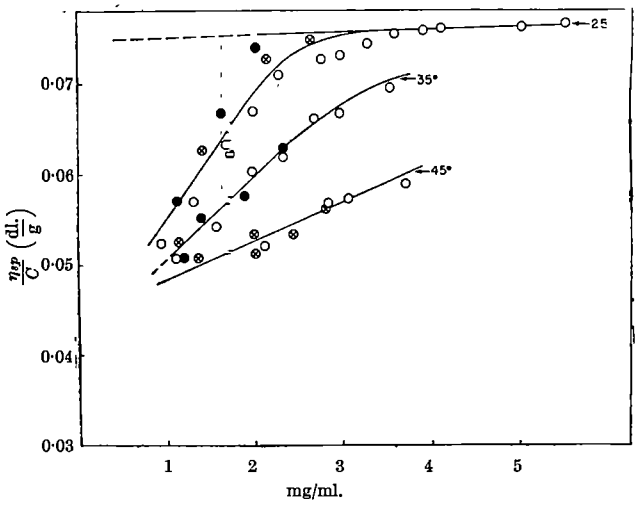


Fig. 2. Determination of the intrinsic viscosity of *s*RNA in 0·01 M potassium acetate, 0·02 M magnesium acetate, *p*H 7·0, at 25°, 35°, and 45° C. ○, *s*RNA IX; ⊗ *s*RNA XI; ●, *s*RNA XII. About 97 per cent reversal was obtained when any of the three preparations used was cooled from 45° to 25° C.

*s*RNA is less than that in moderate concentrations of neutral salt. The imprecision of the viscosity data prevents any meaningful estimation being made of the size of the equivalent ellipsoid of revolution.

If this reduction in effective hydrodynamic volume reflects only a difference in shape of *s*RNA in the two solvent systems, then it is conceivable that the effect of magnesium ions of raising the T_m from 57°–58° C in solutions which are 0·3 molar in potassium chloride and 0·01 molar in potassium acetate (*p*H 7·0)[6] to 77°–78° C in solutions which are 0·02 molar in Mg^{2+} and 0·01 molar in potassium acetate (*p*H 7·0)[1] simply results from a relief of electrostatic stress which compacts the molecule and strengthens secondary forces. Alternatively, a more complex conformational transition cannot be excluded. Finally, it should be noted that the hypothesis outlined here may be restricted to the solvent system described.

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Optical Rotatory Dispersion of Phosvitin

Previous investigations of phosvitin in solution have shown that the specific optical rotation, [α], in the visible and near ultra-violet regions is highly dependent on the *p*H and to a certain extent on the solvent composition. Furthermore, it has been reported that the rotatory dispersion constant of this protein varies with *p*H, λ_c = 225 mμ at *p*H 4·7 and 207 mμ at *p*H 10.75 (refs. 1 and 2).

During a characterization of the chemical properties of phosvitin, prepared according to the procedure of Joubert and Cook[3], the optical rotatory dispersion of this protein was recorded over the wavelength range of 600–190 mμ using a Cary 60 recording spectropolarimeter. In the spectral regions removed from absorption bands, the rotatory dispersions fit the one-term Drude equation.

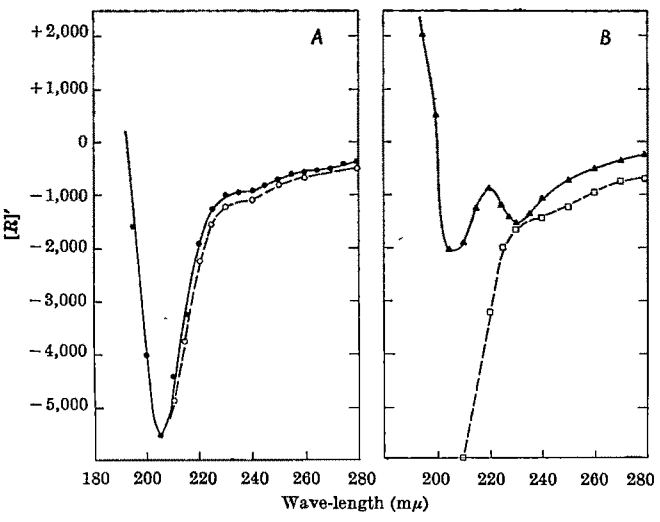


Fig. 1. Far ultra-violet rotatory dispersion of phosvitin. *A*, ●——●, In 0·032 M sodium monohydrogen phosphate–0·004 M sodium dihydrogen phosphate, *p*H 7·7 and ionic strength 0·1; ○— — ○, in 8·0 M urea-phosphate. Protein concentration: 0·01 per cent. *B*, ▲——▲, In distilled water; □— —□, in 0·1 N sodium hydroxide. Protein concentration: 0·04 per cent.

In a sodium phosphate buffer of *p*H 7·7 and ionic strength of 0·1, λ_c = 220 mμ, which agrees well with that found by Jirgensons[1,2]. If the protein is dissolved in 8·0 M urea-phosphate of apparent *p*H 7·7, λ_c = 218 mμ, thus duplicating the behaviour of other proteins with a low dispersion constant where urea has no effect on the specific optical rotation[4]. An intrinsic Cotton effect occurs in the far ultra-violet optical rotatory dispersion of phosvitin. As Fig. 1*A* illustrates, this Cotton effect has an inflexion point at 193 mμ and a minimum at 205 mμ. Such a negative Cotton effect with a trough at 205 mμ has thus far only been reported for collagen[5]. As also indicated in Fig. 1*A*, the residue rotation $[R]'$ is not altered if the protein is transferred from aqueous solution to 8·0 M urea-phosphate. The far ultra-violet dispersion of phosvitin dissolved in distilled water, a solution with resulting *p*H of 3·3, revealed two intrinsic negative Cotton effects with minima at 205 and 232 mμ respectively (Fig. 1*B*). Finally, we note some observations on the Cotton effect of a phosvitin solution on 0·1 N sodium hydroxide at *p*H 12·2. Although it was not possible to record the optical rotation in this solvent below 208 mμ, it is nevertheless apparent from Fig. 1*B* that the residue rotation in 0·1 N sodium hydroxide is larger in magnitude than that in phosphate buffer of *p*H 7·7.

As we have reported elsewhere[6], 56 per cent of the amino-acids of phosvitin are phosphoserine and phosphothreonine, 12 per cent dicarboxylic, 17 per cent basic, and only 15 per cent non-polar amino-acids. Thus the predominance, in this protein, of acidic constituents must considerably influence its conformational pattern. The dependence, therefore, of the macromolecular conformation on *p*H or on solvent composition is not surprising. At *p*H 3·0, for example, not all acidic groups are dissociated, whereas in the *p*H range of 7·0–8·0 some of the acidic groups may be neutralized by the proximity of the basic amino-acids. Furthermore, the possibility of O→N migration of the phosphoryl groups at neutrality[7,8] or β-elimination and formation of dehydroalanine at extreme alkalinity[9] are all factors that have to be considered in an attempt to understand the conformational pattern of this protein. Whatever interpretation of the optical rotatory dispersion curves of phosvitin will finally be established, we feel it is essential at this point to warn against any overemphasis of the rotation measurements in terms of a known conformational pattern unless supplemented by other chemical and physico-chemical methods or by work on known model compounds.

The Cary 60 spectropolarimeter was kindly made available to us by Dr. William F. Harrington, whom we thank for many helpful suggestions. Some of the experiments were carried out at the Marine Biological Laboratory, Woods Hole, Massachusetts.

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Inhibition and Possible Feedback by 6-Methylsalicylic Acid

WHEN commenting generally on secondary metabolic phenomena and on the particular example of the synthesis of 6-methylsalicylic acid (6-MS) in *Penicillium urticae* Bainier (≡*P. patulum* Bainier, etc.), we referred[1] to unpublished evidence suggesting that 6-MS could exert feedback inhibition on its own synthesis. Recently, Arihood and Light have described[2] investigations of this problem, and have reported that the addition of 6-MS, or of certain related compounds, inhibits the incorporation into 6-MS of carbon-14 from exogenous 1-^{14}C-acetate in intact mycelium. Our own work, however, revealed unsuspected complexities in this type of experiment. These have been summarized elsewhere[3], but the publication of Arihood and Light prompts us to record some of our evidence in more detail. The basic experimental techniques and the general dynamics of 6-MS synthesis have already been described[4].

At first we studied the gross levels of 6-MS in the medium of *P. urticae* cultures to which had been added, at various stages, 6-MS in the concentration range $2 \sim 6 \times 10^{-4}$ M (that is, about a tenth of the concentrations used by Arihood and Light). Normally, any mycelial accumulations of 6-MS can be neglected, but this is not so when extra 6-MS is added suddenly. This can be shown by spectrophotometric assay of 6-MS in the aqueous medium; the time-scale of events varies somewhat with the age, phase, and history of the mycelium, but the same qualitative behaviour is consistently observed. For example, on taking a suspension of 13 mg/ml. (wet weight) mycelium in fresh medium and adding 80 μg/ml. of 6-MS, there is a rapid removal of about 25 μg/ml. from the medium, in the first 30–60 min, followed by a slower reappearance over the next 2 h. This occurs even with trophophase mycelium, which has not yet commenced 6-MS synthesis[4]. In similar experiments, using 1-^{14}C-6-MS and recovering the 6-MS by co-crystallization with carrier, the specific activity of 6-MS shows little or no change. Thus, after the initial uptake of 6-MS, its subsequent reappearance does not arise from synthesis of 6-MS but from expulsion of the exogenous material, undiluted, possibly as a result of changes in the internal *p*H of the mycelium. Only in late idiophase mycelium which is actively converting 6-MS into patulin[4] is this picture materially altered.

With this complication added to the fact that even in control cultures the endogenous 6-MS is a reaction inter-

mediate present in fluctuating amounts[4] it was clear that measurements of gross levels of 6-MS following 6-MS addition would not give any information concerning the actual synthesis of 6-MS. Accordingly, we turned to the type of experiment which Arihood and Light have now described[2], in which early idiophase mycelium of *P. urticae* was incubated with added 6-MS and 1-^{14}C-acetate, and the fate of the carbon-14 observed. We found that, even at concentrations of $4 \sim 6 \times 10^{-4}$ M, 6-MS almost completely inhibits the incorporation of exogenous 1-^{14}C-acetate into 6-MS, and this is immediate. The isomeric analogue 3-methylsalicylate (3-MS) is equally effective. Thus far our observations coincide with those of Arihood and Light. We found, however, that the inhibitory effects are actually more widespread. At the same levels of 6-MS and 3-MS there is, for example, a comparably drastic inhibition of the incorporation of exogenous carbon-14 into lipids, mannitol, mycelial insolubles, and respired carbon dioxide, which is observed whether the carbon-14 is supplied as acetate or as glucose. Moreover, there is a comparable inhibition of the total carbon dioxide evolved and of dry weight increase and lipid synthesis during the incubation. In brief, these substances are acting as general inhibitors of metabolism. We are uncertain as to whether or not this effect is related to the effects of salicylates on oxidative phosphorylation.

These effects adequately explain the data of Arihood and Light[2], using rather higher concentrations, and also certain anomalous results which have been described, such as the observations of Tanenbaum and Bassett[5] on the rate of patulin synthesis by *P. urticae* in the presence of ^{14}C-6-MS, and of Packter[6] on the effects of 6-MS on the synthesis of fumigatin in *Aspergillus fumigatus* (allegedly from 6-MS but compare with ref. 7). It is also possible, however, to observe a more specific action of 6-MS on *P. urticae* by operating at somewhat lower levels. At $5 \sim 10 \times 10^{-5}$ M, for example at 7 μg/ml., 6-MS still inhibits the synthesis of 6-MS, as revealed by the recovery of undiluted material after incubations of idiophase mycelium with 1-^{14}C-6-MS for 0·5–2 h, yet at this concentration the more general inhibitory effects are comparatively slight. The analogue 3-MS does not seem to show this selective effect. It is for this reason that we adduced the 6-MS synthesis system of *P. urticae* as a possible example of the operation of product inhibition, or a similar feedback mechanism, in secondary biosynthesis, though a conclusive test must await the description of a reliable procedure for the study of polyketide synthetases in cell-free preparations.

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Effect of Orchidectomy and Ovariectomy on Survival against Lethal Infections in Mice

The reticulo-endothelial system plays a predominant part in the defence of the body against infection. We have previously shown that stimulation of the reticulo-endothelial system leads to raised body defence (indicated by increased phagocytic activity, accelerated antibody production, raised serum gamma globulin and increased protection of experimental animals against virulent infections), and that the strongest reticulo-endothelial stimulants are the oestrogens[1,2]. We have also shown that the action of oestrogen on the reticulo-endothelial system is independent of its action on the reproductive tract, although these two biological activities are contained in the same molecule[3]. We have also demonstrated that cyclical variations in reticulo-endothelial activity occur during the oestrous cycle and during pregnancy in the rat and the mouse[1]. These findings suggest that when protection against infection is required, general and local body defence is increased by raised blood oestrogen levels. We also found that reticulo-endothelial activity falls after ovariectomy in the mouse[4]. These results suggest that oestrogen, especially 17β-oestradiol, is the principal natural stimulant of body defence in both the male and the female—the latter having raised oestrogen levels when protection against infection is normally most needed.

The purpose of this communication is to report the effects of ovariectomy and orchidectomy on resistance to lethal doses of virulent organisms, and to demonstrate the protective effect of oestrogen administration in intact and gonadectomized animals.

Forty male and forty female white mice (*T.O.* Swiss strain) of 20–25 g body-weight were used in the experiment. The orchidectomized and ovariectomized animals were spayed 4 weeks before the experiment started. The mice were divided into eight groups, each containing ten animals, as shown in Table 1. In the oestrogen treated groups, each dose of diethylstilboestrol (0·1 mg) was administered orally by stomach tube in 0·1 ml. of sterile distilled water[5] once daily for 3 days. On the day following the last dose of diethylstilboestrol, all the animals received a single intraperitoneal injection of 0·2 ml. of 1/10 dilution of an 18 h broth culture of *Pneumococcus* Type 1 (about 10,000–100,000 lethal doses). The number of animals surviving was noted at 2-hourly intervals. The mean survival times were calculated according to one of the methods used by Burroughs Wellcome Laboratories[6]. The survival time for each animal was recorded and converted into the reciprocal; the reciprocals were then handled as normal variates and averaged within groups in the ordinary way.

Table 1. Mean Survival Times of *T.O.* Swiss Mice infected with *Pneumococcus* Type 1 following Orchidectomy and Ovariectomy and after Treatment with Diethylstilboestrol

Group	Daily dose of diethylstilboestrol for 3 days before infection (mg)	Dose of infection	No. of animals in group	Mean survival time ± S.E. (h)
Intact males	—	0·2 ml. of 1/10 dilution of 18-h broth culture of *Pneumococcus* Type 1 (about 10,000–100,000 lethal doses) intraperitoneally	10	15·1 ± 0·7
Orchidectomized males	—		10	11·8 ± 0·6
Intact males + diethylstilboestrol	0·1		10	24·9 ± 1·4
Orchidectomized males + diethylstilboestrol	0·1		10	23·2 ± 1·6
Intact females	—		10	15·3 ± 0·8
Ovariectomized females	—		10	11·2 ± 0·9
Intact females + diethylstilboestrol	0·1		10	27·3 ± 1·3
Ovariectomized females + diethylstilboestrol	0·1		10	23·8 ± 1·2

Broth: Oxo Nutrient No. 2 with 15 per cent horse serum.
Mean survival times were calculated according to the method of reciproca survival with observations every 2 h.

The results are shown in Table 1. It can be seen that there was no difference in the mean survival times of the untreated intact males and females. It can also be seen that orchidectomy and ovariectomy significantly reduced the survival time to about the same level. The administration of diethylstilboestrol produced a marked increase in survival time in both the intact and gonadectomized

animals, there being no significant differences between the various groups receiving oestrogen.

The observations recorded in this experiment clearly show that removal of the gonads significantly reduces survival against large doses of highly virulent infection in both sexes. In the human male it is known that considerable quantities of oestrogen are produced by the adrenal cortex and testes, and that, following orchidectomy, there is a marked fall in the urinary excretion of oestrogen[7]. In the human female, increased production of oestrogen by the adrenal cortex compensates, to some extent, for the removal of the ovarian sources of these hormones[8], but the increase is not sufficient to restore body defence to pre-operative levels[4]. Furthermore, it has been shown that, following ovariectomy in women of premenopausal age, there is a significant increase in the susceptibility to certain diseases[9]. Thus, it appears that gonadal oestrogen plays an important part in maintaining body defence in both sexes.

We have previously demonstrated that diethylstilboestrol prolongs survival against a wide variety of virulent Gram-positive and Gram-negative organisms in intact male mice[1]. The present results show that diethylstilboestrol is equally effective in prolonging survival in both intact and gonadectomized animals of both sexes.

The present results provide additional evidence in support of our earlier postulate that oestrogen is the principal natural stimulant of body defence in both the male and the female; and, further, that oestrogen treatment would seem to be of clinical value in the treatment of acute bacterial infections.

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BIOLOGY

Stimulation of Spawning in *Xenopus laevis* by Fowl Manure

SAVAGE[1] found that pondweeds in the presence of light stimulate spawning in *Xenopus laevis* in the laboratory. This finding prompts me to report my own experience with this amphibian under more natural conditions in the Transvaal.

At the Provincial Fisheries Institute, Lydenburg, fishponds that have been dry for the winter are filled with water and fertilized with fowl manure in spring for the breeding of fish. Within 2 or 3 days after fertilization such ponds usually contain large numbers of *Xenopus*, which immediately start spawning, so that by the time plankton has developed the pond is teeming with larvae.

On such a station, these frogs are usually spread over numerous ponds, but fertilized water is so attractive to them that they can be collected simply by fertilizing a pond and netting the frogs from it after a few days. They come from adjacent ponds overnight even when these are not receiving seepage from the fertilized pond. It is suspected that they are able to detect fertilized water by scent when coming up for air or while travelling overland at night.

The fact that they are attracted by fertilized water and spawn before an algal bloom develops suggests that the primary stimulus for spawning under these conditions could be the fertilizer rather than an algal metabolite.

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Coat Structure in Soay Sheep

EARLIER work on the coat of the Soay[1,2] was carried out with "mainland" Soays which stem from stock removed from St. Kilda some 50 years ago. Apparently, all these animals have a woolly, chocolate coloured coat. The designation of St. Kilda as a National Nature Reserve has enabled an investigation to be made of the sheep remaining on the islands by a team organized by the Nature Conservancy[3]. Many of the sheep have a hairy coat, and only 75 per cent are dark brown in colour; the remainder are light brown. The coat structure of these primitive domestic sheep throws light on fleece evolution. This study reveals a lack of heterotype hairs, and a higher secondary/primary follicle ratio (S/P ratio) in the hairy than in the woolly type.

Mid-side coat and skin-snip samples were taken in August 1963 from twenty-four animals removed from St. Kilda that year, and in May 1964 from twenty animals on Hirta, St. Kilda.

Despite gradation in the type of coat, it was possible to divide the samples into hairy and woolly types by eye. Of the dark animals 63 per cent had a hairy coat, compared with 60 per cent of the light animals. The hairy type was characterized by straight hairy fibres projecting beyond the rest of the coat, whereas the woolly type had more definite staples with a curly tip. Most rams had a hairy coat. The hairy fibres are apparently kemps, and although these are finer than those of the wild sheep[4], the coat often resembles that of the Mouflon. No evidence was found for heterotype hairs, that is, hairs that are kemp-like in summer but narrow and non-medullated in winter. Hairy sheep had a longer coat than woolly sheep, and there was a tendency for the dark animals to have a longer coat than the light ones, so that the coat lengths in August (grown since the spring moult) ranged from a mean of 22·5 mm in the light woolly sheep to a mean of 35 mm in the dark hairy ones. This trend was not obvious in the animals with a year's growth of wool (sampled in May); this may have been the result of equalization of hair lengths through wear at the tips, and of shedding of the longest hairs. In only one of these animals did the coat length (80 mm) exceed 50 mm. The maximum length found in mainland Soays was 50–60 mm.

The mean fibre diameter was about 25μ in the woolly sheep, and about 30μ in hairy ones, ranging up to a mean of 35μ in dark, hairy animals. This compares with a mean in some mainland Soays of 23·5μ. There was a "skewed-to-fine" distribution, and the overall diameter range in woolly animals was 12–48μ in August, although some animals had a few hairy fibres up to about 70μ in diameter. In May the range was similar with little apparent reduction in the diameter of either the wool or the hairy fibres. The diameter range in the hairy animals was 12–144μ in August and 10–106μ in May. This contrasts with some spring measurements in the Mouflon

of the outer kemp: 40–190μ (narrowing to shed), mean 144μ; and under wool: 6–18μ, mean 12μ.

Examination of skin sections showed the primary fibres in August to be very variable. At one extreme were coarse fibres with a wide, latticed medulla like that of kemps in cross-section. Some less coarse fibres had either the same extremely wide latticed medulla, or a narrower latticed medulla comparable with that of heterotypes. At the other extreme some fibres were of medium diameter, and had either a non-latticed medulla or no medulla. The central primaries tended to have coarser fibres than the laterals, but not fibres of a different type. There was more variation in fibre type between animals than between different fibres of the same animal. Most coat variation seemed to be the result of differences in the type of primary fibres. There was little apparent variation in the diameter of the secondary fibres, and few exhibited any medullation. A greater density of pigmentation was frequently seen on one side of the secondary fibres than the other[5].

The mean proportion of primary fibres with a latticed medulla was 34 per cent in the dark, hairy animals and 38 per cent in the light, hairy ones. But of these 91 per cent were of the wider type in dark animals, compared with only 67 per cent in the light animals. These figures accord with the tendency for the dark animals to have a coarser coat. The dark and light woolly sheep had a similar proportion (27 per cent and 28 per cent) with a latticed medulla—32 per cent and 39 per cent respectively, having the wider type. The proportion of non-latticed medullation ranged from 10 per cent to 15 per cent in the different groups. The animals sampled at the end of May were about to shed their fleece; by this time most fibres had ceased to have any medullation and many had a brush end. This is in keeping with a complete moult, and supports the identification of the hairy fibres as kemps.

About 75 per cent of both the primary and secondary fibres were pigmented in August, but whereas 63 per cent of the primaries and 49 per cent of the secondaries had dense pigmentation in the dark group, the figures were only 13 per cent and 10 per cent in the light group. The difference in colour between light and dark animals is, therefore, mainly the result of differences in the density of pigmentation, and less the result of differences in the number of pigmented fibres. Pigmentation was not so great in the skin of animals sampled in May, in keeping with preparation to shed the fleece.

Preliminary results indicate that at birth less than 10 per cent of the secondary follicles are growing fibres. Side[6] found that in the Mouflon no secondary fibres were above the skin surface at birth. In contrast, a 135 day Romney foetus had about 80 per cent of the secondary follicles with fibres[7], while at 1 day of age the Herdwick had about 60 per cent[8] and the Suffolk 85 per cent[9]. In the two week old Soay, the proportion of mature secondaries had increased to a mean of 50 per cent and of these as many as 59 per cent had outgrowths like those observed by Ryder[2]. Follicle branching therefore seems to be important in the development of later secondaries in the Soay.

The mean S/P follicle ratio for all sheep sampled on St. Kilda was 3·86/1, compared with 3·59/1 in mainland Soays. The mean S/P ratio of woolly animals on St. Kilda was 3·59/1 (range 3·2/1 to 3·7/1) and that of the hairy sheep was 3·95/1 (range 3·2/1 to 4·7/1), this difference being significant at the 2 per cent level.

This paradox whereby the hairy animals have a higher instead of a lower S/P ratio probably lies in the primitive nature of the Soay coat. Thus the hairy type is probably closer than the woolly type to the Mouflon, which has a relatively high S/P ratio approaching 5/1 (ref. 4). This is higher than in many domestic breeds, for example Scottish blackface, 3–4/1. Animals with a similar S/P ratio can have markedly different coat types resulting from differences in the type of primary fibre (compare the Mouflon and Down fleece, both with an S/P of 5/1). Thus the S/P ratio is not necessarily associated with fineness, and may only become important when the primary fibres are not coarse, and when it exceeds about 5/1. Fineness in the Merino, however, is associated with high follicle density, and so in this breed fineness is not directly associated with a high S/P ratio, which here appears merely to reflect the high follicle density.

The lack of heterotype hairs, and the "skewed-to-fine" distribution of fibre diameter, supports the identification of the Soay with the generalized medium wool type[10], and the hairy type as being a hairy medium wool. The affinity with the Mouflon indicated by this investigation suggests that a gradual evolutionary replacement of kemps with heterotypes did not occur. The heterotype seems to be a specialized and highly evolved fibre type, the origin of which is probably associated with the change from moulting to continuous growth.

In order to investigate the possibility that heterotypes could be produced as a result of a cross, some Merino ewes (fine wool only) were mated with a Mouflon ram (kemp and fine wool). The progeny are now a year old; all their secondary follicles are growing wool, and all their primary follicles are apparently growing kemp. Thus no evidence for heterotypes has yet been found.

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Cytokinins in Ribonucleic Acids

COMPOUNDS which, like kinetin (6-furfurylaminopurine), induce cell division in certain excised plant tissues have been termed cytokinins[1]. A number of synthetic substances (6-substituted purines, 1-substituted adenines and urea derivatives) show this characteristic. The most active natural cytokinin so far discovered is also a substituted purine, termed zeatin [trans-6-(4-hydroxy 3-methyl but 2-enyl) aminopurine]. This was isolated from *Zea mays* seeds[2]. Other natural cytokinins, as yet unidentified, occur in many other plant tissues, but there have been no reports of the occurrence of cytokinins as natural constituents of animal tissues. This communication, however, provides evidence that compounds with cytokinin activity are natural constituents of animal soluble RNA.

Mixtures of highly purified RNA and DNA were prepared (by methods described previously[3]) from rat and sheep liver, and from exponential phase tobacco cells (*Nicotiana tabacum* var. 'Wisconsin 38') grown in cell suspension culture. RNA was hydrolysed in 1 N potassium hydroxide at 25° C for 24 h to give a mixture of 2′- and 3′-ribonucleotides. The hydrolysate was neutralized by passing it through a column of 'Zeocarb 225' (ammonium) cation exchange resin (10 cm × 0·5 cm) and evaporating it to dryness. Ribonucleotides were extracted from this dried eluate with 70 per cent ethanol, *p*H 4. The insoluble

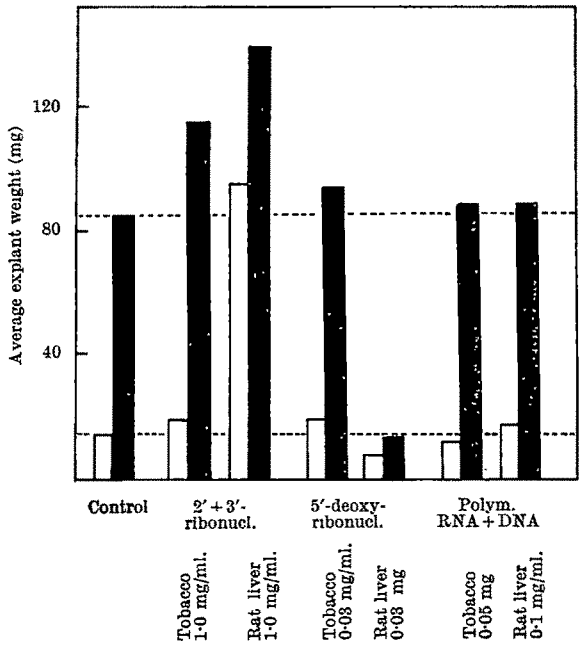


Fig. 1. Average weight of carrot explants after growth in the presence and absence of tobacco cell and rat liver nucleic acids and their hydrolysis products. Kinetin was added to some treatment (0·2 mg/l.). The following fractions were assayed at the levels indicated/ml. culture fluid: 2′- and 3′-ribonucleotides (alkaline hydrolysis); 5′-deoxyribonucleotides (enzyme hydrolysis); unhydrolysed (polymeric) nucleic acids (RNA+DNA); control (indole-3-acetic acid alone). □, Fractions plus indole-3-acetic acid alone; ■, fractions plus indole-3-acetic acid and kinetin.

DNA which remained was digested to 5′-deoxyribonucleotides by treatment with DNase I (deoxyribonucleate oligonucleotido-hydrolase, E.C.3.1.4.5) followed by treatment with purified snake venom phosphodiesterase (orthophosphoric diester phosphohydrolase E.C.3.1.4.1)[4]. RNA and DNA were also hydrolysed to the mixed 5′-ribo- and 5′-deoxyribonucleosides using crude snake venom[5]. Protein was removed from the enzyme hydrolysates by 70 per cent ethanol extraction as before.

To assay for cytokinin activity, nucleic acids and hydrolysates were filter-sterilized, and their effect on the growth of phloem explants[6] was determined. All culture media used contained the growth factors indole-3-acetic acid (2 mg/l.) and myo-inositol (50 mg/l.). All extracts were assayed both in the presence and in the absence of an added synthetic cytokinin (kinetin, 0·2 mg/l.) in order to check for the possible presence of inhibitory compounds or synergistic effects.

Alkaline hydrolysates of sheep and rat liver RNA showed considerable cytokinin activity, while tobacco RNA hydrolysates were inactive. All deoxyribonucleotide preparations were inactive or were inhibitory (Figs. 1 and 2).

Enzyme hydrolysates of sheep liver RNA showed a similar cytokinin activity to that of alkaline hydrolysates of this material (Fig. 2). Thus the activity in alkaline hydrolysates of RNA is not likely to be an artefact caused by the strongly basic conditions of hydrolysis. On the other hand, a mixture of pure ribonucleotides (Pabst Laboratories) was inactive, showing that the cytokinin activity was not due to the four major ribonucleotides found in RNA.

The possibility was considered that the compounds with cytokinin activity may be confined to RNA from one particular part of the cell. Rat liver was therefore homogenized (0·25 M sucrose, 0·025 M potassium chloride, 0·005 M magnesium chloride, 0·05 M *iris*-hydrochloric acid, *p*H 7·8) using a tapered glass homogenizer (Kontes Glass Co.); and the sub-cellular particles were isolated from the resulting homogenate by differential centrifugation. RNA was prepared from each fraction and hydrolysed to 2′- and 3′-ribonucleotides as before. Each fraction was assayed for cytokinin activity. The nuclear, mitochondrial and ribosomal RNA hydrolysates (0·35 OD_{260}/ml. culture fluid) caused moderate growth increments, but RNA from the cytoplasmic fraction (105,000*g* supernatant, principally *s*RNA), though at a lesser concentration (0·21 OD_{260}/ml. culture fluid), caused a growth increment 2·5 times as great. Hence the activity of hydrolysates of *s*RNA far exceeded that of the other ribonucleic acid fractions.

The high degree of purity of nucleic acids used in this investigation[3] implies that RNA products themselves rather than nucleotide contaminants are responsible for the cytokinin activity observed. The absence of activity in enzyme hydrolysates of tobacco RNA, and its presence in similar hydrolysates of sheep liver RNA, suggest that a true component of liver RNA is involved rather than an artefact produced by modification of a common nucleotide.

A possible source of the cytokinin activity in liver RNA is a compound of the methylated purine type. Dunn[7] and Bergquist and Matthews[8] have shown that such compounds are particularly abundant in *s*RNA. A number of these compounds have been tested for cytokinin activity and found to be inactive (9-methyladenine, 6-methylaminopurine, 6-dimethylaminopurine) when assayed alone[9]; though 6-methylaminopurine is active at

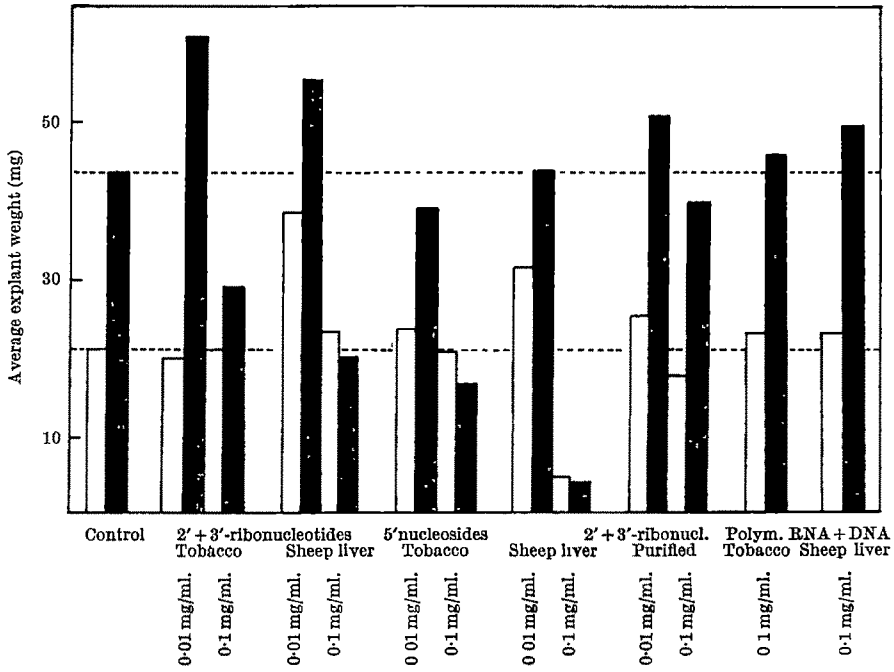


Fig. 2. Average weight of carrot explants after growth in the presence and absence of enzyme and alkaline hydrolysis products of tobacco cell and sheep liver nucleic acids. Kinetin was added to some treatments (0·2 mg/l.). Fractions were assayed at two levels, 0·01 mg and 0·1 mg hydrolysis products/ml. culture fluid. The following fractions were assayed: 2′- and 3′-ribonucleotides (alkaline hydrolysis); 5′-deoxyribo- and ribonucleosides (enzyme hydrolysis); 2′- and 3′-ribonucleotides (purified Pabst products); unhydrolysed (polymeric) nucleic acids (RNA+DNA). □, Fractions plus indole-3-acetic acid alone; ■, fractions plus indole-3-acetic acid and kinetin.

very high concentrations in the presence of adenine[10]. Hall[11] has reported the presence of N^6 aminoacyl adenines in hydrolysates of yeast RNA, but these are alkali-labile and in this study should have been eliminated during hydrolysis procedures. Thus the identity of the active compound or compounds remains unknown.

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Cutaneous Melanocyte System of the Indigo Snake *Drymarchon corais*

FOR many years it has been believed that the cutaneous pigment cell system of the so-called lower vertebrates—fishes and amphibians—is mainly a dermal one. As an effector system its function is primarily to show aggregated or dispersed melanin in different hormonal situations, and it does not significantly donate pigment to other tissues. On the other hand, the pigment cell system of birds and mammals has been considered to have primarily a cytocrine function, and to be effectively resident in the epidermis and its derivatives. Here the melanocytes function by donating melanin to contiguous epidermal cells in the integumental epidermis, or in the matrix of hairs or the collars of feathers. It is difficult to understand how the presumably dermal pigmentation of the ancestral vertebrates produced the very elegant epidermal system of birds and mammals, and little descriptive work on the reptile pigment cell system has proved of value in this connexion.

Routine histological sections of the skin of the indigo snake (*Drymarchon corais*) were taken in the course of another investigation. During their analysis, a very odd and apparently new phenomenon was noted. The melanocyte system appears to be mostly dermal although occasionally melanocytes may be seen in the epidermis. The shed skin is, however, quite darkly pigmented and it was considered that there were insufficient epidermal melanocytes to account for this. Close investigation of the histological sections revealed that many and perhaps most of the dermal melanocytes had fine processes which extended across the dermis above the melanocyte layer, through the basement membrane, and into the basal layer of the epidermis. Because of the fine and highly irregular nature of these dendrites, it is very difficult to demonstrate convincingly that any particular dendrite extends from a certain melanocyte up into the epidermis. Sometimes the thicker dendrites (Fig. 1) may be seen to do this, however, and they are occasionally found complete in one 7μ section.

The significance of this finding is rather difficult to assess. It is tempting to suppose that this condition may represent an intermediate between the situation in fishes and amphibians and that in mammals and birds. It has been shown by Szabo (unpublished) and by Hadley (also unpublished) that there is an epidermal melanocyte system in several North American frogs, so that the situation in this snake may be indicative of no more than a possible evolution from a dermal to an epidermal melanocyte system. Nevertheless it raises a number of questions about the presumed inviolability of the basement membrane of vertebrates, and provides a framework for the consideration of vertebrate pigment cell systems as a whole. Further investigations using electron microscopy are in process.

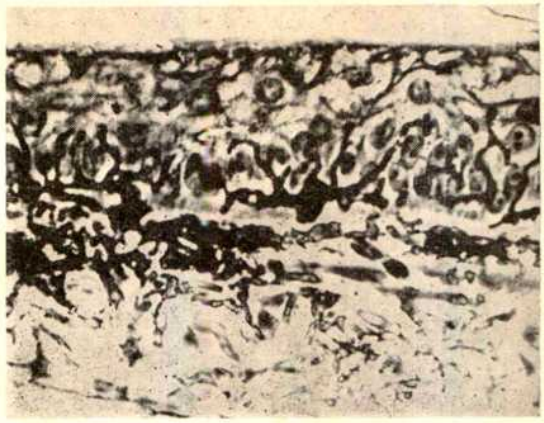


Fig. 1. Part of a 7μ section of *Drymarchon corais* dorsal skin. Dermal melanocytes (*D*) are seen packed with melanin, and parts of epidermal melanocytes (*E*) are sectioned. Note the processes extending across the basement membrane (*BM*)

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Echinoid Coelomic Cells

ECHINOID coelomic cells were first described in detail by Geddes[1], who studied the coelomic fluid of *Paracentrotus lividus* and *Echinus esculentus*. Since then, these cells have been the subject of numerous papers, principally descriptions of the cell-types in various species[2–9].

The main coelomic spaces of a regular echinoid are the perivisceral and lantern coeloms and the watervascular system. The perivisceral coelom alone occupies 40–50 per cent of the volume enclosed by the test of a *Psammechinus miliaris* 2–3 cm in diameter, and 70–80 per cent of an *Echinus esculentus* of about 12 cm diameter. The coelomic cells are suspended in the coelomic fluid within these spaces, but some of the cells are amoeboid and are found in other tissues, notably in those of the haemal system. Coagulation and phagocytosis of "foreign" material are two known functions, and other important functions have been attributed to the coelomic cells; however, as has been recently stated[10], their role has not been entirely elucidated.

The present report is based on a cytological study of the cells found in the perivisceral coelomic fluid of *Diadema antillarum* and *Psammechinus miliaris*. The cells can be divided into three morphological types.

(1) Bladder amoebocytes. These are cells usually about 25μ in diameter, with a central mass of endoplasm containing the nucleus. The endoplasm is surrounded by labile bladder-like processes which become filiform as an irreversible coagulation change. Such cells are phagocytic but, as they are so readily involved in coagulation changes, information on taxes or any locomotor activity is difficult to obtain.

(2) Spherule amoebocytes. These are active amoeboid cells about 35μ long when extended. They contain numerous spheroidal inclusions which move freely in the cytoplasm as does the nucleus. In all echinoids studied there were two types of amoeboid cell, one with unpigmented—termed colourless—inclusions, and one with

predominantly red inclusions. Red pigment extracted from coelomic fluid of *D. antillarum* resembled echinochrome A[11], while that from *P. miliaris* had the characteristics of spinochrome E[12]. Cells with yellow-green inclusions were found in *Echinocardium cordatum*, *Spatangus purpureus*, *Brissopsis lyrifera* and in some specimens of *Arbacia lixula*.

(3) Flagellated cells. These are small cells with a cell-body 5–10μ in diameter. These cells invariably move with the flagellum posterior to the cell-body. The nucleus is anterior in position. Cuénot[13,14] at first believed that these flagellated cells were parasitic and assigned them to the genus *Oikomonas* with the specific name *echinorum*. He later expressed doubts as to the validity of this classification[14] and to the parasitic nature of the cells[15]. His doubts have since been confirmed[16]. Besides the occurrence of these cells in every echinoid examined, the position and form of the flagellum, the form and size of the cell-body and the staining reactions indicate that these cells are echinoid rather than parasitic protozoa. Indeed, detached peritoneal cells closely resemble the flagellated cells, which accords with previous views of their origin[1,6].

The composition of the inclusions of the spherule amoebocytes has been investigated. A modification of Liebman's formol-vapour fixation[6] was used, with a method of identifying cells before and after staining. It was found that, contrary to previous opinion[17,18], the red spherules are not acidophilic. Histochemical methods did not detect lipid or protein in particular association with the red spherules. It is possible that previous authors have not made allowances for the reactivity or tinctorial effects of pigment retained after the use of certain fixatives. The spherules were, however, found to be associated with carbohydrate, probably sulphated mucopolysaccharide. Boolootian and Lasker found glycogen in red coelomocytes of well-fed *Strongylocentrotus purpuratus* and this glycogen content was found to diminish on starvation[10]. Glycogen was not detected in spherule amoebocytes of *D. antillarum* or *P. miliaris*.

Kollmann's conclusion[17] that the colourless spherules are albuminous is substantiated by reactions to tests for amino-acids. There is a strong positive reaction to the Adams[19] test for 3-indolyl derivatives. An indolic compound extracted from perivisceral coelomic cells of *P. miliaris* resembles tryptophan in its chromatographic behaviour. The colourless spherules may also be associated with some phospholipid and/or fatty acid.

Some authors have supposed an inadequacy of vascular function in echinoids. Stott[20] believed that the coelomic amoebocytes are responsible for some intracellular digestion in the stomach lumen, and for absorption and transport by migration through the haemal system. He thought that this might be associated with the poor development of the haemal system and lack of a pumping mechanism, which may now be disputed[21–23]. His conclusions on absorption and transport were based on histological examination after injection of iron saccharate into the pharynx of *Echinus esculentus*. I have never observed coelomic cells in the stomach or intestinal lumen of echinoids, except where insufficient precautions have been taken with dissected specimens to prevent contamination by coelomic fluid. Absorption of iron and tryptophan labelled with carbon-14 can be shown to occur without the initial participation of the coelomic cells in *P. miliaris*. An iron-containing pigment occurs in *D. antillarum*[24]. Control specimens of *P. miliaris* showed the presence of small rounded bodies which stained blue after sulphide and ferrocyanide treatment and emphasized the need for careful interpretation of iron saccharate experiments. It was concluded that the coelomic cells are probably not important in digestion or in the initial absorption and transport of nutrients from the gut of *P. miliaris*. The spherule amoebocytes, however, may act as nutrient distributors, as suggested by Boolootian and Lasker[10] for the red cells, moving either with the surrounding fluid or by active amoeboid progression.

There was an apparent reduction in the indolyl content of the coelomic amoebocytes at the onset of gonad maturation in both sexes of *P. miliaris*. It was thought that this might be an indicator of transfer of material. There was no evidence of direct transfer to the gametes, however, as previously described by Liebman in the ovaries of *Arbacia punctulata*[6]. Holland and Giese[25] did not observe direct transfer in the ovaries of *Strongylocentrotus purpuratus*. Thus, although the spherule amoebocytes appear capable of direct transport and may indeed function in this capacity, it has not yet been demonstrated.

This investigation was conducted under the supervision of Prof. N. Millott during the tenure of a Department of Scientific and Industrial Research studentship.

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Are Pelagic Diatoms Free from Bacteria?

IN attempting cultures of pelagic diatoms it is the common experience that success is more often achieved when the isolating procedures have not been so thorough as to eliminate all bacteria from the cultures. Although direct nutritional dependence on excretions of the bacteria cannot always be ruled out, physico-chemical effects are probably of greater importance. But do bacteria play the same part in nature? The onset of the spring increase of phytoplankton in coastal waters can seldom be traced to simple causes. It is often observed to follow a period of quiet fine weather when a major factor is undoubtedly the absence of vertical mixing. Light may be all that is needed to trigger the diatom growth; but it is also conceivable that the winter water requires "conditioning" before growth can start, and the slight increase of temperature at the surface during a fine spell increases bacterial metabolism sufficiently for this. Such an effect will be greatest when the association between bacteria and diatoms is most intimate—that is, between any epiphytic bacteria and their host cells. It is not clear from the literature, however, how close is the association between bacteria and phytoplankton in the sea. Marine bacteria are nearly always associated with particulate matter[1,2], and there is often a correlation between the abundance of bacteria and living diatoms[2,3]. Nevertheless, live plankton diatoms do not seem to harbour a heavy epiphytic flora, as do many freshwater

algae. On the contrary, the cells usually appear to be remarkably clean. There are, however, no published observations, although other evidence[4,5] suggests that diatoms may have antibiotic activity.

We have attempted by cultivation and by direct observation to determine the extent to which bacterial infestation occurs on the cells of two species of diatom. We picked specimens of the large winter species *Coscinodiscus concinnus* from tow nettings over a period of several months during the winter of 1963, carried them through six washes of sterile sea water and placed them singly into cultures of the diatom *Phaeodactylum tricornutum* in a liver infusion/soil extract/sea-water medium (*E*6, our medium for routine sterility testing of algal cultures[6]). *Ph. tricornutum* was provided as an alternative substratum, since the object was to isolate bacterial strains intimately associated with, and therefore possibly dependent on, the live cells, whereas most *C. concinnus* cells fail to survive the washing procedure. *C. concinnus* is so large that it was not possible to avoid transferring a considerable volume of water along with the cells; we therefore set up controls at the same time by transferring to *Phaeodactylum* cultures a little of the wash water from the final bath, but without the *Coscinodiscus*. The results of this experiment (Table 1) show that, if any bacteria were associated with the diatom, either they failed to survive the transfer, which is improbable, or that the association was loose enough to allow their removal simply by passage through sterile sea water.

Table 1. BACTERIAL CONTAMINATION IN CULTURES OF *Phaeodactylum tricornutum* IN WHICH HAD BEEN PLACED (*a*) SINGLE WASHED CELLS OF *Coscinodiscus concinnus*, OR (*b*) A LITTLE WATER FROM THE LAST WASH BATH

	Presence of bacteria		
	+	−	
(*a*) With *Coscinodiscus*	12	291	303
(*b*) Control	9	221	230
	21	512	533

Satisfactory direct observation to determine the incidence of attached bacteria could not be made on a sufficiently large sample of *C. concinnus*, but we made some phase contrast counts on *Skeletonema costatum* in the following spring. During the diatom increase there were between three and five bacteria attached to each hundred *Skeletonema* cells, while within a week of the diatom maximum the count had gone up to thirty-two per hundred cells. We repeated the phase contrast counts, but in greater detail, the following year, examining some hundreds of cells on each occasion. The result was essentially the same (Fig. 1); the attached bacterial cell/diatom cell ratio remained low and remarkably constant throughout the phase of diatom multiplication and for several days of the stationary phase. These bacteria appear to have been multiplying at the same rate as the diatoms; alternatively, the cells failing to resist bacterial attachment were a constant proportion of the whole. On the sixth day of stationary phase the bacterial count suddenly began to mount, but by then the diatoms had taken on the unhealthy aspect of a population obviously in need of rejuvenation by auxospore formation, and dead cells had begun to appear in the chains, later presumably to be colonized by saprophytic bacteria. Waksman *et al.*[2] have previously recorded an increase in bacterial activity in the later stages of a (laboratory) diatom growth. The spring increase in the Clyde in 1965 was early and rather light, being terminated either by depletion of nutrient or by exhaustion resulting from the absence of auxospore formation, but not by turbulence or by grazing as the water was calm and zooplankton (*Calanus*) were few and had scarcely started to feed.

Both our sets of observations, therefore, lend support to the idea that healthy pelagic diatoms are virtually free from bacteria and they invite speculation about the defence mechanism of the diatom. If our results are typical, epiphytic bacteria are certainly too few to influence the growth of *Skeletonema*. This conclusion, of course, does not touch the wider issues of microbial/diatom interactions in our waters, for *Skeletonema* merely forms the early spring increase, the first wave of diatom growth, whereas the subsequent history of the water is likely to be influenced by the enormous increase in the numbers of the bacteria as the spring advances.

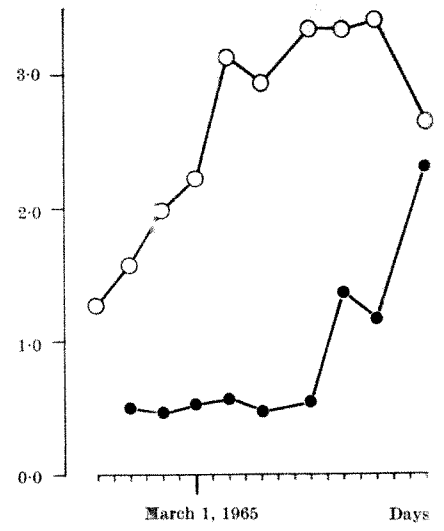


Fig. 1. Time course of the spring increase of *Skeletonema costatum* in the Clyde in 1965. Open circles, $\log_{10}$ diatom cells per ml. from straight samples from Keppel Pier taken at mid tide on the flow. Closed circles, numbers of attached bacteria per ten diatom cells. The water temperature lay between 5·4° C and 6·7° C throughout the period of observation.

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MICROBIOLOGY

D-Glucarate Catabolism by Pseudomonadaceae and Enterobacteriaceae

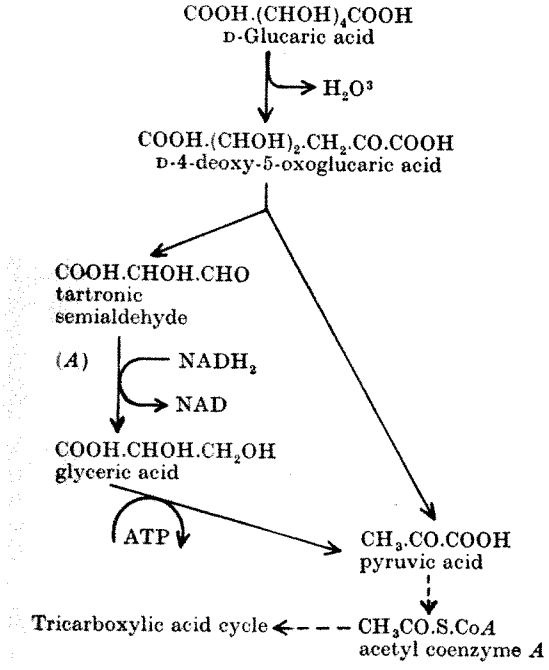
D-GLUCARIC acid, the naturally occurring dicarboxylic acid analogue of D-glucose, can be utilized as the sole source of carbon and energy by many Enterobacteria[1] and Pseudomonads[2]. Blumenthal and Fish[1] found that the induced catabolic pathway shown in Scheme 1 operated in all the Enterobacteria which they investigated (see Table 1).

This pathway (here identified as the glycerate pathway) involves tartronic semialdehyde (HCO.CHOH.COOH) derived from one half of the D-glucarate molecule as a precursor of pyruvate. When cell extracts are incubated with D-glucarate, tartronic semialdehyde accumulates because its conversion to glycerate requires substrate levels of reduced nicotinamide-adenine dinucleotide (NADH). Since tartronic semialdehyde, or its spontaneous decarboxylation product, glycolaldehyde ($HCO.CH_2OH$), forms an α-*bis*-2,4-dinitrophenylhydrazone with a characteristic purple colour in alkali (λ_{max} 550–565 mμ)[5], its accumulation can be detected and used as a diagnostic test for the operation of this pathway in a test organism.

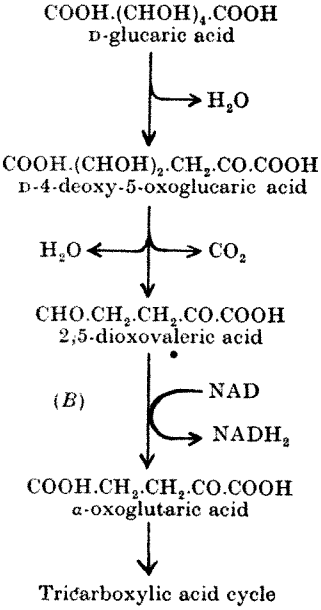
An alternative induced pathway has been reported for a single *Pseudomonas* strain by Dagley and Trudgill[6]. In this pathway, the α-oxo-glutarate pathway, D-glucarate is not cleaved to form two C_3-units but undergoes two dehydrations and a decarboxylation to form 2,5-dioxovaleric acid (Scheme 2).

As the oxidation of 2,5-dioxovalerate to α-oxoglutarate requires substrate amounts of nicotinamide-adenine dinucleotide the 2,5-dioxovalerate accumulates when cell extracts are incubated with D-glucarate[6]. Like tartronic semialdehyde this compound forms a *bis*-2,4-dinitrophenylhydrazone. In contrast to the derivative of tartronic semialdehyde, however, it gives a brown colour in alkali (λ_{max} 433 mμ)[6].

To investigate the frequency of the α-oxoglutarate path way in Pseudomonads, micro-organisms forming morphologically distinct colonies and capable of growth in D-glucarate as source of carbon were isolated by enrichment culture from soil and mud samples gathered in the Aberystwyth area. *Pseudomonas* strains isolated by other workers and found to be capable of growth on D-glucarate were also used. The micro-organisms were grown aerobically in shake culture in a D-glucarate mineral salts medium[6]. They were collected by centrifugation, disrupted by sonication and cell extracts clarified by centrifuging. Finally, extracts were incubated with D-glucarate at 30° C. The pathway followed was elucidated from the nature of the *bis*-2,4-dinitrophenylhydrazone formed by the reaction product. It was found that all the *Pseudomonas* strains investigated utilized the α-oxoglutarate pathway (Table 2), whereas the three strains of *Enterobacteria* which had been isolated followed the glycerate pathway described by Blumenthal *et al.* (Table 1).



Scheme 1. D-Glucarate catabolism by the glycerate pathway (after Blumenthal and Fish (ref. 1)). Dehydration yields predominantly the 4-deoxy-5-oxoglucaric acid (Blumenthal and Jepson (ref. 3)). (*A*) Catalysed by tartronic semialdehyde reductase (ref. 4).



Scheme 2. D-Glucarate catabolism by the α-oxoglutarate pathway (ref. 6). (*B*) Catalysed by 2,5-dioxovalerate dehydrogenase (ref. 6).

Table 1. ORGANISMS UTILIZING THE GLYCERATE PATHWAY OF D-GLUCARATE CATABOLISM

Source	Organism
Soil sample *A*	*Aerobacter aerogenes*
Soil sample *B*	,, ,,
Soil sample *C*	,, ,,
Data of Blumenthal and Fish[1]	*Escherichia coli**
	Escherichia freundii
	Erwinia caratovora
	Aerobacter aerogenes
	Paracolobactrum arizona
	Salmonella typhosa
	Klebsiella pneumoniae

* Nine strains.

Spectrophotometric enzyme assays provided further evidence that the pathways could be attributed to the organisms listed in Tables 1 and 2: only organisms utilizing the α-oxoglutarate pathway possessed high levels of 2,5-dioxovalerate dehydrogenase[6]. Tartronic semialdehyde reductase[4] was present at high levels in those micro-organisms which were operating the glycerate pathway.

Table 2. ORGANISMS UTILIZING THE α-OXOGLUTARATE PATHWAY OF D-GLUTARATE CATABOLISM

Source	Organism*
Soil sample *A*	*Pseudomonas fluorescens*
Soil sample *B*	,, *putida*
Soil sample *C*	,, *acidivorans*
	,, *putida*
	,, *fluorescens*
Soil sample *D*	,, *fluorescens*
	,, *fluorescens*
	,, *fluorescens*
Soil sample *E*	,, *putida*
	,, *putida*
Ref. 7	*Pseudomonas A (acidivorans)*
Ref. 8	,, *C1B (putida)*
Ref. 9	*Pseudomonas fragi (putida)*
Ref. 10	,, *U (putida)*

* Classified by the system of Stanier *et al.* (ref. 2).

Thus the evidence so far accumulated suggests that on these metabolic grounds a division can be drawn along the boundary between the two families of bacteria. Besides the obvious catabolic reaction differences between the two pathways, differences of a more fundamental nature are apparent. The two pathways are in a sense opposites. The glycerate pathway cleaves D-glucarate into two C_3-units both of which yield acetyl coenzyme *A* by way of pyruvate; the necessary provision of C_4-compounds to operate the biosynthetic reactions of tricarboxylic acid cycle is probably achieved by anaplerotic fixation of carbon dioxide on to phosphoenol pyruvate[11]. The formation of α-oxoglutarate in the alternative pathway can provide directly tricarboxylic acid cycle intermediates for biosynthesis. For complete oxidation of the α-oxoglutarate, a proportion of the oxaloacetate formed from it must be converted to acetyl coenzyme A. This is

presumably derived from pyruvate formed by the action of oxaloacetate decarboxylase[12].

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CYTOLOGY

Collagen in Normal Mouse Glomeruli

THE mesangial region of the normal renal glomerulus has been the subject of controversy for many years and is the site of collagen production in some diseases[1,2]. Although there have been many investigations in a variety of animals, we are aware of only one published account which has described collagen fibrils in the normal glomerulus. These were described in the rat[3]. During investigations of experimental renal amyloidosis in the mouse we had cause to examine the glomeruli of normal animals. Careful search of the mesangial regions revealed the presence of typically striated collagen fibrils with periodicity 500–600 Å and showing intraperiod striations. Fig. 1 shows parts of two mesangial cells (the arrows point to collagen fibrils). Fig. 2 is a higher power electron micrograph of the area indicated between the arrows in Fig. 1. The tissue was fixed in buffered osmium tetroxide solution, embedded in 'Araldite', and after sectioning stained for 1 h with 2 per cent phosphotungstic acid dissolved in absolute alcohol.



Fig. 1. Portions of three mesangial cells. Arrows indicate the position of collagen fibrils. (× 10,000.)

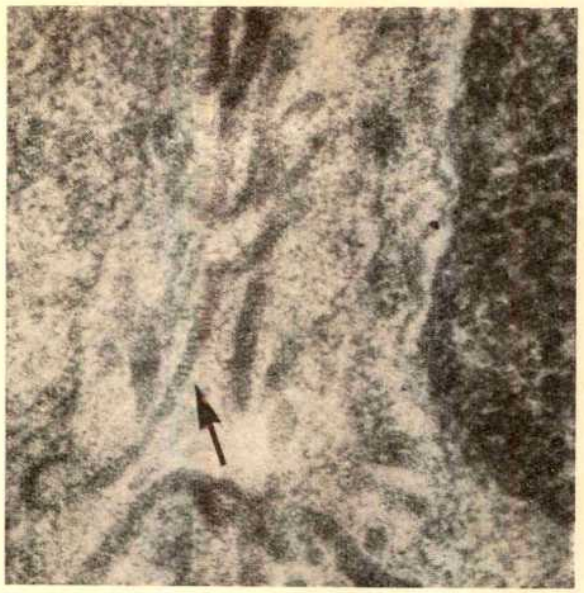


Fig. 2. Higher power electron micrograph of part of Fig. 1. Arrow indicates intraperiod striations. (× 80,000.)

The presence of collagen fibrils within the normal mouse glomerulus establishes that these are not restricted to the rat. This emphasizes the potential functional importance of the mesangial cells. Although such small numbers of collagen fibrils could not offer much structural support to the glomerulus, their presence is indicative of the mesenchymal properties of the mesangial region and its possible role in disease processes.

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Modification of Relative Mutagenic Efficiency in Barley of Mesyloxy Esters by Different Treatments

THE mutagenicity of mesyloxy esters of differing chemical structure has been studied in *Drosophila*, and the marked differences observed have been attributed to differences in the reactive alkyl group and the non-alkylating "prosthetic" parts of the compounds[1]. Relationships between the chemical structure and the biological effects of different monomesylates have also been studied in barley[2]. Although the qualitative and the quantitative differences in the biological effects, including chromosome breakage and mutation rates, produced by this group of compounds have been attributed to a correlation between structure and activity, very little attention has been paid to the physical factors which may influence the reactivity. By "physical" we mean such factors as temperature and hydrogen ion concentration of the treatment solutions, which may influence the reactivity, functionality, stability and the rate of diffusion of a chemical, and which in turn may modify its biological effects. We have therefore studied the modifying effects in barley of temperature and hydrogen ion concentration on various biological effects produced by the three mesyloxy esters—ethyl methane-sulphonate (EMS), methyl methane-sulphonate (MMS) and 1,4-dimethylsulphonoxybutane (myleran). The compounds have different reactive alkyl groups. The present report summarizes data relating to the effect

Table 1. FREQUENCY OF SOMATIC CHROMOSOME BREAKAGE INDUCED BY EMS, MMS AND MYLERAN IN VARIOUS TREATMENT CONDITIONS OF TEMPERATURE AND *p*H (BREAKS/CELL)

		A	*B*	*C*	χ^2
	*p*H	0° C	18° C	32° C	for *A* and *C*
EMS	9·2	0·42	0·42	0·50	0·694
	7·0	0·45	0·44	0·67	4·32
	3·5	0·39	0·43	0·77*	12·44
MMS	9·2	0·41	0·76	0·84	14·78
	7·0	0·58	0·95	1·05	13·55
	3·5	0·59	0·81	1·15†	18·02
Myleran	9·2	0·24	0·53	0·61	16·90
	7·0	0·27	0·50	0·62	13·26
	3·5	0·25	0·46	0·75	2·00

* χ^2_{EMS} for 9·2 and 3·5 *p*H at 32° C = 5·74 significant at the 5 per cent level.

† χ^2_{MMS} for 9·2 and 3·5 *p*H at 32° C = 4·828 significant at the 5 per cent level.

Table 2. CHLOROPHYLL MUTATION RATES INDUCED BY EMS IN VARIOUS TREATMENT CONDITIONS OF TEMPERATURE AND *p*H (AS PERCENTAGES OF M_1 SPIKES SEGREGATING)

	Temperature			
*p*H	0° C	18° C	32° C	Mean
9·2	16·15	21·71	42·19*	26·68
7·0	12·87	23·21	39·18	27·97
3·5	12·45	23·65	32·82	22·97
Mean	13·82	22·85	38·06	

* Effect of *p*H is significant at 32° C.

Table 3. CHLOROPHYLL MUTATION RATES INDUCED BY MMS IN VARIOUS TREATMENT CONDITIONS OF TEMPERATURE AND *p*H (AS PERCENTAGES OF M_1 SPIKES SEGREGATING)

	Temperature			
*p*H	0° C	18° C	32° C	Mean
9·2	9·31	16·34	36·06*	20·58
7·0	8·22	18·15	29·75	18·71
3·5	8·96	17·61	23·52	16·70
Mean	8·84	17·34	29·78	

* Effect of *p*H is significant at 32° C.

of temperature and *p*H on the frequency of chromosome breakage in somatic cells and on chlorophyll mutation rates induced in barley by the three chemicals.

Dry resting seeds of a hulled variety of *Hordeum vulgare* (c.v. *NP* 104) were treated with three mutagen solutions prepared in buffers adjusted to acid (*p*H 3·5), neutral (*p*H 7·0) and alkaline (*p*H 9·2) conditions. Three identical treatments were carried out at 0°, 18° and 32° C for 12 h. Details of treatment and the methods of estimation of chromosome breakage and mutation rates are described elsewhere[3]. Because the comparisons of biological effects could be made for 0·3 per cent of EMS, 0·03 per cent of MMS and 40 mg/100 ml. of myleran at all temperature and *p*H levels, the results for these concentrations alone are summarized in Tables 1, 2 and 3. Myleran induces chlorophyll mutations with very low frequency when dry seeds are used for mutagenic treatments. Thus, for the purpose of comparison, rates observed for previously soaked seeds are given.

A highly significant response to temperature has been observed in all cases, as is evidenced by both chromosome breakage and mutation rates (Tables 1, 2 and 3). The response to *p*H was significant at higher temperatures, especially with EMS and at high concentrations of mutagen. A comparison of the biological effects at different temperature and *p*H indicated that in alkaline conditions the frequency of chromosome breakage is appreciably lower than with acid *p*H, while the frequency of chlorophyll mutations is high (Tables, 1 2 and 3). The converse was observed in acid conditions. This relationship was strikingly similar for all the three chemicals.

The increase in the frequency of chlorophyll mutations in alkaline conditions may be explained by the reduction of injury to the seeds likely to be caused by the acidity of hydrolysed mutagen solutions, which may result in higher recovery.

The specificity of the mutagen enhanced by *p*H, which was evident from the high frequency of chromosome breakage at low *p*H, may also be responsible for the observed differences.

From this study it appears that the production of mutations and chromosome breakage are independent events, and that their relative proportions vary with the treatment conditions to a considerable extent. Analysis of several chlorophyll mutations for their karyotypes indicates no detectable chromosomal change in the complement. The incidence of seed sterility at these doses was roughly 50 per cent in all cases, so that the elimination of mutated cells at low *p*H appears not to be operative.

The three chemicals fall into three distinct categories, on the basis of their ability to induce chromosome breakage and mutations, (*a*) agent that induces a high frequency of mutation accompanied by a low frequency of chromosome breakage (EMS); (*b*) moderately high frequency of mutation accompanied by a high frequency of chromosome breakage (MMS); (*c*) very low frequency of mutation accompanied by a high frequency of chromosome breakage (myleran). It appears that by appropriate manipulation of treatment conditions, any one of these biological effects may be produced in preponderance so as to enhance the mutagenic efficiency of these chemicals.

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PHYSIOLOGY

Slow Response of Crustacean Nerve Fibre in a Solution Deficient in Chloride Ions

IN skeletal muscle fibres the permeability of chloride ions is higher than the permeability of potassium ions[1]. Deficiency of chloride ions leads to a prolongation of the action potential, suggesting that the repolarization phase depends to some extent on anion-current[2]. In the nerve fibre, however, the permeability of chloride ions is relatively low in the resting state and chloride ions are not important for the repolarization phase of the action potential (for example, see ref. 3 for squid giant axon and ref. 4 for Ranvier node of frog).

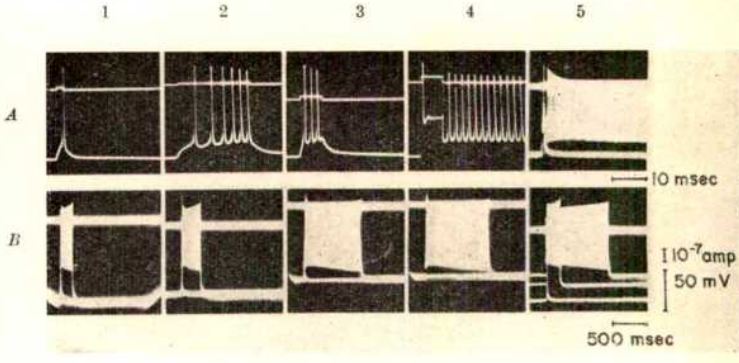


Fig. 1. *A*1 and *A*2, Responses in normal solution (lower traces). 3 min after the chloride had been replaced with ferrocyanide a weak stimulus produced the same response as did the control, but a strong stimulus produced a slow response with repetitive firing (*A*4). *A*5 was taken 5 min after chloride had been replaced with ferrocyanide (the stimulus was the same as that in *A*4, but the record was taken by a sweep speed ten times slower). The membrane remained in a depolarized state even after the repetitive firing ceased. Row *B* shows the effect of hyperpolarization on the slow response. Stimulus intensity and duration were throughout the same as in *A*4 (5×10^{-8} amp, 7 msec). Conditioning hyperpolarization was reduced from *B*1 to *B*4. In *B*5, three responses were superimposed at different hyperpolarization levels. The time scale for *A*5 is 100 msec.

When chloride in the medium surrounding a crustacean nerve fibre is replaced by glutamate, ethanesulphonate or ferrocyanide, it is possible to elicit a slow response to electrical stimulation. The response is a prolonged depolarization similar to that seen in potassium-rich media[5] and is usually accompanied by repetitive spikes. This suggests that there is normally an increase in the permeability of chloride ions and an inward movement of these ions during the falling phase of the action potential. In media free of chloride ions there would seem to be an outward movement of the anion with the formation of a plateau.

Single motor nerve fibres, fast closer or opener fibres, were dissected from the meropodite segment of the walking limb of the crab, *Callinectes sapidus*, collected in the Gulf of Mexico. The techniques used for recording and stimulation were the same as those previously described[6].

Figs. 1 and 2*A* show examples of responses to electrical stimulation in a ferrocyanide solution free of chloride ions, and Fig. 2*B*, those in a glutamate solution free of chloride ions. When the normal solution was replaced with a solution deficient in chloride ions, the membrane potential remained constant for a few minutes, then gradually decreased. The response to stimulation was unchanged for a few minutes (*A*3 in Fig. 1), but then a prolonged depolarization was produced during or after stimulation as shown in *A*4 and 5 in Fig. 1 and Fig. 2*A*. The slow response was usually accompanied by repetitive spike activity. When the magnitude of the slow response was small the spike activity was maintained as long as the slow response continued (Fig. 1 and Fig. 2*A*), but when the slow response was large the spike activity stopped although the depolarization persisted (Fig. 2*B*). The spike shape remained normal throughout these periods of repetitive activity. The peak potential reached during the spike depended on the magnitude of the slow response. As the slow response developed and then decreased, the peak potential decreased and then increased. In a solution free of chloride ions, as the membrane potential fell spontaneous repetitive firing was often initiated at critical firing level and was maintained for long periods of time (Fig. 2, *A*3).

During a slow response without spike activity, cathodal stimulation could no longer produce an active response. A conditioning hyperpolarization, however, restored the excitability of the membrane. Thus the slow response was easily produced by outward current pulses given during the passage of conditioning inward current. As seen in Fig. 2*B*, the repetitive spike activity was reactivated during (*B*1 and 2) or after (*B*3) hyperpolarization. The duration of the slow response depended on the membrane polarization as reported for the slow response observed in potassium-rich media[5]. Hyperpolarization made the response shorter (Fig 1*B*).

The substitution of glutamate and ethanesulphonate for chloride produced more or less similar effects to those of ferrocyanide solution. When thiocyanate was substituted for chloride, neither the slow response nor sustained repetitive firing was seen.

The slow response obtained in solution deficient in chlorine ions cannot result from the decreased calcium ion activity of the solution. Reducing calcium concentration in solution with a normal chloride content did not produce the same kind of response. High calcium concentration, however, reduced the slow response. The slow response in solutions deficient in chloride ions differs from the slow response in solutions rich in potassium ions because the latter response does not show associated repetitive firing.

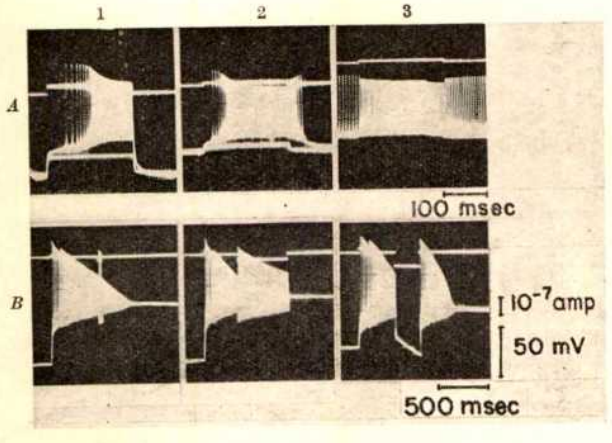


Fig. 2. *A*, The slow responses produced by long stimulating pulses (about 200 msec) in chloride-free ferrocyanide solution. *A*1, 1 min; *A*2, 3 min; *A*3, 5 min after replacing the solution. Repetitive firing was continuous for many minutes in *A*3. *B*, The effect of hyperpolarization during the slow response produced by a 100 msec cathodal pulse in a glutamate solution free of chloride ions. Note reactivation of repetitive spike activity during (*B*1 and *B*2) or after hyperpolarization (*B*3).

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Axon Hillock of Neurones of the Lateral Vestibular Nucleus of the Ox

The axon of vertebrate neurones may arise either from the soma or from a dendrite. When it arises from the soma it may arise directly, as in the Purkinje cells[1], or from a specialized region known as the axon hillock. Little appears to be known regarding the electron micrographic appearance of this region. Electron microscopical studies on isolated neurones facilitate the identification of specific structures, and during our investigations of neurones isolated from the lateral vestibular nucleus of ox (*Bos taurus*) brain we have been able to obtain sections through the axon hillock and the non-myelinated part of the axon.

The neurones were isolated from freshly slaughtered animals by the method previously described[2] and were prepared for electron microscopy in polythene planchets[3]. The observations to be described here were made on cells which had either been isolated in Ringer–Locke solution and fixed in phosphate-buffered glutaraldehyde followed by 1 per cent veronal-buffered osmium tetroxide, or had been isolated in 0·9 per cent sodium chloride containing 0·25 mg, 0·55 mg, or 1·1 mg gangliosides per ml., and fixed in 1 per cent veronal-buffered osmium tetroxide. After dehydration in a graded series of ethanol, the cells were stained with 1 per cent phosphotungstic acid and embedded in 'Araldite'. Isolation, fixation and dehydration were carried out at 4° C.

The axon hillock was identified positively by tracing the origin of the axon from the soma through the non-myelinated part to the beginning of myelination. On account of the size of the structure we have not as yet obtained a section showing the whole of it. Typical pictures of the origin of the axon are shown in Figs. 1 and 2. On the right of Fig. 1, part of the slope of the cone can be seen, and on the left, part of the hillock is shown running into the non-myelinated part of the axon. Fig. 2*a* shows the central core of the axon hillock. The increased density of packing of the neurofilaments, their parallel alignment, and the absence of Nissl substance, characteristics of this region noted by Cajal[4], are clearly illustrated

in the figures. The tubular structures appearing at the bottom of Fig. 1 have been observed frequently in the vicinity of the axon hillock, but we have not as yet been able to determine their relationship to the rest of the structure.

In one case we were able to obtain longitudinal sections through the non-myelinated part of the axon from the point of origin to the start of myelination and thus obtained a measurement of the length of the initial segment (axon hillock plus non-myelinated axon). The initial segment was 50μ long—a figure which is compatible with previous estimations of its length in other neurones[5].

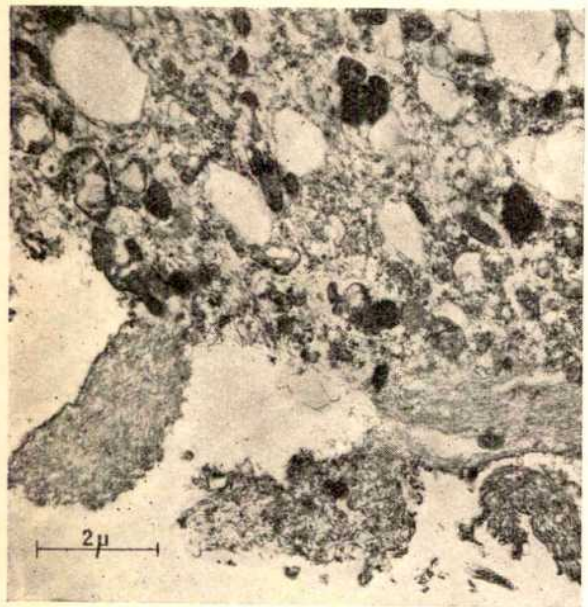


Fig. 1. Part of the cone of the axon hillock.

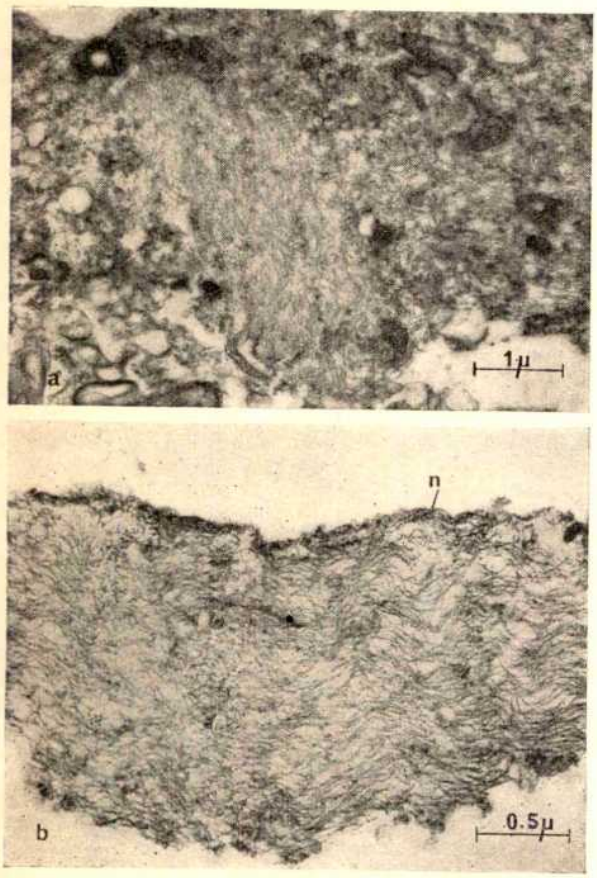


Fig. 2. *a*, Central core of the axon hillock. *b*, Part of the axon hillock at higher magnification showing densely packed neurofilaments (*n*) at the surface.

The most striking electron microscopical feature of the axon hillock is the denseness of the surface. At low magnifications this gives the appearance of a thickened surface membrane (Fig. 1), but at higher magnifications it can be seen that this effect is due to the close packing and alignment of the neurofilaments at the surface (Fig. 2*b*). Unlike the surface of the soma (and most dendrites) which varies in appearance with the composition of the isolation medium, and is very disrupted when cells are isolated in Ringer–Locke solution, this appearance of the axon hillock is consistent. The reason for this is not known. It may indicate an intrinsic difference in the molecular structure of this region, and/or it may be due to there being fewer nerve endings so that the surface is not disrupted by their removal[2,3].

Although it is not known whether the axon hillock of neurones from other regions of the brain presents a similar electron micrographic image, the observations reported here present criteria for the identification of the initial segment in these cells (and possibly others) and should facilitate further observations on this region. Further observations are in progress aimed at clarifying the situation regarding the possible difference in the surface structure, and determining whether or not nerve endings occur on the hillock.

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Oestrus and Ovarian Morphology in Gilts following Treatment with Orally Effective Steroids

THIS communication describes an experiment carried out to evaluate the effect of two combinations of progestin and oestrogen treatments on oestrus and ovarian morphology in gilts.

Attempts to synchronize oestrus in gilts with orally effective progestins have generally resulted in a large proportion of cystic ovarian follicles[1–3]. The level of progestin treatment has also been reported to affect the occurrence of cysts[1,4]. Addition of a gonadotrophin to the treatment regime may be beneficial in reducing cystic conditions[1,4], indicating that an insufficiency of ovulating hormone may be involved in the formation of cysts.

Forty gilts (Duroc, Hampshire, Yorkshire and cross-bred) were used in a trial conducted during May–July 1963. Gilts were checked daily for the occurrence of oestrus with vasectomized boars before treatment. All animals had exhibited at least one oestrous period during this interval, and about half had experienced a complete oestrous cycle (18–23 days in length). The animals were divided into four groups on the basis of breed, weight and stage of oestrous cycle. Two groups were administered daily 25 mg 6-chloro Δ^6-17 acetoxyprogesterone (CAP) per gilt and 1 mg ethynyl oestradiol 3-methyl ether (EE) per gilt. The remaining two groups received daily 125 mg CAP per gilt and 5 mg EE per gilt. These hormones were mixed in 6 lb. of the normal ration and administered for 10 days in individual feeders to ensure that each animal would consume the predetermined amount of CAP : EE.

Three days after termination of CAP : EE feeding, gilts receiving the 25 : 1 combination were injected with 500 I.U. human chorionic gonadotrophin (HCG) and 1 mg oestradiol benzoate (ODB). HCG and ODB were administered intramuscularly in the loin area as separate injections, HCG in 4 ml. physiological saline and ODB in 4 ml. sesame oil. The remaining two groups (125 : 5 CAP : EE combination) received the identical HCG–ODB injections, but the interval between the last CAP : EE feeding and the injections was 5 days. All gilts were checked daily for symptoms of oestrus with intact or vasectomized boars during the experimental period. Animals were slaughtered 7 days after the HCG–ODB injections. The reproductive tracts were then recovered and evaluated.

Both CAP : EE treatment combinations were effective in inhibiting oestrus during the feeding phase. Only half the gilts, however, were observed to be in heat following treatment (twenty out of forty, Table 1). The low level of CAP : EE resulted in nearly twice as many gilts in oestrus as the high level (thirteen compared with seven), but this difference was not statistically significant ($P \sim 0{\cdot}10$). Gilts were observed in oestrus approximately two days following the HCG–ODB injections (range of 1–4 days). Neither the interval to oestrus nor the length of oestrus was significantly affected by treatment.

Examination of the ovaries revealed that 72 per cent of all gilts had one or more cystic follicles (those 15 mm or greater in diameter). Although the number of gilts with cystic follicles did not differ appreciably between treatments, the number of cystic follicles per gilt was significantly different ($P < 0{\cdot}05$). Gilts receiving the higher CAP : EE level had fewer cystic follicles than those receiving the lower level. This difference was also reflected in total ovarian weight ($P < 0{\cdot}05$). The average number of follicles 2–14 mm in diameter was nearly twice as great for the 125 : 5 treatment combination. The average size of these follicles was about 5 mm in both groups. Although the number of these "moderate" sized follicles did not differ significantly between the two treatments, it does suggest that the occurrence of cysts reduces the number of follicles in this classification.

The ovulation rate, as reflected by number of corpora lutea and corpora haemorrhagica, was 13·5 in the 25 : 1 CAP : EE group and 16·2 in the 125 : 5 group. More corpora haemorrhagica were observed in the higher CAP : EE group (not statistically significant), suggesting that ovulatory response was delayed somewhat on this treatment. Results summarized by breed groups are presented in Table 2. The small number of animals involved in two breed groups (Hampshire and crossbred) limits the conclusions which can be drawn concerning breed differences. In general, crossbred gilts differed markedly from the other groups in most of the traits evaluated. Larger numbers would be required to assess the real significance of this apparent difference.

The CAP and EE utilized in this trial were kindly provided by Eli Lilly and Co., Greenfield, Indiana.

Table 1. EFFECT OF CAP : EE TREATMENT ON OESTRUS, OVARIAN MORPHOLOGY AND UTERINE WEIGHTS OF GILTS*

Item	CAP : EE (mg) 25 : 1	125 : 5
No. of gilts	20·0	20·0
No. in oestrus after treatment	13·0	7·0
Days, HCG-ODB to oestrus†	1·9	1·9
(range)	(1–4)	(1–4)
Length of oestrus, days	2·2	1·9
No. of gilts with cystic follicles‡	16·0	13·0
Av. No. of cystic follicles§	6·8	2·0
Av. No. of follicles, 2–14 mm diam.	13·1	23·6
Av. No. of follicles, less than 2 mm diam.	19·5	21·7
Av. No. of corpora lutea	11·6	11·8
Av. No. of corpora haemorrhagica	1·9	4·4
Wt. of ovaries (g)§	71·6	24·5
Wt. of uterus (g)	663·0	642·0

* CAP and EE, administered in feed for 10 days.
† HCG, Human chorionic gonadotrophin; ODB, oestradiol benzoate. All gilts received 500 I.U. HCG and 1 mg ODB intramuscularly following CAP : EE treatment.
‡ Refers to follicles 15 mm or greater in diameter.
§ Significant treatment difference, $P < 0{\cdot}05$.

Table 2. EFFECT OF BREED ON OESTRUS, OVARIAN MORPHOLOGY AND UTERINE WEIGHTS OF GILTS

Breed	Duroc	Hampshire	Yorkshire	Crossbred
No. of gilts	16	6	13	5
No. in oestrus*	9	5	2	4
No. with cystic follicles†	10	4	10	5
Av. No. of cystic follicles	3·7	5·3	3·0	8·8
Av. No. of follicles, 2–14 mm diam.*	19·0	18·8	22·4	5·0
Av. No. of follicles, less than 2 mm diam.*	17·2	28·8	26·8	5·4
Av. No. of corpora lutea*	15·1	9·7	10·2	7·2
Av. No. of corpora haemorrhagica	4·8	0·7	1·0	6·8
Wt. of ovaries (g)	31·7	56·5	21·2	159·9
Wt. of uterus (g)*	671	518	702	625

* Significant difference between breeds, $P < 0{\cdot}05$.
† Refers to follicles 15 mm or greater in diameter.

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Guinea-pig Mast Cells and Age

IN the course of certain experiments on guinea-pigs, it was considered relevant to examine the mast cell population with reference to the age and degree of sexual maturation reached by the animals.

Guinea-pigs were killed by cervical section, and their abdomens opened by a median incision. The greater omentum was spread thinly on a slide and excess fat removed. The slides were then subjected to the technique of Shelley and Florence[1] which involved fixation with ice-cold ethylene glycol monomethyl ether as these mast cells are water soluble, and staining with 0·25 per cent aqueous methylene blue. After dehydration and before mounting in 'Depex', it was necessary to vary the time in xylol depending on the amount of fat present. Mast cells were found in greatest numbers in the perivascular and adjacent fat tissue; ten fields representing 7·35 mm² were counted for these cells. Similar methods were used by Cairns and Constantinides[2], and Koltai, Minker and Deak[3].

The average count with ten female adult guinea-pigs was 37 ± 2·5 mast cells, while immature female animals had an average count of 110 ± 5·9 cells. When bilateral oophorectomy was performed, and counts made 2–3 months later, an average figure of 42 ± 5·5 mast cells was obtained. Statistically, this is not significantly different from the value found for the adult unoperated animals. Comparable results were met in the male animals, adults having average counts of 55 ± 3·2, immature 104 ± 3·6 and orchidectomized guinea-pigs 51 ± 4·5 mast cells. The difference between the figures obtained from normal male and orchidectomized animals was not significant. These findings suggest that the sex hormones are probably not important in this connexion especially as there is no apparent change in the count with sexual maturity. My conclusion that oophorectomy fails to influence the mast cell count is supported by Harvey[4], who also found that oestrogens given to spayed female hamsters did not have any effect on the population of mast cells. Constantinides and Rutherdale[5], working on rats, reported that neither testosterone nor orchidectomy changes the cardiac mast cell population in the male. Similarly, in the female, the cardiac mast cell count is not affected by oophorectomy or by the injection of progesterone.

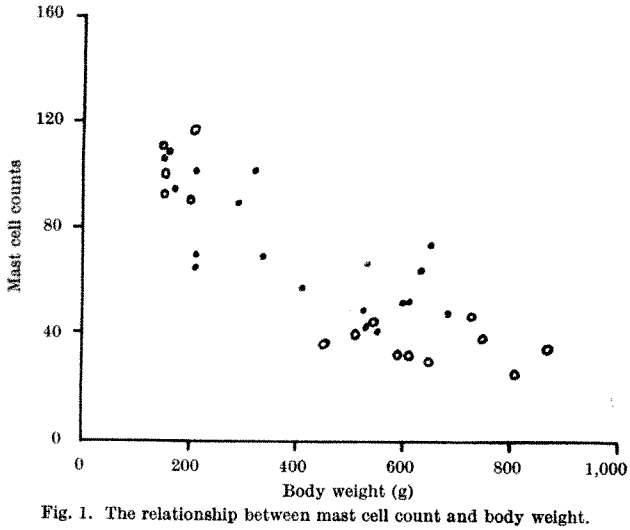


Fig. 1. The relationship between mast cell count and body weight.

When the mast cell counts of the guinea-pigs were plotted against body weight, a rectilinear relationship was found (Fig. 1). The correlation coefficient for the female animals was 0·89 and for the males 0·77. Selye[6], in his intensive bibliography, presents no constant picture of the mast cell count in the ageing process, and makes no mention of it in guinea-pigs. Because weight gives a reasonable estimate of the age of these animals, my results suggest that there is a significant fall in the omental mast cell count with age within the range studied.

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Metabolic Differences between Dietary Liquid Glucose and Sucrose

IN the fifty years since Higgins[1] obtained evidence that fructose has a greater tendency to change into fat in the body than has dextrose, more evidence has accumulated, much of it in the past few years, to show that lipid metabolism in man and experimental animals is affected differently by different carbohydrates[2–5]. Recently, it was found that substitution of sucrose for starch in the diet of weanling and three month old rats caused metabolic changes[6], and that diets containing sucrose, and more especially fructose, increased plasma cholesterol levels and carcass and liver fat of adult rats compared with diets containing dextrose or liquid glucose or a standard laboratory diet[7].

Long-term feeding trials in man under strict experimental conditions are difficult, but studies of primates are desirable to investigate further the possible connexion between dietary sucrose and ischaemic heart disease[8–10]. The susceptibility of baboons to develop atherosclerosis has been demonstrated[11], and at present we are using mature baboons (*Papio anubis* = *P. doguera*) for long-term feeding trials in which high carbohydrate diets are being compared. In preliminary studies with a small group of twelve animals, differences have been found between baboons fed liquid glucose and those fed sucrose; the present findings support the accumulating evidence that dietary carbohydrates are not all metabolically equivalent.

Sexually mature animals weighing about 9·5 kg were fed for 26 weeks on diets containing 74 per cent available carbohydrate, either as spray-dried liquid glucose BPC (ref. 12) (an aqueous preparation of partially hydrolysed starch) or as sucrose, or a control diet containing 47 per cent available carbohydrate mainly as starch but with some sucrose (4·5 per cent) and liquid glucose (4·5 per cent). The results are summarized in Table 1.

Table 1. MEASUREMENTS ON BABOONS FED LIQUID GLUCOSE, SUCROSE OR CONTROL DIETS FOR 26 WEEKS*

	Liquid glucose	Sucrose	Control
Total serum lipid, mean value over 26 weeks (mg/100 ml.)	347 ± 29	458 ± 27	341 ± 22
β-lipoprotein fraction (precipitation index (ref. 13)), mean value over 26 weeks	1·3 ± 0·1	1·8 ± 0·1	1·5 ± 0·1
Results at end of experiment			
Energy value of food consumed (kcal × 10^3)	122·1 ± 4·2	139·1 ± 8·5	103·5 ± 3·1
Weight gained (kg)	1·0 ± 0·57	2·2 ± 0·51	2·6 ± 0·16
Increase in thoracic girth (cm)	0·3 ± 1·41	3·5 ± 1·70	2·6 ± 1·33
Abdominal depot fat + epididymal fat (g)	33·6 ± 7·0	81·5 ± 5·2	38·6 ± 19·7
Lipid content of abdominal depot fat (g/100 g)	60·5 ± 10·3	80·2 ± 1·7	64·3 ± 5·3

* The values are means and standard errors of means for groups of four mature baboons, two male and two female. Differences between sexes were not significant.

The total serum lipid of the baboons fed the three diets increased during the first 2 weeks, and the increase was greatest in the animals fed sucrose; at the end of the experiment, the total serum lipid in these was significantly higher ($P < 0·01$) than in the animals fed liquid glucose or the control diet. The serum β-lipoprotein was also significantly higher ($P < 0·01$) in the baboons fed sucrose than in those fed liquid glucose, while the corresponding value in the control group was intermediate.

Although the animals fed sucrose showed trends towards higher food intake, greater weight gain and larger increase in thoracic girth than those fed liquid glucose, the differences were not statistically significant.

In the animals fed sucrose, the combined weight of abdominal and epididymal fat was significantly greater ($P < 0·01$) than in those fed either of the other diets. The abdominal depot fat from the animals fed sucrose also contained significantly more lipid ($P < 0·01$) than that from the other animals.

Small atherosclerotic plaques were found in some animals, but their frequency was not related to the diet used and the lesions were apparently of long standing.

Our initial results indicate that, compared with a high liquid glucose diet, a high sucrose diet increases the level of serum lipid, and that this increase is associated with an increase in the amount of abdominal fat deposited. If these results with primates are confirmed in tests with larger numbers they could have implications for human diets.

We thank Dr. G. A. Gresham for examining the aortae at post-mortem.

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PATHOLOGY

Breast Cancer in a Tree Shrew (*Tupaia glis*)

On May 29, 1965, a female tree shrew—*Tupaia glis sordida*, according to the system of Lyon[1]—was found in a late stage of pregnancy on Tioman Island off Malaya. A nodular lesion measuring about 4 mm was present beneath the skin of the right thoracic breast near the nipple. No other lesions were found and there were no large ectoparasites. The animal was marked and released in accordance with standard procedure[2]. Three days later, the animal was found dead in a trap; the cause of death was not apparent, and the animal was well preserved and had obviously given birth since it was released. The carcass was injected with 70 per cent alcohol, and a week later placed in a strong solution of formaldehyde, and taken to the United States. On arrival it was washed in water and examined.

The skin was intact except for a reddish-grey, wrinkled, somewhat indurated area of skin surrounding the right thoracic nipple, which measured about 4 mm in greatest dimension. The nipple itself was short and retracted. Beneath the skin about 1 cm to the right of this area and a little below it was a firm nodule 3 mm in diameter, which was not freely movable. A somewhat smaller area of discoloured and indurated skin was found near and above the abdominal nipple. The thoracic and abdominal breast nipples on the left side were prominent and intact, and measured slightly more than 2 mm in length. The skin on the left side showed no lesions and felt soft.

Dissection of the skin from the thorax and abdomen on the left side revealed a layer of enlarged, lobulated, yellow breast tissue extending from the upper thorax to the inguinal region, which measured 5 mm in thickness. Dissection of the skin and underlying breast tissue from the right side was more difficult. In general, the breast tissue on this side was much thinner (less than 3 mm), and less uniformly enlarged than on the left. Breast tissue was almost wholly absent from some parts of the thoracic and abdominal region. The subcutaneous nodule near the right thoracic nipple was found to consist of poorly demarcated, firm, grey tissue. The thoracic and abdominal viscera were grossly normal in arrangement and appearance. The bicornuate uterus was enlarged, and both uterine horns were dilated, which was consistent with the recent pregnancy. The ovaries were small.

Sections of skin and underlying breast tissue from the intact left side of the thorax and abdomen showed well-preserved normal epidermis and dermis and a large amount of normal lactating mammary tissue. Much of the subcutaneous fat and breast tissue from the right side of the thorax and abdomen was replaced by an invasive malignant tumour of epithelial origin.

The subcutaneous nodule consisted of (1) glandular tumour tissue (Fig. 1*a*); (2) extensive intraductal tumour growth with distension and inflammation of ducts; (3) a small amount of normal lactating mammary tissue. The tumour showed areas of acute and chronic inflammation with oedema and fibrosis, especially around the enlarged and greatly distended ducts filled with tumour cells and debris. Many tumour cells showed large secretory vacuoles in the cytoplasm, but only in the more differentiated portions of the tumour. Small calcified concretions were scattered throughout the tumour.

Sampling of the skin from the entire right side showed that the tumour had spread from above the right thoracic nipple to a little below the abdominal nipple. Although the tumour was confined to the subcutaneous tissues and the dermis, leaving the epidermis largely intact, it had invaded lymphatic channels in the dermis and perineural spaces, compressing the nerves (Fig. 1*b*). There was an isolated intraductal papillary lesion in one section of lactating breast tissue which otherwise showed no abnormalities.

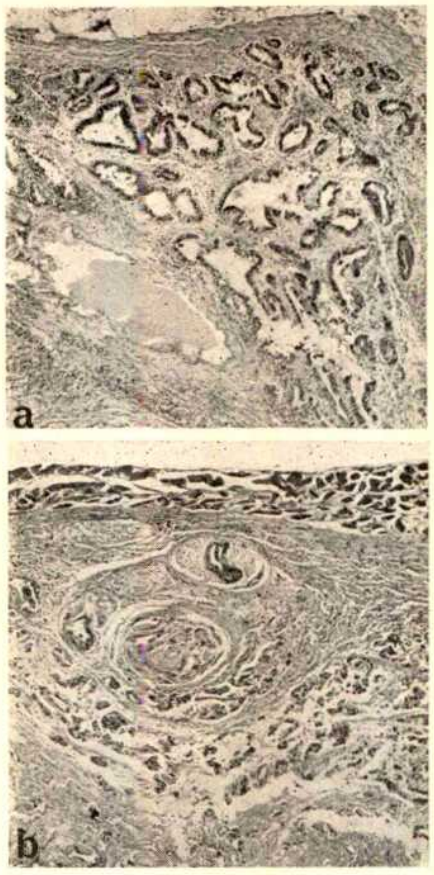


Fig. 1. Adenocarcinoma of breast (*a*) with invasion of perineural spaces compressing nerve (*e*) of a tree shrew (*Tupaia glis*). (× 50.)

A careful search was made for parasites in the tumour and in adjacent breast tissue, but none was found.

This adenocarcinoma is the only tumour which we observed during a study of tree shrews on the Malayan Peninsula over a period of 4 years. About 400 animals were observed carefully enough to ensure detection of tumours or other external abnormalities had they been present. Of these 75 per cent were adults, 55 per cent females and thirty-nine were pregnant. Although many instances of "spontaneous" benign and malignant tumours in sub-human primates have been reported, we have found no reference to neoplastic disease occurring in Tupaioidea[3].

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GENERAL

Evaluation of the Queue Length Distribution for some Queues with Correlated Inputs

THE need for further progress in the analysis of queues with correlated inputs has been referred to by Kendall[1] and Lindley[2]; while systems with randomly delayed regular inputs have been studied[3,4], explicit results do not appear to be available except when the disturbances from regularity are small. Consider the case of a single server queue, with exponential service times $\mu e^{-\mu t}dt$ ($\mu^{-1} = \rho$, the traffic intensity) and an input generated by superposing independent random exponential delays $\lambda e^{-\lambda t}dt$ on a grid of scheduled time-points . . ., −1, 0, 1, 2, ... The characteristics of this system ("model A", say) are so far unsolved, but Kendall has suggested that progress might be possible if the model were modified by redistributing the arrivals randomly within each scheduled interval $(i, i + 1)$ ("model B"). Except when λ is large (the nearly regular case), model B approximates to model A. The purpose of this communication is to show, with examples, that explicit results can be obtained with this type of modified model.

Let x be the number of overdue arrivals and y the number queueing (including the one, if any, being served) at time 1^-, that is to say just before a scheduled point. Then (x) and (x, y) are each Markovian. If m and n are the corresponding values at 0^-, the number of arrivals in $[0, 1)$ is $m + 1 - x = a$, say. Assume that the distribution of these a arrivals over $[0, 1)$ depends only on the values of n and a; this defines a class of models which includes model A, model B and also, for example, model C: all a arrivals at 0^+, and model D: all a arrivals at 1^-.

For the Markov chain (x), the transition probability $p_{x|m}$ is $\binom{m+1}{x} p^{m+1-x} q^x$, ($p = 1 - e^{-\lambda}$, $q = e^{-\lambda}$), and the stationary distribution of x, ω_x say, can be readily computed from the generating function

$$\Sigma\omega_x z^x = \prod_{j=1}^{\infty} (1 - q^j + zq^j)$$

For the Markov chain (x, y), the transition probability $p_{x,y|m,n}$ is of the form $p_{x|m}\, p_{y|a,n}$, vanishes when $x > m + 1$ and also when $x + y > m + n + 1$, and equals $p_{x|m} e^{-\mu} \mu^{a+n-y}/(a + n - y)!$ when $m + 1 < x + y \leqslant m + n + 1$; thus the transition probability matrix takes the form

$$\begin{pmatrix} pN_1 & qN_0 & 0 & 0 & 0 & . & . \\ p^2N_2 & 2pqN_1 & q^2N_0 & 0 & 0 & . & . \\ p^3N_3 & 3p^2qN_2 & 3pq^2N_1 & q^3N_0 & 0 & . & . \\ . & . & . & . & . & . & . \end{pmatrix}$$

where rows are labelled by m and columns by x, each element being a sub-matrix associated with a set of transitions for fixed m, x. The sub-matrices $\mathbf{N}_a$ (in which rows are labelled by n and columns by y) are stochastic. $\mathbf{N}_0$ is the same for all input models of the class, with (n, y)th element 0 for $n < y$, $e^{-\mu}\mu^{n-y}/(n-y)!$ for $n \geqslant y > 0$, and $e^{-\mu}S_n$ for $y = 0$, where S_n denotes $\sum_{r=n}^{\infty} \mu^r/r!$. For $a \geqslant 1$, the $(a + j)$th column of $\mathbf{N}_a$ is the same as the jth column of $\mathbf{N}_0$ when $j > 1$; the distribution of probability among the first $a + 1$ columns of $\mathbf{N}_a$ depends, subject to the stochastic condition, on the particular input model (A, B, etc.). Accordingly, if the stationary distribution of x, y is denoted by c_{xy}, with $(c_{x0}, c_{x1}, \ldots) = \mathbf{c}'_x$, the probabilities c_{xy} satisfy equations of the form

$$\sum_m \binom{m+1}{x} p^{m+1-x} q^x\, \mathbf{c}'_m \mathbf{N}_{m+1-x} = \mathbf{c}'_x$$

It is advantageous to post-multiply by $\mathbf{N}_0^{-1}$, which gives

$$\sum_m \binom{m+1}{x} p^{m+1-x} q^x\, \mathbf{c}'_m (\mathbf{N}_{m+1-x}\mathbf{N}_0^{-1}) = \mathbf{c}'_x\mathbf{N}_0^{-1}, \quad \ldots \quad (1)$$

because one finds that the matrices $\mathbf{N}_a\mathbf{N}_0^{-1}$ take simple forms. $\mathbf{N}_0^{-1}$ itself is given* by $\mathbf{N}_0^{-1}(\mu) = \mathbf{N}_0(-\mu)$. Thus, in the case of model C, $\mathbf{N}_a\mathbf{N}_0^{-1} = \mathbf{U}^a$, where

$$\mathbf{U} = \begin{pmatrix} 0 & 1 & 0 & 0 & . & . \\ 0 & 0 & 1 & 0 & . & . \\ 0 & 0 & 0 & 1 & . & . \\ . & . & . & . & . & . \end{pmatrix}$$

Equations (1) can then be solved iteratively in any given numerical case, providing μ is not too near to 1. For example, when $e^{-\lambda} = 0{\cdot}5$ (that is, mean delay = 1·44 × scheduled interval) and $\mu = 2$ (that is, traffic intensity = 0·5), the stationary distribution is as follows:

x/y	0	1	2	3	4	5	6	
0	0·1657	0·0636	0·0387	0·0151	0·0044	0·0011	0·0002	0·2888
1	0·3490	0·0738	0·0302	0·0085	0·0020	0·0004	0·0001	0·4640
2	0·1803	0·0210	0·0057	0·0012	0·0003			0·2085
3	0·0334	0·0021	0·0004					0·0359
4	0·0026	0·0001						0·0027
5	0·0001							0·0001
	0·7311	0·1606	0·0750	0·0248	0·0067	0·0015	0·0003	1·0000

The known values of $\omega_x = \Sigma_y c_{xy}$ determine at the start of the calculation the effective x-range (for example, for $\lambda = 0{\cdot}1$ one would take, say, $4 \leqslant x \leqslant 16$); the effective y-range for each x is not predetermined but emerges as a by-product of the solution. Beginning with estimates such as $(\hat{c}_{x0})_1 = \omega_x$, $(\hat{c}_{xy})_1 = 0$ $(y > 0)$, one solves each of the equations (1) in appropriate order for the leading $\hat{c}_{xy}$ on the right hand side, takes $(\hat{c}_{x0})_2$ for the next cycle as $\omega_x - \Sigma_{y>0}(\hat{c}_{xy})_2$, and so on. The particular distribution tabulated here, and also the one given below for model B, have been approximately checked by means of equivalent Monte Carlo runs.

For model B, which is of particular interest, the (n, y)th element of $\mathbf{N}_a$ can be shown to be

$$\frac{e^{-\mu}\mu^{n+a-y}}{(n+a-y)!} + \left\{\frac{a!}{(a-y)!\,\mu^y} - \frac{a!}{(a-y-1)!\,\mu^{y+1}}\right\} e^{-\mu} S_{n+a+1}$$

for $y \leqslant a$, $a \geqslant 1$, whence

$$\mathbf{N}_a\mathbf{N}_0^{-1} = \begin{pmatrix} 0 \ldots 0 & -a!\,\rho^a S_{a+1} & a!\,\rho^a S_{a+1} + 1 & 0 & 0 & . & . \\ 0 \ldots 0 & -a!\,\rho^a S_{a+2} & a!\,\rho^a S_{a+2} & & 1 & 0 & . & . \\ \underbrace{0 \ldots 0}_{a-1 \text{ columns}} & -a!\,\rho^a S_{a+3} & a!\,\rho^a S_{a+3} & & 0 & 1 & . & . \\ & . & . & . & . & . & . & . \end{pmatrix}$$

The equations for the c_{xy} can be solved numerically as before. With $e^{-\lambda} = 0{\cdot}5$ and $\mu = 2$, for example, the stationary distribution for model B is as follows:

x/y	0	1	2	3	4	5	6	7	
0	0·0824	0·1026	0·0654	0·0272	0·0085	0·0022	0·0005	0·0001	0·2889
1	0·2371	0·1485	0·0571	0·0164	0·0039	0·0008	0·0002		0·4640
2	0·1450	0·0491	0·0116	0·0023	0·0004	0·0001			0·2085
3	0·0295	0·0055	0·0008	0·0001					0·0359
4	0·0024	0·0003							0·0027
5	0·0001								0·0001
	0·4965	0·3060	0·1349	0·0460	0·0128	0·0031	0·0007	0·0001	1·0001

It is interesting to note that the probability of the server being free at time 1^- is 0·4965, slightly below the overall value $1 - \rho = 0{\cdot}5$; there is a phase effect, the value of any such probability varying with the position in the time-grid at which it is measured. The phase effect is small except for large values of λ. In the extreme case of regular arrivals ($\lambda \to \infty$) re-positioned one in each grid interval according to model B, the probability of the server being free at 1^- can be shown to be

$$1 - \frac{\beta^2(e^{\mu} - 1)}{\beta e^{\mu} - 1}$$

where $e^{(\beta-1)\mu} = \beta$. When $\rho = 0{\cdot}5$ this comes to 0·4739.

Part of this research was done during the summer of 1965 while I was working at the Statistical Laboratory,

* I am indebted to the referee for pointing out that this is a consequence of the fact that $N_0(\mu) = \exp(\mu U')$.

University of Cambridge. I thank Prof. D. G. Kendall for his encouragement and helpful discussions.

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Nature's Time-scale

PROF. MCCORMICK[1] has recently suggested that "all naturally occurring phenomena proceed according to exponential functions of time and each process in Nature conforms to its own unique time scale". The empirical equation:

$$Y = \exp(-kt^{-c})$$

is suggested as an appropriate quantitative formulation, the values of k and c depending on the process considered.

There is no doubt that an extremely diverse series of processes do indeed closely obey a law of the kind considered, so long as they occur under controlled conditions, and it is well worthwhile to direct attention again to this.

It must of course be noted that the formulation does not represent any natural phenomenon if taken literally, that is as valid for all values of t up to infinity, but must be subjected to an arbitrary cut-off at a finite value of t at the end of the experiment or when the subject dies or is thrown away, etc. This objection may fairly be regarded as trivial but even so it is scarcely justifiable to claim that such a formulation is relevant to all natural phenomena. Even if we limit ourselves, from the entire range of nature, to a two-component biological system, there are two common classes which cannot be even approximately represented by such a formula.

With changes only in quantitative properties of the components in such a system, we can illustrate the class which McCormick suggests to be universal, or either of the two quite different classes.

Suppose that we start with a few individuals of a single species of herbivore, with no natural enemies, confined to a limited area on which grows a single species of vegetation.

Case 1. Suppose that the vegetation is a continuously growing perennial type like a turf-forming grass and that the herbivore is a continuously breeding kind, with marked territorial habits, that is in which each breeding pair is able to defend an area adequate for the sustenance of itself and its offspring against other members of the species (as, for example, do grouse). The excess population unable to find unoccupied territory does not breed. Then the system will be well represented by one of McCormick's curves in which there is an initial exponential growth of the herbivore population, which later slows down as the breeding population expands to occupy the whole area available. The population will then tend to a stable equilibrium which will remain unchanged until it is disturbed from outside or until the character of one of the species changes.

Case 2. Suppose now that the herbivore exhibits no territorial behaviour, that the vegetation has a marked seasonal growth and that this seasonal growth continues for a period long compared with the breeding period of the herbivore.

Again, the early part of the curve showing the growth of the herbivore population will be closely in agreement with the early exponential part of one of McCormick's curves. As the growing season finishes, however, the herbivores may have reached numbers which cannot possibly be supported by the dormant vegetation until the next growing season; if they are reasonably efficient at finding residual vegetation they will destroy this and the entire animal population will die, a result which cannot be represented from any modification of the exponential relation already given.

Case 3. Here we will suppose a very large effect of abundance of food on the fertility of the herbivores—whether this is physiological or behavioural is unimportant—together with a maturation period long compared with the recovery rate of vegetation. The latter will be supposed to be continuously growing as in Case 1. Here again the initial rate of rise of population is normally exponential, but as the food supply begins to dwindle the reproduction rate will drop. This will not at first reduce the number of consumers, so that the food and the reproduction rate will dwindle more rapidly still until the population begins to fall as a result of starvation or old age. Eventually a minimum number of animals will be reached which the damaged vegetation can just keep alive. No reproduction occurs in this period, and presently old age will begin to remove further herbivores. The vegetation can then begin to recover and will soon reach a level at which reproduction of the herbivores becomes possible again. Because maturation is slow, however, this does not for a long time appreciably increase the consuming population, and the vegetation is able to escape from its limitation and build up to its original luxuriance.

The herbivores can now begin to expand exponentially again and, in suitable conditions, the process will be repeated indefinitely, giving a stable periodic variation in herbivore numbers of the kind known to engineers as a relaxation oscillation.

Such a periodic variation must be represented by a Fourier series and cannot be even remotely represented by McCormick's "general" equation.

In real life none of these cases will occur in so simple a form, but it would seem to me that a periodic solution of the general type of Case 3 is far more frequently found in nature than either of the other two. The conditions for such oscillations are well known to engineers; they occur whenever a change of any quantity produces effects which will tend to reverse this change but which operate after a time lag. The numerical relations determining the frequency of oscillation are given in any advanced textbook of electrical engineering.

I should like to make a final comment on the proposal for logarithmic scales of temperature or pressure. This, though undoubtedly advantageous in some ranges, is not so over the ranges commonly used. Let us write pT for temperature on a logarithmic scale by analogy with pH; suppose that we take what is now written as 1°K as our "base" where $pT = 0$ so as to avoid negative values over our main working range. Then normal human mouth temperature will be $pT = 2{\cdot}4912$ (36·9° C) while a high fever will be represented by $pT = 2{\cdot}4955$ (40° C). When the temperature falls below $pT = 2{\cdot}436$ there is danger of ice on the roads while on a hot day the temperature might reach $2{\cdot}493$ in the shade. The entire range of biological phenomena will occur between $pT = 2{\cdot}4$ and $pT = 2{\cdot}6$. This is not convenient. Such quantities as specific heat or thermal conductivity would be no longer even approximately constant. The fact is that in most of our ordinary experience, and in much of our scientific work, we are concerned with temperature differences, not ratios, and the former are not readily handled on a logarithmic scale. I would invite Prof. McCormick to rewrite Newton's law of cooling using a logarithmic temperature scale.

In the case of pressure also, there are practical inconveniences in recording small changes; Boyle's law, for example, becomes very awkward unless we also measure volumes logarithmically. Then, indeed, it becomes even simpler than the standard formulation, but it will be difficult to persuade the ordinary man that to order one unit of milk he writes 0 on the slate but to get two units he must write 0·301. Incidentally, it is incorrect

to say that a perfect vacuum has never been obtained. In any radiation-shielded experiment at very low temperatures, say 0·1°K or less, the vapour pressure even of helium is so low that there will be as many as one free molecule of gas in the cooled volume less than once a century.

The logarithmic notation, as in the case of *p*H, or of the decibel used by the electrical engineer, is valuable where very long ranges are habitually used, with only moderate accuracy required. For limited ranges and high accuracies it is not advantageous.

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[1] McCormick, N. G., *Nature*, 208, 334 (1965)

PROF. FREMLIN'S criticism is based primarily on the inability of the equation, $Y = \exp(-kt^{-c})$, to describe adequately natural processes that do not have apparent sigmoidal time-course curves. Of the three hypothetical situations presented, I agree that the first would describe a typical sigmoid curve which can be generated by the basic equation. The time-course of the type of process outlined in the second case would resemble a frequency distribution function, a curve which is generated by the first derivative of the equation[1]. My colleagues and I have suggested that the equation may represent a new type of distribution function[2]. The similarities between the parameters of the equation and those of the Weibull distribution function[3] have been noted[4].

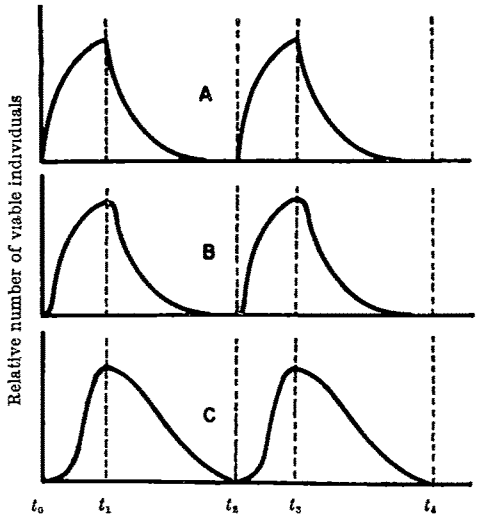


Fig. 1.

Fremlin states that the third hypothetical case gives rise to a curve resembling that known as a "relaxation oscillation", in which instance the resulting curve could not be generated by the equation. He refers to a stable periodic variation which implies to me that each cycle of growth must achieve the same maximum density before it declines into the death phase. If the curve describes a relaxation oscillation it must increase in some exponential manner to a maximum, then decrease in an exponential manner, as in Fig. 1*A*. Any process of growth as it occurs in nature will possess an induction period and the sharpness of the peak will be rounded off as in Fig. 1*B*. In this case the "relaxation oscillation" might be represented by the following series of sigmoidal growth curves and sigmoidal decay curves:

$$\int_{t_0}^{t_1} Y'_a\,dt - \int_{t_1}^{t_2} Y'_b\,dt + \int_{t_2}^{t_3} Y'_c\,dt - \int_{t_3}^{t_4} Y'_d\,dt \ldots \qquad (1)$$

where Y'_a and Y'_c are the rates of increase for the phases of growth and Y'_b and Y'_d are the rates of decline for the death phases. As an alternative, the experimental curve might better resemble a series of distribution functions as in Fig. 1*C*. This curve might be represented by the series

$$\int_{t_0}^{t_2} Y''_a\,dt + \int_{t_2}^{t_4} Y''_b\,dt \ldots \qquad (2)$$

Depending on the values of k and c for each function, either equation can generate a relaxation oscillation in which each successive wave is diminished in amplitude. By proper selection of the limits of integration the duration of each succeeding cycle can be made to increase. These properties are more likely to be found in nature than an oscillation with periods of constant amplitude and equal duration. It may be of some interest that higher order derivatives of the equation generate damped oscillation curves with cycles of decreasing amplitude and increasing duration.

A discussion of the aforementioned points is relevant to the acceptance or rejection of the proposal of exponential time scales[5]. A polemic over the issue of logarithmic scales of temperature of pressure can, however, serve no useful purpose at this time. Fremlin states in several instances that inconvenience is a major reason for not considering these scales. It is my contention that the inconveniences are not insurmountable and are far overshadowed by the possibilities of a better understanding of nature. The main point I am trying to make is the proposal that all processes in nature conform to exponential functions of time, and that the time-scale of each process is exponentially related to the time-scale of every other process. I am proposing a different kind of clock than the one our senses dictate, an inconvenient clock, perhaps, but a clock that affords a different perspective into nature's time-scale.

I would like to point out that the equation applies ideally to a process in a closed system where the time-course of the process is determined by the initial conditions. So long as no outside influence is exerted on the system after time zero (t_0), the process (Process I) flows smoothly and continuously. The instant any condition changes, Process I is terminated and a new process (Process II) comes into existence, with new initial conditions and a new time zero (τ_0). All preceding events experienced by the constituents of Process II are of no consequence because they are all incorporated into the new initial conditions. Thus Process II cannot continue to be timed in reference to the former t_0, but only with reference to the new τ_0 involving its own initial conditions.

My reply to the criticism of the statement concerning a perfect vacuum is that so long as the data must be expressed as the probability of finding a molecule within a specified volume of space at a specified time, I can agree only that the probability approaches zero as the temperature approaches absolute zero and, accordingly, the vacuum approaches a perfect vacuum.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Sunday, September 4—Wednesday, September 7

BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (at Nottingham)—Continuation of 128th Annual Meeting.

Sunday, September 4

At 10.45 a.m.—Official Service at St. Mary's Church. Preacher: Canon D. R. Feaver.

Monday, September 5

At 10 a.m.—Prof. J. S. Weiner: "Aspects of Human Adaptation" (Presidential Address, Section H).

At 10 a.m.—Prof. J. N. Bradley: "Shock Waves in Chemistry and Physics" (Kelvin Lecture).

At 10 a.m.—Dr. D. Noble: "How the Heart Beats" (Darwin Lecture).

At 2.30 p.m.—Prof. R. L. F. Boyd: "The Rocket Age" (Young People's Lecture).

At 8 p.m.—Dr. N. Tinbergen, F.R.S.: "Behaviour as Equipment for Survival" (Evening Discourse).

Tuesday, September 6

At 11.30 a.m.—Prof. R. J. Ball: "Economic Model Building for Control" (Lister Lecture).

At 2.30 p.m.—Prof. A. R. Gemmell: "Biology and Crime" (Young People's Lecture).

Monday, September 5—Friday, September 9

BRITISH PHARMACEUTICAL CONFERENCE (at Manchester)—103rd Annual Meeting.

Thursday, September 8

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 3 p.m.—Meeting on "The Further Education of the Graduate Chemical Engineer". Chairman: Mr. F. E. Warner. Speakers: Prof. Frank Morton, Mr. H. Arthur and Mr. John Kane.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURER or ASSISTANT LECTURER (with good professional qualifications and experience in ophthalmic optics or in a related science) IN THE OPHTHALMIC OPTICS SECTION OF THE DEPARTMENT OF PHYSICS—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4, quoting Ref. 495/2 (September 9).

LECTURER (graduate in science, already with, or wishing to have, experience and research opportunities in virology, including clinical virology) IN THE DEPARTMENT OF BACTERIOLOGY—The Secretary, The University, Aberdeen (September 10).

RESEARCH FELLOW (honours graduate in physics, electronic engineering or geology) at the Tidal Institute and Observatory, to join a research project concerned with the investigation on a regional basis of tides of the solid earth and associated phenomena—The Registrar, The University, Liverpool, 3, quoting Ref. CV/207/N (September 10).

SENIOR RESEARCH ASSISTANT or TEMPORARY ASSISTANT LECTURER IN THE DEPARTMENT OF SOCIOLOGY to contribute to the development of urban sociology and the study of consumer behaviour—The Secretary, University of Edinburgh, Old College, South Bridge, Edinburgh (September 11).

EXPERIMENTAL OFFICER or SENIOR TECHNICIAN IN THE RADIATION PROTECTION SERVICE which provides health physics services for the twenty-one departments in the University in which radioactive substances or ionizing radiations are used—Dr. D. Hughes, Radiation Protection Officer, The University, Leeds, 2 (September 12).

RESEARCH ASSISTANT (with a first- or upper second-class honours degree in botany, and special interests in plant pathology and fine structure of plant cells), and a TECHNICIAN (with experience in preparation, sectioning and electron microscopy of plant material) in connexion with research on electron microscopy of infection of plants by fungi—Prof. B. K. S. Wood, Botany and Plant Technology Department, Imperial College of Science and Technology, London, S.W.7 (September 12).

EXPERIMENTAL OFFICER (with H.N.C. or university ordinary degree in science, and experienced and interested in histological techniques) IN THE DEPARTMENT OF MICROBIOLOGY—The Professor of Microbiology, The Queen's University, Belfast, Northern Ireland (September 15).

TEACHING FELLOW IN MICROBIOLOGY at Rhodes University—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1, or The Registrar, Rhodes University, Grahamstown, South Africa (South Africa and London, September 15).

LECTURER IN THE MECHANICAL AND ENGINEERING PROPERTIES OF MATERIALS (with particular reference to polymeric materials)—The Registrar, The College of Aeronautics, Cranfield, Bedford (September 16).

RESEARCH ASSISTANT (graduate in either science or medicine) IN THE DEPARTMENT OF SURGICAL SCIENCE to work on experimental immunotherapy of cancer, under the direction of Prof. M. F. A. Woodruff—The Secretary, Department of Surgical Science, University of Edinburgh Medical School, Teviot Place, Edinburgh (September 17).

SENIOR RESEARCH FELLOW (graduate in engineering, mathematics or physics) IN THE SCHOOL OF ENGINEERING, to work on an analytical and experimental investigation of rolling contact phenomena—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (September 17).

SCIENTIFIC OFFICER/SENIOR SCIENTIFIC OFFICER (with a good honours degree in botany or biology and preferably postgraduate experience in ecological research) to take part in the ecological studies of the Nature Conservancy's Conservation Research Division at its Monks Wood Experimental Station, Abbots Ripton, Huntingdon—The Natural Environment Research Council (E), State House, High Holborn, London, W.C.1 (September 24).

ASSISTANT BOTANIST (man or woman graduate) at Wisley for duties which will include advisory work, dealing with queries on plant physiology, genetics and chemistry, plant identification, cataloguing and labelling and committee work—The Secretary, The Royal Horticultural Society, Vincent Square, London, S.W.1 (September 30).

CHEMIST (B.Sc. Hons.) IN THE AUCKLAND LABORATORY OF THE DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH, to work with a small group of specialists in the field of industrial chemical research and service, including problems in plastics, waters and corrosion—The High Commissioner for New Zealand, New Zealand House, Haymarket, London, S.W.1, quoting Ref. B13/18/50/3234 (September 30).

JUNIOR DEMONSTRATOR (non-medical) (preferably with experience in some branch of endocrinology) IN THE DEPARTMENT OF PHYSIOLOGY to assist in the practical teaching of undergraduate students—Prof. A. A. Harper, The Department of Physiology, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, 1 (September 30).

LECTURER IN PHYSIOLOGY or BIOCHEMISTRY (with special reference to toxicology)—The Registrar (Room 39, O.R.B.), The University, Reading (September 30).

LECTURER IN THE DEPARTMENT OF PHYSICS—The Registrar, The University of Manchester Institute of Science and Technology, Sackville Street, Manchester, 1 (September 30).

LIBRARIAN to the proposed University of Bradford—The Registrar, Bradford Institute of Technology, Bradford, 7 (September 30).

ASSISTANT LECTURER (primarily interested in experimental psychology) IN PSYCHOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV.216 (October 1).

ASSISTANT LECTURERS (2) in the Bland-Sutton Institute of Pathology—The Secretary, Middlesex Hospital Medical School, Mortimer Street, London, W.1 (October 1).

SECRETARY (with a degree or equivalent qualification in science or engineering and experience of administration) OF THE PHYSICS DEPARTMENT, for duties which will include responsibility for the technical staff of the Department, negotiations about research grants and detailed planning of a new building—Prof. Sir Nevill Mott, Cavendish Laboratory, University of Cambridge, Free School Lane, Cambridge (October 1).

LECTURER IN BIOCHEMISTRY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 10).

DIRECTOR (medical graduate with substantial research experience) OF MEDICAL RESEARCH at the Kanematsu Memorial Institute—H. R. Beer, Chief Executive Officer, Sydney Hospital, Box 1614, G.P.O., Sydney, N.S.W., Australia (October 31).

READER IN HISTORY AND PHILOSOPHY OF SCIENCE—The Secretary-General of the Faculties, University of Cambridge, The Old Schools, Cambridge (November 16).

LECTURER/SENIOR LECTURER (with a degree in veterinary science registrable in the State of Victoria and postgraduate teaching or research experience in veterinary pathology) IN THE DEPARTMENT OF VETERINARY PARACLINICAL SCIENCE, University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 30).

BIOCHEMIST for research on dental caries in relation to dietary factors with particular reference to carbohydrate; and a CHEMIST for research on modified flour and wheat starch and the study of potential commercial application of such products—The Director of Research, Research Association of British Flour Millers, Cereals Research Station, St. Albans, Herts.

BIOCHEMIST to collaborate in research into lipoprotein metabolism in children—The Secretary, Institute of Child Health, 30 Guilford Street, London, W.C.1, quoting Ref. CH/B.

BOTANIST (with a B.Sc. with botany as a main subject and national of, and permanently resident in, the United Kingdom or the Republic of Ireland) IN THE NATURAL CHEMOTHERAPEUTICS RESEARCH LABORATORY, KAMPALA, UGANDA, for work which will involve the identification and evaluation of local medicines for commercial exploitation—Ministry of Overseas Development, Room 404, Eland House, Stag Place, London, S.W.1, quoting Ref. No. RC205/183/030.

DEMONSTRATOR (graduate in biochemistry or chemistry) IN BIOCHEMISTRY—The Acting Registrar, University College of Wales, Aberystwyth.

ELECTRON MICROSCOPIST (with extensive experience in preparing thin sections and familiar with the maintenance and operation of the Siemens electron microscope) in the Electron Microscopy Unit for duties which will include maintenance, demonstrating the equipment to potential microscope users, assistance in the development and application of new cytochemical techniques, and possibly the supervision of other electron microscopes—The Secretary, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1.

EXPERIMENTAL OFFICER (graduate in electrical or electronic engineering) for work in connexion with the University of London Inter-collegiate Research Service for Chemistry and Related Sciences and to be responsible for the running and maintenance of a Decca electron paramagnetic resonance spectrometer under the general supervision of Dr. K. D. Sales—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1.

LECTURER (honours graduate interested in general and social psychology) IN PSYCHOLOGY—The Principal, Lanchester College of Technology, Priory Street, Coventry.

MATHEMATICIANS to work on a wide range of problems arising from research into the utilization and distribution of electricity; topics include: arc physics, electro chemical reaction kinetics, solid state devices, magnetism, electrical distribution systems, heat transfer, and the application of mathematics to life science—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Chester, quoting Ref. N/29.

MEDICAL RESEARCH COUNCIL POSTDOCTORAL FELLOW IN THE DEPARTMENT OF PHYSIOLOGY—Dr. J. C. Brown, Department of Physiology, University of British Columbia, Vancouver B.C., Canada.

PHYSICIST, BASIC GRADE (honours graduate, male or female, in physics) in a newly established medical physics department serving Cornwall and South-west Devon, based in new premises at Plymouth General Hospital—The Hospital Secretary, Plymouth General Hospital, North Friary House, Greenbank Terrace, Plymouth, Devonshire.

PHYSIOLOGIST/BIOPHYSICIST to study physiological response to controlled changes in environmental conditions—D. C. Page, Head of Personnel Services, The Electricity Council Research Centre, Capenhurst, Chester, quoting Ref. N/28.

POSTDOCTORAL FELLOW IN THE DEPARTMENT OF CHEMISTRY for work, supported by the S.R.C., on the photochemistry of aromatic compounds—Prof. D. Bryce-Smith, Department of Chemistry, University of Reading, Whiteknights Park, Reading.

RESEARCH ASSISTANT (graduate in engineering, mathematics, physics or geology, with research experience, preferably in materials science, applied mechanics, structural or engineering geology) to undertake original work on the engineering properties of rocks, the mechanics of rock fracture, the determination of stress and strain in rocks in situ, and the stability of rock slopes and excavations—The Registrar, The University, Sheffield, 10.

RESEARCH ASSISTANT (graduate in metallurgy, chemical engineering or allied fields with previous research experience) IN THE JOHN PERCY RESEARCH GROUP IN PROCESS METALLURGY, to assist in the administration of the research group and to conduct research on his own account (which could lead to a higher degree) in the field of high temperature process engineering applied to metallurgy—Prof. F. D. Richardson, Imperial College of Science and Technology, London, S.W.7.

RESEARCH ASSISTANT (with a university degree in biochemistry, zoology or a related subject) to study aspects of the physiology and biochemistry of less complex nervous systems of lower animals—Dr. G. A. Cottrell, Gatty Marine Laboratory, University of St. Andrews, St. Andrews, Fife, Scotland.

RESEARCH FELLOW (with a postgraduate qualification or experience in chemistry, biochemistry, and the chemistry and technology of adhesives) in DENTAL MATERIALS to participate in a broad programme of work on the development of adhesive dental filling materials under the immediate direction of Dr. D. C. Smith—Prof. E. Matthews, Department of Prosthetic Dentistry, Turner Dental School, The University, Manchester, 15.

RESEARCH OFFICER (graduate, pass or honours, in chemistry, biochemistry or any related field, and preferably some postgraduate laboratory experience) IN THE DEPARTMENT OF BIOCHEMISTRY AND AGRICULTURAL BIOCHEMISTRY, for duties which will include administrative supervision of the day-to-day running of the modern research and teaching laboratories—The Acting Registrar, University College of Wales, Aberystwyth.

SENIOR BACTERIOLOGICAL TECHNICIAN (A.I.M.L.T. or equivalent) IN THE PATHOLOGY LABORATORY—The Establishments Officer, Zoological Society of London, Regent's Park, London, N.W.1.

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UNIVERSITIES UNDER PRESSURE

THE academic year now beginning in Britain is likely to be difficult for the universities, and not merely because there will be some who expect that the dons of Oxford will have spent at least some of the long vacation following their Vice-Chancellor's advice and reading the report of the Franks Commission so as to decide how—if at all—to respond to it. This is also the year in which the level of spending for the new quinquennium will have to be fixed, that in which research budgets will feel the first real pressures of financial restraint, and the year in which the British Government's long flirtation with what is called technology may crystallize into a frankly hostile public attitude towards institutions which are, in the last resort, cheerfully academic in their convictions and intentions. There is, in other words, a danger that, a year from now, British universities will be diminished in freedom, impoverished and robbed of their present pride of place at the apex of the British educational pyramid. As yet, there is no sign that they will lift more than a gentle little finger in their own defence.

Perhaps the most serious difficulty is the uncertainty which has grown up, in the past three years, about the real function of universities on the British pattern. Curious though it may seem, the Robbins Report, which should have helped to define a sense of purpose for the universities, has instead helped to confuse the several issues now crying out for solution. Robbins implied, and everybody seems to agree, that a substantially larger fraction of the British population than at present deserves some form of higher education. But how is this to be accomplished ? Robbins implied, and the universities joyfully agreed, that more universities on the old pattern would be the best way of doing this. Only recently has it become plain that the Robbins solution may not have been the best way of meeting the need which Robbins cogently defined. In particular, it is probably a needlessly expensive solution.

The eagerness with which the Secretary of State for Education and Science has embarked on the development of the polytechnics is one immediate consequence. It seems easier and cheaper to obtain more higher education by building up a second tier of would-be universities than to put more money into the existing institutions. In this atmosphere, however, it is bound to be hard for the universities to lay hands on the funds they need simply to keep ticking over. At the same time there will be created a gulf between the universities proper and the other institutions of higher education. Everything would be simpler if only the universities themselves were to recognize two important sets of problems—the need of something corresponding to a cost-benefit analysis for higher education, and the need of a greater diversity within the system. Unintentionally, the Robbins Report has worked in the other direction, by making existing university practices seem the ideal, and by making it seem that all universities are entitled to the same uniform standards of staffing, accommodation and equipment. It is a step in the right direction that the Committee of Vice-Chancellors plans to support a study of the efficient use of university capital equipment (see page 1113).

The universities as a whole have also been uncommonly slow to give attention to their own curricula and methods of teaching. In half a century, the output of students has multiplied more than ten times, but what is taught and how has not changed anything like as much. Yet it is hard to be sure that the old courses are still the best in circumstances in which the universities must and should be increasingly concerned that graduates go into industry and business. It does not follow that universities should be more concerned with vocational training than they are at present, but there is at least a case to answer in the arguments of those who say that the standard academic diet should be more stimulating of those interests which are most in demand in the modern world. Some universities, not all of them new, are trying out new kinds of courses, but these are only the beginnings of the experiments which circumstances demand. In the same way, though at a more humdrum level, the recent attempt by the universities themselves, under the aegis of the University Grants Committee, to show how better use might be made of audio-visual and other aids to teaching, served principally to show how much remains to be done before the universities will be able to do efficiently whatever job it is that they and the community decide is necessary.

Research comes into this directly and not as icing on the cake. In the past the universities have too often conspired in the belief that the research which their staffs undertake is somehow distinct from the teaching process, and something which must be supported for its own sake. It follows that university research is even more vulnerable than university teaching when public funds are scarce. But in reality, the justification of university research is that it is an essential part of the process of producing competent graduates. In their

own interests, universities should now insist on this connexion, and take some trouble to show outsiders how research fertilizes teaching. For one thing, doing this would help to solve some of the pedagogical problems facing the universities. For another, it would help to bring some sense of realism to those who think the second-tier polytechnics will solve all the problems of higher education at a fraction of the cost of the universities. Most of all, however, a convincing demonstration by the universities that they value the link between teaching and research would help to fend off the attacks, which there are certain to be in the months ahead, on all those activities that are not somehow relevant to the process of keeping Britain solvent.

This is where technology comes in. Just as there is a danger that the pendulum will swing too far against research, so it may swing too far against what is sometimes called pure science. With the Ministry of Technology growing bigger and stronger with the passage of time, and the Department of Education and Science (which is responsible for financing university research) anxious to know where to get money for teachers' colleges, kindergartens and comprehensive schools, it is inevitable that things that smack of being technological should command attention and public support. The fallacy is that technology is not some kind of antithesis of science, but an application of science. To pretend, as some now seem to do, that a country can become prosperous by technology alone is a little like saying that a man can hoist himself off the ground by pulling hard enough on his bootstraps. If the universities were more sure of what they are for in the modern world, they would be able to exert an invaluable influence in this misconceived tug-of-war between science and technology.

But who are the universities? And who speaks for them? This is the root of the trouble. The University Grants Committee has become a kind of government department. The Committee of Vice-Chancellors is still feeling its way, and only time will tell whether it can be an effective initiator of the kinds of self-critical studies of the working of the university system which circumstances demand. (The chance that the Association of University Teachers, torn as always between the roles of trade union and professional organization, will be able to undertake such tasks is small but not to be dismissed outright.) Even within single universities, there is often no means of subordinating the autonomy of individual departments to the need for common policies on matters as innocuous as entrance requirements. If there were time for leisurely solutions, it would be tempting to think of radical innovations in the machinery of university self-government—elected vice-chancellors, for example. But the threats to the character and status of British universities are urgent, and, in the nature of things, the universities will have to make their own defence. It would be ironical if their freedom were to suffer in the months ahead simply because they have exercised their freedom in such a way that there is nobody to defend it.

A POUND FOR A POUND?

THE Consumers' Council is fighting a lonely but entirely just war with the Chancellor of the Exchequer over the decision that Britain should have a decimal currency in 1971. Decimalization as such is not at issue, of course—even the visionaries of the Duodecimal Society, dedicated to what they think of as a reform of the whole of arithmetic, would probably agree that Britain should enjoy the advantages of a decimal currency of some sort in the near future. The only dispute is whether the new coinage should be based, like the present, on the pound sterling as a unit, or whether the new coinage should be based on a unit equal to half the present £. The advantages of keeping the present unit are largely those of familiarity and continuity, although the Halsbury Committee held that overseas confidence in the British economy might be undermined by the disappearance of the pound sterling. The chief disadvantage is that decimalization based on the £1 unit will make it necessary to legislate for half-cent coins, each of them equal in value to 1·2 pence in the present system. By adopting £0·5 as the unit for the new coinage, a cent would have the manageable value of 1·2 pence, but it would also be possible to relate a great many existing coins to sensible decimal fractions of the new unit. In particular, the shilling and sixpenny pieces now in use would become, without change of value, the dime and the nickel of the new currency.

Obviously it is impossible ever to be sure which set of arguments deserves to win the day. It is also somewhat irrelevant of the Consumers' Council to make as much as it does, in its protest to the Chancellor, of the fear that decimalization with the pound sterling as a unit will give shopkeepers a chance to put up their prices; at the worst, that would be only a transitory effect. But it is entirely proper that the council should protest at the irrationality of the Chancellor's reasons for sticking to the £. It is not good enough for him to say that the government "chose to retain the £ only after a full consideration of the many arguments for and against", and then to fall back on the argument that the half-cent coin would be likely to disappear in the course of time. Even if the Chancellor is right in saying that he is counting on prosperity and not inflation to work this vanishing trick, half-cents are likely to survive for long enough to be a galling reminder of the durability of vulgar fractions in British society.

Those who worry about how the pence and halfpennies would vanish should take note of a C.S.I.R.O. training document on *Dollar Currency* prepared for "*C*" day when Australia went decimal and accepted the 1·2*d*. conversion: "It is imperative that on and after '*C*' day cashiers, etc., should accept halfpennies, pennies and threepences only in some combination which is a multiple of sixpence. Halfpennies, pennies and threepences should not be given out in change".

NEWS AND VIEWS

More and Longer Education

CONTROVERSIES may rage over examination systems, school systems and the importance of Oxford to the nation, but two important facts should be kept in mind—Britain is spending a larger amount and a greater percentage on education and more pupils are staying at school longer. These and other interesting facts emerge from the *Statistics of Education* published last week by the Department of Education and Science (H.M.S.O., 19*s*.).

Expenditure by public authorities rose to £1,485 million in 1964–65, nearly three times the figure ten years ago of £513 million, and representing an increase from 3·2 to 5·1 per cent of the gross national product. Of this total, £1,248 million came from the local education authorities and £147 million from the University Grants Committee. More than 60 per cent, or £785 million, of the total went to the primary and secondary education of 8,980,000 children, a 50 per cent increase on the £523 million spent in 1959–60 (during this period the enrolment increased by about 1 per cent). The expenditure and emphasis on new universities are reflected in the 126 per cent increase over the five year period, with £157 million spent last year. Teacher training represented the greatest surge forward with a growth rate of 180 per cent, but this is somewhat misleading for the increase is from £19 million to a slightly improved £53 million; the decrease in the pupil/teacher ratio from 24·8 in 1960 to 23·3 in 1965 is an improvement but does not yet really ease the pressing teacher shortage in Britain.

While there has only been a 53 per cent increase in expenditure on secondary education during the past five years, 22·9 per cent of the 13 year olds in school three years ago were still in school at 16 in 1965, compared with a percentage of 13·3 in 1956 and 17·2 in 1960. The number of sixth-formers in maintained schools rose to 169,000 from 76,000 in 1958—partly because the age-group was larger but also reflecting the tendency to stay in school longer. While the percentage remaining in school drops off rapidly as pupils grow older, 11·4 per cent were still in school at 17 and 4·1 per cent at 18, nearly double the figures for ten years ago. Even the public, non-maintained, schools show a similar, though not as spectacular, increase, with percentages of 69·5, 42·8, and 12·9 for pupils aged 16, 17 and 18, a steady increase from the figures ten years ago of 59·5, 33·1 and 11·4 per cent.

In spite of the increasing emphasis on comprehensive schools, both the number of schools and the total enrolment of grammar and modern secondary schools has increased from 1955. During the same period, it is true, the number of comprehensive schools increased from 16 to 262 with an increase of 224,000 pupils. With more than three million secondary pupils last year, a much more radical increase in comprehensive enrolment must be effected over the next ten years if all schools are ultimately to be made comprehensive.

University Efficiency

AN attempt to study the effectiveness of the use of university buildings and other capital equipment is being sponsored by the Committee of Vice-Chancellors and Principals of Universities of the United Kingdom. The intention is to recruit a person with experience in work study, operational research and the like to carry out a study, lasting two years in the first instance.

C. F. Carter, vice-chancellor of the University of Lancaster and chairman of the sub-committee in charge of the study, called the project "down-to-earth". "We have no large expectations to begin with," he said, "but British universities have been conscious for some time that such a study is needed and there is no doubt that there will be many areas to investigate." The Committee of Vice-Chancellors decided that it would be more economic to make a joint study and agreed to sponsor such a programme.

The programme will start with an efficiency study of lecture rooms in a medium-sized university—how both hours and weeks can be used most effectively. Another area of immediate study will be the use of undergraduate science laboratories. The common practice in British universities of keeping separate laboratories equipped for each year of a degree course invariably leads to much wasted time and space. The sub-committee hopes to determine whether a more intensive use of laboratory space, which would require more technical staff and the moving of equipment, would actually be more economical than the present system. On a wider scale, the study will be concerned with the actual cost per hour of expensive equipment, such as buildings—is centralization an economic and practical solution? No matter what conclusions are reached during the next two years, Mr. Carter feels that there is a permanent need for an advisory staff in this area.

While the study can hardly fail to yield improvements in the use of capital equipment at British universities, it will also have the immediate merit of enabling the universities to refute more easily the criticism that they are unconcerned about the way in which their expensive facilities are used.

Medical Tapes

DR. A. J. HARLEY of the National Lending Library for Science and Technology and director of MEDLARS, the medical information retrieval service, is treating the three year trial period of the service as an experiment. "The most important thing is to determine just how useful the service is—something that has never been studied before for any retrieval system," he said.

MEDLARS—or Medical Literature Analysis and Retrieval System—is an American import from the National Library of Medicine; started in Britain in the spring of 1965, it consists of both a computerized index to medical literature, the *Index Medicus*, and a search service based on the *Index*.

The study in Britain is operated jointly by the Computing Laboratory of the University of Newcastle-upon-Tyne and the National Lending Library for Science and Technology. Copies of the search tapes are flown over from Washington every month and are available at about the same time as the monthly *Index* reaches the libraries; this is the third service to use these tapes—similar services are being studied at the University of Colorado and the University of California. The Computing Laboratory has been given a £27,000 grant for 3 years until the spring of 1968 by the Office for Scientific and Technical Informa-

tion of the Department of Education and Science; they are primarily interested in the computer problems involved and for the past year have been programming the University's English Electric *KDF*9 to carry out searches on the CCF tapes from the National Library of Medicine's Honeywell computer. The National Lending Library is more concerned with training in computer searches and the collecting and editing of requests.

In response to a researcher's request, a list of citations on a specific topic is compiled using the *Index Medicus*. Once a week, searches are processed on the computer; the aim is to provide a comprehensive subject search rather than a rapid answer service. Because the commercial computer time rate for a single search is in the order of £15, MEDLARS can only be economic where a detailed, extensive search involving either a large number of pertinent references or a large number of terms is involved. Since the service came into use 3 months ago, about 220 searches have been carried out and the weekly number has been growing as word has spread. The Library ran a series of four-day courses on the system for librarians and researchers last spring and is starting a new series of two-day courses this autumn. It is hoped that by properly explaining the system it will be possible to eliminate requests that could easily be done by a research worker using the *Index* for half an hour.

"While it is still too early for any analytical results," Dr. Harley said, "it seems that most references could have been found in the *Index* but would almost always have meant more work—say, 2,000 references instead of the 200 searched by the computer." Besides comparing each computer search with a search using traditional methods, Dr. Harley hopes to get feed-back from all research workers using the service; they are requested to grade, on the basis of its usefulness, each reference which they receive. The thirty responses received so far have varied widely; however, some at least have discovered pertinent references that they would either have missed or have spent hours finding. Whether such cases occur sufficiently frequently to justify the cost of the service remains to be seen—Dr. Harley is waiting until next spring to reach any conclusion.

Over the Rainbow

MORE and more young British doctors are avoiding the economic plight of their colleagues by emigration. It is now estimated that about 650 doctors, or the equivalent of more than one-third of the output of British medical schools, emigrated from Great Britain and Ireland in 1965. In a British Medical Association Lecture delivered in Birmingham last spring and published this week in the *British Medical Journal* (ii, 576; 1966), Dr. John Seale of the Middlesex and St. Thomas's Hospitals said that while there was a decline in the rate of medical emigration in 1961 and 1962, from 1963 to 1965 the rate has been higher than ever before.

Based on figures from the medical councils and immigration departments of the Commonwealth countries and the United States, Dr. Seale estimated that emigration from the British Isles has increased from 470 in 1960 to 553 in 1965. "These are estimates," he said, "of the number of doctors leaving each year who will not return to the British Isles for many years—if at all." He added that an earlier study by Professor Brian Abel-Smith and Miss Kathleen Gales (*British Medical Journal*, ii, 53; 1964) showed that Commonwealth and United States estimates should be increased by 17 per cent to give a more accurate estimate of emigration to all countries, thus bringing the 1965 total to 650.

Reflecting this trend over the past few years has been the stagnation of the medical practice in Britain; in the early fifties the number of general practitioners was increasing at the rate of 300–400 each year but in 1965 there were 750 fewer than in 1960. Again, although the number of consultants in England and Wales increased by 1,034 between 1962 and 1965, Dr. Seale noted that this was nearly offset by a fall of 933 in the number of senior hospital medical officers in the same period. Junior hospital staff has been increasing steadily but this is almost entirely due to the large influx of foreign doctors (*Nature*, **211**, 448; 1966).

Halothane Safety

HALOTHANE is a very popular general anaesthetic; it is not inflammable, does not irritate the respiratory tract, and produces little nausea and vomiting and few metabolic side effects. It is therefore not surprising that 5 years after it was introduced in the United States it had become the chosen agent in more than 40 per cent of operations requiring general anaesthesia.

A number of unexplained deaths resulting from liver injury after halothane anaesthesia led to concern about its effects on the liver, and the National Academy of Sciences—National Research Council initiated in 1963 a large-scale investigation of anaesthetics and liver damage. The report of the committee responsible for the survey, under the chairmanship of Dr. J. P. Bunker, has now been published (*J. Amer. Med. Assoc.*; September 5, 1966).

Thirty-four medical centres took part in the survey and contributed histories of 859,515 instances in which general anaesthesia of all types was used. The committee found eighty-two cases of death due to massive hepatic necrosis in the sample. On examination only nine of these were found to be due to "unknown" causes. Seven of the nine dead had been given halothane. The course of the disease and the appearance of the liver in these cases were similar to those in viral hepatitis or to the appearance in chloroform poisoning. This incidence is very small and the committee states that if the drug does cause liver necrosis, it occurs rarely. It failed to establish any causal relation between halothane and hepatic necrosis, and in fact, the survey shows that halothane is, if anything, slightly safer than other common anaesthetics. The report comments that halothane has probably been examined as exhaustively as any drug in the history of medicine, but it is still impossible to give definite answers to a number of outstanding questions about halothane. The committee therefore recommends that this type of survey be applied to drugs as they come into use, through the setting up of a permanent panel of hospitals and institutes. It also calls for statistical surveys of deaths associated with anaesthesia and for a watch to be kept on cases of hepatic necrosis.

An interesting and somewhat disturbing by-product of the survey is the finding of very considerable variations in the death rate after operation between

different institutions in the survey. The highest crude death rate was twenty-three times the lowest, and this cannot be entirely due to differences in the type of patient at the hospitals involved. A study of the reasons for these variations might also produce some interesting results.

Organisms for Biology Teaching

THE boom in biology teaching in British schools, together with the spate of interest in new curricula, has created curious and somewhat unexpected shortages of organisms for students to dissect or otherwise to use. Professor J. W. S. Pringle pointed out, at the British Association last week, that the demands of biology teaching had themselves helped to reduce the size of populations of frogs in Britain, though it is likely that the increasing urbanization of the countryside and the difficulty of persuading men to collect wild frogs have contributed to the difficulties of the biological suppliers in meeting—at an economic price—the demands of the schools. Fortunately there is the promise of a substitute for the frog; the South African clawed toad, *Xenopus laevis*, is as suitable for dissection by juveniles, and has the added virtue that it can be bred in the laboratory, and that the tadpoles—which are almost transparent—are valuable object lessons in development. Precisely these advantages account for the popularity in recent years of the locust as an experimental creature. In the long run, however, the supply of traditional organisms for teaching is likely to be less of a problem than the need to supply unfamiliar organisms. Biological suppliers, and some schools, have been hard pressed in recent years to know how best to handle distinctive mutants of a species of yeast used in a genetical study as part of the biology programme of the Nuffield Science Teaching Project, because one of the mutants tends to revert to the other. Problems like these are bound to become more common in the years ahead.

Fortunately, some of the difficulties seem to have been fully anticipated. The Institute of Biology, independently and through the joint committee on biological education with the Royal Society, is but one of the organizations to have directed attention to the problem. The ideal outcome is a greater awareness among the biological suppliers of the needs of the schools, and a willingness to spend money on development, not merely of methods of culture but techniques of distribution as well. Another prerequisite of good management is that schools and school authorities should be prepared to pay the real cost of the more sophisticated service which the new curricula will require, and not just what it used to cost to supply each half a dozen pupils with a dogfish and a dozen frogs. In the long run, of course, it would be splendid if the universities—which have similar problems on a more exalted level—could keep an avuncular eye on what is happening in the schools.

European Biochemistry

THE Federation of European Biochemical Societies (FEBS) was founded three years ago with the aim of promoting closer contact and co-operation among European biochemists. The federation is governed by a Council of delegates from the constituent societies which meets under the chairmanship of the delegate of the society responsible for organizing the next general meeting. So far, these meetings have been held annually, in London (1964), Vienna (1965) and Warsaw (1966).

The third annual meeting was organized for the federation by the Polish Biochemical Society and was held during April 4–7, 1966, in Warsaw, under the chairmanship of Professor K. Zakrzewski. The meeting was the largest held in Warsaw in recent years and was attended by 1,571 biochemists, of whom 545 came from Poland, 993 from other European countries and thirty-three from overseas, including three from China. The scientific programme consisted of an inaugural lecture by Professor P. Grabar (Paris) entitled "L'immunochimie des protéines", thirty-eight invited lectures given in a symposium on "Properties and Functions of Genetic Elements" and two colloquia, one on "The Biochemistry of Blood Platelets", the other on "The Biochemistry of Mitochondria", and 525 free papers. The contributions to the symposium and colloquia are to be published jointly in November of this year by Academic Press, Ltd., London, and Polish Scientific Publishers, Warsaw. It would be invidious to select for special mention any particular contributions from the large number of papers presented at this meeting. There is little doubt, however, that, provided the present high standard is maintained in future years, the annual FEBS meeting will quickly achieve recognition as one of the most important scientific events for European biochemists.

The federation is also active in directions other than the annual meetings. A programme of summer schools began in 1965 with Professor H. R. V. Arnstein as chairman of the organizing sub-committee. The 1966 programme involved schools at University College, London, on "Mechanisms of Enzyme Action", organized by Dr. B. R. Rabin, and at the University of Uppsala on "Separation and Fractionation of Macromolecules and Particles", organized by Professor A. Tiselius and Professor J. Porath. Financial support for these schools has been given by the European Molecular Biology Organization, the Council of Europe, the Royal Society of London and the British Council. In 1967 there will be two schools, one on "Steroid Biochemistry", organized by Dr. J. K. Grant, which will be held in Glasgow during April 3–14, and the other on "Nucleic Acids", which will be held in Marseilles during September 11–16 under the direction of Dr. M. Grunberg-Manago and Professor R. Monier. Details of the schools are advertised in the bi-annual *FEBS Bulletin*, distributed to all societies in the federation.

The council of the federation, meeting in Warsaw, has taken a major decision to enter the publishing field with a general journal of biochemistry. This is planned to appear early in 1967 and has Sir Hans Krebs as honorary chairman, with Professor C. Liébecq as editor-in-chief, and the support of senior European biochemists as editors and advisers. The appearance of the journal will not however, add to the number of such publications, since an agreement has been reached whereby the new publication will succeed an existing biochemical journal.

Information about the activities of the federation can be obtained from the Secretary-General, Professor W. J. Whelan, Royal Free Hospital School of Medicine, 8 Hunter Street, London, W.C.1.

Changeover at Mt. Stromlo

PROFESSOR OLIN J. EGGEN has now taken up his new post as Director of the Mt. Stromlo Observatory, Canberra. He succeeds Professor Bart Bok, who has been at Mt. Stromlo since 1957 and who has now become Professor of Astronomy at the University of Arizona. Eggen, who is an American by origin, started his distinguished career at Lick Observatory. From there he moved to Canberra when Sir (then Dr.) Richard Woolley was director and when the 74-in. telescope was being built. Since the mid-fifties Eggen has moved between the Royal Greenwich Observatory (which is not at Greenwich but at Herstmonceux) and Pasadena in California. For a time he was assistant to the Astronomer Royal at Greenwich.

Eggen is an astrophysicist who has spent much of his time making photometric observations and has studied motions of stars within our galaxy. He has said in Australia that he intends to concentrate the work of the observatory at Mt. Stromlo on observation and should in this be helped by the care with which Mt. Stromlo is being equipped.

At this stage there is no knowing whether the arrival at Mt. Stromlo of an American astronomer with close connexions in Britain will affect the issue of the Southern Hemisphere telescope. It remains to be decided whether the British Government or the American Government will eventually help with the building of a large telescope (with an aperture of 150 in. or more) in Australia. Astronomers of all nationalities regret the delay there has been.

Railway Relics

NOT only Mr. John Betjeman will be up in arms about the proposal by British Rail to dismantle St. Pancras and Kings Cross Stations, and it is bound to be some time before it is known whether one modern terminal will be built to replace both monuments to the Railway Age. To replace St. Pancras and Kings Cross by a modern terminal would in any case be an unenviable task for the architect chosen, for he could scarcely contrive to improve on their combined merits; the flamboyant self-confidence of St. Pancras, offset by the sterner stuff of Kings Cross. The two buildings epitomize the ambivalence of the Victorian age.

By guile or by accident, however, the railways board has made sure that there will be few technologists among those who will, no doubt, be lying in the road to impede the demolition men. It has been promised that when the soaring Gothic of the station eventually disappears, the arch of the station itself will be exposed for all to see. The arch was built by William Henry Barlow in the continental style in 1868. The vault is vast, and makes great use of cast iron arches and glass, although less elegantly than John Dobson's Newcastle Central Station of many years earlier. The icing sugar hotel façade was added in 1874 to the designs of that high-priest of Victorian Gothic, Sir George Gilbert Scott.

It seems churlish to carp at British Rail when their intention is to cut costs and increase efficiency; a more modern approach to the running of the railways is a right and proper end to pursue, but if St. Pancras and Kings Cross follow the steam engine into oblivion, for many the romantic flavour of the railways will be gone for ever.

University News: The City University

MR. K. J. SHONE has been appointed professor and head of the Department of Management Studies. Mr. M. L. Meyer has been appointed to the second chair of mechanical engineering. Dr. R. N. Cox has been appointed to the second chair of aeronautics. Dr. R. C. Pitkethly has been appointed associate professor in the Department of Chemistry for three years.

Appointments

DR. F. S. DAINTON, vice-chancellor of the University of Nottingham, has been appointed chairman of the advisory committee for Scientific and Technical Information, in succession to Sir James Cook, at present vice-chancellor of the University of Exeter and vice-chancellor designate of the University of East Africa.

DR. C. A. SPOHN has been appointed director of operations of the National Environmental Satellite Center, Environmental Science Services Administration, in succession to A. W. Johnson who has been appointed deputy director of the Department of Commerce facility.

DR. K. O. WRIGHT has been appointed director of the Dominion Astrophysical Observatory at Victoria, B.C., in succession to the late Dr. R. M. Petrie.

Announcements

THE London office of the National Institutes of Health, U.S. Public Health Service, is being closed as from September 12. The functions of this office will be transferred to the N.I.H. European office in Paris. The research grant, fellowship, and other programmes of N.I.H. in the United Kingdom and other areas will continue as before with administrative responsibility consolidated in Paris. Further information can be obtained from Dr. P. G. Condliffe, Chief European Office, National Institutes of Health, 2 Avenue Gabriel, Paris 8e.

THE British Society of Rheology has instituted a gold medal which will be awarded every three years for "the best contribution to the solution of an industrial problem by the application of fundamental rheological knowledge". The first medal will be awarded next year, and the closing date for entries is May 1, 1967. Further information can be obtained from Mr. A. T. J. Hayward, National Engineering Laboratory, East Kilbride, Glasgow.

CORRESPONDENCE

Preprints Galore

SIR,—Your editorial of August 27 will be read with great interest and many biologists will agree with your statement that "The defects of IEG and PIE are no excuse for hiding from the problems which stimulated their emergence". These problems are to be discussed at a conference on "Communication in the Biological Sciences", to be held during December 14–15, 1966, under the auspices of the Biology Group of Aslib and the Institute of Biology. The organizers hope that this conference will agree on the steps needed to deal with the problems of information exchange facing biologists working in laboratory and library. If so, it should guide the various bodies concerned with the publication, dissemination and retrieval of information.

Yours faithfully,

D. J. B. COPP,
General Secretary.

Institute of Biology,
41 Queen's Gate, London, S.W.7.

AGRICULTURE AND CLIMATE

At the end of July an interdisciplinary meeting was held at the University of Reading, to discuss the relationships between climate and agriculture. This symposium was attended by scientists from thirty-six countries, sponsored by Unesco, and organized by Professor A. H. Bunting, professor of agricultural botany at Reading, and Mr. L. P. Smith, president of the Commission for Agricultural Meteorology of the World Meteorological Organization. The conclusions reached at the symposium are summarized here by Mr. L. P. Smith.

THE demand for the correct assessment of climatic factors when planning for the increased food production which is so urgently needed by an expanding world population has become a matter of prime importance to both United Nations technical agencies and individual countries. Time is short, and if the fundamental scientific knowledge is as yet so incomplete that it cannot point immediately to the best solution to any specific problem, at the very least it should be adequate to avoid errors. The cost of the mis-use of land is no longer measured in terms of money, but in human lives.

It is not surprising that agroclimatology is in an imperfect form. Both biology and meteorology are partly understood sciences of infinite complexity, so that the links between the two and the responses of a plant or animal to its physical environment are bound for the most to be tenuous and empirical, or, at the very best, semi-empirical. Empiricism, when applied by skilled brains, is an excellent tool with which to attack practical problems, but its limitations have always to be borne in mind, otherwise considerable dangers can arise.

The validity of any agroclimatological analysis depends basically on the depth of agrometeorological knowledge or, in other words, the way in which any of the manifold agronomic factors, growth, pest and disease incidence, depend on the weather. Considerable progress has been made in the past decade in several such problems. This progress has been made both on the micro-meteorological scale and in field research on a meso- or macro-scale. As a result, much more confident deductions can now be made regarding agricultural potential by the use of weather parameters than was hitherto possible.

The increasing accuracy, on a practical scale, of such bio-climatic "models" leads to a greater demand for data. In the past, meteorological data, albeit of a fairly simple character, have been more plentiful than biological data, but in the future it is clear that progress must be made on both sides. For the meteorologist more radiation data are required, and problems of the routine measurements of evaporation or transpiration, and of soil moisture, with the attendant instrumental errors, exposure errors and sampling errors are nowhere near a satisfactory solution. In many areas, almost any biological or agricultural data would be an advance, provided that a degree of accuracy and representativeness is attained. An increase in plot-trial data is attractive but recent micro-meteorological research casts doubts on the ways in which such trials can be interpreted meteorologically. The climate in which a plant grows depends greatly on the nature of the ground surface up-wind; this takes a considerable distance to become established so that small plots are not all experiencing the same weather on a micro-scale, especially when they have been randomized in conformity with the statistician's designs. The science with the all-embracing brief, ecology, would appear to be suitable to play a leading part in satisfying this need for more biological data, and yet it has provided its exponents with so many fascinating by-ways that so far it seems to have made little progress along the main road to agronomic assessment. Perhaps the old practice of phenology needs reviving in terms of functional agriculture.

In the absence of measurement of the precise meteorological parameters required, principally radiation and evaporation or transpiration, many ways are now available to estimate such parameters, using data of a simpler nature which are more readily measured. Radiation estimates can be made from sunshine or cloud amount observations which, on average, are not much greater in error than direct measurements often made by inaccurate or poorly maintained instruments, but with transpiration the position is more difficult. So far as potential transpiration is concerned, as was pointed out at the Reading symposium, there now exist almost as many meteorological formulae as there are religions. This comparison is not so ridiculous as it sounds, for each of these formulae have their extreme supporters who will hear no ill of their chosen idol and lose no opportunity to heap scientific scorn on the beliefs of others. Fortunately this frustrating combination of blind trust and illogical intolerance is not common to all scientists and with careful choice of formula appropriate to the conditions under review it is possible to obtain a close approximation to the potential transpiration factor.

Further troubles arise, however, in the search for an estimate of actual transpiration, which is extremely difficult to measure accurately in natural conditions. Here again "models" proliferate, and the results of small-scale experiments, both in the laboratory or in the field, tend to confuse rather than clarify. Progress can nevertheless be expected in this difficult aspect of the subject, for there are quite a few distinguished micro-meteorologists and research plant physiologists carrying out research in

several countries. Many of them are associated with the production processes section of the International Biological Programme, and the World Meteorological Organization has recently formed a small working group to facilitate collaboration and the cross-fertilization of ideas and techniques.

The correct interpretation of climate in terms of agriculture has always been one of the most important aspects of the problems of land-use in semi-arid zones. Three United Nations technical agencies, Unesco, F.A.O. and W.M.O., have recently joined together in sponsoring agroclimatological surveys of the semi-arid zones in the Levant and in the area of Africa lying to the south of the Sahara; one is an area of limited winter rainfall, the other of limited summer rainfall. These surveys, each carried out by a team of a meteorologist and an agronomist, were discussed in detail at Reading. The methods used received general commendation from the meeting, especially when the limitations of time and data were considered. Sound precedents for such work were lacking, so that the degree of scientific caution which had been exercised met with general praise, in that no conclusion was reached or method of argument used without corroboration from the limited evidence available. In particular the treatment of the water balance was approved; this involved the choice of Penman's formulae for calculation of potential transpiration, in that it was the least likely to introduce major errors in this fundamental factor.

It was not suggested that these studies were either perfect or complete and more elaborate analysis was needed on a more local scale. Such analysis should include the theoretical investigation of the water balance for alternative systems of cropping. This follow-up action demanded increased co-operation with the existing research stations in the area and increased scientific activity by the countries concerned both in meteorology and agronomy, and, most important of all, greater collaboration between the two sciences.

This collaboration is possibly the key to more rapid progress in agrometeorology but it is easier to specify than to bring about. It is always evident at international meetings, and Reading was no exception, that there is rarely a balance between the two sciences in terms of the numbers of participants. In only a small minority of countries does there exist a tradition of active co-operative research or investigation. The reason for this might with justice be traced back to the schools, where biology is often taken as an alternative to physics or mathematics, and few universities include adequate teaching of meteorology in any course of agriculture or horticulture.

The result is that, at postgraduate stage, there is no convenient common scientific language in which ideas can be exchanged. Beyond the universities, it is not common to find active collaboration between research stations dealing with agricultural problems and professional meteorologists, although Britain is one of the conspicuous exceptions, and the closing of this gap is not made easier by the preoccupation of weather bureaux with the treadmill of forecasting and the fulfilling of insistent aviation demands for meteorological aid.

It is clear that a radical review of scientific education is needed in almost every country, but in the meantime there is an increasing tendency for agricultural meteorologists to work within the biological field. This process is resulting in more knowledge being gained in the basic problems and more reliable practices being advocated in agricultural advisory and extension services. A similar evolution is taking place in hydrology where the scientist is coming more and more to the aid of the water engineer. Indeed, as one of the clues to greater food production lies in the increased use of skilled irrigation, it may be said that agrometeorology and hydrometeorology must advance together, quite apart from the fact that both applied sciences are fundamentally concerned with the water use of crops and ground cover.

The Reading symposium did not overlook the secondary effects of weather and the part which climate plays in determining the incidence and epidemiology of pests and diseases, both during growth and in storage. It also touched on the modifications of climate which are within the scope of the farmer. Irrigation and shelter have been used since time immemorial to ameliorate a difficult climate; in modern times, science is replacing art as the prime mover in such management.

Climate assessment is also a factor in determining correctly the economics involved in seasonal decision-making in practical agriculture. The risks, climatically speaking, of early or late planting of a crop can be interpreted in terms of economic risk, if the crop–weather relationships are known. The difficulty arises, however, in determining the variability of the climate. It is often thought, without much justification, that while weather is variable, climate is constant, but nothing could be more misleading.

The climate of most areas of the world is constantly changing, and it is a matter of mortification that such changes, or climate trends, are rarely identified in meteorology until they have almost ceased to be effective in terms of agriculture—or, on the other hand, agriculture may have adjusted itself, by force of circumstances such as three successive crop failures, before any scientific guidance can be given.

This points to the need for a relatively new line in meteorological research, backed by modern data and machine analysis, with the aim of attempting seasonal forecasting or even forecasts of climatic change. There is no immediate hope of such aids to agronomical planning becoming available in the near future, but, as more is learnt of world circulation patterns and the forces behind them, then the more distant future may see a new scale of weather forecasting.

In the past, agroclimatology has been a creature of a world more academic than practical, more of the lecture-room than the field, and bedevilled by the enthusiasm of the scientifically ill-equipped. Climatic classifications have been proposed, with criteria which owe more to the ingenuity of the originator than to any basic scientific truth, and have been applied on a world scale with a degree of labour and devotion that seems largely misapplied. The gibe of "little lines on little maps, drawn by little men with little brains" is not entirely devoid of truth. Simplicity has often demanded, and been granted, the sacrifice of validity. While the world, and world science, owes much to its enthusiasts and non-conformists, the energetic worker with the second-class mind can cause infinitely more trouble than the gifted idler.

The originator of any new technique, designed to make a practical approach to resolving an area of ignorance, is often aware of its limitations. His disciples, fired by the new possibilities, sometimes disregard these zones of uncertainty and apply new ideas rigidly and with limited imagination. The result can be chaos, and we would be well advised to remember the dictum of Descartes: "Nothing must be accepted as true till it has been clearly recognized as being so; which means that every care must be taken to avoid haste and prejudice."

Haste, however, is inevitable in modern times, and the future of agroclimatology must take this into account. It must help to provide reliable answers to land-use problems, and provide them quickly. It must not only improve its own methods and techniques, but also must ask agrometeorologists to provide more reliable aids. Thus on one hand it must look to the furtherance of fundamental research on the micro-scale; on the other it must make the most use of macro-data.

The data themselves must be improved and greater care must be devoted to establishing the radii of error involved. Short-term field experiments must be better

planned and the results interpreted with care. In far too many cases experimenters tend to find the answers which they have (perhaps subconsciously) determined before starting the practical proving test.

Interchange of ideas between biologists, physicists and mathematicians is essential; one of the major advantages of meetings such as the symposium at Reading lies not in the papers presented or the proceedings published, but the endless open-ended discussions which take part between scientific workers in different fields and from different parts of the world.

The international technical agencies provide, at little enough cost, an immense service to humanity in organizing these meetings. An idea, picked up at Reading, may lead to a completed research in Tokyo which could resolve a practical problem in Argentina. The future, however, demands more than these interim aids to the improved application of science. Education, balanced in nature and pruned of its archaic inaccuracies, has to be advanced at all levels. Not only must the scientist of tomorrow be better armed at all points for his attack on ignorance, but the farmer of tomorrow must be better equipped mentally to make use of new discoveries.

It is probably true to say that a very small proportion of existing knowledge is properly applied to practical agriculture on a world scale. Errors are still being made in land-use that were made by the Romans two thousand years ago. Peasant wisdom tends to be forgotten, sound technical advice does not advance rapidly enough to replace it.

Unsound pseudo-scientific advice, unhappily, sometimes appears to advance very rapidly indeed and for this reason agroclimatology must maintain a very high degree of scientific honesty Even though the demands are urgent, facile approaches cannot be tolerated unless every precaution has been taken to eliminate error.

The participants at Reading clearly realized these principles and, while it would be untrue to say that every view advanced could be accepted without reservation, there was a conspicuous absence of anything resembling charlatanry. If we are to survive on this planet, every advantage must be taken of the natural resources to produce more food, and we can no longer rely on the old-time, hit and miss, trial and error methods of a more leisurely age. It is vital that agrometeorology and agroclimatology should advance.

PREDICTION OF ECLIPSES

By D. H. SADLER

Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, Sussex

THE main object of this article is to review the most recent contribution to the prediction of solar eclipses, namely the *Canon of Solar Eclipses*[1] prepared by J. Meeus and colleagues. Before doing so it may be of interest to give some background and historical information about the prediction of eclipses in general.

There are two essentially different methods of predicting eclipses: they can be predicted, without any real knowledge of the theories of the motions of the Sun and Moon, on the basis of mean periods derived from investigations of past records; and they can be calculated in advance, to almost any desired degree of precision, from theories of these motions. Gravitational theories of the motions of the Sun and Moon only became available in the past three hundred years, but they rapidly improved to the point at which predictions adequate for all practical purposes could be made without any difficulty of principle; the computational difficulties continued to be considerable, and it is only in recent years that electronic computers have made it possible to calculate the detailed circumstances of all eclipses over several thousand years.

At the instants of new moon (for solar eclipses) and full moon (for lunar eclipses) the Sun, Moon and Earth are aligned in longitude (that is, lie in the same plane perpendicular to the ecliptic). Eclipses occur at such times when the Moon is sufficiently close to the ecliptic in latitude. The Moon moves in an orbit which is inclined at an angle of about 5°·15 to the ecliptic, and its latitude is sufficiently small for eclipses to occur only when it is close to the "nodes" in which its orbit intersects the ecliptic. "Ecliptic limits" (of latitude, angular distance from the nodes, or more crudely of time from the nodal passage) can be specified within which eclipses must or may occur and outside which they cannot occur. These limits vary slightly, mainly on account of the varying distances of the Sun and Moon, but may be treated as fixed for general descriptive purposes. The important periodicities are therefore the synodic month or lunation (the interval between successive new or full moons) and the nodical or draconic month (the interval between successive passages of the Moon through the nodes of its orbit).

The main features of the occurrences of eclipses (as contrasted with the detail of times, magnitudes and visibility) can be derived from a consideration of the mean values of these periods which are, at the present time

lunation, $L = 29{\cdot}53059$ days
draconic month, $D = 27{\cdot}21222$ days

Starting from a full or new moon, at which the Moon is a fraction f_0 of a revolution past a node, it will be $f_n = f_0 + 2{\cdot}170392\,n$ revolutions past a node after n lunations; eclipses must, may, or cannot occur according to whether the decimal part of f_n is within prescribed limits. Thus, provided an initial value of f_0 is known, the main features of the occurrences of eclipses can be obtained in a simple calculable sequence. With the introduction of refinements arising from the consideration of the relationship to the year and to the anomalistic month (the interval between successive passages of the Moon through perigee—or its closest point to the Earth), more detailed circumstances of eclipses (such as the nature of the eclipse, durations and areas of visibility) can be given. Such methods have been extensively developed and analysed by G. van den Bergh[2].

Most interest, however, attaches to the rational approximations to the decimal fraction 0·170392; a converging sequence (1/5, 1/6, 7/41, 8/47, 15/88, 23/135, 38/223, ...) is immediately obtainable from its representation as a continued fraction. The ratio 38/223, corresponding to the Saros of 223 lunations and 242 draconic months, is in error by only 0·000012, the equivalent of 0·04 days after a cycle of 6585·32 days. Eclipses therefore are repeated, but not at the same times of day, at intervals of 223 lunations. Such regular cycles, and even the more complicated patterns that occur over shorter intervals, can

be derived from an investigation of the occurrences of eclipses, without any knowledge of the underlying reasons; the Saros was so derived by the Chaldeans in about 750 B.C., and was probably used for the famous prediction of the eclipse of Thales which is now considered to be that of May 28, 585 B.C.

In view of the recent suggestions that Stonehenge was designed and built to serve as an aid for the prediction of eclipses, it is of interest to examine how these "simple" cycles are related to the more complicated procedures put forward. Neglecting complicated refinements, there are six periodicities involved, only three of which are independent; these are the periods of revolution of the Earth about the Sun, of the Moon about the Earth, and of the nodes of the Moon's orbit

the year,	Y	=	365·24220 days
the month,	M	=	27·32158 days
the nodal period,	N	=	6798·37 days

The derived periods are the lunation (L = 29·53059 days), the draconic month (D = 27·21222 days) and the eclipse year (the interval between successive passages of the Sun through the nodes of the Moon's orbit, E = 346·62005 days). Using lower-case letters for the daily motions in revolutions (for example, $l = L^{-1}$, $d = D^{-1}$), the following exact relationships hold

$$l = m - y;\ d = m + n;\ e\ (= d - l) = y + n$$

For the prediction of eclipses, the only periods required are the lunation L and the draconic month D, or its equivalent; the nodal period N enters only in so far as it is necessary to derive D from other periods.

G. S. Hawkins[3–5] has suggested that Stonehenge was designed to mark the extreme and mean azimuths of the rising and setting of the Sun and Moon, corresponding to declinations of 0°, ±5°·2, ±18°·3, ±23°·5, ±28°·6; although the evidence is far from conclusive, this suggestion is astronomically acceptable. He goes on to suggest that the purpose of this construction was to facilitate the prediction of eclipses, using a combination of observations over the markers with a simple computer based on the fact that the number (fifty-six) of Aubrey holes is about three times the nodal period N measured in terms of years Y (N = 18·613Y). F. Hoyle[6] elaborates the method of use, and his method may be briefly summarized as being equivalent to predicting the relative longitudes of the Sun, Moon and node by using

$$13\ y = 2/56;\ m = 2/56;\ n = 3/56\ y$$

The approximations to the periods are poor but they are only required to serve for short intervals, being monitored by deductions made from the observation of the azimuths of the rising and setting of the Sun and Moon.

Both Hawkins and Hoyle base their suggestions on the number fifty-six and its relationship to the ratio N/Y, presumably on the grounds that the independent periods Y, M and N can be derived from the observations of azimuths; this is certainly true for Y and M, but some care is required to derive N (though not to the accuracy required) because of the possible error of 0·5 days in the time, and a corresponding error in the declination, of moonrise or moonset. As has been seen, however, the only relationship required for the prediction of eclipses is that between L (which is the first and most obvious periodicity to be observed) and D. A simple calculation, or even better a reference to a list of eclipses, since there is no change of principle over the centuries, provides the sequence of full moons at which lunar eclipses, visible from Stonehenge, occurred. Because of the gaps this sequence is very complicated, but the Saros is readily seen; moreover, there are several recognizable shorter cycles, particularly that of forty-seven lunations, though it is questionable whether such would have been recognized by a primitive people. The sequence of risings or settings of the full moon in directions opposite to those of sunset or sunrise would fill the gaps, and, apart from solar eclipses, there are other, less likely, methods of determining the relationship between L and D from observation. Such information might suggest a single shift, at each lunation, of four stones in a circle of forty-seven; this would predict eclipses more simply and more accurately than Hoyle's method.

One further point should be clarified: with three independent periods, there are two independent ratios; one of these, L/D, gives rise to the Saros in which

	223 lunations	(L)	= 6585·32 days
	242 draconic months	(D)	= 6585·36 days
giving	19 eclipse years	(E)	= 6585·78 days

The other ratio, L/M, provides the Metonic cycle in which

	235 lunations	(L)	= 6939·69 days
	254 months	(M)	= 6939·68 days
giving	19 years	(Y)	= 6939·60 days
but	255 draconic months	(D)	= 6939·12 days

In his book[5] Hawkins refers to the Saros, which is fundamental to the prediction of eclipses, as "the saros of 18 years" and misleadingly refers to the Metonic cycle as an eclipse cycle because it operates as such over a very limited range; it is in fact only as accurate as the 13/12 ratio for L/D. Hoyle[6] uses the relationship 223 L = 19 E with the comment "This is probably the method of the Saros used in the Near East". F. Wheeler[7] refers to the 18·6 year "Metonic cycle" and later to "the nodes circle the Earth once every 19·61 years—the Metonic cycle...". The nodal period N of 6798·37 days (= 18·61 years) is not an eclipse cycle and has no simple ratios with L, M or D.

Clearly no definite answer can be given as to whether Stonehenge was designed and used as suggested; it appears that it could have been so used, but it is strange that so complicated a procedure should have been used when apparently simpler methods were available.

It is nevertheless interesting to note that Hoyle propounds the concept of an all-powerful god at the nodes of the Moon's orbit. Can he not know of the well-known[8] origin of the term "draconic" for the nodical month, or of the dragon that supposedly eats up the Sun or Moon at the nodal points ? god eats dragon ?

A great deal has been written[9] about ancient eclipses and about the methods used by the ancient civilizations for their prediction; the Assyrians, the Babylonians, the Chaldeans, the Chinese, the Egyptians, the Greeks and the Romans were all able, in lesser or greater degree, to make predictions. And, generally, the basis was the Saros or an equivalent cycle. Hipparchus, who flourished in the second century B.C., is credited with the discovery of the nodal period, as a result of investigating the Chaldean and Babylonian records of observed eclipses; he was aware of all the principal periods involved. With this knowledge, the prediction of eclipses, though still an esoteric art commanding the greatest respect, became less specialized.

With the introduction of the theory of gravitation, the prediction of eclipses became the supreme test of the theories and tables of the motions of the Sun and Moon; and it was for long regarded as an intellectual triumph to be able to predict eclipses to within a few minutes, and later seconds, of time. Apart from the technical and computational difficulties, which were considerable, precise times and local circumstances were limited by the

irregular rotation of the Earth; predictions over long periods were also uncertain on account of the unknown secular accelerations of the Sun and Moon, as a result of the dissipation and interchange of energy within the Sun–Earth–Moon system. The astronomer's interest in ancient eclipses lies primarily in their use in determining such accelerations[10], and much research into old records has been done for this purpose.

In 1829 F. W. Bessel introduced the geometrical method of prediction that bears his name; this was used, in conjunction with the best available ephemerides of the Sun and Moon, for the predictions in the national ephemerides and is still so used. Meanwhile in 1887 Th. Ritter von Oppolzer produced his famous *Canon der Finsternisse* which gives the main circumstances of all (there are a few missing!) solar eclipses from 1208 B.C. to A.D. 2161 and all lunar eclipses from 1207 B.C. to A.D. 2163. The basis of this enormous work consists of approximate tables of the motions of the Sun and Moon, in which only the prinicpal terms are included; the Saros was used to indicate the occurrence of the eclipses. Elements of the eclipses are given in an abbreviated form of Hansen's method, and, together with the charts showing the central lines of total and annular solar eclipses, provide the data for its two main objects: to give the occurrence and the circumstances of future eclipses to a precision that is adequate for planning purposes; and to provide material for the identification and investigation of ancient eclipses. For long a carefully guarded scarce treasure, it has recently been made generally available by the publication of a most excellent reprint[11].

The general introduction, in 1960, of the concept of ephemeris time and of the ephemeris meridian made it possible to predict all the circumstances of eclipses without reference to the irregularities in the Earth's rotation; a final reduction to the Greenwich meridian can be made simply at the time of the eclipse. Computer programs for these calculations, requiring only the date and the positions of the Sun and Moon (from other programs) as input data, were written at the U.S. Naval Observatory (which is responsible for the calculation of eclipses under international agreements) and elsewhere.

This then is the background to the publication of the *Canon of Solar Eclipses*[1]. In this magnificent volume, J. Meeus, who is an amateur astronomer, has given essentially all required data for the 1449 solar eclipses occurring between July 1898 and March 2510 with almost the maximum possible precision. Whereas Oppolzer used very crude tables of the motions of the Sun and Moon, Meeus has used modifications (based on his own tables[11]) of the best currently available theories which considerably reduce the amount of calculation while preserving the high precision, of order 1″; also, because positions are required only near the times of new moon, many terms dependent on the elongation of the Moon from the Sun can be greatly simplified. With ephemerides of such high precison, Meeus and his colleagues have calculated, using rigorous formulae, and tabulated: (*a*) the general circumstances (date, time, nature, magnitude) in Part I; (*b*) the Besselian elements for each hour covering the whole eclipse in Part II; (*c*) all data for the central line of the track on the Earth's surface for central eclipses in Part III. These central lines are also plotted, as precisely as the scale will allow, on a series of charts similar in concept and design to those so usefully given by Oppolzer in his *Canon*. All the calculations have been carried out in terms of ephemeris time.

The data given are not sufficiently precise for the rigorous comparison of observation with theory, but they are almost as good as many observations are likely to be and they are certainly far more than adequate for all other purposes. With one important reservation, the volume thus pre-empts the data on solar eclipses to be tabulated in future editions of *The Astronomical Ephemeris* (*A.E.*). Ruefully it has to be admitted that a discrepancy between the figures in the *A.E.* and the *Canon* for the annular eclipse of July 31, 1962, arises from an error in the *A.E.* (in the initial data, not in the eclipse calculation itself). At the request of the International Astronomical Union, detailed predictions of eclipses have been made available ten years in advance for the planning of eclipse expeditions, and so many years in advance of the systematic calculations for the ephemerides; the data available in Meeus's *Canon* now meet this requirement and will make unnecessary the always dangerous practice of special non-systematic computations.

A thorough investigation into the precision of Meeus's ephemerides and eclipse data is unnecessary, as anything less than the rigorous evaluation of the internationally adopted theories is unsuitable as a reference standard; apart from the approximations there are also a few minor differences in the constants used, but the main limitation, over the period of 600 years, is certainly the uncertainty in our knowledge of the secular accelerations. The eclipse data in the *A.E.* must, therefore, be continued and must be used in the reduction of observations.

All the calculations were performed on the *IBM* 1620 computer at the Computing Laboratory of the University of Ghent; this is a relatively small machine and nine separate programs were necessary, involving much handling of cards. The theory, the planning, the programming, the execution and the production reflect the greatest credit on Meeus and his co-workers; as one example, the data in Part I are given for the time of closest approach rather than for the traditional time of conjunction. Meeus also contributes a long introduction containing (in addition to all necessary explanations of the data and methods of their calculation and use) much information of interest regarding eclipse cycles, comparison with Oppolzer's *Canon*, etc.; his enthusiasm and his energy appear inexhaustible.

His enthusiasm has, however, overstepped his understanding of the relationship between ephemeris time (E.T.) and universal time (U.T.). The difference, ΔT = E.T. − U.T., which depends on the departure from regularity of the Earth's rotation, cannot be predicted; in fact estimates based on all available observations in 1957 were 2^s in error by 1960. Anxious to have values of ΔT in order to use Greenwich longitudes for the plots of the central lines, Meeus has used an incomplete formula for extrapolating values of ΔT; his values after 1963 are likely to be increasingly in error. Since his values of ΔT can result in a shift of up to 6½° in longitude at A.D. 2500, the tracks of the central lines as plotted on the charts may be significantly in error as given—though easily corrected when better values of ΔT become available. The tabulated ephemeris longitudes should be used, with E.T. as the time argument; the tabulated values of ΔT and the deduced Greenwich longitudes should be ignored after 1963.

Meeus's *Canon* is none the less a monumental work, extremely well produced; and it certainly is the "last word" in eclipse prediction. Could it possibly be that Stonehenge was the first ?

[1] Meeus, J., Grosjean, Carl C., and Vanderleen, Willy, *Canon of Solar Eclipses* (Pergamon Press, London, 1966).

[2] van den Bergh, G., *Periodicity and Variation of Solar (and Lunar) Eclipses* (H. D. Tjeenk Willink and Zoon N.V., Haarlem, 1955).

[3] Hawkins, G. S., *Nature*, **200**, 306 (1963).

[4] Hawkins, G. S., *Nature*, **202**, 1258 (1964).

[5] Hawkins, G. S., and White, J. B., *Stonehenge Decoded* (Souvenir Press, London, 1966).

[6] Hoyle, F., *Nature*, **211**, 454 (1966).

[7] Wheeler, F., *New Scientist*, **31**, 251 (1966).

[8] Berry, A., *A Short History of Astronomy*, 48 (1898); and repeated in many text-books and histories.

[9] See article "Eclipse" in *Encyclopedia Britannica*, and in almost all histories of astronomy.

[10] Jeffreys, H., *The Earth*, third edition, 222 (1952).

[11] Oppolzer, Th. Ritter von *Canon of Eclipses*, translated by Owen Gingerich (Dover Publications, 1962).

[12] Meeus, J., *Tables of Moon and Sun* (published privately, 1962).

BOOK REVIEWS

MOONS, MOTORS AND MARS

Advances in Space Science and Technology

Vol. 8. Edited by Frederick I. Ordway, III. Pp. xiv+396. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1966.) 128*s*.

PREVIOUS volumes in this series have provided lengthy reviews of known techniques or speculative essays on future developments in space science and technology. The present volume conforms to this pattern and consists of four lengthy contributions.

The first is a persuasive discourse in support of the volcanic theory of lunar craters, by G. J. H. McCall. The author is a geologist, and his arguments depend largely on detailed examination of the pattern of the craters and the way in which the smaller bite into the walls of the larger. He also produces some interesting new comparisons with terrestrial features. His thesis, though it may have little impact on exponents of the meteoric school, really leaves them with a great deal to explain away.

In Chapter 2, which runs to 130 pages, the structure of lunar craters has yielded place to the structure of solid-fuel rockets. The case of a solid-propellant rocket not only houses the propellant but also has to withstand the hot pressurized gases and carry the flight loadings due to aerodynamic forces; furthermore, its weight has a critical effect on the rocket's performance. The authors of this chapter, C. W. Bert and W. S. Hyler, provide a very thorough survey of the subject, covering stresses, structural design, reliability, forms of fracture, fatigue behaviour, environmental effects and other factors, with 317 references to support their case.

The third and longest chapter, by G. W. Morganthaler and G. E. Fosdick, is devoted to the future exploration of the solar system. Readers may quail when faced with a speculative preview extending to 150 pages. And they may also be irritated by the assumption that up to 1 per cent of the gross national product of the U.S.A. will continue to be devoted to space exploration: anyone with the smallest concern for the happiness of mankind could find better uses for $\$10^{10}$ per year, or even $\$10^{9}$; and if that concern is absent, is our way of life worth exporting to the planets? However, any such irritation tends to evaporate as one reads on. Although the authors are prone to quote 'authorities' of doubtful worth, their survey is impressive in its scope and detail. They provide extensive tables giving a complete space programme, with costs yearly for the next three decades, including manned spacecraft for landing on Mars and fly-by of Venus. It is quite a relief to find that the total cost is only about $\$10^{11}$.

In the final contribution, which is almost an appendix to the preceding one, E. A. Steinhoff considers the logistics of exploring Mars and its satellite Phobos. In particular he discusses how far to exploit extra-terrestrial resources instead of bringing all the required materials from earth.

In its printing, production and price, as well as its contents, the book maintains the high standard of its predecessors.

D. G. KING-HELE

GYRO-MECHANICS

Mechanics of Gyroscopic Systems

By A. Yu. Ishlinskii. Translated from the Russian by A. Barouch. Edited by T. Pelz. Pp. iv+313. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 135*s*.

THIS is primarily a textbook on the geometry and kinematics of gyroscopic systems. As such it is excellent, particularly in the development of mathematical expressions where the author applies approximations immediately they become relevant and not, as is commonly done, at a stage long after the less mathematical reader has lost the trend of the argument.

The scope of the book, however, is largely limited to geometry and kinematics. This, to some extent, is explained by the date of the first edition (1952) and that this is the first revision. Before 1950, the use of unmonitored gyros was almost entirely restricted to marine applications with a few exceptions such as the twin gyro guidance system of the German *V*2 rocket. The philosophy of this latter guidance system is clearly explained in the book. The continuous rolling and pitching of a ship makes gimballing errors of major importance, and a proper understanding of these, as is given in this book, is essential to the marine gyro designer. Since 1950 a vast amount of research and development has been done on gyros in connexion with inertia navigation. In this field, where the premium on space limits gyro angular momentum to values two or three orders of magnitude less than those of marine gyros, investigations have of necessity been directed towards ways and means of minimizing random precession of the gyro, arising from such sources as instability of the position of the centre of gravity, frictional torques in the gimbal suspensions and anisoelasticity of the rotor bearings. These topics are touched on in one chapter of the book and the few examples given are well chosen, but the treatment is too limited in view of the vast strides which have been made in the past two decades in the development of new suspension systems and in the development of single axis rate and rate integrating gyros.

I consider this book excellent in its analysis of the geometry and kinematics of gyro mechanics but I hope that in a future edition the appendix on inertial navigation will be brought into the body of the book so that the mechanical design difficulties of small angular momentum gyros and the methods adopted to overcome them could be analysed and thereby add to what is already an excellent textbook.

A. I. STEVENSON

EMIL ARTIN, MATHEMATICIAN

The Collected Papers of Emil Artin

Edited by Serge Lang and John T. Tate. Pp. xvi+560. (Reading, Mass., and London: Addison-Wesley Publishing Company, Inc., 1965.) 102*s*.

EMIL ARTIN was born in Vienna in 1896. His father was an art dealer and his mother an opera singer. Artin himself

was a keen musician, playing the flute and several keyboard instruments.

His interest in mathematics, he tells us, was not awakened until he was 16 years of age. But after that his rise to fame was very rapid. Although his studies were interrupted by military service in the First World War, he was appointed to the Faculty at the University of Hamburg in 1923 and elected to a chair in 1926. Escaping from the tyranny of Nazi Germany, Artin emigrated to the United States in 1937. His stay in America, and especially the twelve years he spent at Princeton, had a profound effect on the development of mathematics. Many mathematicians of many nations were thus given the opportunity of benefiting from Artin's brilliance both as a research worker and as a teacher. Artin returned to Hamburg in 1958 and remained there until his death in 1962.

Artin will rank as one of the great mathematicians of this century. His work is admired for its depth and for its elegance, and it is distinguished by an aesthetic approach to mathematics which is communicated in lucid and eminently readable expositions, however technical their contents, and whether composed in German or in English. It was his basic conviction that "we all believe that mathematics is an art" and that "the author tries to convey the structural beauty of mathematics to his readers".

This volume is edited by Serge Lang and John T. Tate, who have prefaced the work with a short biography and an interesting discussion of some of Artin's conjectures, several of which had not been previously published. They have also added a list of text-books and lecture notes which are not included in this volume. All Artin's papers are reproduced in this collection. They are arranged according to topics, the chronological order being preserved in each section, beginning with the doctoral thesis on quadratic fields. This is followed by the section on class field theory, which includes Artin's celebrated discoveries on L-series and the general reciprocity law. The other headings are algebraic number theory, real fields, algebra and number theory, topology, and miscellaneous, 44 titles in all. Finally, there is a set of five general papers dealing with various aspects of mathematics, historical, educational and critical, which are addressed to a wide audience. Every mathematician, whatever his field of interest, will treasure this volume, which enshrines the work of a genius whose inspiring influence will extend far beyond his own generation.

W. LEDERMANN

WHITEHEAD DEFENDED

Experience and Conceptual Activity

A Philosophical Essay based upon the Writings of A. N. Whitehead. By J. M. Burgers. Pp. vii+277. (Cambridge, Mass.: The M.I.T. Press, 1965.) $7.50; 57*s*.

THE purpose of this essay is to indicate a system of thought in which notions concerning values can find a place along with ideas of causal relationships which are applied in the physical sciences. It stems from Prof. Burgers's admiration of A. N. Whitehead, and from his belief that the views of the great philosopher deserve re-interpretation and extension because of their complexity and because Whitehead was not always the clearest of communicators. The basis of Whitehead's doctrine is that in every act occurring in the universe, experience derived from that which has gone before is integrated with conceptions concerning possibilities. To this, Burgers grafts on the theme that throughout the universe there is an underlying purpose and design and that this can be discerned in the inanimate and animate worlds; in the latter, this is reflected in conceptual activities even in the lowest animal orders. Although Burgers recognizes the difficulty of ascribing values to any but human activity, he is "convinced that values, intentions and anticipation are not features of human life alone. I therefore believe that biological research can not come to terms with life unless it recognizes the effectiveness of such features in the activities of living organisms".

Such is the thread which winds through a book of vast scholarship supported by copious annotation. Its main defects are that argument fails to convince if premises are not acceptable—and Burgers's are founded on assumptions which are not capable of substantiation in much human experience—and that the attempt to elucidate Whitehead might have been more successful with less turgid prose, greater use of orthodox grammatical construction, and the avoidance of words whose coinage seems to have come from an overheated furnace. Inadvertently, however, the book may achieve its aim of drawing renewed attention to Whitehead's philosophy; it should lead many students to re-read his works in the original.

T. H. HAWKINS

DIFFRACTION AND COHERENCE

Diffraction : Coherence in Optics

By M. Françon. Translated from the French by Barbara Jeffery. Translation edited by J. H. Sanders. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies.) Pp. ix+139. (London and New York: Pergamon Press, Ltd., 1966.) 20*s*. net.

THIS is a book for students, based on the courses given by the author in the University of Paris. It contains a fairly detailed discussion of a great number of special cases of diffraction, together with an elementary discussion of coherence. Prof. Françon is known as a masterful lecturer, and it is therefore somewhat surprising that his course-book makes rather dry reading and has the character of a manual rather than a text-book. I suspect that to save space he has left out many of the illuminating remarks with which he illustrates his lectures, leaving only a skeleton of formulae. It is also somewhat surprising that in a book on diffraction phenomena which are all within the scope of Kirchhoff's integral, that general formula cannot be found and even Kirchhoff's name is not mentioned. But, lest one might accuse the author of nationalism, Fresnel zones are not mentioned either.

This makes the treatment rather too mathematical. I suspect that the beginner will have difficulties because of the reluctance of the author to introduce Dirac functions on pages 32–34, for example. Surely, it would help the beginner simply to state that the Fourier transform of an infinite sine wave is a Dirac function. It would also help him if he were to have been told, on page 51, that the Fourier transform of a periodically repeated signal goes over into a Fourier series, especially after such pains have been taken to work it out for one, two, . . . repetitions. It was probably the preoccupation of the author with physiological optics which made him introduce the resolving power of an optical instrument, which after all is an objective phenomenon, as something relating to the perception by an eye with a pupil of 0·6 mm diameter and a contrast threshold of 0·02. This too, I am afraid, can scarcely fail to confuse the beginner.

All this had to be said in the hope that in the next edition the author will make this a much better book, but even as it stands, it is a valuable introduction for second and third year physics students into this very important field. The many excellent illustrations make up for much that appears to be lacking in the text.

D. GABOR

SOVIET QUANTUM RESEARCH

Methods of Quantum Chemistry

By M. G. Veselov. Translated by Scripta Technica, Inc. Translation edited by S. Chomet. Pp. xi+181. (New York: Academic Press Inc.; London: Academic Press Inc. (London), Ltd., 1965.) $7.50.

Advances in Quantum Chemistry

Vol. 2. Edited by Per-Olov Lowdin. Pp. xi+372. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) 116s.

THE current research literature of the U.S.S.R. can nowadays be read translated into English; text-books, research monographs, and even review periodicals cannot. A few books recognized as outstanding, like the works of Landau and Lifschitz, have appeared in translation several years after first publication, but most are never seen at all in western countries, and one has little idea of the character and scale of such secondary publishing in comparison with the bewildering abundance in the West. Do scientists in the U.S.S.R., in reflective assessments of recent work, assign importance to the same discoveries? Do they have distinctive scientific attitudes to the role of theory in chemistry? Is there a distinguishable official "line" informing their more critical writings and tending to influence the course of original work? On present-day evidence, supported by the evidence of *Methods of Quantum Chemistry*, there are no such differences. Indeed, there seems no particular reason why this book, which is a collection of reviews, should have been singled out for translation because most of the material, while perfectly soundly presented, is widely available in English language review publications. This is true of "Semi-empirical Methods in the Theory of Small Molecules" (T. K. Rebane), of the chapters on molecular orbital methods (Mestechkin), and theoretical reactivity studies in conjugated systems (Tupitsyn and Adamov). Nikitin's article on "Interelectron and Non-Adiabatic Interaction in Long Molecules", and to a lesser extent Rebane's on finite difference methods in molecular orbital theory, have greater interest on account of their discussions of the application of less familiar theoretical methods. M. G. Veselov's connexion with the book is that of editor, a fact which is not disclosed on the cover and title page. It is regrettable that he is not also one of the authors.

The second *Advances in Quantum Chemistry* is well up to the standard of the first. The articles are by Boys and Rajagopal, Fischer-Hjalmars, Blinder, McWeeny and Steiner, Jansen, Jehle and Löwdin. Good reviews are always to be welcomed; and as the original literature slips out of reach by its sheer quantity, recovery in digests and comprehensive reviews as good as in these *Advances* is the more worth while and essential.

D. P. CRAIG

ONE-DIMENSIONAL FLAMES

Flame Structure

By R. M. Fristrom and A. A. Westenberg. (McGraw-Hill Series in Advanced Chemistry.) Pp. xiii+424. (New York: McGraw-Hill Book Company, Inc.; Maidenhead: McGraw-Hill Publishing Company, Ltd., 1965.) $17.50; 140s.

A FLAME is, at first sight, the obvious medium for the experimental investigation of high temperature processes. It may be time-invariant phenomenon, almost completely free from surface effects and cheap to set up. Flames can be produced with a wide range of temperature and composition. However, the disadvantages of flames—having many components and large temperature and concentration gradients in the reaction zone—have caused many research workers interested in physical and chemical processes at high temperatures to dismiss flames as too complex to be suitable media for research. The authors of *Flame Structure* believe that this view is too pessimistic and their belief is fully substantiated by this book. There is no denying, of course, that flames are more complex than some other experimental media and that the steep gradients which exist in them make the analysis of flame data to obtain chemical kinetic information more difficult. These gradients can, however, be measured accurately and taken into account in many cases and the expenditure of effort involved is amply repaid by the results obtained.

This book is intended for combustion research workers, in particular for those working in the field of gas phase reaction kinetics. It is concerned primarily with measurements of the properties of pre-mixed, laminar flames and with the interpretation of these measurements to obtain information on fundamental physical and chemical processes in flames. This type of flame is chosen because it approximates closely to a one-dimensional flow system and is therefore the most amenable to quantitative analysis. It is also the best type of flame to use to assess the validity and accuracy of some of the experimental techniques commonly used in combustion studies.

The book begins by dealing with the basic equations needed in discussing flame processes, together with an essential amount of relevant information on transport processes, thermodynamics and chemical kinetics. The basic equations for the conservation of energy and the conservation of species are given with emphasis on the forms of the equations applicable to flat and spherically symmetrical flames. The information which can be obtained from these equations is discussed as are the flame variables which have to be measured to do so.

The second, and principal, section deals with the practical measurement of the properties of one-dimensional flames. It begins with a description of different types of burners and of the ancillary flow metering and pumping equipment. This is followed by accounts of experimental techniques used to obtain flame velocities, temperature profiles, the concentration profiles of stable species (by probe sampling), the concentration profiles of free radicals and ions and by accounts of techniques for measuring distances and positions in flames, and obtaining flame photographs.

Throughout this section careful attention is paid to essential practical details and clarity of presentation. Each technique is illustrated by numerous excellent diagrams—somewhat over-elaborate in some cases—and photographs. In addition there are several compact summaries of useful practical data, numerous references and a critical evaluation of the accuracy of the results obtained by different methods of measuring the same physical quantity. Most of these techniques are not, of course, limited in their usefulness to one-dimensional flames but can be used in many combustion systems. Because of this, *Flame Structure* will also appeal to research workers who study flames other than the idealized ones with which the book is mainly concerned. The weakest parts are those dealing with spectroscopic measurements of flames. Descriptions of spectroscopic methods of determining flame temperatures and species concentrations are not given at the same depth and with the same amount of detail as are descriptions of the other techniques.

The chapter summarizing values obtained experimentally for the diffusion coefficients and thermal conductivities of binary mixtures of gases is particularly valuable. Included also are the means for calculating the values of these transport quantities at flame temperatures either from the kinetic theory of gases or from values which have been obtained at lower temperatures. As the authors say, "The magnitude of the diffusion velocities

found in typical cases may seem surprising to anyone inclined to ignore diffusion and look upon a flame as a purely thermal phenomenon". The importance of diffusion in sub-atmospheric flames is brought out very clearly here.

The final two chapters deal with the information which can be deduced by applying the conservation equations to data on laminar flames. The two chapters cover the results on physical properties of flames and chemical reaction rates in flames, respectively; the latter subject is generally the principal reason for studying flames of this type. The chemical reactions considered are only those which are thought to occur in normal flames and the detailed descriptions of low temperature or "cool" flame kinetics which are commonly substituted for a discussion on combustion chemistry are not included. An account of the analysis of a fairly complete set of data on methane–oxygen flames is given as an example of the kind of information which can be obtained. Included also are summaries of the values of rate constants for elementary reactions in flames, given in the form of Arrhenius plots whenever a sufficient range of values is known.

Within its framework this book is first rate and will be found very valuable by research workers interested in high temperature gas phase kinetics and by combustion engineers who need to make measurements of flame properties. Unfortunately, as a good deal of the material which appears here has been published previously, it is likely that many who would like to have their own copy of this book will find the price too high.

D. R. JENKINS

PROGRAMMING AND FLOW

Programming, Games and Transportation Networks

By Claude Berge and A. Ghouila-Houri. Translated by Maxine Merrington and C. Ramanujacharyulu. Pp. x + 260. (London: Methuen and Co., Ltd.; New York: John Wiley and Sons, Inc., 1965.) 55*s*. net.

THIS book consists of two parts of nearly equal length. The first part, by Prof. Ghouila-Houri, is entitled "General Theory of Convex Programming", and devotes its first three chapters to an account of the pure mathematics needed later in the book. The topics considered include vector spaces, linear manifolds, convex sets and convex functions, open, closed and compact sets, continuous and semi-continuous functions, convex hull of a set, intersections of convex sets, Von Neumann's minimax theorem and the Farkas–Minkowski theorem. The presentation is clear and is adequate for a reader well grounded in a modern approach to pure mathematics, but those who do not possess such a background and are more interested in applications than in mathematical foundations would find the reading severe. The next two chapters are concerned respectively with programming and with convex programmes with linear constraints, and are still mainly of a theoretical nature. The final chapter of Part 1, the shortest in the book, deals with games of strategy and is at a less abstract level than the preceding chapters.

The second half of the book, by Prof. Berge, is entitled "Problems of Transportation and Potential". The first two chapters give the necessary theoretical background of graph theory and a general theoretical study of flows and potential differences. The central theme is that of duality, first between cycles and co-boundaries of a graph, then between trees and co-trees, continuing with a planar graph and its dual graph, strongly connected graphs and graphs without circuits, flows and potential differences, cyclomatic and co-cyclomatic matrices, and finally between the transportation problem and the problem of potential. The whole treatment has unity and coherence and is extremely elegant.

The remainder of the book is entirely concerned with applications. A large number of algorithms for solving various flow problems are given and there is a chapter devoted to problems related to the transportation problem. The detailed examples illustrate methods due to Dantzig, Ford and Fulkerson, Hoy, Minty, and others.

The book will be read with interest and pleasure by those concerned with theoretical aspects of the subject, and with profit by those interested in the applications. Although a number of errors were noted, the presentation is of a high standard and the translation reads well. There is an excellent index. H. O. FOULKES

A USEFUL TECHNIQUE

Agar Gel Electrophoresis

By R. J. Wieme. Pp. xiii + 425. (Amsterdam, London and New York: Elsevier Publishing Company, 1965.) 110*s*.

SOME years ago, in my review of Dr. Wieme's thesis *Studies on Agar Gel Electrophoresis*, I expressed the hope that it would form the basis of a more widely presentable work. This hope has now been realized. Agar gel is, of course, widely used for immunoelectrophoresis, but it is also a useful medium for simple zone electrophoresis, having a separating power between paper or powder slabs and starch or acrylamide gel, and it is to this aspect of its use that *Agar Gel Electrophoresis* is mainly devoted.

The first chapter provides an excellent account of the physics underlying electrophoretic processes in non-convective media and compares the properties of agar with those of other gels. (Incidentally it scarcely seems appropriate to give details in this chapter of the preparation of acrylamide gels, which are not complete anyway.) The next chapter surveys the various techniques used for agar gel electrophoresis. This is followed by a chapter which chooses the three techniques which the author considers to be of most general usefulness. These are a macrotechnique using gel on long glass plates, a microtechnique on microscope slides and a special procedure designated "fluid film" which uses dilute agar. The practical details are very adequately described. In the next chapter quantitative aspects are fully discussed, as are the properties of different varieties of agar, its chemistry and purification and the preparation of agarose (the representation of the linear agarose molecule is not correctly given). There is also a good discussion of various other properties of agar gel—endosmosis, interactions with electrophoretic substances and ion exchange and molecular sieve effects. Next comes a survey of the methods for rendering substances visible following electrophoresis, which provides full details of reagents for proteins, lipids, carbohydrates, nucleic acids and so on. Of particular interest is the description of reagents for substances having enzymatic activity. There is a short but useful section on immunoelectrophoresis giving useful details of preparation of immune sera, electrophoretic procedures and interpretations.

The sixth and final chapter is the longest, occupying seventy-one pages. It is a detailed review of the many applications of simple agar electrophoresis and covers a very wide field. Though it contains much useful information, this chapter suffers from being written in the style of a thesis where everything is included, important or not, and where arguments sometimes verging on the polemical are indulged in over matters not always worth arguing about. This does not help to make for easy reading, neither does the occasionally obscure English. Since 1959, when Dr. Wieme's thesis was published, the applications of agar electrophoresis have grown enormously,

but so have those of other types of electrophoresis, not to mention non-electrophoretic methods. Consequently to concentrate on one technique, as this chapter does, carries the danger of giving the erroneous impression that this technique has played a major part in advancing knowledge in some important fields. Some examples are the isolation and properties of human β_2 serum globulins, structural studies of immunoglobulins (it is admitted in this section that "analysis on Sephadex columns also proved fruitful" for this purpose !), human haemoglobin varieties and the study of plasmocytoma. Many of the detailed discussions of these and other subjects are scarcely relevant to a book of this nature and publications about them exist which cover all the analytical methods. This chapter does show that agar-gel electrophoresis is a useful complementary analytical technique, however, and the full coverage of the literature will be of great value to those who may want to use it for their own particular problems.

Agar gel electrophoresis is probably not so widely used in Britain as it is in the author's own country and other Continental countries. It has been used with great success in some important fields, a good example being isoenzyme studies. In general this book, well printed and illustrated, provides excellent guidance on the methodology of agar gel electrophoresis and should encourage its wider use. R. CONSDEN

VANISHING PEOPLE

Documents Anatoliens sur les Langues et les Traditions du Caucase

Par Georges Dumézil. 3: Nouvelles Études Oubykh. (Université de Paris. Travaux et Mémoires de l'Institut d'Ethnologie, 71.) Pp. 269+6 planches. (Paris: Institut d'Ethnologie, 1965.) 60 francs.

THE Ubykh are a small but important branch of the Caucasian family of peoples, closely related to the Circassians and the Kabardians. Since Greek and Roman times, they are reported as inhabiting a stretch of the Black Sea coastline between Abkhazia and the Crimea, not far from the modern Sochi. In 1864, the Russian conquest of the Caucasus resulted in the total dispersion of the Ubykh people, one segment being deported to the province of Kostroma, the other preferring to emigrate *en bloc* to the Ottoman Empire, to join forces with their Muslim co-religionists. In both cases, linguistic and cultural assimilation proceeded apace. To-day, Ubykh is spoken and understood only by a handful of old men, and these are rapidly dying out.

The interest presented by the Ubykh and their language was appreciated by scholars over the past century; the Russian Baron Uslar, the German Dirr and the Hungarian Mészáros all produced valuable pioneer studies. Much remained to be done, however, towards recording the vocabulary and phonology of this unique language before its final disappearance. Prof. Georges Dumézil, of the Collège de France, whose publications in the Caucasian field cover more than 30 years, has for several years been making annual trips to the remaining Ubykh villages in Turkey to preserve and interpret the vestiges of Ubykh folklore and oral tradition. This latest volume in his series of Ubykh publications commemorates the centenary of the annihilation of the Ubykh nation by Tsarist troops. Opening with a historical narrative of the last years of the independent Ubykh people, and biographical notes on Ubykhs whom the author has known, the book continues with nearly 150 pages of fresh Ubykh texts—traditional stories, anecdotes and personal reminiscences, of Caucasian and Turkish provenance—given with French and Kabardian translation. There is also an analysis of Hans Vogt's Ubykh dictionary, published at Oslo in 1963, as well as miscellaneous notes on Ubykh grammar and personalia. With six plates depicting the life of the survivors of this dying warrior nation, the book is a worth while addition to all libraries of ethnography anthropology and linguistics. DAVID M. LANG

The Principal Diseases of Lower Vertebrates

By H. Reichenbach-Klinke and E. Elkan. Pp. xii+600. (London: Academic Press, Inc. (London), Ltd.; New York: Academic Press, Inc., 1965.) 126*s*.

THIS book fills a gap in the literature and brings together a vast amount of information on the pathology, bacteriology, zoology and parasitology of fishes, amphibians and reptiles. It is not a "popular" book but can be recommended to students.

Although the cover citation states that the book "is designed to help owners of such animals to maintain healthy stock, to afford some explanation of the losses they invariably suffer and to suggest the means of avoiding their occurrence", I consider it to be much too advanced for the ordinary owner. It will, however, find a place in laboratories which conduct post-mortem examinations and carry out investigations into losses of animals in these three groups.

The problem of diseases in these animals is immense, and this book emphasizes the difficulties of diagnosis, particularly in fishes. The present outbreak of disease among salmon in the Republic of Ireland is a case in point. Despite weeks of careful investigation, no clear pronouncement on the cause of the trouble has yet been made. To a pathologist this book emphasizes the unity of pathology in all species. The diseases and parasites of domestic animals have their counterparts in the lower animals and the means of investigation are not dissimilar.

Part I deals with fishes. Methods of investigation, infectious diseases, non-infectious diseases, environmental factors, healing of wounds and regeneration are adequately covered. Chapter 6 is devoted to lampreys, and Chapter 7 deals with fish as carriers of human disease. In Chapter 8 is given a useful review of the main symptoms and localization of fish diseases. Chapter 9 deals with treatment but emphasizes the importance of hygiene. Treatment at the moment does not seem too promising and prevention is obviously to be aimed at. Part II deals with amphibia, and Part III with reptilia, on similar lines to those on fishes.

Altogether this book is most interesting and the authors are to be congratulated. H. G. LAMONT

Advances in Chemotherapy

Vol. 2. Edited by Abraham Goldin, F. Hawking and Robert J. Schnitzer. Pp. x+330. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) $13.

LIKE its predecessor, this book devotes nearly half its space to the chemotherapy of cancer, but the emphasis is perhaps more derived from the volume of work than the rate of advance in this field. What advances have been made in the recent past stem not from new drugs but from the more skilful clinical application of those drugs already available. This is described with a fascinating precision by Frei and Freireich in their account of the four-drug attack on leukaemia being conducted in the United States. Wallace Fox's chapter on "Tuberculosis" presents a most interesting comparison. He also describes a pattern of combined therapy with streptomycin, *p*-aminosalicylic acid and isoniazid, and even though the antitubercular drugs available can achieve cures—which are most rare in the leukaemias—the author comments that "100% success has only been

achieved in the best hands". This chapter as a whole, excellently written though it may be, fits oddly into the series. Much of it is devoted to discussion of the relative merits of institutional and domiciliary treatment and this surely is much more a medico-social question than a problem of chemotherapy.

The two chapters, one on "Cephalosporins" and the other on "Olivomycin, Mithramycin, Chromomycin: Three Related Cancerostatic Antibiotics", are perhaps the best models for collections of this sort. They deal most readably with limited topics, authoritatively enough for the expert while still maintaining interest for the general reader. To a slightly lesser extent, the same is true of the very different chapter on "Systemic Control of Plant Nematodes". But of the chapter on "Purine and Pyrimidine Analogs" one can only agree with the authors themselves when they say that "To some extent . . . readability has been sacrificed to logic. . . ." This proves a severe disadvantage in a book that has been produced to be read. WALTER DAVIS

Human Nutrition and Dietetics

By Stanley Davidson and R. Passmore. Third edition. Pp. xii+864. (Edinburgh and London: E. and S. Livingstone, Ltd., 1966.) 95*s*. net.

IN bringing this invaluable text-book on human nutrition up to date the authors have revised all the sections and brought the bibliography up to 1965 to include most recent revisions of recommended allowances of nutrients and other such important material. Chapters on the composition of the body, appetite and satiety, proteins, fats, carbohydrates, poisons, various deficiency diseases and the population problem have been completely rewritten.

In Part IV of the book, which is concerned with diet and physiological stress, the chapter on nutrition and care of the aged is considerably revised and new statistical data are included as well as a brief comment on social aspects of the problems of the increasing numbers of aged members of the population. This is only one example of the way in which the text has been improved since the first edition in 1959. The book continues to be indispensable for all students and teachers in the field of nutrition and dietetics as it brings together in one volume the whole subject of food for man. It is written by medical authors but their language, style and approach are such as to be fully understood by both medical and non-medical readers. In fact, most of the text makes easy reading for the student or the layman. For those who need deeper knowledge of particular problems the references give proper leads into the relevant literature. References to recent reviews are especially useful and are given in many sections where only a brief treatment of a subject is indicated.

A. M. COPPING

Embryology of the Ovary and Testis

Homo sapiens and *Macaca mulatta*. By Gertrude van Wagenen and Miriam E. Simpson. Pp. xvii+256. (New Haven and London: Yale University Press, 1965.) 56*s*.

Dr. Gertrude van Wagenen and Dr. Miriam E. Simpson have provided a valuable illustrated atlas which compares successive stages in the development of the ovary and testis in Man and the Rhesus monkey. Half the volume consists of plates of photo-micrographs, and for this reason alone its price is very moderate. Unfortunately the text is limited, both in scope and in the quality of the interpretation. The descriptions are on simple classical lines, and the study extends neither to an electron microscopic analysis of the kind with which students of gametogenesis are now becoming familiar, nor to a quantitative analysis of the stages of development which are described. Students will, none the less, find the book valuable, since even if it only confirms the classical picture of the development of the gonad, it provides useful background material with which those new to the subject will be able to orientate themselves in their more detailed studies.

Insects in Southern Africa

How to Control Them—a Handbook for Students, Health Officers, Gardeners, Farmers. By Bernard Smit. Pp. xiv+399. (London and Cape Town: Oxford University Press, 1964.) 63*s*. net.

No text-book of economic entomology has been published in southern Africa since C. K. Brain produced his *Insect Pests and their Control in South Africa* in 1929. Since that time many new crops and new pests have been introduced; and the method of control, whether by cultural and biological means or by the use of modern insecticides, have been transformed. During the past forty years Dr. Bernard Smit has been engaged in economic entomology in South Africa and has indeed been responsible for many of the advances that have been made. It is most valuable to have all this accumulated experience, much of which has hitherto been reported only in science bulletins and leaflets, brought together in book form. The book is wholly practical. Introductory matter is reduced to a minimum; no attempt is made to give more than the barest elements of insect anatomy, and insect physiology does not come into consideration. Clear and homely line drawings serve for identification. The insects are given their scientific, English and Africaans names; their life history and habits are clearly described; and measures of control are set out in a simple and effective style. The book is intended "for students, health officers, gardeners, farmers". It will fulfil this purpose admirably and can be warmly recommended. It may even find use outside South Africa. V. B. WIGGLESWORTH

Physical Properties of Martensite and Bainite

(Report of the Proceedings of the joint conference organized by the British Iron and Steel Research Association and the Iron and Steel Institute, held at Scarborough, 10–12 May, 1965. Special Report 93.) Pp. 211. (London: The Iron and Steel Institute, 1965.) 100*s*.

THE physical properties of martensite and bainite are of great technological importance to the steel industry and also provide challenging topics of investigation for research workers in industry, the universities and research associations. This volume reports the proceedings of a conference attended by representatives from these different backgrounds and successfully reflects the diversity of interest in the crystallography, morphology and kinetics of these classes of phase transformations. Eighteen papers, which review the field and describe recent research, are included. These were circulated to participants before the meeting and this has helped to produce an informed discussion which provides a major and valuable section of the book. Although the martensite and bainite reactions in steels are given prominence, work on non-ferrous metals and alloys is also described, emphasizing the close relationship which exists between transformations in a wide range of materials. The most striking feature of the book is the wealth of experimental information on the internal structure of transformation products which has been revealed by thin foil electron microscopy in recent years. This should provide a stimulus to the further development of theories of martensite crystallography which have undergone only minor modifications in the past decade.

The papers and discussion are of a generally high standard and the presentation is attractive. In particular the Iron and Steel Institute are to be congratulated on publishing this report less than five months after the conference was held. A. G. CROCKER

MEETINGS

PLANT TERPENOIDS

A SYMPOSIUM on "Terpenoids in Plants" was held in conjunction with the annual general meeting of the Phytochemical Group at the Department of Agricultural Biochemistry, Aberystwyth, during April 12–14, 1966, under the chairmanship of Professor E. Lederer (Gif-sur-Yvette). The symposium covered aspects of the occurrence, chemical structure, biosynthesis, and biological function of the major groups of terpenoids.

In the opening lecture Professor T. W. Goodwin (University College of Wales, Aberystwyth) reviewed some possible biological functions of terpenoids in plants, in particular the role of abscisin in the control of leaf abscission, the control of carotenogenesis in the (—) strain of *Blakesleea trispora* by trisporic acid and the stimulatory effects of sterols on oospore production by *Phytophthora* spp. and by *Pythium* spp.

The biogenetic isoprene rule was reviewed by Professor D. Arigoni (Federal Technical College, Zurich). In the past few years a variety of sesqui-, di-, and triterpenes have been found which have structures which were not expected from the biogenetic rule. These apparent anomalies, however, can now be explained by several types of intramolecular arrangements which were outlined. Recent work on conformational analysis of terpenoids and steroids was described by Dr. J. S. E. Holker (University of Liverpool). He also showed how the behaviour of terpenoid molecules during chemical reactions was correlated with differences in their molecular geometry.

Methods of synthesis of mevalonic acid labelled with carbon-14 or tritium, or deuterium in specific positions were described by Dr. G. Ryback (Shell Research, Sittingbourne); these compounds can be used in the elucidation of the biochemical mechanisms involved in the biosynthetic conversion of mevalonate to terpenoids. The biosynthesis and metabolism of monoterpenoids was described by Dr. W. D. Loomis (University of Oregon). From the results of experiments in which the incorporation of mevalonic acid and $^{14}CO_2$ into monoterpenes was compared, it was suggested that mevalonic acid was formed near the site of monoterpene synthesis, presumably from sugars. The monoterpenes seem to be metabolically interconverted and these reactions are influenced by illumination and by night temperature.

Dr. A. C. Oehschlager (University of Strasbourg) described *in vitro* transformations, and particularly acid-catalysed stereospecific cyclizations of diterpenes; in many of the transformations the sequence of arrangements follows that of the proposed biogenetic pathways, such as in the acid-catalysed stereospecific cyclization of all-*trans* isoprenoid alcohols to *trans* decalins and the conversion of pimaladiene to abietadiene.

The chemistry of sesquiterpenes was outlined by Dr. M. O. Sutherland (University of Brisbane). Although the sesquiterpenes are derived from farnesyl pyrophosphate, the reactions involved in the further transformations are not clear. For example, the hydrocarbon geijerene which has two asymmetric centres is isolated in racemic form and, therefore, cannot be derived as an artefact of isolation from elemol. The toxic principles of *Myoporum deserti* contain sesquiterpene furanoid ketones, related to myoporone, which seem to arise by extensive allylic oxidation of unsaturated precursors.

The chemistry of triterpenes was reviewed by Dr. T. G. Halsall (University of Oxford). Recent structural determinations on more complex molecules and also on small amounts of compounds have clarified views on triterpene biogenesis. It is now clear that, after the methyl and hydrogen shifts accompanying the initial biosynthetic cyclization of squalene, oxidation occurs which leads not only to ketones and acids, for example, but also to loss of carbon, to bond fission, to further carbon–carbon bond rearrangement and even to new carbon–carbon bond formation. Such reactions were illustrated by reference to a number of triterpenes including platanic acid, nycthanic acid, limonin, swietenine, ceanothic acid and related compounds.

The biosynthesis of phytosterols, reviewed by Dr. L. J. Goad (University College, Aberystwyth), appears to be similar in many respects to that of cholesterol in animals. The extra C_{24}-alkyl groups arise by transmethylation probably shortly after the cyclization of squalene. In plants it seems that lanosterol is not in the biosynthetic sequence but that its place is taken by *cyclo*-artenol which has recently been found always accompanying the plant sterols.

Professor R. Tschesche (University of Bonn) described recent experiments on the biosynthesis of steroid sapogenins and related compounds. 21-^{14}C-Δ5-pregnene-3β-ol-20-one glucoside is converted, in leaves of *Digitalis lanata*, to radioactive digitoxigenin, digoxigenin and gitoxigenin. Similarly, 4-^{14}C-cholesterol glucoside is converted to tigogenin, gitogenin and diosgenin. There must, therefore, be a hydrogenase which saturates the Δ5 double bond to give a *cis* configuration in ring *A/B* and hydroxylases which can introduce hydroxyl groups at C_{11} and C_{16} of digitoxigenin and C_2 of tigogenin.

The introduction of infra-red, nuclear magnetic resonance and precision mass spectrometry into structural studies of carotenoids was described by Professor B. C. L. Weedon (Queen Mary College, University of London). Even if the older degradative methods have to be used they can now be combined with chromatographic analysis of the products so that as little as 5 or 10 mg of carotenoid can be used. The newer methods were used to determine the structures of capsanthin and capsorubin, and also more recently the structures of fucoxanthin and foliaxanthin, both of which are shown to possess allene groupings, have been investigated.

Dr. J. F. Pennock (University of Liverpool) reviewed the chemistry and occurrence of the four major groups of terpenoid quinones, namely the vitamins K, plastoquinones, ubiquinones, and tocopherol quinones. These are all associated with intracellular organelles and their functions are associated with hydrogen transport by conversion to the corresponding hydroquinones although the possibility of biological reactions involved in cyclization to the chromanol forms may also be important.

The biosynthesis of the terpenoid quinones was reviewed by Dr. D. R. Threlfall (Aberystwyth). The three major distal precursors of the quinones appear to be mevalonic acid which gives the terpenoid side-chain, shikimic acid which gives the aromatic nucleus of the *p*-benzoquinones, and methionine giving the ring methyls and methoxyls. Permeability barriers across organelles has hampered many of the biosynthetic studies. It is not yet clear whether prenyl phenols, analogous to 2-decaprenyl phenol, are universal precursors of the terpenoid quinones.

Dr. F. W. Hemming (University of Liverpool) described the recent isolation from green leaves of long chain polyisoprenoid alcohols with chain lengths up to 110 carbon atoms. Each of these so-called prenols has more *cis* than *trans* isoprene residues and some of them resemble the pig liver dolichols because the double bond normally β— to the hydroxyl group is saturated. The *cis–trans* prenols occur as a mixture of isoprenologues, varying with the species, the concentration in green leaves increases with age and the bulk of them is associated with the osmiophilic globules of the chloroplast; the rest occurs outside the chloroplast.

J. FRIEND

HAEMORHEOLOGY

THE First International Conference on Haemorheology was held at the University of Iceland, Reykjavik, during July 10–16, 1966.

Haemorheology—the study of the flow of blood and its components, of its coagulation and lysis and of the mechanical properties of the vessel walls—is of such practical importance that it was felt worth while to organize a series of conferences on the subject and to have a society responsible for such organization and international co-ordination. Before the conference started, an International Society of Haemorheology was founded. The Officers and Council of the Society were elected but it was left to the Council to draw up a constitution. The conference chairman, Professor A. L. Copley (United States), was unanimously elected president. Should an international society of rheology as a whole, or of biorheology, be formed at some future date, it was agreed that the new Society would consider some form of affiliation.

After a formal inaugural session at which the conference was welcomed by Professor A. Snaevarr, Rektor of the University, the Minister of Education and Dr. A. Brekkan of the Icelandic Medical Association, there were three short addresses. The first, by Professor A. L. Copley (United States), was an inaugural address covering the general purposes of the conference. This was followed by short lectures on applications of haemorheology to the practice of medicine by Professor H. Hartert (West Germany) and on the link with physics by Dr. G. W. Scott Blair (United Kingdom). More than sixty papers were read during the conference and in the closing session short appraisals of the achievements in the various fields covered were given by specialists.

Dr. A. Silberberg (Israel), in a summary of the papers on haemorheological theory, pointed out the difficulties involved in applying precise mathematical expressions unless certain biological processes are ignored. He noted with approval the paper by Professor W. G. Frasher *et al.* (United States), who combined a rheologically well defined system with the immediate return of the blood to the living animal. A further complication is the two-phase nature of blood which makes the experimentation depend on the dimensions of the apparatus. Casson's very popular equation, for which the square roots of stress and of shear rate are plotted for capillary flow, may be regarded as sound if it is used to study the process of flow in one direction only. Dr. Silberberg also discussed the theoretical implications of an assumed yield value for blood and closed by emphasizing the considerable progress which has recently been made in the haemodynamics of pulsatile flow.

Work on haemorheological techniques was summarized by Dr. M. Joly (France). Of special interest were Professor Frasher's work (already referred to) and a paper by Professor H. Wayland (United States), who emphasized the importance of the exact determination of three-dimensional geometry in *in vivo* studies. The use of cinemicrophotography was emphasized by Professor H. Bloch (United States), and Professor H. R. Jacobs (United States) described studies on meniscal resistance. Thrombodynamography, a technique developed by Professor H. Hartert (West Germany), was now widely used clinically.

Model theory and phase separation were discussed by Professor G. Bugliarello (United States). The models proposed during the conference covered a very wide range from Dr. A. Silberberg's physico-chemical boundary process to Dr. E. O. Attinger's (United States) model of peripheral circulation. Proposed systems included hydrodynamic, mathematical, electrical and visual models. Model suspensions for blood continue to be improved, and the importance of adsorption of molecules on the vessel wall in regulating the flow in small vessels and also bifurcations as controls for the haematocrit in downstream vessels are increasingly being appreciated.

Professor S. Witte (West Germany) summarized work on cellular and fibrin clotting. The two most important questions concerning clotting were undoubtedly the production of fibrin and the aggregation of platelets. Professor M. E. Leroux (France) discussed the effect of platelets on retraction in terms of Professor H. Hartert's thrombelastograph and retractograph techniques. Retraction starts much earlier than was previously supposed. Professor A. Copley (United States) reported investigations of an intermediate product during fibrin development when only the fibrino-peptide *A* is split off from the molecule. Fibrinolysis was also much discussed. This is inhibited by the presence of platelets. The building of platelet aggregates is important for haemostasis and for thrombosis; however, it seems to bear no relation to vascular changes in the early stages of arteriosclerosis, as was shown by Professor W. E. Stehbens (Australia). Dr. G. W. Scott Blair (United Kingdom) made a preliminary attempt to propose basic equations for flow, coagulation and fibrin softening but such generalizations are still at an early stage.

Dr. R. L. Whitmore (United Kingdom) discussed the findings of the conference on plasma expanders. All dextrans of molecular weight greater than about 20,000 tend to cause some aggregation of red cells. The addition of dextrans with molecular weights of 40,000 to suspensions which have been strongly aggregated by fibrinogen or dextran of high molecular weight gives no better flow behaviour than is obtained with those diluted with albumin or saline. The addition of dextrans of high molecular weight to dogs *in vivo* leads to a fall in haematocrit and a dramatic decline in cardiac output. The improvement in the dispersion of red cells following infusion of dextran of 40,000 molecular weight is probably a result of an increase in the shearing forces to which the blood is exposed. Interaction between dextran and platelets on the vessel walls must also be taken into account.

Professor M. G. Taylor (Australia) summarized papers on *in vivo* work but he first emphasized the importance of Poiseuille's work on water and other simple liquids flowing in wide artificial capillaries, which is the basis of all studies of flow under *in vivo* conditions. Conditions in very small vessels, such as were discussed by Professor C. A. Wiederhielm (United States), are particularly complex. Dr. A. C. Groom (United Kingdom) described a muscle preparation giving transit times of cells and muscles through the vascular bed.

Professor A. C. Burton (Canada) discussed the papers on blood cellular elements. He emphasized the importance of microscopy in such studies. "There is no substitute for observation of the shape and general appearance of the erythrocytes." "Black-box" types of apparatus have their uses but one must look inside "to see the wheels of transformation turning". The electron microscopist may miss irregularities in the living membranes of the cells that may be all-important. Other methods, such as the measurement of birefringence, as described by Dr. H. H. Pfeiffer (West Germany), can also be very helpful.

Finally, Professor L. E. Gelin (Sweden) appraised the clinical value of the conference. He emphasized especially the value of current work in relation to the study of shock. The importance of the viscosity of blood and plasma is increasingly realized but viscosity in non-Newtonian systems is itself complex. In the field of blood clotting, the importance of platelet aggregation is increasingly appreciated and also the effect of split products (peptides) on clotting. The importance of fat in the circulation was also discussed.

Continued on next page

OBITUARIES

A. T. Glenny

ALEXANDER THOMAS GLENNY died on October 5, 1965, at the age of eighty-three. He was born in Dulwich on September 18, 1882, and brought up—though he never joined the sect—as a Plymouth Brother. He was educated at Alleyn's School, Dulwich, and when in 1899 Dr. W. Dowson, the director of the Wellcome Physiological Research Laboratories, asked the headmaster of the school to recommend two boys as possible laboratory assistants, Glenny was one of those chosen. His association with the Laboratories lasted for forty-eight years, as laboratory assistant and as head of the Department of Immunology.

Much of Glenny's work was done in collaboration, but the ideas are in general his or follow from his own. Apart from a few miscellaneous papers, his output forms a connected whole which deals with a wide variety of problems concerned with immunization of man and animals—the production, purification and assay of antigens, the increase in their potency, and the basic ideas underlying their use in immunization; the characteristics of passive immunity, and its effect on active immunization; the assay of antisera and the nature of the toxins present in bacterial filtrates. In much of this work he was a pioneer, and to everything he worked on he added fundamental observations and ideas.

Some of his earliest papers deal with the basic and almost universal responses to antigens—the primary and secondary response. These and a consideration of the nature of normal antibodies in animals led him to lay down the ideal conditions for immunization—few injections with long rests between them. To this fundamental work he added the production of new antigens—diphtheria and tetanus toxoids, diphtheria toxin precipitated by alum and toxoid antitoxin floccules—and methods for their assay and use. Not only did he work out the characteristic phases of passive immunity, but he also applied the information to the natural case of homologous passive immunization of the human foetus *in utero*. Here he showed that the immunity transferred passively from the mother may interfere with active immunization of the newborn child, and that, on the average, the interference falls almost to nothing by 6 weeks after birth.

Glenny also showed that combined antigens were a practical proposition, but that they had to be made and used intelligently. Above all, he showed that basic immunity to one antigen in a mixture may reduce antibody response to other components of which the subject has had no experience—a development of earlier work in which he had shown that horses sensitive to horse serum respond poorly to diphtheria toxin-(horse)-antitoxin floccules. By all these methods Glenny laid down the rules that govern modern immunological practice, especially against those diseases in which bacterial toxins are important.

He made considerable advances in assaying antitoxins, both by introducing new methods or improvements on old ones, and, most important, by determining what was being assayed. To this end he developed many of the means of detecting multiple toxins in bacterial filtrates, and of assaying antitoxins against them singly. Besides this, he did excellent work on the avidity of antitoxins—the intensity with which they are bound to their toxic antigens. Most of this work was carried out while he was in charge of the production and testing of antitoxins and prophylactics; it is an astonishing performance for a man so strenuously occupied.

He was not easy to get on with; shy, diffident, but very conscious of his own capacity, and (often justifiably, but not always sensibly) very critical of that of others. He was almost unbelievably thorough in his own work, tidy, and constantly occupied with the best and most useful form of recording for routine immunization and testing and experimental work.

He was elected Fellow of the Royal Society in 1944, and awarded the Addingham Gold Medal and the Jenner Medal in 1953. He married Emma Blanche Lilian Gibbs in 1910; she and two of their three children survive him. He retired from the headship of the Department of Immunology at the Wellcome Physiological Research Laboratories in 1947, but continued working there until 1959.

C. L. OAKLEY

W. R. Spurrell

WALTER ROWORTH SPURRELL, emeritus professor of physiology in the University of London, died at his home in Dorset on June 7, 1966, at the age of sixty-nine.

He belonged to the generation which was depleted of so many of its best men in the First World War, but Spurrell survived three years of active service as a gunner officer and returned to his medical studies in 1919. His career as a student was brilliant. He carried off all the best prizes at Guy's Hospital Medical School and found time to take an honours degree in physiology before he qualified M.B., B.S., with the university gold medal. He then embarked on a career of surgery, gained an M.S. and his F.R.C.S., and within a few years his scientific training and his clinical excellence marked him out as a surgeon of the highest promise. But a long illness forced him to give up his clinical career.

With characteristic courage and enthusiasm he turned to physiology and, after spending some happy years in McSwiney's department in the University of Leeds, he was invited to return to his old school to take charge of the Department of Physiology at Guy's when M. S. Pembrey retired in 1933. Spurrell retired from his chair in 1962, so that for 30 years he directed the teaching of physiology; no one ever did more to bring together the pre-clinical and clinical activities of Guy's Hospital and its School. His experience and understanding of patients which he gained as a surgeon in his earlier years gave him a unique insight into the teaching of fundamental physiology to medical students.

He had a simplicity of expression which came from profound knowledge and clarity of thought and the most difficult subjects seemed simple when he expounded them. His own work included subjects as diverse as gastric function and endocrinology. He generously encouraged the lecturers in his department and guided them past fallacies to sound work. In the development of the Medical School at Guy's he was a uniting force. His own personal modesty and unselfishness gave him an authority that was often the means of settling conflicting views and interests. His wise advice was sought by individuals in their personal problems no less than by committees in their difficulties over big issues.

G. F. GIBBERD

Continued from previous page

At a special session, a gold medal, cast in honour of Poiseuille, "the first haemorheologist", was presented to Professor Robin Fåhraeus (Sweden) in recognition of his pioneer work on blood flow and sedimentation.

The secretary of the new International Society of Haemorheology is Professor George Bugliarello, Department of Civil Engineering, Carnegie Institute of Technology, Pittsburgh, Pennsylvania.

G. W. SCOTT BLAIR

A MODEL FOR VARIABLE EXTRAGALACTIC RADIO SOURCES

By PROF. H. VAN DER LAAN*

National Radio Astronomy Observatory†, Charlottesville, Virginia

THE recently discovered[1] temporal variations in the millimetre and centimetre radiation from a number of quasi-stellar radio sources and one Seyfert galaxy[2-5] provide radio astronomy with a potentially rich source of information on the physics of non-thermal radio sources. It has been suggested[5,6] that the varying components are sources of synchrotron radiation which are initially optically thick but, as they expand, become optically thin at progressively longer wavelengths. Indeed, data now available[5] suggest that the variability in the high frequency part of the radio spectrum has a systematic character. The purpose of this article is to present a quantitative discussion of the evolving synchrotron self-absorption model. The time-dependence of the radio spectrum is derived, and it is shown how variations observed at one frequency may be used to predict the behaviour at any other frequency.

The attitude here is to admit the present mysterious nature of the initial event(s) giving rise to the relativistic electron flux. Rather than add to the multi-parameter speculation concerning such events, a definite model for the subsequent evolution of the radiation source is considered, which has a number of features accessible to direct observational tests. In this manner it may be possible, as additional and refined data are obtained, to approach the initial event by imposing well defined boundary conditions and other requirements.

Properties of the model. Consider a uniform and spherical cloud of radius r, expanding at the rate $\dot{r}$, filled with a flux of electrons at relativistic energies. Let these electrons have an isotropic velocity distribution and an energy distribution $N(E)\mathrm{d}E = K(t)E^{-\gamma}\mathrm{d}E$ (cm^{-3}) confined to a range $E_1(t) \leqslant E \leqslant E_2(t)$. They radiate as they are accelerated in a magnetic field of strength B gauss (G). Initially, the cloud is sufficiently compact to be optically thick at all radio frequencies. The condition that the brightness temperature of the radio source cannot exceed the equivalent temperature of the electrons chiefly responsible for the synchrotron radiation at any given frequency yields the well known relation[7,8]

$$\nu_m = CB^{1/5}\,\theta^{-4/5}\left\{S(\nu_t)\,[\nu_t/\nu_m]^{(\gamma-1)/2}\right\}^{2/5} \qquad (1)$$

where ν_m is the frequency at which the spectral curve reaches a maximum, ν_t is some frequency at which the source is transparent, $S(\nu_t)$ is the flux density at frequency ν_t, θ is the angular diameter, and C is a numerical constant dependent on the units used. For synchrotron radiation the volume emissivity $p(\nu) \propto \nu^{-(\gamma-1)/2}$ while the absorption coefficient $\mu(\nu) \propto \nu^{-(\gamma+4)/4}$, and for this reason it follows that the frequency dependence of the spectral curve takes the form

$$S(\nu) \propto \frac{p(\nu)}{\mu(\nu)}\,[1-\mathrm{e}^{-\tau(\nu)}] \propto \nu^{5/2}\,[1-\mathrm{e}^{-\tau(\nu)}] \qquad (2)$$

where the optical depth $\tau(\nu) = \int\mu(\nu)\mathrm{d}l \propto \nu^{-(\gamma+4)/2}$.

For frequencies $\nu \ll \nu_m$ the flux density is given by

$$S(\nu) = k_1 B^{-1/2}\,\theta^2\,\nu^{5/2}. \qquad (3a)$$

while at $\nu \gg \nu_m$

$$S(\nu) = k_2 K B^{(\gamma+1)/2}\,\theta^2\,\nu^{-(\gamma-1)/2} \qquad (3b)$$

where k_1 and k_2 are constants.

The parameters change as in the adiabatic expansion model first proposed by Shklovsky[9] to represent the early phase of supernova remnant evolution. Magnetic flux is conserved

$$B = B_0(r/r_0)^{-2} \qquad (4a)$$

and the relativistic gas cools adiabatically, so that for an individual particle

$$E = E_0(r/r_0)^{-1} \qquad (4b)$$

while the angular diameter, of course, varies as

$$\theta = \theta_0(r/r_0) \qquad (4c)$$

In the model, particles neither enter nor escape the reservoir after the initiating event, so that

$$\frac{\mathrm{d}}{\mathrm{d}t}\left\{r^3(t)K(t)\int_{E_1(t)}^{E_2(t)} E^{-\gamma}\,\mathrm{d}E\right\} = 0 \qquad (4d)$$

The subscript 0 denotes parameter values at a specified instant. From relations (3) and (4) it follows that for $\nu \ll \nu_m$

$$S(\nu,\rho) = S_0(\nu)\,\rho^3 \qquad (5)$$

and for $\nu \gg \nu_m$

$$S(\nu,\rho) = S_0(\nu)\,\rho^{-2\gamma} \qquad (6)$$

where $\rho = r/r_0$ is the relative radius of the source. From relations (1), (4) and (6) the dependence of ν_m, the frequency of maximum flux density, on radius is seen to be

$$\nu_m(\rho) = \nu_{m0}\,\rho^{-(4\gamma+6)/(\gamma+4)} \qquad (7)$$

From equations (7) and (5) or (6) the variation of the maximum flux density follows:

$$S_m(\rho) = S_{m0}\,\rho^{-(7\gamma+3)/(\gamma+4)} \qquad (8)$$

The evolution of the spectrum is thus seen to be very simple. The spectral curve, as given in the usual $\log S(\nu) - \log\nu$ plane, does not change, but moves down and towards the left, that is, to lower flux densities and lower frequencies. Observing such a source at one frequency will show the flux density to increase rapidly at first, reach a maximum and then decrease more gently. Simultaneous observation at a lower frequency will show the same fractional rate of increase, but the maximum will be reached later and have a smaller value.

Secular variations take the form

$$\dot{\nu}_m/\nu_m = -\left(\frac{4\gamma+6}{\gamma+4}\right)\dot{r}/r;\quad \dot{S}_m/S_m = -\left(\frac{7\gamma+3}{\gamma+4}\right)\dot{r}/r \qquad (9)$$

and for $\nu \ll \nu_m$ $\quad \nu \gg \nu_m$

$$\dot{S}/S = 3\dot{r}/r\ ;\ \dot{S}/S = -2\gamma\dot{r}/r \qquad (10)$$

To calculate $S(\nu,\rho)$, the flux density at a given frequency and radius, use expressions (2), (7) and (8). It is noted that the spectral curve has no identifiable features other than the curvature associated with the turnover, which

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† Operated by Associated Universities, Inc., under contract with the National Science Foundation.

provides the point of reference. The flux density and frequency are therefore given relative to their values at the spectral maximum when $\rho = 1$. Then

$$S(\nu,\rho) = S_m\left(\frac{\nu}{\nu_m}\right)^{5/2}\left[\frac{1-\exp(-\tau)}{1-\exp(-\tau_m)}\right]$$

$$= S_{m0}\,(\nu/\nu_{m0})^{5/2}\,\rho^3\,\frac{\left[1-\exp\left\{-\tau_m\left(\frac{\nu}{\nu_{m0}}\right)^{-(\gamma+1)/2}\rho^{-(2\gamma+3)}\right\}\right]}{[1-\exp(-\tau_m)]} \qquad (11)$$

where τ_m, the optical depth corresponding to the frequency at which the flux density is a maximum, is the solution of

$$e^x - \left(\frac{\gamma+4}{5}\right)x - 1 = 0 \qquad (12)$$

which is the condition for expression (2) to be a maximum.

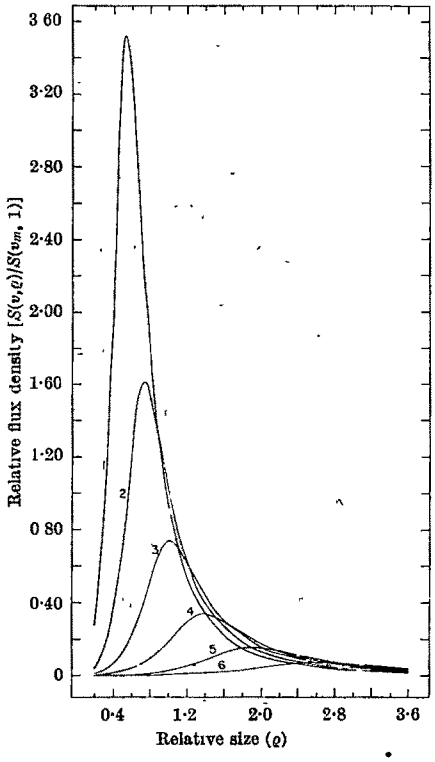


Fig. 1. The radial dependence of flux density. The frequencies of successive curves differ by a factor of two. The spectral index (α) in this case is 0·25. Values for the various curves are ν_{m_0}/ν : (1) 1/2; (2) 1; (3) 2; (4) 4; (5) 8; (6) 16.

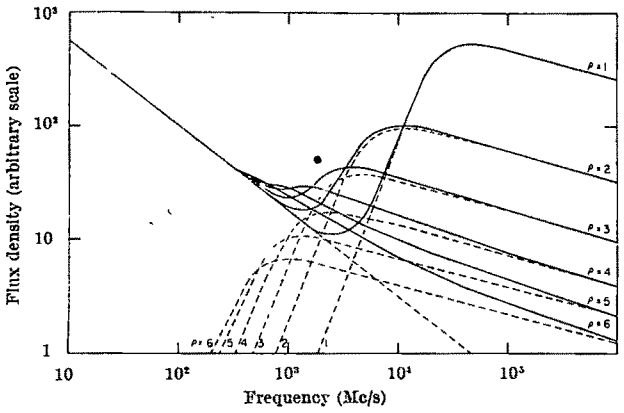


Fig. 2. The evolution of a typical variable radio source spectrum. The variable source spectrum is added to that of the constant source and the resultant is shown at successive integer values of ρ.

Flux density as a function of frequency and time. In order to obtain the flux density at any frequency as a function of time, it is necessary to specify the time dependence of the expansion velocity. Let $t_0 = r_0/\dot{r}_0$ be the "apparent age" of the source when its radius is r_0. Suppose $\rho = (t/\beta t_0)^\beta$ and define βt_0 as the "expansion age" at radius r_0. (The "true age" is not defined when the circumstances that give rise to the source are unknown.) Two cases will be referred to in particular: (*a*) constant expansion velocity, that is, $\beta = 1$; and (*b*) deceleration in the manner of a relativistic gas cloud expanding into a uniform gaseous medium[10], asymptotically $\beta = 2/5$.

The function $S(\nu,\rho)/S_{m_0}$, as given in equation (11), is presented graphically in Figs. 1 and 2 for several values of ν/ν_{m_0} and a range of ρ. These curves were computed using a spectral index $\alpha = (\gamma - 1)/2 = 0{\cdot}25$. If the frequency of maximum flux ν_{m_0} is known and S_{m_0} is obtained from measurement or from equation (11), then the vertical scale of curves such as in Fig. 1 can be specified in flux units and each curve can be assigned a definite frequency.

The time scale factor t_0 can be determined from a measurement of one observable and its rate of change. Any of the equations (9) and (10) is an expression for the apparent age when $1/t_0$ is substituted for $(\dot{r}/r)_0$. If, for example, the flux density and its rate of change are measured at a frequency $\nu_0 \ll \nu$

$$t_0 = 3S_0/\dot{S}_0 \qquad (13)$$

In order to relate radius and age, let the time t' be measured from the instant $t' = 0$ when S_0, $\dot{S}_0$ and ν_{m_0} are specified. Then

$$\rho = (1 + t'/\beta t_0)^\beta \simeq 1 + t'/t_0 \text{ for } |t'| \ll \beta t_0 \qquad (14)$$

The function (14) is sketched in Fig. 3 for several values of the deceleration parameter β. For times small compared with βt_0, before and after the instant $t' = 0$, the model can be compared with given sources at any frequency and time. For times separated from the instant $t' = 0$ by intervals of order βt_0 the curves in Fig. 3 serve to relate ρ and t' for a chosen β.

If the maximum of the spectral curve is at frequency ν_1 when $t' = t_1$, then this maximum reaches another frequency ν_2 at

$$t'(\nu_{m_2}) \equiv t_2 = (\beta t_0 + t_1)\,(\nu_1/\nu_2)^{(\gamma+4)/\beta(4\gamma+6)} - \beta t_0 \qquad (15)$$

If at $t' = t_1$ the maximum flux is $S_m(\nu_1)$, then at the time given by relation (15) the flux density maximum is at ν_2 and is given by

$$S_m(t_2) = S_m(t_1)\left(\frac{\beta t_0 + t_2}{\beta t_0 + t_1}\right)^{-\beta(7\gamma+3)/(\gamma+4)} \qquad (16a)$$

or the equivalent expression

$$S_m(\nu_2) = S_m(\nu_1)(\nu_1/\nu_2)^{-(7\gamma+3)/(4\gamma+6)} \qquad (16b)$$

These relations together can be applied to any source the spectrum of which is variable and known over a fraction of the expansion age. Spectral changes in the immediate future are then predictable; for later times they depend on the expansion mode. Prolonged observations can be expected to provide information about the dynamics of expansion and to yield a value for β.

The applicability of the model *überhaupt* can be tested by the requirement that the expressions for t_0 equivalent to expression (13), given by each observable and its rate

of change as given in equations (9) and (10), result in values equal within experimental errors. Data now available are spread too thinly in time and wavelength to attempt as yet quantitative tests; qualitatively the model conforms to observed radio source behaviour[1,4,5].

There are ways other than synchrotron self-absorption which can give rise to a turnover in the spectral curve[11]. A magneto-ionic medium dense enough to cause significant dispersion leads an otherwise inverse power law spectrum to decrease sharply on the low frequency side of $\nu = 20\, n_e/B$ Mc/s where n_e is the free electron number density in cm^{-3} (Tsytovich effect). In that case the adiabatic expansion model without synchrotron self-absorption gives

$$\nu_m \propto n_e/B \propto \rho^{-1}$$

Qualitatively the alternatives are similar, but quantitatively they differ considerably. Compare, for example, the following ratio in the two cases:

$$\left\{\frac{\dot{S}(\nu_m)/S(\nu_m)}{\dot{\nu}_m/\nu_m}\right\} = (3\gamma + 1)/2 \text{ (dispersive medium)} \qquad (17a)$$

$$= (7\gamma + 3)/(4\gamma + 6) \text{ (synchrotron self-absorption)} \qquad (17b)$$

Because for most sources $\gamma \simeq 1{\cdot}5$–$2{\cdot}5$, spectral information over a time $\sim t_0$ will enable such cases to be distinguished.

Multiple bursts. Although current spectral data are incomplete, in a few instances the spectral information is sufficient to indicate that the observed spectrum is a superposition of two or more time-varying remnants of "bursts" and a constant component with the usual inverse power law spectrum. Such is the case for 3*C* 273 and 3*C* 279, for example. Very homogeneous data, at closely spaced time and frequency intervals, will make it possible to separate the contributions of each component, to which the method here outlined can be applied to predict the evolution of the composite spectrum.

The presence of more than one variable component in a single radio source has interesting implications. It cannot be ruled out *a priori* that the magnetic field is an attendant result of the event that produced the relativistic particles, but it is simpler to regard the former as an ambient field, present before the event in which energy is released. If this were indeed the case, then the spectra would indicate that the events occur at spatially separate points. Repeated outbursts at the same position would result in the following sequence of events: the first burst sweeps out the surrounding magneto-ionic medium, leaving an explosion vacuum. Particles produced in a second burst at that point would travel in a vacuum and not radiate until they entered the expanding shell owing to the preceding event. The result would be a shift in the spectral curve towards higher frequencies and an intensity increase. The observed spectra show, however, that the frequency spectra rather than the energy spectra are superimposed. It is therefore probable that regions exist in the quasi-stellar radio sources where conditions favour the occurrence of many independent outbursts. The available data show separate components which become observable only 2–5 years apart[6]; the regions of explosive activity may therefore be only a fraction of a kiloparsec in extent. The cumulative effect of many outbursts is expected to be a non-varying radio source with a conventional spectrum[12], resulting when the expansion volumes overlap and the energy spectra are superposed.

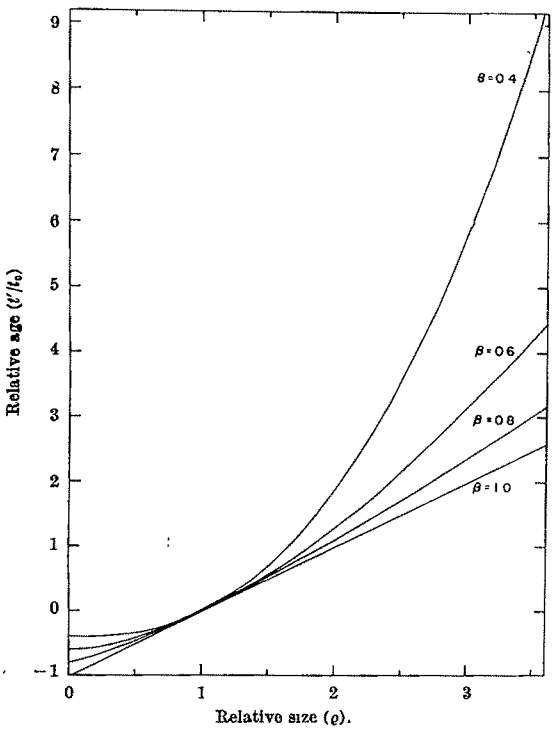


Fig. 3. The relation between the radius and the age of the source for several values of the deceleration parameter β.

Discussion. In the first instance the model is intended to account for and to predict the gross features of early radio source evolution. Departures from the predicted behaviour will occur to the extent that the complexity of the radio source exceeds that of the model. Such discrepancies will either show that the model is not related to the phenomenon observed or suggest what additional structure the model requires for a more adequate representation.

It should be noted that the higher the frequency at which the variation is detected, the shorter the time scale factor t_0. This shortens the time interval over which predicted variations can be compared and different expansion modes can be distinguished, proportionately. Millimetre and centimetre observations will yield the parameter values required to predict expected variations at longer wavelengths. Because the measurements cannot be repeated, it is highly desirable that at least two instruments are used for simultaneous observations at (nearly) equal frequencies. Systematic monitoring of the variable sources at many frequencies is necessary to acquire homogeneous data[13], so that theoretical suggestions can be tested. Optical and X-ray data are equally necessary; correlated variations at widely separated regions of the electromagnetic spectrum could shed much light on the nature of these sources. Such correlations are expected for plausible parameter ratios, where some radio photons, by inverse Compton interaction with the relativistic electrons, are converted to hard radiation.

Homogeneous radio data will make it possible to obtain reliable life times and linear diameters of the varying components. This information may then enable radio interferometry with baselines of several thousand kilometres to settle the controversial question of quasi-stellar distances.

I thank Drs. K. I. Kellermann and I. I. K. Pauliny-Toth for informative discussions and a preprint of their paper. I also thank Dr. D. S. Heeschen, director of the National Radio Astronomy Observatory, Charlottesville, for the hospitality of this institution.

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[13] Kellermann, K. I. (personal communication) has initiated efforts to observe variable radio sources systematically on an international scale.

ORIGIN OF METEORITES, IN THE LIGHT OF RECENT ROCKET PHOTOGRAPHY OF THE MOON AND MARS

By PROF. G. MUELLER
University of Concepcion, Chile

THE theories of the origin of meteorites have been summarized by Mason[1] and Anders[2]. Parent bodies of cometary, asteroidal or moon dimensions are visualized in the most generally discussed up to date hypothesis of genesis. Urey[3] suggests a "complex" origin through the break-up of a moon-sized object and the re-formation of smaller bodies within the range of dimension of the asteroids. Other possible theories suggesting a "complex" or "multiple" origin are (*a*) that diverse types of meteorites (irons, mesosiderites, chondrites, carbonaceous chondrites, achondrites) originate from two or more distinct types of parent bodies of markedly different dimensions; (*b*) that parent bodies of different dimensions may still produce identical or very similar types of meteorites, because certain geological processes may occur over a considerable range of conditions.

The presence of brecciated pieces of two or more of the main types within the same stone, and the existence of remarkably well defined interrelations between the volatile constituents within the stony meteorites[4,5], seem to favour, by and large, origin of all meteorites from a group of parent bodies of rather closely comparable dimensions and cosmological histories.

Attempts have been made to reconstruct the dimensions and cosmological histories of the hypothetical parent bodies on the basis of published work and a variety of observations. This article deals with certain features which, in my opinion, have been illuminated by recent rocket photography.

Implications of the observed lack of water-deposited sediments on the Moon and Mars. The absence of water-deposited sedimentary structures is, perhaps, the least controversial feature of the petrography of meteorites. This indicates that the parent body must have been too small to hold free water for prolonged periods. The actual critical gravitational mass below which water is not retained in adequate quantities and for an adequate time, under the climatological conditions which prevailed in our region of the Solar system, is not known. Preliminary analysis of the recent photographs of Mars produced by the *Mariner* space craft revealed no sedimentary structure which could readily be detected in the same scale on our planet. No trace of any layered rock appears on the photographs of the Moon from *Rangers* 7, 8 and 9, down to a resolution of some 25 cm (refs. 6 and 7). It appears, so far, that volcanic and impact features predominate on both Mars and the Moon and sediments are very subordinate or not present at all. These observations seem to indicate that a "random" assemblage of fragments from the Moon or even from Mars, of 6·620 km diameter, could resemble the meteorites in our hands, at least in respect of absence or relative scarcity of water-deposited sediments.

Comparison of statistical petrography of the Moon with that of the meteorites. It appears that the distribution of inferred rock-types on the Moon surface differs to a considerable degree from that represented in our present collection of meteorites.

Nothing is seen of the high albedo of nickel-iron or even of material of mesosideritic composition on any of the lunar photographs, down to stones 10 in. across. This would seem to indicate that nickel-iron may be present on the surface of the Moon either in fractional percentages or not at all. As against this, the parent body—or mean of parent bodies—produced an overall percentage of approximately 6 per cent nickel-iron and 2 per cent mesosideritic fragments[1].

The literature would seem to lead to the conclusion that the chondritic meteorites have a tuff-like structure, formed through processes resembling terrestrial eruptions of ash or welded tuff, at least to the degree of the accumulation of finely divided matter from a gaseous suspension, that is, a cloud. The achondrites, on the other hand, seem to originate from molten lavas or intrusions. 954 chondrites and 64 achondrites are known to date[1], the latter forming only about 7 per cent of the stony meteorites. According to Kuiper[6], it appears from photographs of the Moon that its surface has lava flows far in excess of the 7 per cent indicated, as a first approximation, for the parent body of the meteorites. In this connexion it should be mentioned that the fact that the "fragments" may originate from locations deeper in the parent body, which may afterwards have become covered by debris, or from rare but exceptionally violent volcanic activity, may tend to change the proportion of the petrographical types, compared with their distribution on the surface. Judging from the terrestrial features of igneous structures, however, we would anticipate an even greater proportion of lavas from the deeper zones of the parent body.

It seems that the tuff lava ratio on the Moon may prove to be intermediate between that of the Earth and that indicated for the parent bodies of meteorites. An explanation of this might be that the parent bodies of the meteorites were objects smaller than the Moon. It may be expected that with the decreasing dimensions of a celestial body, its tuff lava ratio would tend to increase (*a*) if cooling occurred relatively soon to temperatures below the melting-point of silicates, before degassing reached an advanced stage, or (*b*) if escape of gases from the interior is impeded by the lower gravitational field. The second factor caused the formation of better preserved, solidified spray particles, the chondri[4].

Kuiper[6] showed that collapse structures are prominent in the higher-resolution photographs of the Moon. This indicates that the material just beneath the surface of the Moon consisted of numerous cavities and the partial collapse of these formed the karst-like structures. Although most of the meteorites contain a considerable volume of microscopic pores, no larger partially collapsed vesicles are seen in any of the achondrites although such structures abound in all the terrestrial lavas and even the shallow intrusives.

Some of the lunar lava flows are markedly red (apart from the observed red phosphorescence), indicating the presence of tri-valent iron. None of the thousand or so available meteorites contains enough tri-valent iron to account for the observed red colour which is characteristic of a significant percentage of the total lunar surface.

Mechanical properties of lunar rocks. It was estimated by Kuiper[6], from the size and apparent partial burial of loose rocks on the lunar surface, that the average lava flow floor of the maria supports about 1 ton/ft.^{2}. In this connexion it is worth noting that great diurnal changes of temperature and bombardment by micrometeorites may cause the fragmentation of rocks, leading to the impression of a deeper burial than the original penetration of the surface, and it is possible that the lunar surface in question may be stronger. The same factor may also contribute to the apparent rounding of the fissures, rilles, which were interpreted by Kuiper[6] as indications of a rather softer surface material.

It would appear that experiments on the behaviour of diverse terrestrial igneous rocks and meteorites in re-

peated changes of temperature of the lunar range must be completed before we can obtain the necessary information regarding the mechanical properties of the different types of surface of the Moon. Another experiment, which seems technically feasible in the future, could be the ejection through a recoil mechanism of a small explosive charge some 10–30 sec before the impact of the space craft. If the charge leaves the space craft in the direction of its motion with, say, an additional velocity of 1 km/sec, the results of the impact and explosion of the charge could be photographed with the subsequent frames of the space craft. Photographs of the explosion of similar charges in different types of rocks and meteorites, or objects with simulated mechanical properties of these, may give us a clearer impression of the mechanical properties of the surface of the Moon, and also which rock or meteorite these resemble the most closely.

Contributions to the problem of origin of carbonaceous chondrites. The smooth transition between chondrites and carbonaceous chondrites as well as the considerable uniformity of the group seem to indicate[4] that these latter may have formed in the close-to-surface zones of the parent bodies of the rest of the meteorites. They subdivide into three rather well distinguishable zones, the zone of the parent body closest to the surface producing the Type 1 carbonaceous chondrites, which are characterized by the highest volatile content with 3·54 per cent carbon and 20·08 per cent water. Type 2 stones, with 2·46 per cent carbon and 13·35 per cent water, and Type 3 stones, with 0·46 per cent carbon and 0·99 per cent water[5], may originate from successively deeper zones of the parent body, which were heated to correspondingly higher temperatures in the course of their cosmological history. All the twenty-five or so stones hitherto recorded consist of a porous ground mass of hydrated silicates and carbonaceous matter in which are embedded the mainly globular grains of high temperature minerals: olivine, pyroxene, magnetite, troilite, some nickel-iron, etc. It is possible that their markedly loose consistency causes a high proportion of these stones to disintegrate during fall and the overall percentage in space may be considerably larger than the 2 per cent attributed to them on the basis of their observed falls.

The Type 1 stones, as well as some of the Type 2, contain some magnesium sulphate and also some gypsum, the salts of which readily form white efflorescences on the surface of the stones. In this connexion, the white snow-like substance on the peak of the Alphonsus crater was interpreted by Kuiper[6] as a fumarola product which traversed the apparently tuff-like material of pyroclastics. Kuiper further drew attention to the interesting terrestrial analogy of a white efflorescence after the eruption of the Laimana volcano on Hawaii, which proved to consist of gypsum and "a compound of calcium oxide". It is also worth noting that the salts in Type 1 meteorites were interpreted[4] as of fumarolic origin on the grounds that, like terrestrial fumaroles, they contain minute grains of iron sulphides, mainly FeS in the meteorites and mainly FeS_2 in terrestrial rocks, as a result of reactions between the Fe^{++} of the ash and the hydrogen sulphide and sulphur vapours which traverse it. In the terrestrial "evaporite" type, salt deposits such as crystals of iron sulphide are not at all characteristic. It appears that these salt-impregnated ashes can be found in abundance on all these relatively small celestial bodies, in which there is inadequate water to leach them out and eventually to re-deposit them in oceans or in smaller accumulations of water. It would appear that this feature, which is common to certain lunar rocks and the carbonaceous chondrites, does not necessarily indicate a lunar origin for the chondrites.

A high proportion of the Type 2 and Type 3 carbonaceous chondrite material contains too low a percentage of salts for these to appear as efflorescences. Certain vague criteria may, however, enable us to distinguish some indications for carbonaceous material. For example, certain types of carbonaceous complexes might be expelled through solar distillation, and the resulting more graphitized residues would give the rock a darker appearance, or, if the carbonaceous material were highly volatile, the rock might be leached. There is hardly a carbonaceous rock on the surface of our planet which would not show some change of shade on exposure. In the case of the Moon, portions of the craters and other inclinations which are more shaded or more heated by the Sun may show different shades, but such features are not evident from the lunar photographs taken. Another point worth noting is that the basic silicates in a finely divided form, that is, as pyroclastics, are markedly lighter than their fused (lava) equivalents, whereas carbonaceous rocks do not show such a strong tendency to become lighter in shade with sub-division. The fact that some of the minor craters on the floors of maria are seen surrounded by considerably darker ejecta seems to indicate, rather, more intensive darkening with fusion, characteristic of silicates without a high proportion of carbonaceous matter.

Conclusions. It appears that not all or even the majority of the meteorites are of lunar origin; however, it should be emphasized that the evidence noted and discussed here is of an inconclusive nature. The main aim of the present article is rather to create interest in the interpretation of rocket photographs of the Moon and other celestial bodies, in considering the problem of the origin of meteorites. It is possible that the petrography and other geological aspects of celestial bodies may prove to depend essentially on a few parameters such as mass, temperature and other properties of the material in the course of condensation, etc. We must look for additional clues in the specification of the parent bodies of meteorites to help us to establish the main laws governing the condensation of matter, that is, what we may term "cosmic geology".

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ELECTRIC AND THERMAL CONDUCTIVITY OF A MEDIUM CONTAINING ARRAYS OF SPHERICAL AND CYLINDRICAL PARTICLES

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THE problem of finding the effective electric conductivity of an infinite medium loaded with spherical or cylindrical particles was proposed by Clerk Maxwell[1]. Interest in the problem has revived recently because of its wide applicability[2,3]. For a cubic array of spherical particles (conductivity σ_1) embedded in a matrix (conductivity σ_0) Maxwell obtained the expression

$$\sigma/\sigma_0 = \frac{(2-2f)\varkappa + 1 + 2f}{(2+f)\varkappa + 1 - f} \qquad (1)$$

for the effective conductivity σ of the composite medium. Here f is the fractional volume occupied by the particles and $\varkappa = \sigma_0/\sigma_1$. Putting $\varepsilon = (1 - f)/f$ where ε is the "voids-ratio" (ratio of the matrix volume to particle volume in a unit cell) equation (1) reduces to

$$\sigma/\sigma_0 = \frac{2\varepsilon\varkappa + 3 + \varepsilon}{(2\varepsilon + 3)\varkappa + \varepsilon} \qquad (2)$$

The same expression has been derived by other methods[2].

Maxwell emphasizes that equation (1) is essentially true only for small values of f (large dilutions). The equation was improved on by Lord Rayleigh by taking into consideration the effect of neighbouring particles and further by Meredith and Tobias[3].

Equations (1) and (2) and their improved versions fail to reveal the essential discontinuity in σ for perfectly conducting spheres as $f \to \pi/6$, that is when the gaps between the spheres tend to zero. J. Keller[4] re-examined the problem and derived the result, valid near the singularity, that

$$\sigma/\sigma_0 = -(\pi/2)\ln[\tfrac{1}{2}(4\pi/3f)^{\frac{1}{2}} - 1] + \ldots, (\pi/6) - f \ll 1 \qquad (3)$$

For $f = 0{\cdot}5161$, equation (3) yields $\sigma/\sigma_0 = 7{\cdot}65$ which agrees with the experimental value (7·6) of Meredith and Tobias. (Their calculations give 5·8, Rayleigh's 4·6 and equations (1) or (2) 4·3 for this case.)

For a square array of perfectly conducting cylinders (Fig. 1) Keller derived an analogous expression, namely,

$$\sigma/\sigma_0 = \pi[(\pi/f)^{\frac{1}{2}} - 2]^{-\frac{1}{2}} + \ldots, (\pi/4) - f \ll 1 \qquad (4)$$

He also proved the general theorem that for any value of f, σ/σ_0 for a medium containing a square array of non-conducting cylinders is the reciprocal of σ/σ_0 for the same array of perfectly conducting cylinders.

H. B. Keller and Sachs[5] modified equation (4) by estimating its second term, which they state as "approximately $-1{\cdot}95$". They also attacked the present boundary value problem by the finite difference method and calculated a table of σ/σ_0 using the IBM 7090 computer.

Neither Keller nor Keller and Sachs cite any experiments on cylindrical particles in support of their calculations. This test has been undertaken in our laboratory and the results are discussed below.

Curve 1 in Fig. 2 shows σ/σ_0, calculated by Keller and Sachs, for a medium containing non-conducting cylinders. The abscissa in the figure is the ratio y/a (gap/radius, compare Fig. 1). We have $f = \pi a^2/(y + 2a)^2$ where a is the radius of the cylinders. Curve 2 is drawn after the Keller-Sachs modified form of equation (4). For $y/a < \sim 0{\cdot}3$ it differs insignificantly from curve 1. Strictly speaking, curve 2 cannot represent a correct solution in any range since its asymptote for $y/a = \infty$ is 0·27 instead of 1·00.

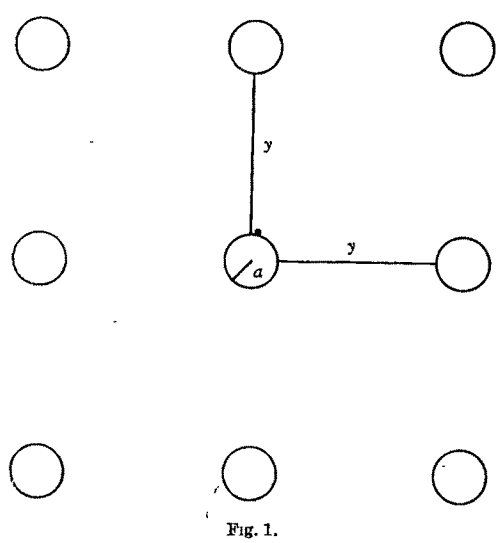


Fig. 1.

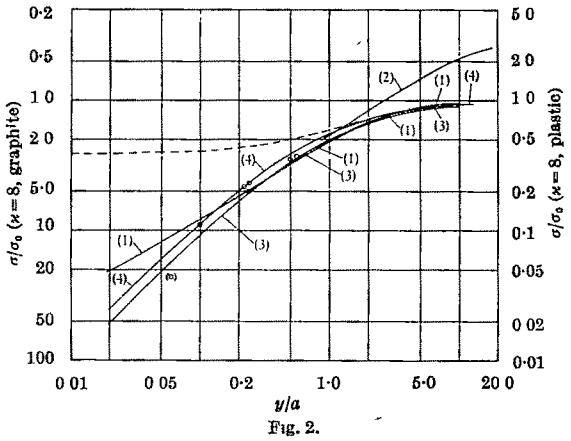


Fig. 2.

The experimental points obtained in the laboratory in measurements on blocks of accurately machined plastic and graphite rods (diameter 10 mm, mounted in a square array on a basal plate) are shown in Fig 2 by circles. (The results on plastic and graphite rods may be plotted on the same diagram in view of Keller's theorem.)

It should be noted that the various points do not fall on curve 1 and it seems that the observed σ/σ_0 for $y/a < \sim 0{\cdot}2$ is systematically less, and for $y/a > \sim 0{\cdot}2$ systematically greater, than the value calculated by Keller and Sachs. The position of the point for $y/a = 0{\cdot}06$ is uncertain since the gap in this case could not be measured accurately Also, there is some doubt whether all the gaps in this block were absolutely equal, although metal foils of appropriate thickness, which were later removed, were inserted as usual between the rods during the construction of the block. If the observed point for $y/a = 0{\cdot}06$ is to fall on curve 1 the nominal width of the gaps must be larger than the effective mean width by a factor of between 3 and 4. An error of this size is conceivable but unlikely.

As an alternative approach to explaining the experimental results the conductivity formula derived[2] by the "self-consistent" method was tried. For a medium containing cylindrical particles we have:

$$\sigma/\sigma_0 = \frac{\varepsilon\varkappa + 2 + \varepsilon}{(\varepsilon + 2)\varkappa + \varepsilon} \qquad (5)$$

For $\varkappa \to 0$ (perfectly conducting cylinders) equation (5) reduced to

$$\sigma/\sigma_0 = 1 + (2/\varepsilon) \qquad (6)$$

It is obvious furthermore that the solution (5) satisfies Keller's theorem since σ/σ_0 for $\varkappa = \infty$ (non-conducting cylinders) is equal to $\{1 + (2/\varepsilon)\}^{-1}$.

At first sight it seems plausible to put $\varepsilon = (1/f) - 1 = 4\pi^{-1}\{1 + (y/2a)\}^2$; however, this substitution in (6) yields the broken curve in Fig. 2 which is definitely not the correct solution. The following explanation can be adduced for this failure.

The assumption concerning the voids-ratio ε implies a current flow that is perpendicular to the plane of Fig. 1 (along the axial direction of the cylinders). But ε as experienced by a current which is flowing perpendicular to the axial direction must be of the form $\varepsilon = \alpha_1 (y/a) + \alpha_2(y/a)^2 + \ldots$ where α_1, α_2, ... are factors of a geometrical nature. For cylinders in contact with each other, this expression yields $\varepsilon = 0$ and hence the correct limit $\sigma/\sigma_0 = \infty$.

Retaining only the first term in the proposed expression for ε the curves 3 and 4 in Fig. 2 were calculated by postulating $\varepsilon = 2(y/a)$ and $\varepsilon = (8/\pi)(y/a)$. Thus

$$\sigma/\sigma_0 = 1 + 1/(y/a) \text{ for curve 3} \qquad (7a)$$

$$\sigma/\sigma_0 = 1 + (\pi/4)/(y/a) \text{ for curve 4} \qquad (7b)$$

The two curves enclose a narrow strip. Moreover, all the observed points except the doubtful one for $y/a = 0{\cdot}06$ lie between them. It appears then that the mean of the

conductivities given by the simple semi-empirical formulas (7*a*) and (7*b*) is as good a practical approximation to the effective conductivity of a medium containing a square array of perfectly conducting cylinders, as the value obtained[5] from the considerably more complicated expressions of Keller and Sachs, Rayleigh, and Runge. The position of the point for $y/a = 0{\cdot}06$ seems to indicate that equations (7*a*) and (7*b*) may even be nearer to the truth, over the entire range of y/a, than any of the expressions proposed so far. This question cannot be settled, however, without measurements on arrays of nearly touching parallel cylinders.

It was also of interest to look for the physico-geometrical significance of the two postulates $\varepsilon = 2\ (y/a)$ and $\varepsilon = (8/\pi)\ (y/a)$.

The gist of the self-consistent method is that we fix attention on a single particle of the array and consider it to be uniformly coated with a film (of conductivity σ_0) the thickness of which depends on the voids-ratio ε, and the composite particle is supposed to be embedded in a homogeneous medium of conductivity σ, which is to be determined from the boundary conditions at the sphere-film and film-medium interfaces. The details are given in ref. 2.

Let b be the radius of the film coating the cylindrical particle of radius a so that the thickness of the film is $t = b - a$. Then the voids-ratio $\varepsilon = (b^2 - a^2)/a^2$, from which $t = \{a^2(1 + \varepsilon)\}^{\frac{1}{2}} - a$.

For $\varepsilon = 2y/a$, $t = \{a(a + 2y)\}^{\frac{1}{2}} - a$. In Fig. 3, RP = a, MR = y, and PQ is perpendicular to RR′ meeting the semicircle with RR′ ($= 2y + 2a$) as diameter. Since PR′ $= 2y + a$, PQ $= \{a(a + 2y)\}^{\frac{1}{2}}$. Hence SQ = S′Q′ represents the thickness t of the fictitious cylindrical film by which a single particle may be supposed to be coated in being embedded in a medium of conductivity σ.

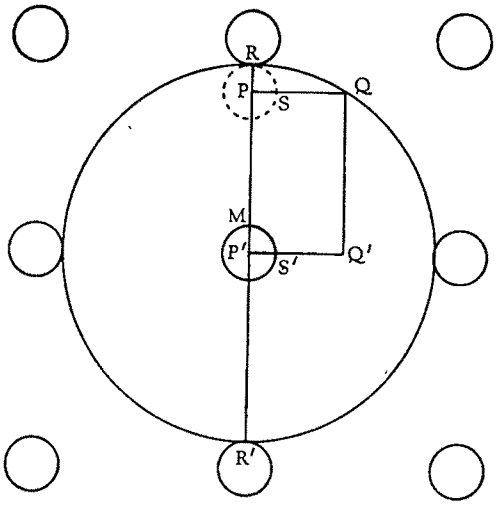


Fig. 3.

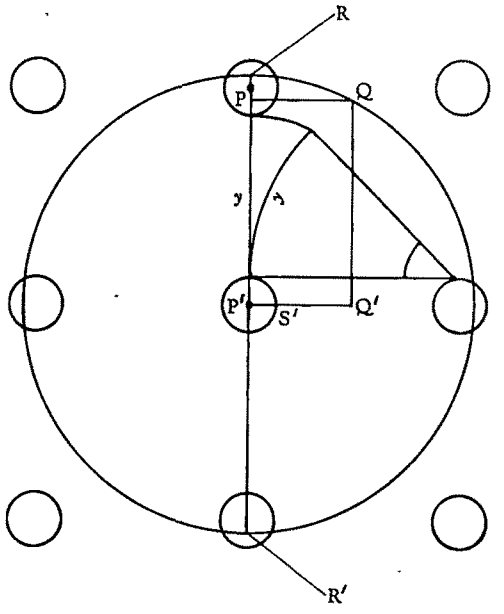


Fig. 4.

For $\varepsilon = (8/\pi)\ (y/a)$, $t = \{a(a + 8y/\pi)\}^{\frac{1}{2}} - a$. The construction in Fig. 4 is similar to that in Fig. 3 except that MR $= (4/\pi)y$ and hence is equal to the radius of the circle in which an arc of length MR subtends an angle of $\pi/4$ at the centre.

There is no potential-theory explanation for the apparently plausible forms postulated for ε. In any case, the agreement with experimental results indicates that the coefficient of y/a in equations (7*a*) and (7*b*) lies between 1 and $4/\pi$.

A simple modification of equation (2) (for spheres) on these lines does not, on the other hand, lead to any improvement over the expression obtained by Meredith and Tobias[3] for large gaps and by J. Keller[4] for vanishingly small gaps between the spheres. Moreover, equation (2) yields the result that for an array of perfectly conducting spheres σ/σ_0 is the reciprocal of the same array of non-conducting spheres for all ε. This is obviously false. A more sophisticated form of the self-consistent method is probably needed in this case.

The calculations and experiments reported here were made in the course of scale model work on resistivity prospecting methods, the expenses of which were defrayed from a grant from the State Technical Research Council in Sweden.

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PRESSURE DISTRIBUTION IN A PLANE FLUID FILM SUBJECTED TO NORMAL SINUSOIDAL EXCITATION*

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Consider a plane circular bearing comprising two parallel disks of radius R, distance h apart. A lubricating fluid at atmospheric temperature and pressure completely fills the space between the disks.

* The following notation is used throughout this article: R, bearing radius; p, pressure, u, v, w, linear velocity components; r, z, cylindrical polar co-ordinates, μ, absolute viscosity; ν, kinematic viscosity, ϱ, density; h, film thickness: ω, frequency of excitation; a, amplitude.

Then using cylindrical polar co-ordinates with axial symmetry the equations of motion for an element of the incompressible fluid are

$$\frac{\partial u}{\partial t} + u\frac{\partial u}{\partial r} + w\frac{\partial u}{\partial z} = -\frac{1}{\rho}\cdot\frac{\partial p}{\partial r} + \nu\left(\nabla^2 u - \frac{u}{r^2}\right) \quad (1)$$

$$\frac{\partial w}{\partial t} + u\frac{\partial w}{\partial r} + w\frac{\partial w}{\partial z} = -\frac{1}{\rho}\cdot\frac{\partial p}{\partial z} + \nu\nabla^2 w \qquad (2)$$

and the continuity equation is

$$\frac{\partial u}{\partial r} + \frac{u}{r} + \frac{\partial w}{\partial z} = 0 \qquad (3)$$

Eliminating p between 1 and 2

$$\frac{\partial}{\partial t}\left(\frac{\partial u}{\partial z} - \frac{\partial w}{\partial r}\right) + u\frac{\partial^2 u}{\partial r\partial z} + \frac{\partial u}{\partial r}\cdot\frac{\partial u}{\partial z} + w\frac{\partial^2 u}{\partial z^2} + \frac{\partial w}{\partial z}\cdot\frac{\partial u}{\partial z} - u\frac{\partial^2 w}{\partial r^2} - \frac{\partial u}{\partial r}\cdot\frac{\partial w}{\partial r} - w\frac{\partial^2 w}{\partial r^2} - \frac{\partial w}{\partial r}\cdot\frac{\partial w}{\partial z} = \nu\left[\nabla^2\frac{\partial u}{\partial z} - \frac{1}{r^2}\cdot\frac{\partial u}{\partial z} - \nabla^2\frac{\partial w}{\partial r}\right] \qquad (4)$$

Now consider one surface to be moving normally in such a way that its position ε at any instant is given by

$$\varepsilon = h + a\sin\omega t \qquad (5)$$

Thus the boundary conditions are

$$\left.\begin{aligned} u = w = 0 \text{ at } z = 0 \quad &\text{(i)} \\ u = 0,\ w = a\,\omega\cos\omega t \text{ at } z = \varepsilon \quad &\text{(ii)} \end{aligned}\right\} (6)$$

From 6(ii) it can be seen that an r independent form for w is required, so that the equation

$$\frac{\partial}{\partial r}\left(\frac{\partial u}{\partial r} + \frac{u}{r}\right) = 0$$

has to be satisfied.

The general solution is $u = Ar + B/r$. But at $r = 0$, $u = 0$, and therefore $B = 0$.

So let $u = \dfrac{\partial}{\partial z}u_0(z,t)\,r$

Therefore, $w = -2u_0$ from 3.

Expanding 4 in full and ignoring second-order terms

$$\frac{\partial}{\partial t}\cdot\frac{\partial^2 u_0}{\partial z^2}\cdot r - 2u_0\frac{\partial^2 u_0}{\partial z^2}r = \nu\left(\frac{\partial^3 u_0}{\partial z^3} + r\frac{\partial^4 u_0}{\partial z^4}\right) \qquad (7)$$

Comparing coefficients of r

$$\frac{\partial}{\partial t}\cdot\frac{\partial^2 u_0}{\partial z^2} - 2u_0\frac{\partial^3 u_0}{\partial z^3} = \nu\frac{\partial^4 u_0}{\partial z^4} \qquad (8)$$

It is necessary to make a tentative solution for u_0. Since u_0 and $\dfrac{\partial u_0}{\partial z}$ both vanish at $z = 0$, z^2 is a factor of u_0 and so

$$u_0 = z^2\varphi_0 + z^3\varphi_1 + \ldots. \qquad (9)$$

where φ is a function of t only. Equation 8 may therefore be expanded in full, and comparing coefficients of z,

$$\left.\begin{aligned} 2\dot{\varphi}_0 &= 24\,\nu\,\varphi_2 \quad &\text{(i)} \\ 6\dot{\varphi}_1 &= 120\,\nu\,\varphi_3 \quad &\text{(ii)} \\ 12\varphi_2 - 12\varphi_0\varphi_1 &= 360\,\nu\,\varphi_4 \quad &\text{(iii)} \end{aligned}\right\} (10)$$

The boundary condition at $z = \varepsilon$ has still to be satisfied. Suppose for the time being that the successive terms of the series decrease in magnitude and solve for φ_0 and φ_1 by equating u_0 to $\frac{1}{2}a\,\omega\cos\omega t$ and $\dfrac{\partial u_0}{\partial z}$ to zero. Thus

$$(h + a\sin\omega t)^2\varphi_0 + (h + a\sin\omega t)^3\varphi_1 = \tfrac{1}{2}a\,\omega\cos\omega t$$

and

$$2\varphi_0 + 3(h + a\sin\omega t)\varphi_1 = 0$$

whence

$$\varphi_0 = \frac{3a\,\omega\cos\omega t}{2(h + a\sin\omega t)^2}$$

and

$$\varphi_1 = \frac{-a\,\omega\cos\omega t}{(h + a\sin\omega t)^3}$$

Therefore $\varphi_2 = \dfrac{-a\omega^2}{8\nu}\left[\dfrac{h\sin\omega t + a(1 + \cos^2\omega t)}{(h + a\sin\omega t)^3}\right]$

Now each term in u_0 achieves a maximum value when $z = \varepsilon$ and thus the magnitude of the third and first terms is, relatively,

$$\left|\frac{a\omega^2}{8\nu}[h\sin\omega t + a(1 + \cos^2\omega t)](h + a\sin\omega t)\right|$$

and

$$\left|\frac{3}{2}a\,\omega\cos\omega t\right|$$

Now write $\dfrac{h}{a} = \lambda$ and then consider the maximum value of

$$V = (\lambda\sin\theta + 1 + \cos^2\theta)(\lambda + \sin\theta)$$

so that $\dfrac{dV}{d\theta} = \cos\theta\,(\lambda^2 + 2 - 3\sin^2\theta)$

Now since $\lambda > 1$, $\lambda^2 + 2 > 3$ and therefore the factor in brackets can never be zero, and the maximum and minimum values of V occur when $\cos\theta$ is zero, in which case $\sin\theta = \pm 1$. The maximum is clearly when $\sin\theta = 1$ so that $V_{max} = (\lambda + 1)^2$. Consequently the ratio of the maxima of the third to first terms is $(\omega/12\,\nu)(h + a)^2$, which is certainly small in this case.

Similar arguments apply to the higher terms in the series for u_0. Thus those terms in the expression for u_0 may be safely neglected so that

$$u_0 = \tfrac{1}{2}a\,\omega\cos\omega t\left[\frac{3z^2}{(h + a\sin\omega t)^2} - \frac{2z^3}{(h + a\sin\omega t)^3}\right] \qquad (11)$$

$$\text{Thus } u = \tfrac{1}{2}a\,\omega\,r\cos\omega t\left[\frac{6z}{(h + a\sin\omega t)^2} - \frac{6z^2}{(h + a\sin\omega t)^3}\right] \qquad (12)$$

Substituting in equation 1,

$$\frac{1}{\rho}\cdot\frac{\partial p}{\partial r} = \frac{3r(a\,\omega\cos\omega t)^2}{(h + a\sin\omega t)^2} - \frac{6a\,\omega\,r\,\nu\cos\omega t}{(h + a\sin\omega t)^2}$$

Integrating

$$p_{max} = \frac{\frac{3}{2}R^2\rho(a\,\omega\cos\omega t)^2}{(h + a\sin\omega t)^2} - \frac{3a\,\omega\,R^2\mu\cos\omega t}{(h + a\sin\omega t)^3} \qquad (13)$$

The maximum value of the second term in this expression is very much greater than the corresponding maximum of the first term, so that when maximum pressures are considered the second term only need be taken into account.

This can be written

$$\frac{p_{max}}{\mu\omega} = -3\left(\frac{a}{h}\right)\left(\frac{R}{h}\right)^2\cos\omega t\left[1 - 3\left(\frac{a}{h}\right)\sin\omega t\right] \qquad (14)$$

Now if $\dfrac{p_{max}}{\mu\omega}$ is plotted against ωt for a fixed value of $\dfrac{R}{h}$ (= 1,000) and varying values of $\dfrac{a}{h}$, Fig. 1 is obtained. It can be seen that for all values of $\dfrac{a}{h}$ the greatest negative pressure occurs, at 330°, and the greatest positive pressure at 210°. So that the maximum pressures are given by

$$\frac{p_{max}}{\mu\omega} = \pm 2{\cdot}6\left(\frac{a}{h}\right)\left(\frac{R}{h}\right)^2\left[1 + 1{\cdot}5\left(\frac{a}{h}\right)\right] \qquad (15)$$

When the non-dimensional factor $\frac{p_{max}}{\mu\omega}$ is plotted against $\frac{a}{h}$ for varying values of $\frac{R}{h}$ Fig. 2 is obtained.

From a practical point of view the two extreme values of p_{max}, the maximum positive and maximum negative values, are of interest. Apart from the obvious examples of thrust and collar bearings, situations similar to that considered theoretically can arise in gear teeth and journal bearings. Consider a pair of meshing gear teeth under load separated by an oil film of 10^{-4} in. If the natural frequency of one of the gear teeth is 3 kc/s, typically, the dimension R for an equivalent circular area of contact is 0·25 in., the lubricating oil viscosity is 5×10^{-6} Reyn and the ratio of vibration amplitude to oil film thickness $\frac{a}{h}$ is $\frac{1}{10}$, then from Fig. 2, p_{max} is 24,000 lb./in.^{2}. This pressure on the tooth face is unlikely to cause damage, but it can be seen that any slight variation in any of these parameters could bring the stress level up to that which could cause a short fatigue life. Before an amplitude/thickness ratio of $\frac{1}{10}$ is reached, however, an even more dangerous condition could be encountered, that is the condition of cavitation in the fluid. In this case cavitation will commence when $\frac{p_{max}}{\mu\omega} = 10^3$. It can be seen from Fig. 2 that cavitation would occur with $\frac{a}{h}$ ratios of much less than 10^{-3}. This means that cavitation and the subsequent fatigue damage would, in practice, be unavoidable in this particular case.

Experimental Work

It was decided to check the validity of equation 15 by observing the onset of cavitation in a fluid in circumstances similar to those outlined in the analysis. A mild steel plate of $\frac{3}{8}$ in. diameter polished to 2μ in. was rigidly fixed. A similar mating plate was attached to the thrust rod of an electromagnetic vibrator in such a way that it was parallel to the fixed plate and that its distance from the fixed plate could be varied within the range 0–0·010 in. The two plates were immersed in a liquid contained in a transparent vessel. It was intended to observe the onset of cavitation characterized by the formation of bubbles in the fluid at the centre of the bearing by the naked eye. It was found that for film thicknesses less than 2×10^{-3} in. the onset of the formation of bubbles could not easily

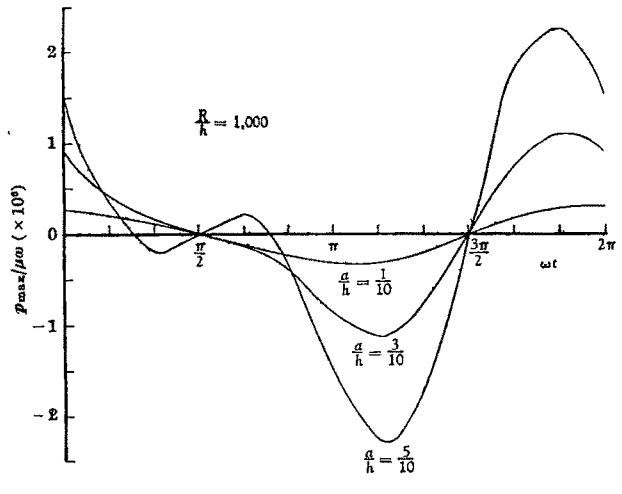


Fig. 1.

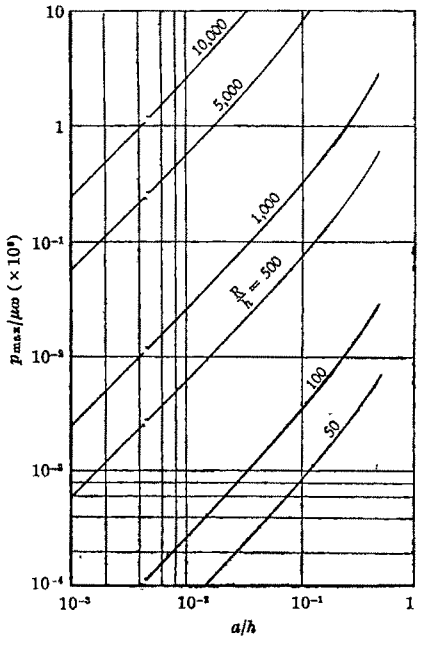


Fig. 2.

be distinguished. It was therefore out of the question to test, by this method, films of the thicknesses actually found in gears. It was decided, however, that the curves in Fig. 2 could still be checked experimentally over a useful range. The film thicknesses and amplitudes of vibration were measured by capacitance probes. The fluids used in the experiment were 'Shell Tellus 27', 'Shell Vitrea 79', gas oil, liquid paraffin (B.P.) and tap water. The viscosities of these liquids are evenly spaced throughout the range 10^{-1} to $10^2 \times 10^{-6}$ Reyn at 20° C.

It was found that the experimental values of $\frac{a}{h}$ at which cavitation occurred tended to be up to 20 per cent higher than the theoretical values at the lower ratios. This is because the criterion for the onset of cavitation was that cavitation was deemed to occur at that amplitude at which a bubble would span the gap between the plates. It is undoubtedly true that cavitation had occurred before this stage was reached, but it was not measurably evident.

After allowing vigorous cavitation to ensue for about 10^6 cycles of lateral excitation extensive "pitting" damage was present on both plate surfaces. The moving plate was replaced by a $\frac{1}{2}$ in diameter steel ball at a later stage in the experimental work; this enabled a gap of 10^{-4} in. to be easily maintained. Cavitation could not be observed in such a small gap, but by allowing vibration to proceed for 10^6 cycles again extensive damage was caused to the mating surfaces.

The intention now is to increase R, μ and ω so that stress levels high enough to cause surface damage will be imposed on the plate centres. But, even so, it may be difficult to decide whether damage is caused by the high stress levels or by cavitation though possibly at high frequencies the onset of cavitation may be suppressed. It is also intended to produce more realistic gear tooth conditions by using a two-disk machine, one disk of which would be caused to vibrate in a diametral direction. In this way the normal excitation of very thin films can be studied. In order to improve the accuracy of the detection of cavitation an attempt will be made to detect the noise which ensues when a bubble collapses.

I thank the Science Research Council for financial support and Mr. A. J. Sherin of the Dynamic Analysis Laboratory, University of Southampton, for practical assistance.

BINDING OF CALCIUM, YTTRIUM AND THORIUM TO A GLYCOPROTEIN FROM BOVINE CORTICAL BONE

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THERE is a considerable radiobiological interest in those elements which, when they reach the blood stream, have an affinity for bone. These have been called "bone-seeking" isotopes and include strontium[1], yttrium[2], americium[3], radium[4], plutonium[3,5] and thorium[6]. The exact distribution of these elements in the bone varies. Strontium and radium are concentrated on active surfaces beneath the osteoid border and are spread diffusely throughout the bone mineral, probably replacing calcium isomorphously in the growing apatite lattice[7]. Americium and yttrium, on the other hand, are mainly taken up on the resting and resorbing surfaces of the bone[3,8] and plutonium is concentrated particularly on the endosteal resorbing surfaces[3,5]. However, the surfaces on which yttrium, americium and plutonium are deposited all react strongly in the periodic acid–Schiff (PAS) staining procedure. It has been suggested that this procedure is specific to materials containing carbohydrates[9] and consequently the binding of these elements in bone has been linked with the possible presence of a carbohydrate moiety in the matrix at the resting and resorbing surfaces[10].

Herring and Kent[11] have isolated four fractions containing carbohydrate residues from bovine cortical bone, one of which is a glycoprotein containing about 20 per cent by weight of sialic acid, which has been named bone sialoprotein. The chemical[12,13] and physical properties[14] of this substance have been described. We now report studies of the binding of bone-seeking metal ions to this sialoprotein, extracted from bone by the ethylene diamine tetra-acetic acid method[12].

The binding of yttrium and calcium has been studied by a titration method similar to that reported by Gilbert and Myers[15]. The titration curves of bone sialoprotein were determined in the presence of increasing concentrations of yttrium chloride (0·1–20 mM) and at one concentration of calcium chloride (40 mM), with sufficient sodium chloride present to bring the ionic strength up to 0·2. Bone sialoprotein (about 6 mg in 5 ml.) solutions were titrated under an atmosphere of nitrogen within the *p*H range 2·2–10·5 (accuracy of *p*H measurement ± 0·002 units). Water corrections were made and the hydrogen ions bound to bone sialoprotein were calculated. All titrations were found to be completely reproducible and reversible. Titrations in the presence of Y^{3+} were only carried out in the range *p*H 5–2·2 because at *p*H > 5 there was precipitation of either hydrated oxide or a complex of yttrium and the glycoprotein. The depression of *p*H, ΔpH, caused by the metal ions, was taken to be the result of competition between metal ions and hydrogen ions for negative sites on the glycoprotein, that is, carboxylate anions, and depends on the degree of binding. The degree of binding, a_m, defined as the fraction of total carboxyl groups to which metal ions were bound, was calculated from the value of ΔpH at constant hydrogen ion binding and this was determined as the difference, parallel to the *p*H axis, between the titration curves in the presence of Ca^{2+} or Y^{3+} and that in the presence of 0·2 *M* sodium chloride alone. Two assumptions were made: (i) that the titration of carboxyl groups could be represented by the expression

$$pK_0 = p\mathrm{H} - \log\frac{\alpha}{1-\alpha} + f(Z, \mu, s)$$

where K_0 is the intrinsic dissociation constant of the bone sialoprotein carboxyl groups, α is the average degree of dissociation and f (Z, μ, s) is a function of charge (Z), ionic strength (μ), and a shape factor (s): (ii) that binding of a metal ion caused a neutralization of charge on bone sialoprotein equal to its own charge. Calculation of the binding is possible because $[f(Z, \mu, s) - pK_0]$ can be determined empirically from the titration curves, typical examples of which are shown in Fig. 1.

The dependence of the binding (a_m) of yttrium and calcium on *p*H is shown in Fig. 2, and it can be seen that

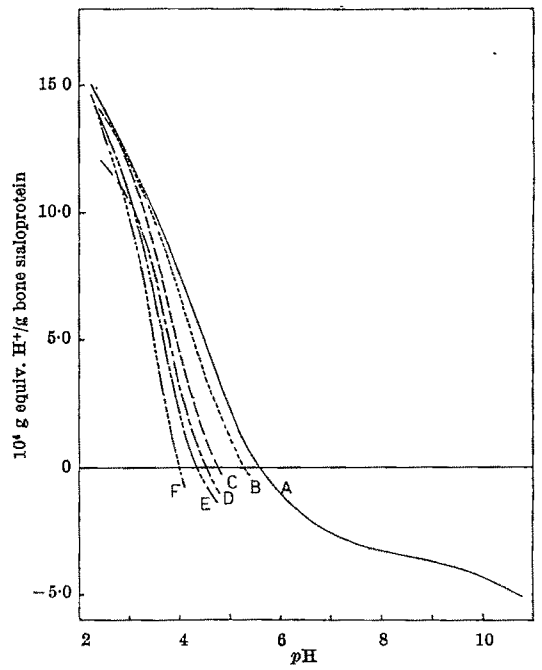


Fig. 1. Titration curves of bone sialoprotein at 25° C. Ionic strength 0·2. *A*, 0·2 M sodium chloride; *B*, 0·1 mM yttrium chloride + 199 mM sodium chloride; *C*, 0·5 mM yttrium chloride + 197 mM sodium chloride; *D*, 1 mM yttrium chloride + 194 mM sodium chloride; *E*, 3·25 mM yttrium chloride + 180 mM sodium chloride; *F*, 20 mM yttrium chloride + 80 mM sodium chloride.

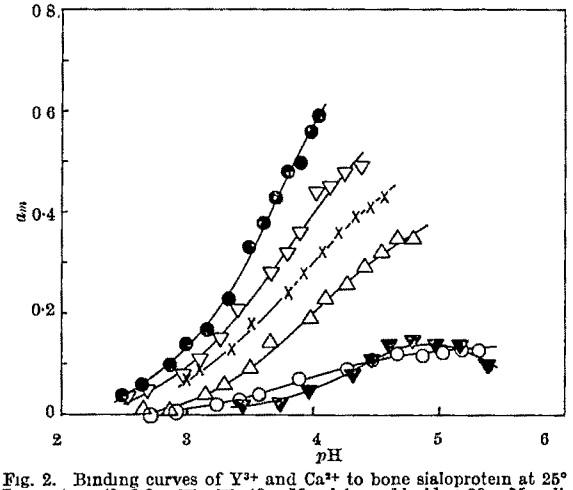


Fig. 2. Binding curves of Y^{3+} and Ca^{2+} to bone sialoprotein at 25° C. Ionic strength 0·2. ▼—▼, 40 mM calcium chloride + 80 mM sodium chloride; ○—○, 0·1 mM yttrium chloride + 199 mM sodium chloride, △—△, 0·5 mM yttrium chloride + 197 mM sodium chloride; ×—×, 1 mM yttrium chloride + 194 mM sodium chloride; ▽—▽, 3·25 mM yttrium chloride + 180 mM sodium chloride; ●—●, 20 mM yttrium chloride + 80 mM sodium chloride. Ordinate: a_m, fraction of carboxyl groups involved in binding.

almost the same curves are obtained with calcium at a total concentration of 40 mM as with yttrium at a total concentration of 0·1 mM.

Bone sialoprotein contains about fifteen sialic acid residues, fifteen aspartic acid residues and twenty glutamic acid residues per molecule[13]. To determine if the binding of yttrium was specific to any of these carboxylic acids, three model systems were titrated in 0·2 M sodium chloride, in 20 mM yttrium chloride + 80 mM sodium chloride*, and in 40 mM calcium chloride + 80 mM sodium chloride. These model systems were sodium poly-L-glutamate, sodium poly-L-aspartate and sodium *N*-acetyl neuraminate. The two polyamino acids showed the same gross effects as bone sialoprotein, ΔpH being large for Y^{3+} but small for Ca^{2+}, whereas the ΔpH of the sialic acid solutions was small in the presence of Y^{3+} or Ca^{2+}, and almost the same with the two ions. This suggests that the sialic acid was not involved in the stronger binding to bone sialoprotein of Y^{3+} compared with Ca^{2+} and this inference was confirmed by removing the sialic acid residues from bone sialoprotein (by hydrolysis in 0·1 N hydrochloric acid for 1 h at 80° C). The titration curve of the resulting de-sialated glycoprotein was displaced by Y^{3+} and Ca^{2+} to the same extent as was that of the original bone sialoprotein. Thus binding was not diminished by loss of the sialic acid, and was therefore mainly on the carboxylate anion of the acidic amino-acid residues.

A similar series of titrations were carried out with crystalline bovine serum albumin (Fig. 3) and crystalline ribonuclease and also with ribonuclease which had been treated with mercaptoethanol in 6 M urea, followed by iodoacetic acid to convert the disulphide bonds to —S—CH_2COOH groups (this treatment destroyed the native secondary structure of the enzyme and increased the number of carboxylate groups available for titration). In no case was the depression of *p*H caused by the 20 mM yttrium chloride + 80 mM sodium chloride solution greater than 0·3, and the 40 mM calcium chloride + 80 mM sodium chloride did not alter the titration curve within the experimental errors.

Because of the ease of hydrolysis of thorium, even in acid solutions, the binding of thorium had to be measured in a different way. By adding acid to a 20 mM solution of thorium nitrate and comparing the results with the addition of acid to 0·2 M sodium chloride, it was found that below *p*H 2·35 the two solutions behaved identically, but above *p*H 2·35 the change of *p*H with added acid was less for the thorium solution than for the water. Therefore, for titration purposes only at *p*H $\leqslant$ 2·35 could the thorium be considered ionic. On mixing 3 ml. of 20 mM thorium nitrate (μ 0·2) at *p*H 2·35 to 2 ml. of a bone sialoprotein solution in 0·2 M sodium chloride also at *p*H 2·35, the *p*H dropped to 2·207 and a diffuse suspension was formed. From the titration of an identical solution of bone sialoprotein with 3 ml. of 0·2 M sodium chloride added in place of the thorium, it was calculated that the drop in *p*H was equivalent to the release of $1{\cdot}22 \times 10^{-5}$ moles of H^+; moreover, the amount of hydrogen ions bound by the sialoprotein from *p*H 7·0 to 2·35 was also $1{\cdot}22 \times 10^{-5}$ moles. This indicates that all the hydrogen ions bound to carboxylic acid groups at *p*H 2·35 are released on addition of thorium, and that the thorium therefore binds to all the carboxylic acid groups on the molecule. This binding is apparently much stronger than that of Y^{3+}, which has already been shown to be stronger than that of Ca^{2+}.

The common structural factor in the macromolecules which, by this criterion, are able to bind Y^{3+} strongly, namely, bone sialoprotein, de-sialated bone sialoprotein, poly-L-glutamate and poly-L-aspartate, is a high density of distribution of carboxyl groups along the protein chain (42 per cent of the amino-acids in bone sialoprotein are either aspartic or glutamic acids[13]). Proteins which have a much lower proportion of acidic amino-acids (bovine serum albumin, ribonuclease), do not bind Y^{3+} strongly. This suggests that the highly acidic protein component in bone sialoprotein is mainly responsible for its binding of the highly charged ions of the bone-seeking elements.

* + Indicates that the solution is 20 mM in yttrium chloride and 80 mM in sodium chloride. This notation is used throughout the article.

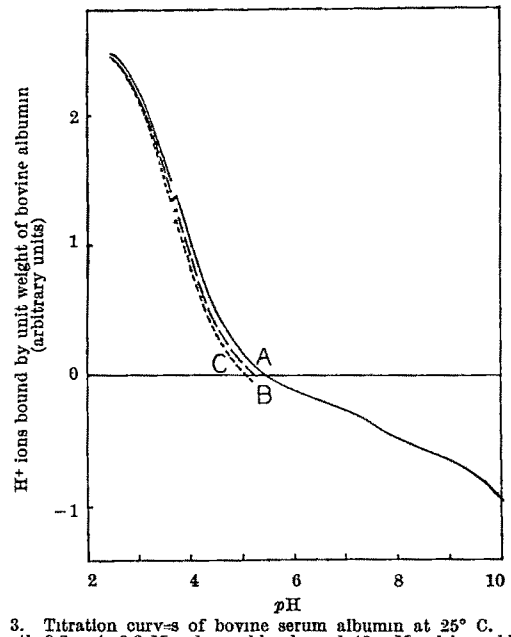


Fig. 3. Titration curves of bovine serum albumin at 25° C. Ionic strength 0·2. *A*, 0·2 M sodium chloride and 40 mM calcium chloride +80 mM sodium chloride; *B*, 5 mM yttrium chloride +170 mM sodium chloride; *C*, 20 mM yttrium chloride + 80 mM sodium chloride. Ordinate: Gram-equiv. H^+ bound by unit weight of bovine serum albumin (arbitrary units).

Preliminary experiments show that the chondroitin sulphate-protein fraction isolated from bovine cortical bone by Herring[16] also binds yttrium strongly, and calcium to a lesser extent, although the relative binding of the two appears to differ from that of bone sialoprotein.

Because bone sialoprotein stains in the PAS reaction and also binds yttrium (and thorium) more strongly than calcium it is likely to contribute substantially to the uptake of those bone-seeking elements which are preferentially deposited at the resting and resorbing surfaces.

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DISSOCIATION OF SODIUM CARBONATE AND SODIUM SULPHITE IN ALCOHOL PLATING SOLUTIONS

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THE electrodeposition of inorganic compounds from alcohol and other organic media has found few if any applications in industry. The main reason for this is that it is frequently difficult to obtain a coating of pure metal on the surface of the material to be plated[1]. There are, however, applications where this need not be a disadvantage—for example, in the nuclear sciences where thin layers of radioactive material are frequently required. Usually, in such situations the major concern is the production of a homogeneous layer of metal, and not the chemical form of the material[2–6]. The successful electrodeposition of layers of actinium and thorium has been reported by Haissinsky[7], and by Cottelle and Haissinsky[8], who used acetone as well as alcohol solutions. In this instance, however, ordinary electrolysis was reported and a voltage of about 10–12 V, d.c. was used. (Parker *et al.*[9] were unable to obtain the near quantitative results reported by these workers. Yields were found to vary between 45 per cent and 60 per cent.)

Following a series of experiments aimed at the preparation of colloidal solutions[10], Parker and Falk[11] found that with the help of high voltage (> 500 V, d.c.), almost any water-soluble compound could be electrodeposited from isopropanol on to the cathode. The material was deposited, not as the metal, but in a more basic form. The process proved to be quantitative for a given amount of material, and was found suitable for the production of thin layers of radioactive material of excellent uniformity. As a result of these observations we have named the process "molecular plating". In other reports we have described the application of molecular plating to experiments in nuclear physics[12–16] and also to the preparation of electron microscope specimens[17]. (A general review is given in ref. 6.) The experimental results which now follow are those obtained from an investigation of the nature of the deposits formed and of the degree of dissociation which ensued.

Two chemical compounds were chosen for this study: sodium carbonate and sodium sulphite. Both compounds are soluble in water but are somewhat less soluble in alcohol. They have the additional advantage of lending themselves to radioactive tracer studies in the form of $^{24}Na_2{}^{14}CO_3$ and $^{24}Na_2{}^{35}SO_3$. Chemical methods and quantitative titration analysis were used to determine the nature of the material deposited at the cathode.

The radioactive tracer studies were divided into the following groups: (1) $Na_2{}^{14}CO_3\beta^-$; (2) $^{24}Na_2CO_3\gamma,\beta^-$; (3) $Na_2{}^{35}SO_3\beta^-$; (4) $^{24}Na_2SO_3\gamma,\beta^-$; (5) $^{24}Na_2{}^{14}CO_3\gamma,\beta^- + \beta^-$; (6) $^{24}Na_2{}^{35}SO_3\gamma,\beta^- + \beta^-$.

For the preparation of plating solutions of (1) and (3) the radioactive material was obtained from the Radiochemical Centre, Amersham. Solutions of (2) and (4) were prepared by irradiating quantities of (1) and (3) for 5 h periods with slow neutrons in the *R*1 nuclear reactor at Stockholm. Because of the short time of irradiation and because slow neutron flux was used, sulphur-35 was only produced in very small quantities. For the same reasons, the quantities of $^{34}S(n,p)^{34}P$ were also very small. Finally, solutions of (5) and (6) were prepared by simply mixing equal proportions of (1) and (2), and (3) and (4), respectively.

Measurements of the β-ray activity were made by means of a liquid scintillation counter, while γ-ray activity was determined by means of a γ-scintillation spectrometer. The two counting solutions used for the measurement of sulphur-35 and carbon-14 were originally described by Eastman *et al.*[18] and had the following composition: for sulphur-35, 1·5 g of 2·5-diphenyloxazole and 0·08 g of 2·2-p.-henylene-*bis*-(5-phenyloxazole) were dissolved in a mixture of 100 ml. of dimethylformamide and 200 ml. of anisole; for carbon-14, 15 ml. of the same solution as used for sulphur-35 was mixed with 1 ml. of methanol. In all the measurements reported here 15 ml. counting vials were used. For measurement of the γ-rays a thallium doped sodium iodide crystal (2 in. × 2 in.) was used together with a 400 channel analyser. All combinations of labelled compounds which we tested in this investigation were subject to the same experimental conditions during electrodeposition; only the final measurements differed.

Stock solutions of the various labelled combinations were prepared with concentrations of 10 mg/ml. in water. When preparing the plating solution, a 50 μl. portion of a given solution was added to 25 ml. of isopropanol and thoroughly mixed. A 50 μl. portion of this (*a*) was added to a 15 ml. vial of the appropriate counting solution, and the plating solution then transferred to a plating cell (Fig. 1) provided with a platinum anode and stainless steel cathode. A high voltage (1,000 V, d.c.) was connected across the cell and the electrodeposition was allowed to proceed for 30 min. For an anode/cathode distance of 30 mm a current of about 250 μamp passed through the cell. During the last few seconds of the deposition period another 50 μl. portion (*b*) was taken from the solution and added to a second counting vial. The platinum anode was withdrawn from the solution without disconnecting the high voltage, and the solution was rapidly poured off. On removal, the cathode disk was

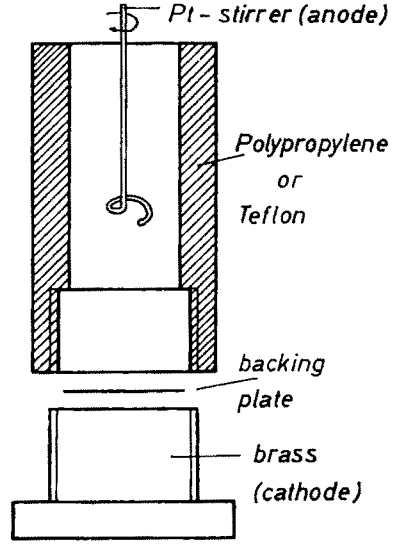


Fig. 1. Diagram of the electrodeposition cell used for the preparation of the $^{24}Na_2{}^{14}CO_3$ and $^{24}Na_2{}^{35}SO_3$ samples. The samples were first subject to measurement by means of a γ-ray scintillation spectrometer. After decaying, the deposits were washed into vials and the β-activity measured by a liquid scintillation technique

quickly washed with alcohol and allowed to dry. The plated area (10 mm diameter) was dissolved in 50 μl. of water and washed into a counting vial (*c*) with the appropriate amount of counting solution. For labelled compounds[5] (5) and (6), the comparatively short-lived sodium activity was measured first and after a suitable decay period (8 days) the relative sulphur and carbon activities determined in the same way.

Measurement of more than thirty samples of each of (*a*), (*b*) and (*c*) showed that in all cases 32 per cent of the total radioactive material in solution was deposited at the cathode after 30 min and that the ratio between sodium and carbon, and sodium and sulphur, remained the same provided the water content did not increase to more than 2 or 3 per cent of the total cell volume. This was verified as follows: A 500 μl. portion of the stock solution in question was thoroughly mixed with 150 ml. of isopropanol and transferred to a 200 ml. platinum beaker. The beaker constituted the cathode, and a platinum wire in the form of a spiral acted as the anode. The experimental arrangement is shown in Fig. 2. A current of about 4 m.amp was passed through the cell at a voltage of 1,000 V, and each deposition run lasted for 30 min. The anode wire was then removed from the solution and the plating solution rapidly poured off. Any deposits obtained were washed quickly but carefully in a few millilitres of isopropanol, and after drying were dissolved from the beaker with about 10 ml. of distilled and boiled water.

The dissolved deposits of sodium carbonate gave an alkaline reaction and also precipitated barium carbonate from a solution of barium chloride. The samples were analysed by quantitative titration with 0·05 N hydrochloric acid, both with and without the removal of any barium carbonate present. The dissolved deposits of sodium sulphite gave an alkaline reaction and precipitated barium sulphite from a solution of barium chloride: they also reduced a solution of potassium permanganate. The samples of sodium sulphite were analysed quantitatively by titration with hydrochloric acid and with sodium hydroxide after they had been subjected to oxidation with hydrogen peroxide and passed through a column of 'Dowex 50' (H^+ form). In the case of the sodium carbonate samples, analysis showed that they contained a mixture of sodium carbonate and sodium hydroxide in the ratio 1 : 2. Likewise the sodium sulphite samples were found to be mixtures of sodium sulphite and sodium hydroxide in a molecular ratio between 1 : 0 and 1 : 2.

These findings indicate that dissociation does not occur during electrodeposition of sodium carbonate and sodium sulphite from isopropanol solution when high voltage is used—provided the water content of the solution is kept to a minimum. More recent investigations have shown

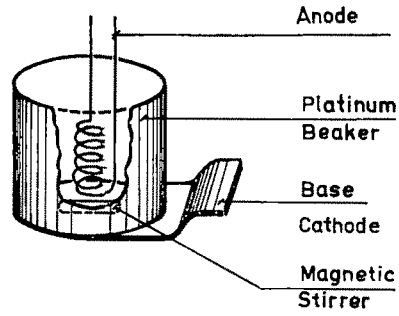


Fig. 2. Experimental arrangement used for plating milligram quantities of inactive sodium carbonate and sodium sulphate used for the chemical analysis described in the text.

that when the water content is increased to about 4–5 per cent, the deposition ratios between sodium and carbon and sodium and sulphur tend to increase in favour of the sodium. At higher levels, dissociation becomes complete, that is, the deposition of carbon and sulphur ceases, and in the case of sodium sulphite, sulphur is liberated at the anode as sulphur dioxide.

In future attempts to achieve a better understanding of the phenomena involved we intend to use tritium-labelled water and isopropanol.

We thank Prof. Nils Ryde for his interest and support in this work. We also thank Mr. H. Riedl of the Institute of Technical Chemistry for his help with the chemical analysis. The liquid scintillation equipment was kindly lent to us by LKB Produkter *AB*, Stockholm.

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GROUPING OF NOCTURNAL MIGRANTS

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"ONLY birds and fools fly—and birds don't fly by night" was one of the sayings of the men of wartime Bomber Command. The first and, perhaps, the most surprising discovery made by the radar method of bird watching introduced by Lack and Varley in 1945 (ref. 1) refuted this statement—birds do fly by night and sometimes in vast numbers. By means of radar it has now been established that many of the largest mass bird movements of the spring and autumn take place by night[2,3]. Nevertheless, many problems of bird flight at night remain unresolved—including the question of whether a migrant stream is composed of flocks of birds, each of which constitutes one of the "angel echoes" seen on the plan position indicator of a radar, or whether a random distribution of individual birds only is involved, each bird flying in the general direction of migration.

Many pictures have been published of bird migration recorded from the plan position indicator of a surveillance radar, and these illustrate the fact that discrete angels occur both by day and by night (see, for example, ref. 4). It is well known, however, that in daytime many species, such as the chaffinch (*Fringilla coelebs*), migrate in large flocks, the members of which move together in a coherent group by keeping each other in sight. Direct association of such visible bird flocks with diurnal radar angels has been reported[5,6]. The possibility therefore exists that

nocturnal angels can be similarly attributed to real groups of birds.

If this proves to be the case, then it would be interesting to know how the birds which compose such a group are able to stay together at night, and whether there is any form of social relationship between them. On the other hand, the possibility that radar angels are merely a result of the imperfect resolution by the radar of a mass of randomly distributed birds must also be considered.

The spatial resolving power of a pulsed radar signal depends on the pulse length and the shape of the beam radiated by the antenna. If τ is the time duration of the transmitted pulse, then targets lying on the same radius, and separated by less than $\frac{1}{2} c\tau$, will be shown as a single echo on the plan position indicator (c is the velocity of light). Similarly, if θ and φ are the horizontal and vertical beam-widths, then the limits of linear resolution in the azimuthal and meridional planes are $r\theta$ and $r\varphi$, respectively, where r is the range from the radar. It follows that targets contained within the so-called pulsed-volume $\frac{1}{8} c\tau r^2 \theta \varphi$ at range r will be unresolved and will be recorded on the plan position indicator as a single point echo. For the surveillance radar at the Marconi Experimental Station at Bushy Hill, Essex, which has been used for many years to observe bird migration across the North Sea, $\lambda = 23$ cm, $\tau = 5$ μsec, $\frac{1}{2} c\tau = 2{,}450$ ft., $\theta = 0.8°$ and φ is expressed by the vertical performance diagram of the radar given in Fig. 1. The inner contour of limiting visibility on Fig. 1 is plotted for a target of cross-sectional area 2 cm², which corresponds to a small bird such as a warbler; the outer contour refers to a target having an echoing area of 100 cm², which would be roughly the echoing area produced by a flight of about twenty starlings (*Sturnus vulgaris*). In such a random formation of birds the radio energy is scattered incoherently and the effective echoing area of the group is the arithmetic sum of the echoing areas of the individual birds.

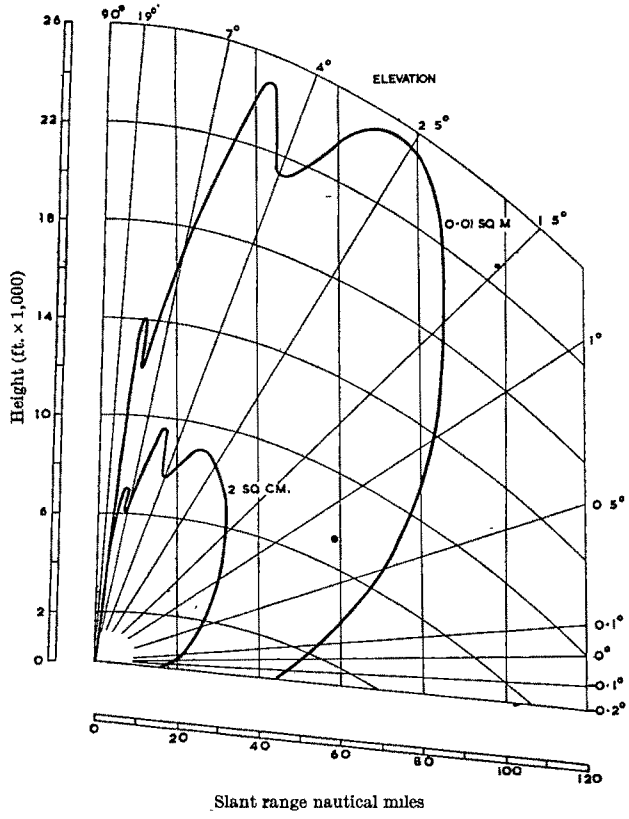


Fig. 1. Cover diagrams, Bushy Hill radar ($\lambda = 23$ cm).

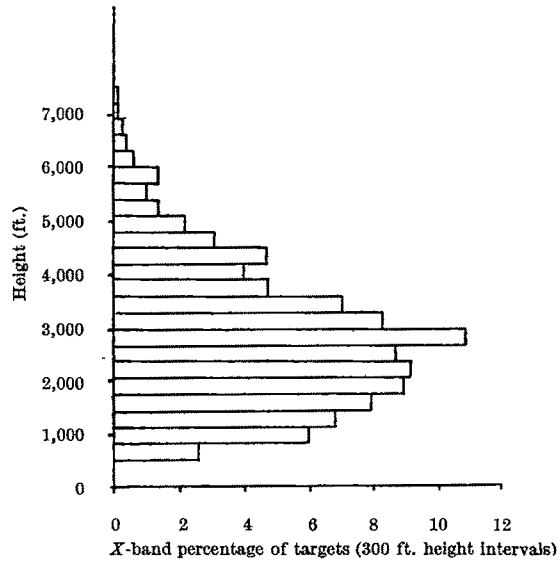


Fig. 2. Height distribution histogram. $\lambda = 3$ cm, 3,562 observations in 32·5 h in spring 1966.

For bird migration over the North Sea, the vertical resolution limit of $r\varphi$ is not reached owing to the limited height of the birds[7]. The easterly passage of nocturnal migrants at the Essex coast during the period January–May 1966 was observed from Bushy Hill using a high resolution 3 cm radar which had its beam directed vertically upwards. This radar was recording the passage of single birds through the beam, and frequencies of transits were measured as well as altitudes. The pattern of the height distribution is given in Fig. 2, which shows that the most frequently occurring altitude band was 2,700–3,000 ft.; very few birds were observed above 7,000 ft. and 90 per cent were below 4,500 ft. Fig. 1 indicates that birds flying at 4,500 ft. will cross the radio horizon at 77 nautical miles, and so the magnitude of the pulse volume occupied by birds will not vary with the range in accordance with the foregoing simple expression, but will pass through a maximum before decreasing to zero at $r = 77$ nautical miles.

As all the birds contained within the pulse-volume contribute to the echo signal it will be seen that the granular appearance of an angel display could arise from the interaction between the polar diagram of the aerial, the parameters of the radar and a random distribution of migrants within the permitted altitude band. According to this view, nocturnal angels would not correspond to true groups of birds, but would merely be the echoes from those pulse-volumes in which the number of birds happened to provide a total echoing area sufficient to produce a signal at the radar, exceeding the receiver noise level (4×10^{-15} W) by about 10 dB. The inevitable diminution of angel density at longer ranges is a consequence of the pulse-volume effect, combined with the rapid fall-off of signal power with range as expressed by the radar equation ($1/r^4$).

Examples of radar displays of birds which show the pulse volume effect are not lacking. Warblers on migration have the appearance on the plan position indicator of a hazy continuum of light in which are embedded transient echoes of low intensity[4]. A similar diffuse display is also typical of night ascended swifts (*Apus apus*) during late June[8]. These displays are in marked contrast with the assembly of bright point echoes which are characteristic of discrete angels. It seems fairly certain that such a diffuse type of display is caused by incoherent scattering from the mass of small targets contained within the pulse-volume; together, these provide a fluctuating, weak signal that only occasionally exceeds the noise level. Sutter[6] reported that his nocturnal angels

appeared weaker and less sharp than the diurnal angels; this, he explained, is because the birds are grouped into tight flocks by day but not by night. Lack[2] also thought that the evidence did not point to the existence of night groups; on the other hand, he also reported tracking strong, discrete nocturnal angels at long range. Bergmann and Donner[9], however, in their study of the migration of the common scoter (*Melanitta nigra*) and longtailed duck (*Clangula hyemalis*) in the Gulf of Finland, considered that these birds were grouped into flocks by night as well as by day.

Observations were made from Bushy Hill during 1964–66 in the hope of resolving the nature of nocturnal angels. Various radar equipments were used which operate at wavelengths of 23 cm, 10 cm or 3 cm (*L*, *S* and *X* microwave bands, respectively). Use was also made of the signature analysis technique—the study of the pulse-to-pulse variations in amplitude of the signal received from a target in order to deduce information about its scattering characteristics. A number of strong, discrete, nocturnal angels were examined with the aid of a steerable, *L*-band tracker radar the beam-width of which was 1·8°. Signature analysis shows that the amplitudes of successive pulses from such angels followed a Rayleigh distribution typical of a target composed of a number of scattering centres moving randomly with respect to each other—that is, the strong angels in the vicinity of East Anglia were usually composed of a number of birds. In contrast, signature analysis applied to the signals received by the *X*-band radar showed them to be typical of single-bird targets—this was to be expected in view of the high resolution of the equipment and the short range at which the observations were made (the pulse-volume used was small).

The average echoing areas of a number of angels were also measured with the *L*-band tracker, and the echoing area distribution function which was obtained for both day and night angels of this type is shown in Fig. 3. The distribution pattern is not symmetrical and suggests that angels having echoing areas of 500 cm² or more are markedly more rare than those having echoing areas less than this. If we take the most probable value of the echoing area suggested by this figure to be about 70 cm² it is possible to estimate the average number of birds per angel between five and thirty-five, because the echoing area of those birds likely to be airborne at the time of the measurements would probably range from 2–14 cm².

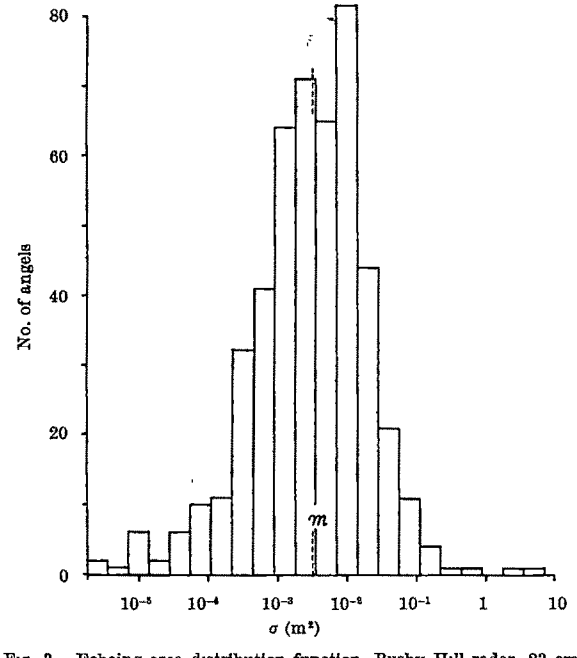


Fig. 3. Echoing area distribution function, Bushy Hill radar, 23 cm; July 1962.

A different method was used to estimate the average bird content of nocturnal angels observed during the easterly emigration from East Anglia of winter visitors such as the blackbird (*Turdus merula*), redwing (*T. musicus*), fieldfare (*T. pilaris*), lapwing (*Vanellus vanellus*), chaffinch (*Fringilla coelebs*) and starling. An *L*-band surveillance radar supplied data on angel densities at a known range, while the rate of passage and density of birds overhead were derived from the *X*-band radar counts. After making appropriate correction for the thinning of angel densities with range, in a manner similar to that described by Nisbet[10], the number of birds per angel appeared to lie between four and twenty.

The application of a mixed radar and Moon-watching technique by Nisbet[10] showed that the average number of birds per nocturnal angel which he observed during the autumn migration in the region of Cape Cod was usually between two and twelve. After a careful review of the data, and noting particularly the fact that the average composition of an angel increases only very slowly with the migration density, Nisbet concluded that many birds migrate in well-defined groups.

Bearing in mind the uncertainties attached to radar measurements of this kind, it will be seen that the number of birds per angel determined by Nisbet does not differ significantly from the value suggested by the two independent methods of assessment used at Bushy Hill. But this rough agreement does not prove the group nature of the angels observed in the two regions. One method of testing the existence of such angel groups would be to record and to compare the angels which occur in a particular region by means of two radars with different pulse-volumes over the area examined. Real groups of birds should then be seen as such on both radars. This experiment was, in fact, performed by Eastwood and Rider, when angels observed on the plan position indicator of an *L*-band surveillance radar were also photographed simultaneously from the display of an *S*-band nodding height-finder. It can be concluded from this varied evidence that many of the strong angels must correspond to groups of birds. Such a conclusion is supported by the comparatively long lives which many angels often possess on films of the plan position indicator when viewed by the time compression method, and the long distances, often 10–20 miles, over which they may be tracked. The fact that angels on a magnified plan position indicator display rarely split further supports the conclusion; this would be surprising if angels were only a pulse-volume effect[11].

Do these data suggesting the existence of groups of migrant birds at night necessarily contradict the idea of migration as made up of random distributions of birds? Counts made with the *X*-band radar at Bushy Hill showed that the rate of passage overhead of individual birds during nights of the spring migration was far from uniform. While observations were being made it was particularly noticeable that the number of transits during successive 15 min intervals changed greatly, and even that the rate of passage could vary from minute to minute. In other words, the birds tended to make their appearance in bursts, so that their spatial distribution was very granular and areas of concentration were present among the general spread of the migrants. The distances between the members of such closely packed sections were well within the pulse-volume of the *L*-band surveillance radar and would have been seen as a single angel on the plan position indicator. If such a local concentration of birds were to remain contained within the gradually expanding pulse volume during the outward flight, then the single angel which would result could be tracked on the plan position indicator, even though the birds might not constitute a true group—there being no form of social relationship between them.

That this possibility can indeed happen is supported by other radar evidence. Time compression films of the surveillance radar plan position indicator have shown that the mass autumn departure of warblers at night from the south-eastern region of England can cause the outlines of the Essex, Kent and Sussex coasts to advance out to sea like a wall in the direction of migration, which is towards the south-south-east. Although these warbler echoes almost certainly arise from a pulse-volume effect there is a continuity to the geographical distribution of the birds at take-off which is maintained during the first part of the migratory flight and takes a surprisingly long time to disappear[4]. Similarly, films of the vesper flights of swifts show that the denser regions which correspond to the places of origin of the birds continue to show for quite a long time, presumably because the individual birds perform similar tight spiral movements[8].

This continuity of pattern is even more marked in the night movement of waves of starlings which were reported by Lack and Eastwood[4]. Furthermore, the dispersals of starlings from their roosts at sunrise to join the migrant stream to the continent showed that the parallel waves of birds, sometimes extending over a front 20 miles long in a north–south direction, moved towards the east but were still distinguishable as waves an hour later, and some 60 miles to the east[12].

These examples suggest that some form of station-keeping is practised by the birds which can supplement the migration direction "programme" with which all the birds are endowed. This station-keeping would ensure the continuity of their initial distribution pattern over quite long periods. More probably, the birds perform similar movements in response to the same stimuli. But there will inevitably be scatter in respect of speeds flown by individual birds and on the headings which they take up. The effect of these variances will cause the initial distribution to become blurred and more extended as the flight proceeds. Finally, the pattern will merge with the general background of birds—which is, in fact, observed in all the foregoing examples.

The radar evidence as a whole suggests that while some of the nocturnal angels which give good echoes at long range are probably true groups of birds analogous to those observed by day, others, and perhaps the majority, are pseudo-groups which result from the pulse-volume effect. Even the "true groups" may only be groups in the sense that the birds composing them have taken off from a common locality or a common roost; the group form is roughly maintained because the birds fly at roughly the same speed and in the same direction. Nevertheless, the well known visual acuity of the birds may serve to bind them into some sort of a unified group; the well known phenomenon of acoustic calling on the wing would also assist in this. If such were indeed the explanation of nocturnal angels it would mean that the appearance of discrete angels only differs from the appearance of warblers by reason of the echoing areas of the birds involved and the intensity of the distribution.

Radar is perhaps the only method available for counting the numbers of birds flying by night, but it is still in need of refinement before it can be regarded as a precise counting instrument. One further tentative result which has emerged from this investigation is of sufficient interest to be mentioned here; this concerns an estimate made of the total number of birds taking part in the springtime migratory movement which corresponds to the return to the Continent of many of the winter visitors to these islands. Lack[2] has shown that this is the major migration of the spring in the south-eastern region of England. Undoubtedly the most accurate way of counting the passage of migrant birds by night is to use the vertical incidence *X*-band radar technique. This method gives an accurate value for the rate of passage of birds across a 1 mile front at the position occupied by the radar, but it cannot supply information about the flow of birds at points remote from the radar. Many such radars would be needed to cover an extended front and the method would be prohibitively expensive to apply to a broad front migration. The *L*-band surveillance radar, however, can map the general progress of angel activity across a front extending from the north coast of Norfolk down to the Pas de Calais. Combination of the *L* and *X* radar techniques translates the angel echoes into numbers of birds and permits useful estimates to be made of the rates of flow of birds across the whole of this front. When these average rates are associated with the pattern of activity for the spring migration as a whole, which was established by Lack for this easterly movement, it becomes possible to estimate the total number of birds involved as 2×10^7. These heavy migrations do not denude the countryside of birds, of course, and their place is largely taken by the summer visitors which come to these islands to breed. The figure of 2×10^7 may be compared with Harper's[5] estimate of 3×10^6 birds taking part in a heavy migration over Hertfordshire in one spring night.

We thank Mr J. D. Bell, Mr. N. R. Phelp and Mr. J. Franklin, of the Marconi Research Laboratory, for assistance in making the measurements.

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GROWTH AND STRUCTURE OF THE SHELL OF LIVING ARTICULATE BRACHIOPODS

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The relationship between the triple-layered shell and the underlying outer epithelium of the mantle of living articulate brachiopods has been known for some time[1]. It involves the production of epithelial cells in a generative zone at the mantle edge and the secretion of each of the three layers in turn by the cells as they become incorporated within the expanding mantle. The morphological consequences of this process have not hitherto been completely established, but electron microscope studies of the shell fabric of the terebratulides, *Macandrevia* and *Terebratulina*, and the rhynchonellides, *Hemithyris* and *Notosaria*, have now shown the full sequence of events.

The site of the generative zone for each valve is a circumferential groove dividing the mantle edge into two lobes. Newly proliferated cells, which are ultimately added to the outer epithelium, first become part of the

inner surface of the outer lobe and are then conveyed forward towards the tip of the lobe by the addition of new cells behind them. At this juncture they exude the outermost shell layer, the periostracum, composed exclusively of protein[2]. The previously reported[1] differential thickening of the periostracum is related to the external morphology of the cells, in that thickened bands forming a reticulate mesh on the inner surface of the periostracum usually correspond to boundaries between cells.

When cells reach the mantle tip they start secreting calcite crystallites between the periostracum and the underlying cell walls. This crystallite mat is the first formed part of the calcareous primary layer. It is built up more or less continuously across intercellular boundaries and bears no recognizable imprint of cell outlines. But its strongly pitted appearance at the shell edge, with pits up to 0·4μ in diameter, indicates that the outer surfaces of the cells responsible for deposition of calcite bear microvilli intruding into the primary layer. Thus the cells are already anchored in position relative to one another on the calcareous exoskeleton, and any differential movement away from these original loci of attachment depends on variation in the rate of secretion for each cell. The rigidity and continuity of the calcareous primary layer also control the disposition of the outer mantle lobe; enlargement of the shell is thus effected by a "conveyor belt" system whereby proliferated epithelial cells forming the inner surface of the outer lobe are successively brought into position to extend the primary layer by extracellular calcareous secretion.

The thickness of the primary layer is a function of the number of rows of epithelial cells involved in its secretion, with the innermost, oldest cells underlying the thickest part of that layer at the shell edge. In general, the number of concentric rows making up this zone in adult shells is fairly constant; in *Terebratulina*, for example, there are about fifteen, so that the thickness of the primary layer underlain by secondary shell does not vary very much. This stability is maintained because each row of cells undergoes profound changes in secretory habit when, through addition of new cells at the tip of the outer lobe, they come to form the innermost rows underlying the primary layer. The changes are two-fold. They involve a reversion to the exudation of protein, now restricted to narrow arcs more or less coincident with the anterior intercellular boundaries, and also the secretion of calcite crystallites as a uniformly continuous plaster laid down within the protein boundaries. At this stage the cells, each with a convex anterior boundary, alternate in slightly overlapping rows so that their intercellular boundaries approximate to an anterior semicircle subtended between two adjacent arcs which are shared with the cells immediately behind (Figs. 1 and 2). The protein strips underlain by the boundaries in common with the two older cells are usually completed, at least as contiguous strands, before traces of protein exuded by the anterior boundary can be seen. Plastering of secondary calcite over the comparatively porous primary layer usually starts in the re-entrant angle between the two older cells and soon coalesces with any isolated deposits that may appear elsewhere within the protein boundaries. In this way the secondary calcareous layer of the articulate brachiopod shell is formed by cells that were initially responsible for the secretion of the periostracum and then for the calcareous primary layer (Figs. 3 and 4).

Continuous deposition of protein along the intercellular boundaries segregates the calcite of the secondary shell into fibres, each of which is contained in a protein sheath with only rare lacunae. Thus each fibre is controlled in its further growth by the outer surface of a cell which is characteristically concave outwards and more pronouncedly so towards the front, so that the internal surface of the fibre is convexly lobate with an outline determined by its protein boundary (Fig. 4). In this manner, the inner surface of the secondary shell, unlike that of the primary layer, is fashioned into the so-called shell mosaic (Figs. 1 and 2) which is in reality a protein–calcite cast of the outer surface of the epithelium[3]. This pattern is so consistent that even cell division taking place after the deposition of secondary shell can be identified by variation in the morphology of the mosaic.

The mosaic has a discernible lineation reflecting the growth relationship between fibres at the moment of death, which can be graphically represented by plotting the long axes of exposed parts of fibres as growth vectors. Such growth charts show that, in convex valves at least, an extension of fibres normal to the shell edge is only characteristic of a narrow peripheral zone, because the overlapping ends of fibres comprising the inner part of the mosaic are rapidly re-orientated more or less parallel to the valve margin. Thus each fibre tends to grow in a spiral and is directed clockwise in the right half of a valve and anticlockwise in the left half. Some modification of the mosaic occurs when the fibres form skeletal outgrowths, but in general the pattern is blurred only when

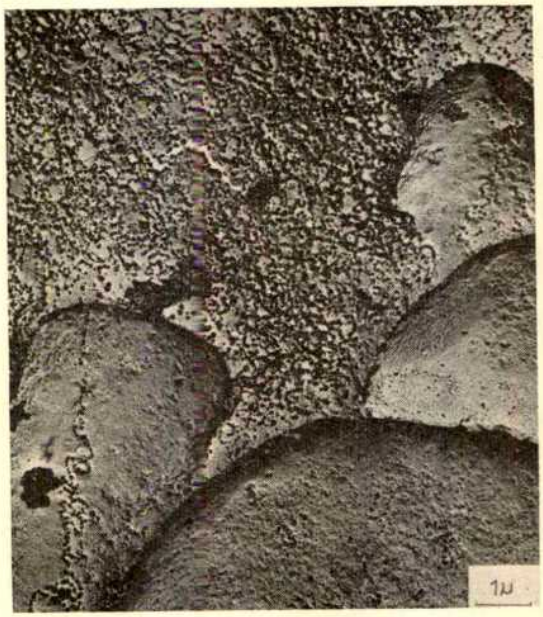


Fig. 1. Single-stage negative replica of the internal shell surface at the junction between the primary and secondary layers of *Terebratulina caput-serpentis* (Linn.): shadowed with gold-palladium at 1 in 1.

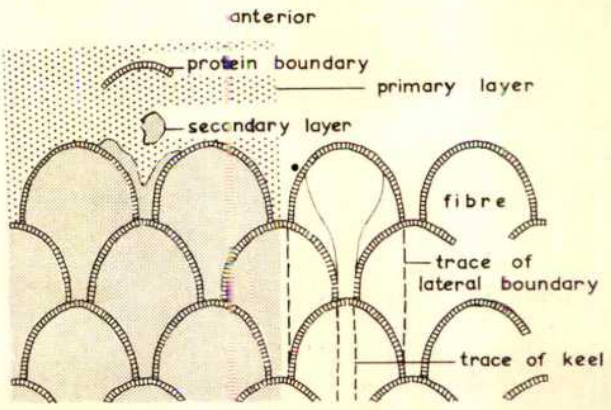


Fig. 2. Diagrammatic internal surface view of the junction between the primary and secondary layers of the articulate brachiopod shell.

excessive calcite deposition interrupts the laying down of protein boundaries as, for example, in muscle bases.

The arrangement of the cells of the outer epithelium relative to one another is also responsible for the distinctive stacking of fibres as seen in any random section of the shell. In longitudinal section, fibres appear as blades gradually increasing in width towards the inner surface of the valve and, when not involved in accommodating puncta or contributing to the formation of internal features, are inclined at about 10° to the inner surface of the primary layer (Fig. 3). A transverse section of a typical fibre, however, shows that the inner surface is flatly to roundly convex inwardly, usually with a medial keel, while the outer surface is made up of two lateral areas joined by a medial saddle, all concave outwardly (Figs. 5 and 6). The inner surface corresponds to the outwardly concave, anterio-median surface of a cell, the lateral areas to the two contiguous halves of alternating cells in the immediately younger row, and the saddle to the keel laid down by a cell belonging to the next younger row (compare Figs. 1 and 5). This arrangement facilitates the overlapping growth of fibres. It also enables any shell fragment to be orientated because all concave surfaces face outwards.

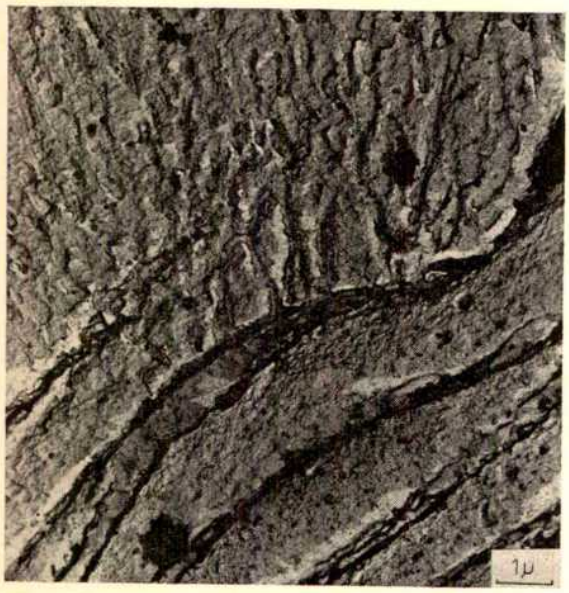


Fig. 3. Single stage negative replica of a longitudinal section of the junction between the primary and secondary layers of *Laqueus californicus* (Koch): shadowed with gold-palladium at 1 in 1.

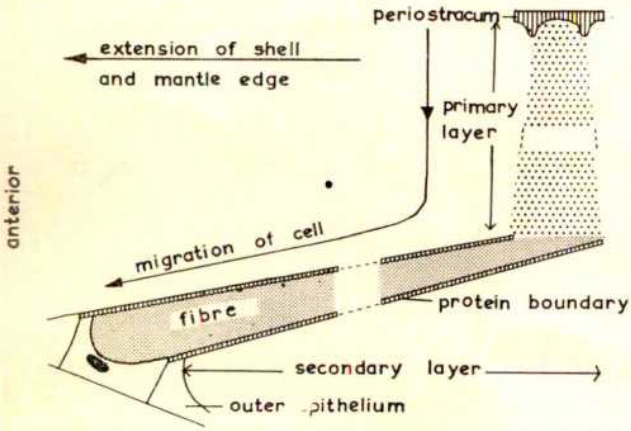


Fig. 4. Diagrammatic longitudinal section showing the relationship between an outer epithelial cell and the three layers of an articulate brachiopod shell.

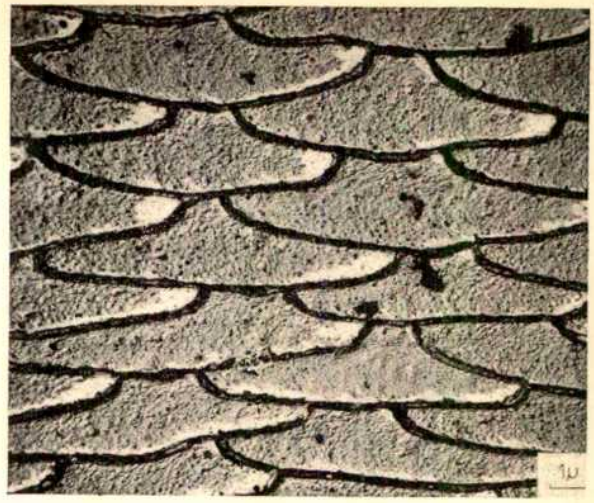


Fig. 5. Single stage negative replica of a transverse section of the fibres of the secondary layer of *Macandrevia crania* (Muller): shadowed with gold-palladium at 1 in 1.

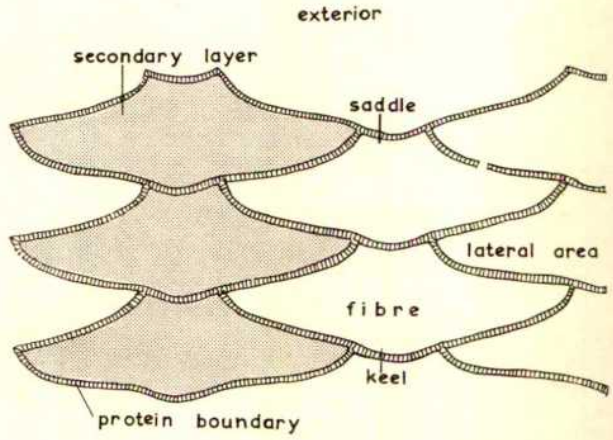


Fig. 6. Diagrammatic transverse section showing the relationship between fibres of the secondary layer of an articulate brachiopod shell.

The different appearances of transverse and longitudinal sections, the incremental increase in size and the spirally arcuate traces of fibres growing away from the primary layer have led to misinterpretations of sections of the brachiopod shell. Thus Sass, Monroe and Gerace[4] were puzzled by their electron micrographs of sections of the terebratulids *Gryphus* and *Pictothyris*. In fact, a segment of any section through the shell will normally show an inward increase in size of fibres because those adjacent to the primary layer will have been deposited by small epithelial cells at the mantle edge, whereas the inner fibres will have been secreted by progressively older, larger cells as successively overlapping extensions from their original loci of attachment. Moreover, in segments of longitudinal sections away from the shell edge, it is likely that fibres immediately underlying the primary layer will be seen in transverse section, and those towards the inner surface of the shell in oblique sections which may be parallel for part of their lengths with the long axes of the fibres.

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MODEL OF THE MEMBRANE OF SMOOTH MUSCLE CELLS OF THE GUINEA-PIG TAENIA COLI MUSCLE DURING TRANSMISSION FROM INHIBITORY AND EXCITATORY NERVES

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THE changes in membrane potential which occur in the smooth muscle cells of the guinea-pig taenia coli muscle during transmission from inhibitory and excitatory nerves have been described recently[1-4]. This smooth muscle was selected for these investigations because the ionic properties of the membrane, the ionic content of the cell, and the effect of transmitter substance on the cell membrane had already been examined[5-12]. On the basis of these investigations a tentative model of the membrane during transmission to smooth muscle cells may be proposed.

Membrane theory. Before a model of transmission can be presented, the ionic properties of the smooth muscle membrane need to be known. An attempt has therefore been made to determine if the membrane theory of Boyle and Conway[13], which has been shown to hold for the skeletal muscle membrane[14,15], also holds for the membrane of the smooth muscle cells of the taenia coli.

From the theory of Boyle and Conway

$$(\mathrm{Na})_i + (\mathrm{K})_i + (\mathrm{Cl})_i + \frac{D}{V} = C \qquad (1)$$

$$(\mathrm{Na})_i + (\mathrm{K})_i - (\mathrm{Cl})_i + \frac{DZ}{V} = 0 \qquad (2)$$

$$(\mathrm{K})_o\,(\mathrm{Cl})_o = (\mathrm{K})_i\,(\mathrm{Cl})_i \qquad (3)$$

From (1) and (2):

$$(\mathrm{Na})_i = \frac{(\mathrm{K})_i\,(Z-1) + (\mathrm{Cl})_i\,(1+Z) - ZC}{(1-Z)}$$

if $Z = -1$ (ref. 16).

$$(\mathrm{Na})_i = \frac{C}{2} - (\mathrm{K})_i \qquad (4)$$

where i refers to intracellular concentrations; o refers to extracellular concentrations; D is the number of indiffusible molecules inside the cell; Z is their average valency; V is the cell volume; and C is the total concentration of all the molecules in the external solution.

In skeletal muscle the Donnan equilibrium exists for external potassium concentrations above 10 mM[14,15], and so

$$E_m = E_{\mathrm{K}} = \frac{RT}{F} \ln \frac{(\mathrm{K})_o}{(\mathrm{K})_i} = E_{\mathrm{Cl}} = \frac{RT}{F} \ln \frac{(\mathrm{Cl})_i}{(\mathrm{Cl})_o} \qquad (5)$$

where E_m is the membrane potential; E_{K} and E_{Cl} the equilibrium potentials for potassium and chloride ions, respectively; and R, T, F have their usual meaning. Hodgkin and Horowicz[14] have shown that in a chloride-free solution the deviation of the membrane potential from the potassium electrode for $(\mathrm{K})_o$ greater than 10 mM can be predicted by the Goldman[17] equation

$$E_m = \frac{RT}{F} \ln \frac{(\mathrm{K})_o + \alpha(\mathrm{Na})_i}{(\mathrm{K})_i + \alpha(\mathrm{Na})_i} \qquad (6)$$

where α is the ratio of the permeability coefficient of sodium to that of potassium. Hence

$$\alpha = \frac{(\mathrm{K})_o - (\mathrm{K})_i\, 10^{\frac{E_m}{62}}}{(\mathrm{Na})_i\, 10^{\frac{E_m}{62}} - (\mathrm{Na})_o} \qquad (7)$$

The investigations of the smooth muscle membrane of the taenia coli in high-potassium solutions $(\mathrm{K})_o > 25$ mM and in chloride-free solutions $(\mathrm{Cl})_o = 0$ made by Holman[9] and Kuriyama[10] can now be interpreted in terms of equations 1–7.

$(\mathrm{K})_o$ greater than 25 mM. In this case it has been assumed that the potassium and chloride ions were in a Donnan equilibrium at the highest potassium concentration, and from equations 3 and 5 $(\mathrm{K})_i$ and $(\mathrm{Cl})_i$ have been obtained. Then for lower concentrations of potassium, if Donnan equilibrium continued to hold, potassium and chloride ions should have moved across the membrane in equal numbers until equation (3) holds. Therefore, equation (3) has been used to find the new values of $(\mathrm{K})_i$ and $(\mathrm{Cl})_i$, and if the assumption of a Donnan equilibrium is correct then the predictions of the value of the potassium electrode from equation (5) should agree with the measured value of the membrane potential. Tables 1 and 2 summarize the values of E_m, $(\mathrm{K})_o$ and $(\mathrm{Cl})_o$ taken from Holman's[9] results on page 470 of her paper and Kuriyama's[10] results on page 17 of his paper. All values are expressed in mM or mV. The predicted value of E_{K} agrees well with the value of E_m measured with microelectrodes. It is therefore probable that in high potassium concentrations, potassium and chloride ions are in Donnan equilibrium across the membrane.

Table 1

E_m	$(\mathrm{K})_o$	$(\mathrm{Cl})_o$	$(\mathrm{Cl})_i$	$(\mathrm{K})_i$	E_{K}
−20	100	240	120	200	−19
−30	50	190	65	145	−30
−33	40	180	54	134	−33
−42	25	165	35	115	−42

Table 2

E_m	$(\mathrm{K})_o$	$(\mathrm{Cl})_o$	$(\mathrm{Cl})_i$	$(\mathrm{K})_i$	E_{K}
−17·5	118	258	137	220	−17
−27·5	59	199	74	157	−26·5
−40	29	169	40	123	−39

According to the Boyle and Conway theory, there should be little or no change in the intracellular concentration of sodium when the potassium and chloride concentrations outside the cell are changed, since the membrane is supposed to be largely impermeable to sodium. If this is the case, then $(\mathrm{Na})_i$ in equation (4) should remain constant with changes in $(\mathrm{K})_i$ and in the total external concentrations of ions, C. The $(\mathrm{K})_i$ at various values for C can be obtained from Tables 1 and 2, and these are summarized in Tables 3 and 4. $(\mathrm{Na})_i$ in each table is fairly constant at different $(\mathrm{K})_o$, and both tables show that the value of $(\mathrm{Na})_i$ is about 40 mM.

Table 3

E_m	$(\mathrm{K})_i$	C	$(\mathrm{Na})_i$
−20	200	480	40
−30	145	380	40
−33	134	360	44
−42	115	330	45

Table 4

E_m	$(\mathrm{K})_i$	C	$(\mathrm{Na})_i$
−17·5	220	516	38
−27·5	157	398	33
−40	123	338	36

Chloride-free solution. If the chloride ions normally present in Krebs solution are replaced by sulphate ions, keeping the potassium concentration constant, there will be a decrease in osmotic pressure outside the cell due to the decrease in the net number of ions, C. Ions will then leave the fibre together with water until osmotic equilibrium is regained and there will thus be a decrease in $(\mathrm{K})_i$. Kuriyama[10] has performed this experiment, as well

as changing the $(K)_o$ in a chloride-free Krebs solution. The changes in $(K)_i$ with elevation of $(K)_o$ in sulphate Ringer can be predicted by equation (4) with $(Na)_i$ = 40 mM. Table 5 summarizes values for E_m, $(K)_o$ and C from Kuriyama's results and the theoretical predictions of E_K. The membrane potential is shown to be close to the value of the potassium electrode for each value of $(K)_o$. It seems likely then that the chloride ion is in electrochemical equilibrium with the membrane potential for this range of potassium concentrations.

Table 5

E_m	$(K)_o$	C	$(K)_i$	E_K
−4	118	387	153	−6
−17·5	59	298	109	−17
−32	29	253	86	−30
−45	12	228	74	−49

For low values of $(K)_o$ the membrane potential E_m deviates from the potassium electrode, E_K, in sulphate solution. If we assume that this deviation is due to a small permeability to sodium, then the value of α should be the same over this range of membrane potentials. Using equation (4) to obtain $(K)_i$ and equation (7) to obtain α, Table 6 shows that α does remain constant at 0·05 for the two values given by Kuriyama[10] for E_m, $(K)_o$ and C.

Table 6

E_m	$(K)_o$	C	$(K)_i$	$(Na)_i$	$(Na)_o$	
−47·5	6	219	70	40	140	0·05
−53	3	214	67	40	140	0·05

The deviation of the membrane potential of smooth muscle from that predicted by the potassium electrode, which was reported by Holman[9] and Kuriyama[10], is probably due to their adding potassium chloride salt directly to the Krebs solution. This would probably increase the osmotic pressure outside the cells, so that potassium and chloride ions would move into the cell until osmotic equilibrium was regained. If allowance is made for this increase in $(K)_i$, it has been shown that the membrane potential they measured does obey the predictions of the potassium electrode. It is interesting that the normal $(K)_i$ predicted for the cell was about 120 mM, which agrees with the value of 120 mM obtained by photometric analysis by Freeman-Narrod and Goodford[5].

The concentration of intracellular sodium in the smooth muscle of the taenia coli is much larger than that found in nerve or skeletal muscle, being about 37 mM. This value can be obtained from the value of 70 mM of sodium/kg wet weight of muscle found by Goodford[6], assuming the extracellular space is 333 ml./kg wet weight of muscle[7] and that the intracellular space is 600 ml./kg wet weight of muscle[5]. This value of $(Na)_i$ agrees well with the value of about 40 mM obtained using the Boyle and Conway theory. The value of the intracellular sodium concentration remained constant with changes of the membrane potential, a result to be expected if the membrane remains mostly impermeable to sodium.

There was no deviation of the membrane potential from the potassium electrode for membrane potentials less than −40 mV. In high-potassium solutions, therefore, chloride ions are probably in electrochemical equilibrium with the membrane potential. The intracellular chloride concentration of 35 mM predicted on this basis agrees well with the value of 30 mM obtained by Daniels[8] using photometry techniques, this value being calculated on the basis of an intracellular volume of 333 ml./kg wet weight of muscle.

It is therefore concluded that for potassium concentrations above 25 mM, potassium and chloride are in a Donnan equilibrium, and for potassium concentrations less than 25 mM there is a small sodium permeability.

Transmission theory. Eccles[18] has suggested that transmission to the motoneurone can be represented by the sudden short-circuiting of the total membrane resistance and capacitance by a low resistance which selectively allows the ion species which carries the synaptic current to flow through it. The post-junctional membrane changes which occur during transmission to smooth muscle can be represented by an electrical model similar to that used for transmission to the motoneurone[18], if the following assumptions are made: the length of a single taenia coli smooth muscle cell is less than the length constant[19], each smooth muscle cell has receptors for both inhibitory and excitatory transmission[3,11], and the concentrations of transmitter artificially applied to the muscle are sufficient to maintain the maximum permeability changes at the receptors.

The electrical circuit model of the post-junctional membrane is shown in Fig. 1, where V is the membrane potential at time t; C_m the total membrane capacitance; G_m the total membrane conductance; G_i the maximum conductance at the inhibitory receptor region; G_e the maximum conductance at the excitatory receptor region; E_i the reversal potential for the ions which carry the inhibitory current; E_e the reversal potential for the ions which carry the excitatory current; and E_m the resting membrane potential.

Since there is no external source of current across the cell membrane

$$C_m V' + V(G_m + G_i + G_e) - (G_m E_m + G_i E_i + G_e E_e) = 0 \quad (8)$$

where $V' = \frac{dV}{dt}$, and since only steady membrane potential are considered, $V' = 0$.

Therefore

$$V = \frac{E_m + \frac{G_i}{G_m} E_i + \frac{G_e}{G_m} E_e}{1 + \frac{G_i}{G_m} + \frac{G_e}{G_m}} = \frac{E_m + aE_i + bE_e}{1 + a + b} \quad (9)$$

$$\text{where } a = \frac{G_i}{G_m} \text{ and } b = \frac{G_e}{G_m}$$

From (9)

$$(V - E_m) = \frac{aE_i + bE_e}{(1+a+b)} - \frac{E_m(a+b)}{(1+a+b)} \quad (10)$$

Equation (10) gives the maximal change in the membrane potential when the inhibitory and excitatory transmitters are simultaneously applied to the cells.

When the inhibitory transmitter is applied alone, $b = 0$ in (10) and:

$$(V - E_m) = \frac{aE_i}{(1+a)} - \frac{aE_m}{(1+a)} \quad (11)$$

When the excitatory transmitter acts alone, $a = 0$ in (10), and

$$(V - E_m) = \frac{bE_e}{(1+b)} - \frac{bE_m}{(1+b)} \quad (12)$$

Equations (10), (11) and (12) are shown plotted in the graph of Fig. 2.

Bulbring and Kuriyama[11] have examined the effects of adrenaline (10^{-7} g/ml.) on the membrane potential of cells with different initial resting potentials. Their results are

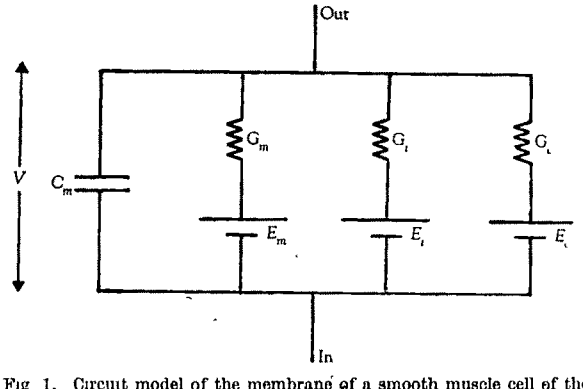


Fig 1. Circuit model of the membrane of a smooth muscle cell of the taenia coli during transmission from inhibitory and excitatory nerves.

summarized in the plot of $(V-E_m)$ versus E_m in Fig. 3. There is a linear relation between the change in membrane potential due to adrenaline $(V-E_m)$ and the initial resting potential E_m, the product moment correlation coefficient being 0·71. The regression line for these results is $(V-E_m) = -27 - 0{\cdot}37\ E_m$, and when this is compared with equation (11), the values of E_i and 'a' are found to be −73 mV and 0·6, respectively. This means that the reversal potential for inhibition is about −73 mV and that the ratio of the maximum conductance at the inhibitory receptors to the total membrane conductance is 0·6.

Bulbring and Kuriyama[11] have also examined the effects of acetylcholine (ACh) (10^{-6} g/ml.) on the membrane potential of cells with different initial resting potentials. Their results are summarized in the graph of $(V-E_m)$ versus E_m of Fig. 3. A linear relation exists between the changes in membrane potential to ACh $(V-E_m)$ and the initial resting potential E_m, the product moment correlation coefficient being 0·97. The regression line for these results is $(V-E_m) = 20 - 0{\cdot}77\ E_m$, which when compared with equation (12) gives E_e and b as −26 mV and 3·3, respectively. Thus the reversal potential for excitation is about −26 mV and the ratio of the maximum conductance at the excitator receptors to the total membrane conductance is about 3·3.

The values of a, b, E_e and E_i have been determined, and the graph of equation (10) can thus be drawn as in Fig. 3. This shows the membrane potential changes to be expected when both adrenaline and ACh are simultaneously applied to the muscle cells. Fig. 3 shows that simultaneous application of both transmitters brings the membrane potential from its resting value to a potential between −30 and −40 mV.

Recently, Jenkinson and Morton[12] have shown that noradrenaline produces an increase in the potassium permeability of smooth muscle cells of the taenia coli. If noradrenaline is the sympathetic inhibitory transmitter, then the equilibrium potential for sympathetic inhibition is about −80 mV. This value is close to the equilibrium potential of −73 mV determined by the present theory. The transmitter released from intramural inhibitory nerves to the taenia coli smooth muscle cells has not been identified[20,21]. This transmitter also increases the permeability of these cells to potassium ions[21], however, and therefore probably also has an inhibitory equilibrium potential of about −80 mV.

If potassium ions carry the inhibitory current, then according to equation (11), adrenaline will only cause a hyperpolarization of the membrane when the magnitude of the equilibrium potential for potassium (E_K) is greater than the resting potential (E_m). It has already been shown that in high external potassium solutions there is no difference in the value of E_K and E_m when the resting

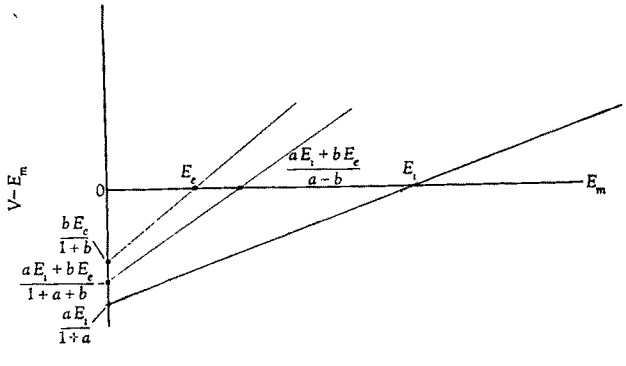


Fig. 2. Theoretical predictions of the relationships between the membrane potential change caused by the application of a transmitter substance to the smooth muscle cells of the taenia coli and the initial resting potential of these cells. Abscissa, E_m is the initial resting potential. Ordinate, $V-E_m$, is the membrane potential change produced by the transmitter substance For definition of symbols, see text.

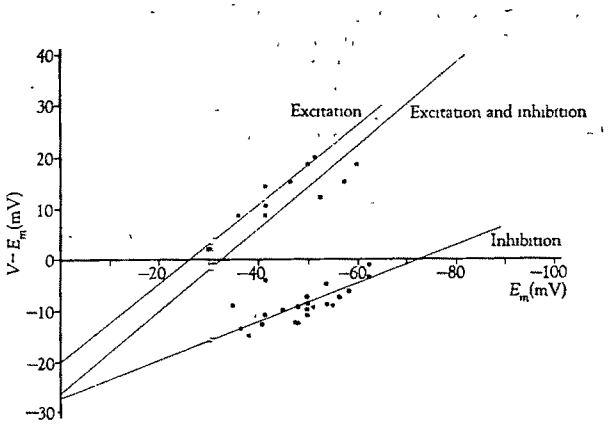


Fig. 3. Experimentally determined relationship between the membrane potential change caused by the application of a transmitter substance to the smooth muscle cells of the taenia coli and the initial resting potential of these cells. Co-ordinates as in Fig. 1. Closed squares, application of acetylcholine. Closed circles and open triangles, application of adrenaline. Curve marked "excitation" regression line for closed squares. Curve marked "inhibition" regression line for closed circles. The three closed squares from −50 to −60 mV are not included in the calculation of the regression line for excitation.

potential is smaller than about −40 mV. Hence, adrenaline should have no effect on the membrane potential when it has been depolarized beyond −40 mV by high external potassium solutions. This explains why Kuriyama[10] was unable to hyperpolarize the cells of the taenia coli with adrenaline when they had been depolarized beyond −40 mV with high external potassium solution.

Takeuchi[22] has shown that the end-plate current is carried by sodium and potassium ions. The reversal potential for the end-plate potential is therefore intermediate between the potassium electrode and the sodium electrode, being approximately −15 mV. In the case of the taenia coli the reversal potential determined for excitation was −26 mV, which is also intermediate between the sodium and potassium electrodes. It seems likely then that the excitatory current of the smooth muscle cells is also carried by both potassium and sodium ions.

If the external sodium concentration is increased, then the value of the sodium electrode will be increased. Since the excitatory junctional currents probably depend on the value of the sodium electrode, they will increase with an increase in external sodium concentration. This effect probably explains why Bulbring and Kuriyama[11] observed a potentiation of the depolarizing action of ACh when the external sodium concentration was increased.

If the external potassium concentration is increased, then the amplitude of the potassium electrode will decrease and so will the membrane potential. As the reversal potential for excitation probably depends on both potassium and sodium electrodes, high potassium solutions will enable the reversal potential more nearly to approach 0 V. Thus the action of high potassium will be to depolarize the membrane potential and shift E_e nearer to 0 V. There will be no excitation when $E_e = E_K = E_m$. This explains why Bulbring and Kuriyama[11] were unable to obtain any depolarizations of the membrane potential with ACh when the membrane had been depolarized to about −14 mV with high external potassium solutions. In this case $E_e = E_K = E_m = -14$ mV, which is smaller than the normal value of $E_e = -26$ mV.

This model has successfully predicted a near linear relation between the resting potential and the membrane potential changes caused by excitatory and inhibitory transmitter substances. It has also shown that the equilibrium potential for inhibition is close to the potassium electrode. The reversal potential for excitation has been shown to be close to that for the end-plate potential. The validity of the model remains partly in question, however, until the predictions of the membrane potential

changes during simultaneous application of both inhibitory and excitatory transmitters are verified experimentally.

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TRANSPORT OF ALKALI IONS IN A MODEL SYSTEM DRIVEN BY WATER FLOW

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IT has been suggested that water movement in living systems might be an immediate driving force leading to unequal distribution of non-reactive substances with different mobilities[1-11]. Starting from this hypothesis, the problem of the distribution of alkali ions has been examined with the aid of relatively simple models, in which selective distribution of ions has been brought about[7-11]. Attempts to apply such models to biological problems indicate, however, that further investigations are required of certain characteristic properties of systems of this type.

In this work the rate of attainment of the stationary state for the distribution of ions as a function of water flow has been examined both experimentally and theoretically. The sodium/potassium selectivity coefficient values have been determined at high water velocities in order to ascertain whether the values in this range are in agreement with the earlier theoretical results[10]. Furthermore, values reported in the literature as having been obtained in a series of experiments with living cells have been compared with the theory.

Rate of attainment of the stationary state. The model system under consideration (Fig. 1) consists of two compartments, α and β, connected by a porous membrane. A constant flow of water is supplied to the smaller compartment α, which passes through the membrane to compartment β. At the moment $t = 0$, sodium chloride is added to the larger compartment β, which initially contained pure water. The concentration of the electrolyte in β, c^β, is kept constant during the experiment. The electrolyte diffuses through the membrane from compartment β to compartment α. The concentration of the electrolyte in the membrane is denoted by $c = c(x,t)$; thus $c(0,t) = c^\alpha$. The diffusion obeys the equations

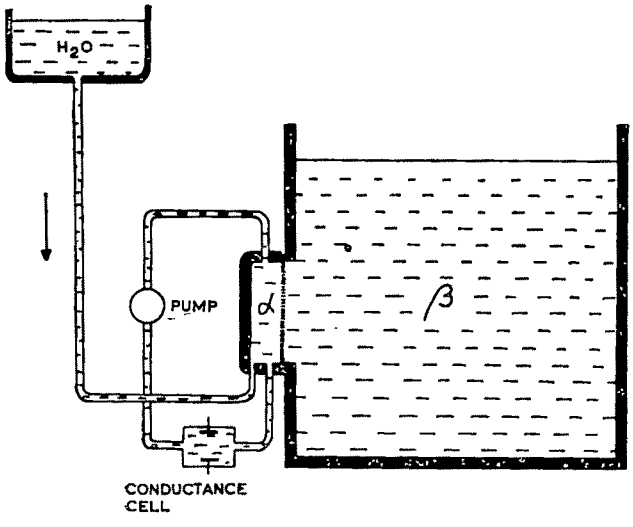


Fig. 1. The model system.

$$c(v_1 - v) = -D\frac{\partial c}{\partial x} \tag{1}$$

and

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - v\frac{\partial c}{\partial x} \tag{2}$$

where v_1 is the velocity of the electrolyte, and v (constant) the velocity of water. D is the diffusion coefficient of the electrolyte, which is assumed to be a constant. The problem is to solve c^α as a function of time t from equations (1) and (2) with the initial and boundary conditions given.

Using the transformation

$$c = c^* \cdot \exp\left(\frac{v}{2D}(x - l) - \frac{v^2 t}{4D}\right) \tag{3}$$

equation (2) can be expressed in the form

$$\frac{\partial c^*}{\partial t} = D\frac{\partial^2 c^*}{\partial x^2} \tag{4}$$

The following initial and boundary conditions are valid in the system

$$\begin{aligned} c(x,0) &= 0 && 0 < x < l \\ c(l,t) &= c^\beta = \text{constant} && t > 0 \\ \frac{\partial c(0,t)}{\partial t} - \frac{DA}{V}\frac{\partial c(0,t)}{\partial x} &= -\frac{vA}{V}c(0,t) && t > 0 \end{aligned} \tag{5}$$

where A is the effective area of the membrane, and V the volume of compartment α. Equation (5) is obtained by substituting equation (1) into equation (6), which gives the time dependence of the concentration in compartment α

$$\frac{\partial c(0,t)}{\partial t} = \frac{dc^\alpha}{dt} = -cv_1\frac{A}{V} \tag{6}$$

The initial and boundary conditions corresponding to transformation (3) are respectively

$$c^*(x,0) = 0 \qquad 0<x<l$$
$$c^*(l,t) = c^\beta . \exp(v^2t/4D) \qquad t>0 \qquad (7)$$
$$\frac{\partial c^*(0,t)}{\partial t} - \frac{DA}{V}\frac{\partial c^*(0,t)}{x} = c^*\left(\frac{v^2}{4D} - \tfrac{1}{2}\frac{vA}{V}\right) \qquad t>0$$

The solution for $x = 0$:

$$c^\alpha(t) = c(0,t)$$
$$= c^\beta . \exp\left(-\frac{vl}{D}\right)\left[1 - \exp\left(-\frac{Avt}{V}\Big/\left(1 - \exp\left(-\frac{vl}{D}\right)\right)\right)\right] + \sum_{n=1}^{\infty} F_n(v,t,D,.....) \qquad (8)$$

where we have returned to the original variables, and where the initial and boundary conditions are satisfied by equation (7), was obtained by using the Laplace transformation. The last term can be neglected if the "free" volume of the membrane is small compared with the volume of compartment α, or $Al \ll V$, and we can write

$$c^\alpha(t) = c^\beta . \exp\left(-\frac{vl}{D}\right)\left[1 - \exp\left(-\frac{Avt}{V}\Big/\left(1 - \exp\left(-\frac{vl}{D}\right)\right)\right)\right] \qquad (9)$$

Equation (9) can also be obtained directly by assuming that a quasi-stationary state continually exists in the membrane. If $vl \gg D$, the exponent in parentheses in equation (9) takes the form $-\frac{Avt}{V}$ where Avt gives the volume of water that has flowed through the membrane. It is noticed from equation (9) that the decisive factor determining the rate at which the stationary state is attained is the ratio between the volume of water flowing through the membrane and the volume V of compartment α, the half-time being $t_{\frac{1}{2}} = \ln 2.\frac{V}{Av}$. When the volume of water flowing through the membrane exceeds the volume of compartment α by three to five times, the stationary state is virtually (95–99 per cent) attained.

The rate at which the stationary state could be reached was also studied experimentally at 25° C in the system depicted in Fig. 1. The electrolyte used was sodium chloride. The changes in the concentration of sodium chloride in compartment α were followed by conductance measurements. The porous membrane used in these and following experiments was '*MF* 30' (Membranfiltergesellschaft, Göttingen). Equation (9) was verified within the limits of experimental error. The results of one typical series of experiments are represented graphically in Fig. 2.

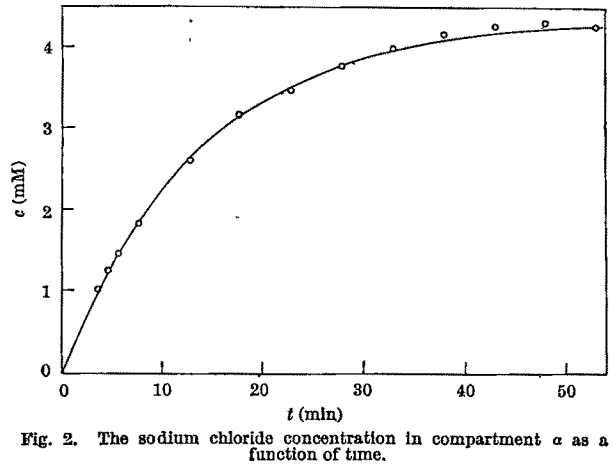


Fig. 2. The sodium chloride concentration in compartment α as a function of time.

The coefficients Av/V and vl/D can be obtained from equation (9) by substituting the experimental results. If the values of V, l, D and c^β are known, the values of v and A can be obtained. The values of l, A and v in the experiments were as follows: $l \sim 10^{-2}$ cm, $A \sim 2$–3 cm^2, and $v \sim 10^{-3} - 10^{-2}$ cm/s.

If the system contains several electrolytes, its exact mathematical treatment becomes complicated. In an approximate treatment the mutual effect of the electrolytes on their diffusion coefficients can be neglected. In this case every electrolyte obeys equation (9) independently, and the characteristic quantity that determines the rate at which the stationary state is reached, is still the ratio between the volume of water flowing through the membrane and the volume of compartment α.

Distribution of sodium and potassium ions in the stationary state. For the stationary-state distribution of sodium and potassium ions the following equation has been derived[12,3,10]:

$$\ln r_{Na,K} = \ln\left(\frac{c^\beta_{Na}}{c^\beta_K}\Big/\frac{c^\alpha_{Na}}{c^\alpha_K}\right) = 2\left(\frac{1}{D_{NaCl}} - \frac{1}{D_{KCl}}\right)vl \qquad (10)$$

where D_{NaCl} and D_{KCl} are the diffusion coefficients of sodium and potassium chlorides, and $r_{Na,K}$ is the selectivity coefficient, as defined in equation (10). This equation has been verified by the experiments of Nims and Thurber[8] with a similar system in the range $r_{Na,K} = 1$–4. The system lithium chloride–potassium chloride has been investigated by Rapoport[11] in the range $r_{Li,K} = 1$–7. In living systems, however, larger selectivity coefficients ($r_{Na,K}$ up to 2,000) are found. Thus it is of interest to examine the validity of the equation at higher values of $r_{Na,K}$. As can be seen from equation (10), if the diffusion coefficients and the thickness of the membrane, l, are constants, the selectivity coefficient $r_{Na,K}$ depends solely on the velocity of water.

Experiments were performed with the apparatus described in Fig. 1 at 25° C. The concentrations of sodium and potassium chlorides in compartment β were kept constant by adding calculated amounts of concentrated solutions. The concentrations of sodium and potassium ions were analysed with a flame photometer at the higher concentrations, and with neutron activation analysis at lower (10^{-5} moles/l) concentrations.

From equation (11):

$$D_{NaCl} \ln \frac{c^\beta_{Na}\, c^\beta_{Cl}}{c^\alpha_{Na}\, c^\alpha_{Cl}} = D_{KCl} \ln \frac{c^\beta_K\, c^\beta_{Cl}}{c^\alpha_K\, c^\alpha_{Cl}} \qquad (11)$$

derived previously[7,10] and from equation (10), it can be seen that the concentrations of sodium and potassium chlorides in compartment α decrease strongly as the water velocity v increases. This places an upper limit to the range at which $r_{Na,K}$ could be experimentally studied in the present system. When the value of $r_{Na,K}$ approached 100 the concentration of sodium ions became of the order of 10^{-6} moles/l., which is of the same order as the sodium impurities in the water used.

Fig. 3 shows that log $r_{Na,K}$ depends linearly on the rate of water flow, as equation (10) presupposes. The concentrations of the electrolytes should also obey equation (11). If we calculate the ratio

$$\mathrm{Log}\left(\frac{c^\beta_{Na}\, c^\beta_{Cl}}{c^\alpha_{Na}\, c^\alpha_{Cl}}\right)\Big/\mathrm{Log}\left(\frac{c^\beta_K\, c^\beta_{Cl}}{c^\alpha_K\, c^\alpha_{Cl}}\right)$$

from the experimental values (Table 1), we obtain values varying in the range 1·17–1·20. For the ratio D_{KCl}/D_{NaCl} we obtain the theoretical value 1·24, if we use the limiting values of diffusion coefficients at 25° C ($D_{KCl} = 1{\cdot}99 \times 10^{-5}$ cm^2/s, $D_{NaCl} = 1{\cdot}61 \times 10^{-5}$ cm^2/s).

These experiments demonstrate that in the system sodium chloride–potassium chloride–water it is possible, by increasing the value of the term vl, to obtain a high value for the selectivity coefficient $r_{Na,K}$. This happens,

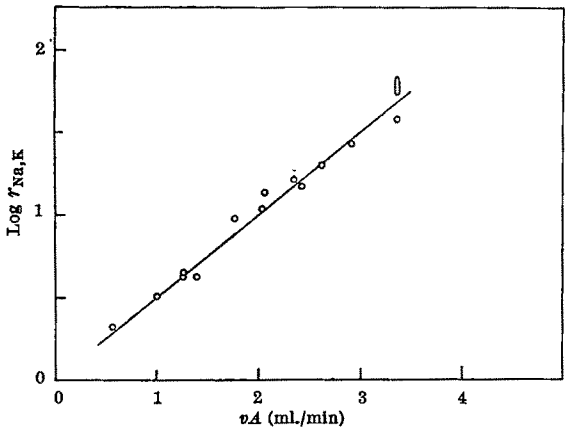


Fig. 3. The logarithm of the sodium/potassium selectivity coefficient as a function of the water flow rate.

however, at the expense of the decline of the concentrations not only of sodium ions but also of potassium ions in compartment α. Thus water flow alone cannot lead to the phenomenon characteristic of living cells, that is, accumulation of potassium ions with the concomitant extrusion of sodium ions. Equation (10) is valid even if compartment α contains ions other than sodium, potassium and chloride; however, the concentrations of sodium and potassium ions in compartment α do not now accord with equation (11)[10]. In connexion with the water flow, the hydrochloric acid in compartment α can lead to transport of sodium and potassium ions in opposite directions against their concentration gradients[9,10]. This effect may become pronounced when there are "fixed" anions in compartment α and when the concentration of hydrogen ions is low. Then the "fixed" anions determine the lowest concentration of the sodium and potassium ions in compartment α, regardless of magnitude of the water velocity and the membrane thickness, and thus the selectivity coefficient $r_{Na,K}$.

If the system under consideration contains more than two species of alkali cations, it is still possible to calculate approximately the selectivity coefficient between any two cations by inserting their mobilities into equation (10). Since, for example, the mobilities of rubidium and caesium ions are slightly higher than that of potassium, the selectivity coefficients between sodium and rubidium ions $r_{Na,Rb}$, or between sodium and caesium ions, $r_{Na,Cs}$, will become higher than the selectivity coefficient between sodium and potassium ions in the same system.

Biological applications. If compartments α and β are taken to represent intracellular and extracellular phases, respectively, and if the selectivity coefficients of alkali ions in the living systems are determined by equation (10), it is possible, with the aid of this equation and the experimental value given for $r_{Na,K}$, to calculate the expected values for $r_{Na,Rb}$ and $r_{Na,Cs}$ in living systems.

Relman *et al.*[13] have determined the distributions of sodium, potassium, rubidium and caesium ions between the intracellular and extracellular compartments of the rat. For 2 or 3 weeks, the test animals were given food

Table 1. STATIONARY STATE CONCENTRATIONS OF SODIUM AND POTASSIUM IONS AS FUNCTIONS OF WATER FLOW RATE

c^{α}_{Na}	c^{α}_{K} (mmoles/l.)	c^{β}_{Na}	c^{β}_{K}	vA (ml./min)	$r_{Na,K}$
58	4·8	1,040	42	0·56	2 1
19	2 4	1,040	42	1·00	3·2
8·6	1·6	980	40	1·26	4·5
8 8	1 5	975	40	1 26	4·2
4·9	0 83	1,040	42	1·40	4·2
1 24	0 47	1,040	42	1·76	9 5
0 38	0·18	860	37	2 04	11
0 44	0 23	880	34	2 05	14
0·14	0 087	880	34	2 34	16
0 10	0·066	860	37	2·42	15
0 04	0 032	1,020	41	2·62	20
0 02	0·022	930	39	2·92	27
0·004	0·006	900	37	3·35	37
$3–4 \times 10^{-3}$	0 008	840	32	3 35	52–70

in which half the potassium was replaced with rubidium or caesium ions, after which the alkali ion distributions were analysed. The term vl in equation (10) was calculated from the concentrations reported for sodium and potassium ions in the extracellular and intracellular compartments, and further, by insertion of the diffusion coefficients[14] for rubidium chloride, $D_{Rb} = 2{\cdot}05 \times 10^{-5}$ cm^2/s, and caesium chloride, $D_{Cs} = 2{\cdot}04 \times 10^{-5}$ cm^2/s, into equation (10), the theoretical values for $r_{Na,Rb}$ and $r_{Na,Cs}$ were calculated.

Table 2 shows that in the case of sodium, potassium and rubidium ions the calculated selectivity coefficients are in satisfactory agreement with the experimental results, while a certain discrepancy exists in the case of sodium, potassium and caesium. It is to be noted that the diffusion coefficients used in the calculations are those of dilute aqueous solutions.

Table 2. SELECTIVITY COEFFICIENTS* BETWEEN SODIUM AND OTHER ALKALI IONS CALCULATED FROM THE EXPERIMENTS OF RELMAN *et al.* (REF 13) AND A COMPARISON WITH THOSE OBTAINED FROM EQUATION (10)

c_{Na^+}	c_{K^+}	c_{Rb^+}	c_{Na^+}	c_{K^+}	c_{Rb^+}			
		Plasma (mmoles/l.)		Muscle		$r_{Na,K}$ exper.	$r_{Na,Rb}$ exper.	$r_{Na,Rb}$ theor.
141·4	1 9	0·86	4·2	75·4	90·7	1,340	3,540	3,300
140 4	1·2	0 83	3·4	63·2	100·5	2,210	5,000	5,700
		c_{Cs^+}			c_{Cs^+}		$r_{Na,Cs}$ exper.	$r_{Na,Cs}$ theor.
144·9	2·0	0·55	9·8	75 1	96 8	558	2,600	1,600

$$^{*}r_{Na,X} = \frac{c_{Na\ plasma}}{c_{X\ plasma}} \Big/ \frac{c_{Na\ muscle}}{c_{X\ muscle}} \quad (X = K^+, Rb^+, Cs^+)$$

According to equation (9), the water volume flow rate required to maintain a stationary state can be calculated from the half-exchange times for alkali ions and from the effective volume of the cell. Using a half-exchange time of 35 h for potassium in red blood cells[15], and assuming that the effective volume, V, is equal to the total cell volume, we obtain water volume flow rates of the order of 3×10^{-2} ml./ml. cells.h. These values, however, are several hundred times greater than the water volume flow rates which result from oxidative processes, as calculated from the measured oxygen consumption rates of red blood cells of 10–100 μl./ml. cells.h[16,17]. Thus the suggestion[9] that the cations could be driven by metabolic water seems to require that the effective volume of the cell should be several hundred times smaller than the total cell volume. It is unlikely that this condition would ever arise, and thus metabolic water seems to be inadequate as the sole source for the water volume flow rates required according to the theory. It is possible, however, that there is circulation of water both into and out of the cell. A metabolically induced water inflow along positively charged cation-impermeable sites might lead to a water outflow along the anionic "Na,K sites" and to a cation separation according to equation (10).

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INDUCTION OF TOLERANCE IN ADULT MICE TO ALLOGENEIC RED CELLS

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It is now well established that specific immunological unresponsiveness—"tolerance"—can be elicited in normal adult animals by treatment with the specific antigen. Although tolerance to simple chemicals or soluble proteins is more easily produced in adult animals[1–5], the prolonged acceptance of skin homografts has been accomplished in mice by means of pre-treatment with large and/or repeated inocula of viable or disrupted spleen cells[6]. Recently, Rowley and Fitch[7] have demonstrated a state of partial unresponsiveness to sheep erythrocytes in rats exposed to a small and then repeated injections of this antigen. Examination of these reports reveals several variables which could well prove critical to the initiation of the non-reactive condition. First, the administration of a small quantity of specific antigen before or near the usually immunizing inoculum facilitates the development of tolerance[3–5]. Second, in addition to the small inoculum of antigen, a second larger injection incorporated in adjuvant partially eliminates the need for repeated closely spaced treatments to establish and perpetuate the tolerant state[3–5]. Third, the route of antigenic administration has been an important factor in some investigations[1,2].

This article describes the induction of tolerance to intravenously administered red blood cells labelled with chromium-51 in *H*-2 incompatible mice when the animals have been pre-treated with multiple intraperitoneal injections of adjuvant–antigen emulsions.

C57BL/6 female mice 12–16 weeks old served as hosts and *C3H/Anf* mice of the same age as donors throughout the investigation. These mice were purchased from the Cumberland View Farms, Oak Ridge, Tennessee, and maintained on the 'Rockland Mouse Diet' and water *ad libitum*. *C3H/Anf* red blood cells suspended in saline or incorporated in incomplete Freund's adjuvant (Difco) were administered to *C57BL/6* mice according to the following procedure. After separation of the plasma, donor *C3H/Anf* erythrocytes were washed three times and adjusted to a 50 per cent suspension in buffered saline. Some groups of *C57BL/6* recipients were injected with 0·2 ml. of this suspension subcutaneously or intravenously. Other groups were inoculated intraperitoneally, intradermally, or subcutaneously with 0·2 ml. of 50 per cent suspension of *C3H/Anf* red cells emulsified in 0·2 ml. incomplete Freund's adjuvant. Control mice received equal volumes of saline incorporated in adjuvant by these same routes, or saline injections alone subcutaneously and intravenously. The injections were repeated every 2 weeks for a total of 8 weeks.

Two weeks after the last injection, all the immunized and control recipients were injected with *C3H/Anf* erythrocytes labelled with chromium-51. The methods of chromation and estimation of survival of the donor labelled erythrocytes were identical to those previously reported[8]. Each mouse received 0·15 ml. of the labelled cells intravenously and samples were obtained at intervals thereafter and analysed for radioactivity in a Packard 'Autogamma' counter. In addition to these experimental groups, the survival of *C3H/Anf* and *C57BL/6* erythrocytes was determined in syngeneic recipients. The primary response of normal *C57BL/6* mice to labelled *C3H/Anf* red blood cells was also demonstrated by this same technique

Fig. 1 illustrates the survival of *C3H/Anf* and *C57BL/6* red cells in syngeneic recipients. The time at which 50 per cent of the radioactivity remained ($T\frac{1}{2}$) for *C3H/Anf* cells by this method was about 13 days while that of the *C57BL/6* cells was 15 days. In addition, Fig. 1 demonstrates the survival of *C3H/Anf* red cells in allogeneic *C57BL/6* hosts. For 6 days there was no evidence of decreased survival, but from this point accelerated elimination of the allogeneic red cell occurred. Thus, the primary response by *C57BL/6* mice to allogeneic *C3H/Anf* red cells was indicated by a reduced $T\frac{1}{2}$ of 8·5 days and by the fact that only 13 per cent of the labelled cells remained in circulation 20 days after they had been injected.

The pre-treatment of *C57BL/6* hosts with saline or saline–adjuvant emulsions administered by the aforementioned routes did not significantly alter the primary response and as such is not separately illustrated.

Fig. 2 demonstrates that some *C57BL/6* mice pre-treated with intraperitoneal injections of *C3H/Anf* red cells emulsified in incomplete Freund's adjuvant developed almost complete tolerance to this antigen. The survival of the labelled *C3H/Anf* erythrocytes in these

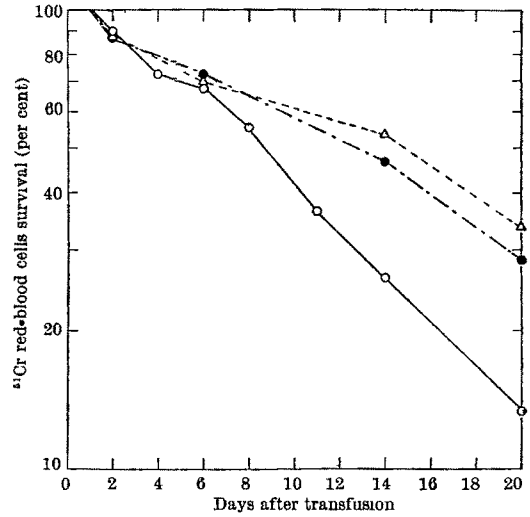


Fig. 1. The survival of *C3H/Anf* and *C57BL/6* erythrocytes labelled with chromium-51 in syngeneic recipients and of *C3H/Anf* erythrocytes in allogeneic *C57BL/6* hosts. △, *C57BL/6* red blood cells in *C57BL/6* hosts (fifteen mice), ●, *C3H* red blood cells in *C3H* hosts (eighteen mice); ○, *C3H* red blood cells in *C57BL/6* hosts (twenty-five mice).

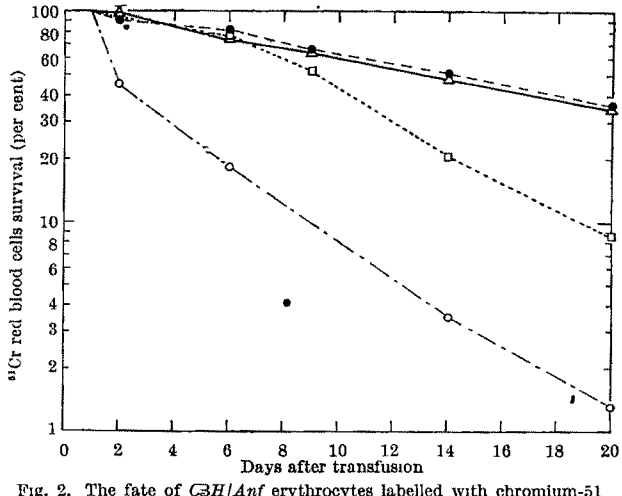


Fig. 2. The fate of *C3H/Anf* erythrocytes labelled with chromium-51 in syngeneic recipients and in allogeneic *C57BL/6* hosts pre-treated with multiple intraperitoneal or subcutaneous injections of *C3H/Anf* red blood cells incorporated in incomplete Freund's adjuvant ●, Normal *C3H* hosts (five mice); □, control *C57BL/6* hosts (ten mice), ○, *C57BL/6* hosts pre-immunized subcutaneously with *C3H* blood–incomplete adjuvant (eleven mice), △, *C57BL/6* hosts pre-immunized intraperitoneally with *C3H* blood–incomplete adjuvant (eleven mice).

allogeneic hosts was identical to that in syngeneic recipients. It should be emphasized that this represents the results in one experiment and that this non-reactive state did not occur in every mouse so treated. The induction of fully tolerant mice has varied in several experiments from 40 to 90 per cent. The remaining mice which did not develop tolerance fall into two categories. A few behaved as if this were the first exposure to the antigen, that is, the survival of the chromated red cell followed the pattern of a primary response, or they responded by accelerated immune elimination of the erythrocytes. The majority of the *C57BL/6* mice that were intradermally or subcutaneously inoculated with the antigen–adjuvant emulsion developed immunity, and no tolerant mice were produced with this procedure. The prior injection of red cells without adjuvant, either intravenously or subcutaneously, yielded a somewhat intermediate response between that of a primary and an immune reaction. Within each of these groups considerable variability occurred, but in no instance was a state of complete tolerance demonstrated.

In order to investigate further the condition of the immunized and tolerant *C57BL/6* host, serum and spleen cells were transferred to normal and sublethally (450 r.) irradiated syngeneic recipients. The methods of X-irradiation and the preparation of spleen cells have already been described[9]. Each recipient received either 0·2 ml. of Hanks's solution intravenously as the control, a suspension containing 25 million nucleated spleen cells in Hanks's, or 0·2 ml. of serum obtained from the appropriately indicated donors. Half the recipients were irradiated 48 h before the transfer of cells or serum and 24 h after the transfer all were injected with *C3H/Anf* erythrocytes labelled with chromium-51.

The mean survival in each group on the nineteenth day after transfusion is summarized in Table 1. At this time the primary response to *C3H/Anf* erythrocytes was reflected by a survival of 16·3 per cent in the untreated non-irradiated hosts. It is clear that 450 r. effectively blocked this response because *C3H/Anf* red cells survived fully as well in the irradiated *C57BL/6* hosts, 40·6 per cent, as in the control group of syngeneic recipients, 38·6 per cent. Serum obtained from normal, immune and tolerant *C57BL/6* mice had no effect on the survival of allogeneic red cells in irradiated and non-irradiated hosts.

Whereas the transfer of normal spleen cells had no additional action in the non-irradiated recipients, they were able to partially reconstitute the primary response in the irradiated hosts as was indicated by the fact that the survival of the allogeneic red cell was reduced to 24·3 per cent. Spleen cells collected from mice which had been exposed to the antigen on only one occasion, primary response spleen cells, produced a somewhat accelerated rejection, 13·1 per cent survival in the non-irradiated recipients, and 7·5 per cent in the irradiated recipients. Spleen cells obtained from immunized *C57BL/6* recipients also evoked accelerated elimination.

The observation that the cells transferred from tolerant mice behaved quite differently in the non-irradiated as compared with the irradiated hosts is of particular interest. They produced only partial reconstitution of the immune mechanism when transferred to irradiated recipients. Yet administration of these same cells to non-irradiated hosts caused an accelerated rejection—6 per cent survival of the labelled erythrocytes, which is an effect at least as potent as that obtained by the spleen cells collected from immune mice.

Thus, these results confirm the observation that the appropriate manipulation of several factors may be critical to the development of either the tolerant or hyperimmune state. The fact that neither condition could be elicited by the subcutaneous or intravenous injection of the same quantity of antigen in saline indicates that the antigen–adjuvant emulsion was one important factor. Furthermore, in preliminary investigations not reported here, the induction of immunological unresponsiveness was more easily accomplished with incomplete rather than complete Freund's adjuvant. The latter agent tended to further the production of immunity rather than tolerance. This may prove to be an important distinction because splenomegaly and reticulo-endothelial hyperplasia were greater in those mice injected with an emulsion of antigen in complete Freund's adjuvant than in those treated with emulsions lacking the mycobacteria.

The route of administration of the antigen–adjuvant emulsion appears to be another important factor. The fact that the subcutaneous and intradermal routes tend to favour the initiation of immunity rather than tolerance cannot be adequately explained at the present time. Perhaps the intraperitoneal route favours the early release into the circulation of a small quantity of antigen. If this were true, the early antigenic stimulation might act in concert with the other sustaining properties of the antigen–adjuvant emulsion to promote tolerance[3–5].

The mechanism whereby antigen, particularly in small amounts, may facilitate the development of immunological unresponsiveness has not been definitely ascertained, but the demonstrations by Uhr and Bauman[10] and Neiders, Rowley and Fitch[11] that passively transferred antibody depresses the primary response to antigen later administered may offer an explanation. It follows that the first inoculum of antigen elicits a humoral response which in turn serves to regulate the limits of the reaction to antigen later administered. Rowley and Fitch[7] also conclude that the persistence of antigen is important in the maintenance of tolerance because it may interfere with either the division or maturation of cells responsible for the formation of antibodies. They have demonstrated that repeated injections of sheep red cells given at short time intervals resulted in the stabilization of the number

Table 1. Effect of Immune and Tolerant Spleen Cells and Serum on the Survival of *C3H/Anf* Blood labelled with Chromium-51 in Normal and Sublethally Irradiated *C57BL/6* Mice

Group *		Host	Irradiation	Treatment †	19 day survival (per cent)
I		*C3H* mice	None	None	38·6
II	*A*	*C57BL/6* mice	None	None	16·3
	B	"	450 r.	None	40·6
III	*A*	"	None	Normal serum	18·4
	B	"	450 r.	" "	46·6
IV	*A*	"	None	Immune serum	18·1
	B	"	450 r.	" "	42·3
V	*A*	"	None	Tolerant serum	17·8
	B	"	450 r.	" "	41·4
VI	*A*	"	None	Normal spleen cells	15·8
	B	"	450 r.	" " "	24·3
VII	*A*	"	None	Primary response spleen cells	13·1
	B	"	450 r.	" " " "	7·5
VIII	*A*	"	None	Immune spleen cells " "	7·3
	B	"	450 r.	" " "	5·5
IX	*A*	"	None	Tolerant spleen cells	6·0
	B	"	450 r.	" " "	21·0

* Each group was composed of between four and eight mice.

† Serum recipients each received 0·2 ml. intravenously. Spleen cell recipients each received intravenously 25×10^6 nucleated cells. Primary response spleen cells refer to cells collected from mice reacting to *C3H/Anf* blood for the first time. Immune or tolerant spleen cells refer to cells collected from mice exhibiting either an immune or tolerant response to *C3H/Anf* blood.

of antibody forming cells in the host rat spleen, but as soon as the interval was lengthened a progressive increase in the number of antibody producing cells occurred. Adjuvant; by retarding and yet sustaining the release over prolonged periods, may accomplish this same purpose.

Although the survival of chromated red cells represents a useful method of detecting immunological phenomena[8,12], it does not help to ascertain whether the removal of the antigen is brought about by cellular or humoral reactions. Moller[13] has reported that the immune elimination of *H*-2 incompatible mouse erythrocytes may not occur in circumstances where both haemolytic and haemagglutinating isoantibodies are readily demonstrated *in vitro*. We can confirm that the passive transfer of serum from immunized mice was totally incapable of accelerating the elimination of labelled allogeneic red cells in normal and irradiated recipients; however, spleen cells collected from immunized mice were active in both the normal and X-irradiated syngeneic recipients. The ability of spleen cells transferred from tolerant mice to convey fully adoptive immunity on normal, but not on sublethally irradiated syngeneic recipients is of particular importance. This suggests that the inductive phase of immunization was intact in the tolerant spleen cells, but that the productive phase was limited. This observation does not agree with the findings of Dietrich and Weigle[5], Argyris[14] and Friedman[15], who indicate that the transfer of tolerant cells conveys adoptive tolerance on the recipient. Nossal and Larkin[16], however, have reported that the transfer of rat spleen and bone marrow cells made tolerant to mouse red cell antigens lost their tolerance on transfer to irradiated rat recipients. At the present time it is not possible to explain the difference.

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EFFICIENCY OF SCINTILLATION COUNTING OF TRITIUM LABELLED DNA-PROTEIN IN THE MIXED LEUCOCYTE REACTION *IN VITRO*

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IN the course of experiments on the incorporation of tritiated thymidine (thymidine methyl-^{3}H, specific activity 12·8 c./mmole, Nuclear Chicago Corporation) into deoxyribonucleic acid (DNA) in mixed leucocyte cultures[1] it was observed that the efficiency of scintillation counting varied considerably with the method of preparation of the sample. Investigations were therefore carried out to determine the reasons for this variation.

Mixed leucocyte cultures were prepared according to the method previously described[2], except that *TC* 199 (Grand Island Biological Co., Grand Island, New York) was substituted for *TC* 109 and 20 per cent plasma was used instead of 40 per cent. Three-millilitre cultures containing 1×10^6 cells ($0{\cdot}5 \times 10^6$ from each donor) were incubated for up to 7 days before adding tritiated thymidine (Th-^{3}H). In later experiments the cultures were usually collected on the sixth day, because under the experimental conditions described maximum DNA synthesis occurred at this time, and declined thereafter[1]. Radioactive samples were obtained by first incubating the cells for 6 h with Th-^{3}H (3 μc. per 3 ml. culture), then washing the cells three times in ice-cold 0·9 per cent saline before precipitation of the tritium-labelled DNA-protein by addition of excess ice-cold 5 per cent trichloroacetic acid (TCA). In some experiments precipitates were filtered on to membrane filters (Gelman 'Alpha-6-Metricel', pore size 0·45μ (about 0·3μ when wet), thickness about 70μ), and then dried (but not desiccated) overnight in room air (21° C). After this the precipitates were immersed in 0·5 ml. 0·6 N Nuclear Chicago 'Solubilizer' (NCS) and 0·3 ml. of spectroanalysed toluene and the surface precipitate was dissolved by shaking in a water bath for 4 h at 65° C. In a later group of experiments, the precipitates were not filtered, but were dissolved in 0·5 ml. of 0·6 N NCS, followed by gentle agitation for a few seconds in a stream of hot tap water before being transferred to a counting vial. Ten millilitres of scintillator fluid (100 mg 1,4-*bis*-2-(5-phenyloxazolyl)-benzene (POPOP), 4 g 2,5-diphenyloxazole (PPO), 1 l. spectroanalysed toluene) was then added to each counting vial. "Blank" vials were made up in an identical manner, but in these the DNA-protein precipitate was omitted. In some experiments DNA was extracted from TCA precipitate by heating in 5 per cent TCA for 15 min at 90° C or in 0·5 N perchloric acid for 20 min at 90° C; 100 μl. portions of the extract were then added to counting vials containing 0·5 ml. NCS and 10 ml. fluor. Radioactivity was measured in a Nuclear Chicago 720 series liquid scintillation counter at ambient temperature and counting efficiency was determined by the channels ratio method[3,4] from a standard quenching curve prepared in the laboratory using chloroform-quenched samples of known radioactivity.

To facilitate analysis, results of efficiencies for dry solubilized (dry-sol) and wet solubilized (wet-sol) precipitates were grouped separately and then arranged in classes depending on the radioactivity (d.p.m.) of the sample. Data from extracted DNA were included in the wet-sol groups. There were 285 dry-sol and 110 wet-sol samples.

It was found that the calculated counting efficiency for wet-sol precipitates was consistently higher than that of dry-sol precipitates; the differences were most marked in the low count range of 0–500 d.p.m. (Fig. 1). The distribution of counting efficiencies for wet-sol and dry-sol samples was determined and, by applying the χ^2 test for goodness of fit, it was found to differ significantly from normal in both cases ($P < 0{\cdot}001$). In each case the distribution was skewed (wet-sol $\sqrt{b_1} = 0{\cdot}999$, $P < 1$ per cent, dry-sol

Table 1. SIGNIFICANCE OF DIFFERENCES BETWEEN COUNTING EFFICIENCIES OF WET-SOL AND DRY-SOL SAMPLES IN GROUPS SELECTED FOR RADIOACTIVITY (D P.M.)

d.p.m.	(Wilcoxon 2-sample test) K^*	Probability
0– 500	−6·628	< 0·001
500–1000	−4·682	< 0 001
1000–2000	−2 327	0 02
2000–4000	−3·120	0 002
> 4000	−6·469	< 0·001

* K is the standardized statistic used in the Wilcoxon 2-sample test (ref. 6).

$\sqrt{b_1} = 0{\cdot}692$, $P < 1$ per cent). Further analysis showed that differences in efficiencies for wet-sol and dry-sol samples between classes selected on the basis of count rate were highly significant (Wilcoxon 2-sample test, Table 1).

It was found possible to count wet-sol samples and extracted DNA after short (3–4 h) stabilization periods, whereas dry-sol samples could not be counted for as long as 2 days after preparation because of prolonged spurious activity. It was at first suspected that the increased counting efficiency of wet-sol samples resulted from the presence of small quantities of acid, and samples of known radioactivity were therefore made up to contain either 5 per cent TCA, 0·5 N PCA, or hydrochloric acid in increasing volumes from 0 to 100 μl.; addition of volumes larger than 100 μl. resulted in cloudiness owing to phase separation. Controls consisted of NCS–PPO–POPOP–toluene without radioactive material. All samples were then counted at intervals for several days at ambient temperature. It was found that 50–100 μl. acid not only markedly increased the counting efficiencies of test samples but also led to rapid loss of spurious activity in controls. To determine whether acidification alone was responsible, the experiment was repeated using distilled, de-ionized water. Results were found to be the same as for acidification and are shown in Fig. 2. Small increments in efficiency (< 0·5 per cent) were noted for all samples when they were counted after several weeks. The effect of hydration was tested further by the addition of 50 μl. of water to dry-sol samples which had been counted previously; efficiency increased—notably in samples of low activity. It was found, however, that hydration of chloroform-quenched samples did not increase their counting efficiency.

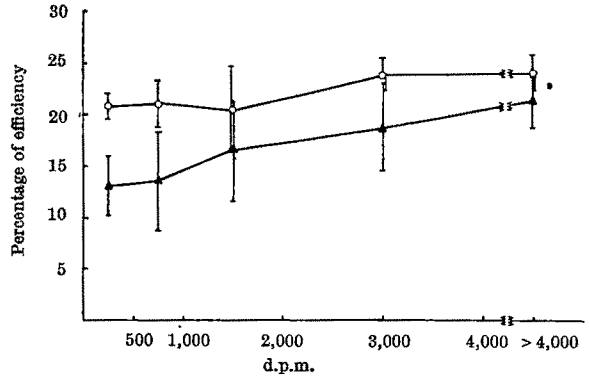


Fig. 1. Comparison of means and standard deviations of wet-sol (○) and dry-sol (▲) precipitates of increasing radioactivity. I = ± 1 *S.D.*

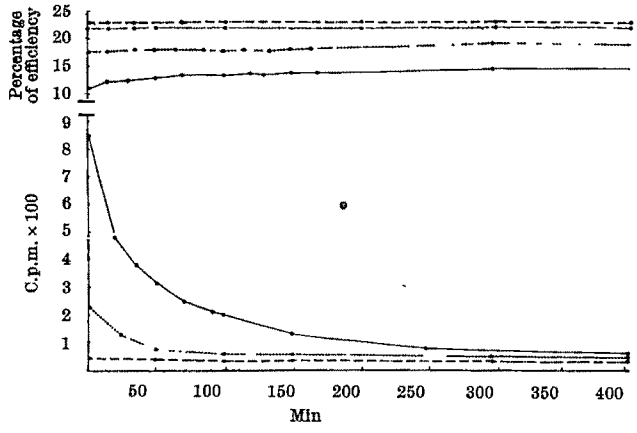


Fig. 2. Upper curves, counting efficiency of hydrated samples of known equal radioactivity. Lower curves, loss of spurious counts following hydration. —·—·—, 50 and 100 μl. water; ·····, 25 μl. water; – – – –, 10 μl. water; ————, no water.

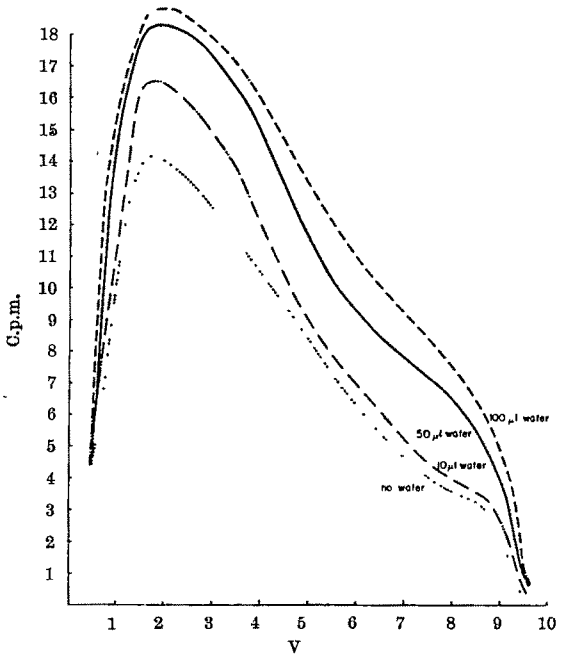


Fig. 3. Pulse height spectra of samples of equal radioactivity subjected to varying degrees of hydration. ....., No water, — — —, 10 μl. water, ————, 50 μl. water, — —·—, 100 μl. water.

In connexion with the deterioration in counting efficiency resulting from quenching, Baillie[3] has pointed out that, probably unique to tritium, small shifts in the pulse height spectrum result in large changes in counting efficiency when this is measured by the channels ratio technique. The possibility that this phenomenon was responsible for the increased efficiency observed was examined by scanning the spectra of increasingly hydrated samples containing equal amounts of tritiated thymidine but no protein or DNA, using a moving window between level 3 and level 5 (channel A and channel B). The results, which are presented in Fig. 3, indicate that hydration did not cause obvious major spectral shifts, but rather increased the total number of counts recorded, the principal increment being in the number of low-energy emissions recorded (Fig. 3, 3–10-V window). From this observation it seems likely that the increased number of low-energy emissions recorded resulted from enhanced sensitivity of the scintillator. Further, increased sensitivity was accompanied by early stabilization of the count rate. It is known that water acts as a quencher when its concentration in the scintillator is high[5], but to our knowledge a gain in efficiency resulting from the addition of small quantities of water up to (but not exceeding) the point of phase separation has not previously been recorded. Additional points of practical importance are that the count rate remains stable for long periods (more than 2 months) and does not appear to be adversely affected by long exposure to fluorescent light. Moreover, it is unnecessary to cool the samples in order to obtain normal background counts.

In conclusion, following the observation that tritium-labelled DNA protein samples could be counted at room temperature with greater efficiency if acidified, preliminary investigations suggest that addition of water alone to the NCS–PPO–POPOP–toluene scintillator system stabilizes the scintillator and enhances counting efficiency by increasing the total number of emissions recorded, the greatest relative increase being in the low energy part of the tritium spectrum. The site of action in the scintillator of small quantities of water is unknown, but is apparently different from that of chloroform and possibly other quenching agents. Small amounts (< 3 mg) of DNA labelled with tritiated thymidine have been counted within a predictable narrow range of efficiency despite wide variations in sample activity. We have so far applied this technique only to the measurement of radioactivity in the mixed leucocyte reaction and antigen stimulated cultures, but it is possible that it may find wider application in liquid scintillation counting of tritium.

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FAILURE TO DEMONSTRATE BLOCKING OF I ANTIGEN BY *Mycoplasma pneumoniae* IN VITRO AND IN VIVO

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Infection with *Mycoplasma pneumoniae* in man gives rise, in a large proportion of cases, to transiently elevated titres of cold agglutinin anti-I[1,2]. The chronic cold agglutinin syndrome, which is characterized by a prolonged elevation of anti-I level, is sometimes seen in association with malignant lymphomata[2]. Such observations have suggested a possible relationship between blood group I, mycoplasma infection and the malignant haemopoietic disorders. Furthermore, the blood of leukaemic patients has been reported to contain structures resembling mycoplasmas[3], and tissue cultures inoculated with leukaemic material have yielded mycoplasma species[4,5]; in addition a mycoplasma has been isolated directly, on agar, from a human leukaemic bone-marrow[6]. McGinniss *et al.* reported diminution of I antigen in fifteen out of forty patients with leukaemia[7] and suggested that this probably resulted from blocking or destruction of the I antigen by an infective or neoplastic agent. Further work apparently demonstrated that anti-I activity could be inhibited by treatment of normal I-positive red cells with various mycoplasmas[8]; *M. pneumoniae* was among those which produced "strong" inhibition.

M. pneumoniae is so far the only human mycoplasma of undoubted pathogenicity in man; the part played by other mycoplasmas in human disease is uncertain. We therefore chose to study the effect of this organism on the I antigen *in vitro* as well as *in vivo*.

In the investigations reported here we used red cells from a healthy group OI subject, from a healthy group Ai donor and from four group A patients with respiratory infections due to *M. pneumoniae*. The diagnosis in these latter four patients was based on the development of a four-fold or greater rise in titre of complement-fixing antibody to *M. pneumoniae* in association with a transient rise of cold agglutinin titre during the illness. We used anti-I sera from the following three persons, (*a*) a group A patient with *M. pneumoniae* infection who was bled at the time of a transient rise of her cold agglutinin titre to 128 at 4° C (titre 32 at 20° C), (*b*) a group A patient with the chronic cold agglutinin syndrome who had a cold agglutinin titre of 1,024 at 4° C (titre 256 at 20° C) and (*c*) a healthy donor of group Ai who had a cold agglutinin titre of 8 at 4° and at 20° C.

The strain of *M. pneumoniae* used by us was obtained from Dr. Sheila Stewart (Wellcome Laboratory, City Hospital, Edinburgh). In our laboratory it was maintained by weekly passage on solid media and when necessary reclaimed from cultures stored in sloppy pleuropneumonia-like organism (PPLO) agar at −30° C. Difco PPLO agar and Difco PPLO broth were used as basal culture media. To 70 ml. of each of these were added 20 ml. unheated horse serum, 10 ml. 10 per cent Oxoid yeast extract and 1 ml. 0·2 per cent calf thymus DNA (B.D.H.). Thallium acetate (final concentration 1/4000) and penicillin *G* (final concentration 50 units/ml.) were included as bacterial inhibitors. The inoculum was either 1 ml. of a 3 day broth culture or three blocks, 1 cm square, from solid cultures. The culture was incubated aerobically for 5 days at 36° C and then centrifuged at 12,000 r.p.m. at 4° C for 30 min. The deposit was resuspended in physiological saline to achieve a hundred-fold concentration and frozen and thawed twice before use. Before and after centrifugation subcultures of the material were prepared on agar medium and after incubation they were examined for typical colonial morphology, β-haemolysis of sheep red cells and for haemadsorption, and they were also tested against mycoplasma rabbit antisera using a paper disk technique to confirm the specific identity of the mycoplasma. The concentrated mycoplasma suspension contained between 10^7 and 10^8 colony forming units per ml. and it was shown to be capable of fixing complement with serum known to contain complement fixing antibody to *M. pneumoniae*. Uninoculated medium was incubated and concentrated in an exactly similar manner for use as a control suspension.

To test the effect of *M. pneumoniae* on the I antigen, one volume (measured as drops from a 0·025 ml. dropping pasteur pipette) of mycoplasma suspension was added to one volume of a 2 per cent saline suspension of washed OI cells. The mixture was incubated at 20° C for 30 min or at 4° C overnight, washed thrice in 5 times its volume of normal saline and reconstituted to a 2 per cent red cell suspension. As controls, 2 per cent suspensions of OI cells were similarly treated with a concentrate of uninoculated PPLO medium and with normal saline. Then the I antigen on the red cells was measured quantitatively by comparing the cold agglutinin titres of the three anti-I sera using control OI cells (incubated with saline or with a concentrate of uninoculated medium) with the titres obtained when OI cells were treated with *M. pneumoniae* suspension. For the titration of the anti-I sera glass precipitin tubes and 0·025 ml. dropping pasteur pipettes were used. The anti-I sera were initially diluted with normal saline so that serial two-fold dilutions of them would produce OI cell agglutination in 3–5 tubes (Table 1).

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Table 1. DEGREE OF AGGLUTINATION* WITH ANTI-I OF CONTROL OI RED CELLS AND OF OI CELLS TREATED WITH *M. pneumoniae*

Treatment of OI cells	Agglutination of red cells (reciprocal of serum dilution)				
	Anti-I from a case of *M. pneumoniae* infection				
	(8)	(16)	(32)	(64)	(128)
OI cells + saline	++++	++++	+++	+++	++
OI cells + medium	++++	++++	+++	+++	++
OI cells + *M. pneumoniae*	++++	++++	+++	++	++
	Anti-I from a chronic cold agglutinin syndrome				
	(64)	(128)	(256)	(512)	(1024)
OI cells + saline	+++	++	++	+	+
OI cells + medium	+++	+++	+++	++	+
OI cells + *M. pneumoniae*	+++	+++	++	++	±
	Anti-I from group Ai donor				
	(2)	(4)	(8)	(16)	
OI cells + saline	+++	+++	+	±	
OI cells + medium	+++	+++	++	−	
OI cells + *M. pneumoniae*	+++	++	+	−	

Agglutination tests performed at 4° C.

* ±, 2 or 3 cells sticking together; +, agglutinates of 4–12 cells; ++, agglutinates of 12–20 cells; +++, large agglutinates but some free cells remaining, ++++, complete agglutination with no free cells.

To one drop of each dilution of anti-I serum one drop of red cell suspension was added and readings for grading of agglutination were performed under the low power of a microscope after incubation at 4° C for 2 h. In order to achieve an accurate comparison between the mycoplasma-treated and control cells, these agglutination tests were performed at the same session.

Table 1 shows that treatment of OI red cells with *M. pneumoniae* suspension did not affect the activity of any of the three anti-I sera. Furthermore, overnight incubation at 4° C of normal red cells with mycoplasma suspension did not affect the titre of these sera. Because Schmidt *et al.* performed their tests of anti-I activity at 22° C, we performed additional tests at this temperature; inhibition of anti-I activity was again not observed. In addition, the *F.H.* strain of *M. pneumoniae* provided by Dr. D. Taylor-Robinson failed to inhibit the anti-I activity of the three sera.

The agglutinability (I status) of red cells from patients with *M. pneumoniae* infection was compared with that of red cells from healthy OI and Ai donors in the following manner. Anti-I serum from the patient with the chronic cold agglutinin syndrome was initially diluted 1/32 and then serial two-fold dilutions were made to a dilution of 1/1024; to one volume of each dilution of anti-I serum an equal volume of a 2 per cent suspension of test red cells was added. After 2 h of incubation at 4° C the results of the tests were read microscopically and recorded as shown in Table 2.

The anti-I serum agglutinated the normal OI cells to a titre of 512. It reacted only weakly with the Ai cells, indicating the lack of I antigen in this donor. The cells of the patients with *M. pneumoniae* infection reacted with the anti-I serum in a manner indistinguishable from the normal OI cells. In other words infection with *M. pneumoniae* did not cause blocking of the I antigen *in vivo*.

The red cells of the patients with *M. pneumoniae* infection were obtained during the third to the seventh weeks of illness. Since none of these patients showed overt evidence of haemolytic anaemia during the course of their disease it would be expected that any destruction of the I antigen by the mycoplasma during the early phase of the disease would have persisted and would have been detectable at the time of the test. We have, in fact, tested red cells obtained from eight patients during the first week of illness due to *M. pneumoniae* infection. These were stored in glycerol at −20° C and were shown to be of normal I status.

Table 2. COMPARISON OF I STATUS RED CELLS FROM PATIENTS WITH *M. pneumoniae* INFECTION WITH THAT OF CELLS FROM A HEALTHY OI AND A HEALTHY Ai DONOR, USING ANTI-I SERUM FROM A PATIENT WITH CHRONIC COLD AGGLUTININ SYNDROME

Type of red cell tested	Agglutination* (reciprocal of serum dilution) (32)	(64)	(128)	(256)	(512)	(1,024)
Normal OI	++++	++++	++++	++	++	−
Ai	+	+	−	−	−	−
M. pneumoniae infection						
Patient 1	++++	++++	++++	+++	++	±
Patient 2	++++	++++	++++	+++	+	−
Patient 3	+++	+++	+++	+++	++	−
Patient 4	+++	+++	+++	+++	+	−

All agglutination tests were performed at 4° C and at same session.

* Grading of agglutination as in Table 1.

The cause of the discrepancy between our *in vitro* results and those of Schmidt *et al.*[8] is difficult to explain. The three anti-I sera used in our experiments also failed to detect loss of I antigen in patients with acute leukaemia[9]. During the preparation of this report we noted that Ducos *et al.*, using a quantitative technique, have also failed to detect I antigen loss in leukaemic patients[10].

Apart from our *in vitro* results, we have also failed to demonstrate the blocking effect of *M. pneumoniae in vivo*. Indeed, it would be difficult to envisage a mechanism whereby mycoplasma could block the I antigen of red cells and at the same time stimulate the production of I-specific cold agglutinins.

We thank Dr. W. J. Jenkins for the cells from an Ai donor, Dr. J. Wallace for the anti-I serum from an Ai donor and Dr. R. G. Sommerville for the *M. pneumoniae* complement fixation tests. We also thank Dr. D. Taylor-Robinson for his helpful criticism.

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A THEORY OF THE NATURE OF MEMORY

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IT appears to be generally believed that no really simple theory of memory is possible, which is based on synaptic facilitation, is physiologically plausible and is in agreement with the most basic properties of conditioned reflexes[1–3]. This article describes a mechanism which, although undoubtedly speculative, does seem to possess all these requirements.

It seems desirable not to have too narrow a view of the problems connected with memory. The nervous system of an animal can learn to do new things, but it also has a very considerable stability of organization. The slow, partial restoration of reflex action after transection of the spinal cord (recovery from "spinal shock") is a good example of this[4]. Whether such a phenomenon

should be called stability or learning is an irrelevant matter of semantics, but it is clear that any theory of memory should find a natural place for it. Even in the special case of human memory for material experienced consciously, it can be argued that some sort of stability criteria are operating. People forget. There is a tendency to think of long-term memory as indelibly engraved on the brain, but the experimental evidence suggests that it decays, although whether completely or not is uncertain[5]. Even our memories from early childhood are usually, if not always, reinforced by being "inspected" from time to time. The reciprocally related processes of learning and forgetting, as of damage to the nervous system and restoration of function, show the characteristics of the components of a servomechanism. I shall deal with this theme in this article.

I have investigated the proposal that learning is based on changes of threshold or synaptic linkage following the joint firing of two neurones and have found myself naturally led to quite a comprehensive theory of the basis of memory. This theory is much influenced by the earlier physiological discussions of the problem of memory[1,6-9], especially that of Burns[2], and by those which take into account our present knowledge of the biochemical organization of cells[10-13]. I shall not discuss the idea that memory is encoded in the nucleotide sequence of RNA or DNA. However, *m*RNA may be a necessary intermediate at one point. On account of the confused experimental position, no discussion will be entered into on the reports of transfer of learning between animals[14,15]; it will be apparent, however, that the present theory is not inconsistent with the transfer of some relatively unspecific effects.

Control of Synaptic Linkage

One way in which learning could operate is as follows. If a neurone A has a synapse from its axon to a cell B, and A and B fire together, then the synapse is strengthened. Hence the next time A fires, B is more likely to do so. It was pointed out by Burns[2] that, although this particular model of learning is popular among theorists, it does not really satisfy the requirements of psychologists. I shall retain it for the time being, however, because Burns's criticism can be met. A more serious point is that most experimental evidence, especially in the peripheral nervous system, suggests that use weakens synapses rather than strengthens them[16]. Because of this, I shall consider the more general question: how can the synaptic strength be altered as a consequence of the joint firing of neurones A and B?

The first point to note is that the alteration process involves a mechanism which only works when both neurones A and B have fired, otherwise it would not be specific for the relationship of A to B. This means it cannot depend simply on the transmitter substance ejected from A. For similar reasons, a mechanism assuming a regulatory action on enzyme synthesis[17] in the nucleus of either cell A or cell B is unsatisfactory. For example, supposing, through the effect on its nucleus, the threshold of B is lowered. Then B is fired more easily by all cells having excitatory synapses with it, and not just by A. On the other hand, an effect on the nucleus of A, which might be difficult, anyway, because of the expected axoplasmic flow down the axon[18,19], would affect all the synaptic terminals, not just those connecting with B.

These considerations suggest that the change must occur directly, not indirectly, at or near the synapse. It occurs just when or after the surface membrane on the two sides of the synaptic cleft has been disturbed (depolarized) and must involve both cells. This seems to suggest strongly that something passes between cell A and cell B, that this something can only pass through the two membranes when both have been recently disturbed, and that the "something" forms the basis of learning and memory. Let us call this "something" F. F passes between A and B and then alters the synapse, either by causing it to grow or atrophy or perhaps by affecting the enzyme-controlled production of transmitter[10]. Which way does F pass? There are several reasons for thinking it more likely to be from B to A. Synapses are usually on or near the cell body of the receiving cell B, but far from the body of the transmitting cell A. Thus the chief manufacturing district of B is near the synapse, while the synaptic region of cell A is distinctly isolated from its nucleus. Next, once F gets through into the axonal tip of cell A, it will tend to stay in that tip because of the axoplasmic flow already mentioned. Were the passage in the other direction, there would be less reason for F to remain in the tip (although there could, of course, be specific sites which would absorb it irreversibly). Finally, it is believed that in the formation of the nervous system cells find their correct partners by a process of axonal growth under the stimulation of some substance emitted from another cell[20,21]. All these things suggest, although not conclusively, that F passes from cell B to cell A and, having arrived there, stimulates or depresses the growth of the axonal tip.

Peripheral Learning

Much reliable information is available about learning at the cerebral level, but it does not relate to cellular change. It is therefore better to start with the peripheral system, where the experimental evidence appears to be clear: use causes atrophy, disuse causes growth[16]. If the argument of the previous section is accepted, then this evidence implies that there is a substance E produced in the receiving cell B which, on joint firing of A and B, passes across the synaptic cleft and into the adjacent axonal tip of A. E inhibits the growth of that axonal tip. On the other hand, the axon was provoked to grow there in the first place by an exudate from cell B. Thus it becomes necessary to postulate also a growth substance, F, which passes continually across the synaptic cleft from B to A. The maintenance and growth of the axonal tip are assumed to depend partly (or even wholly) on this supply of F. We also suppose that the rate of passage of F per unit area of synaptic cleft depends only on the metabolic activity of cell B but not directly at all on the number of times A and B fire jointly.

This gives a simple servomechanism which, provided A receives its normal input, keeps the rate of firing of B fairly constant even if the average input to B from other sources should change. It is instructive to write down an equation governing it, assuming that E and F compete in a proportional and additive manner. We can then write

$$\mu\alpha + \frac{d\alpha}{dt} = \lambda\alpha - \nu\alpha \qquad (1)$$

Here α is the synaptic area, μ the maintenance requirement of the axonal tip for F per unit area per unit time, and λ, ν are the rates of flow of F and E, respectively, across unit areas of the cleft. It is quite in accord with present-day theories of control mechanisms in cells[17] to suppose that the concentration of E and F within cell B is held constant, at least unless there is excessive demand for them. Hence we take λ as constant.

ν must depend on the rate of joint firing of the two cells, however, and hence on the area β of any other synaptic linkages to cell B. It also depends on the rate of bombardment of those other linkages, which for simplicity of exposition we suppose to be constant. Then ν is proportional to p, the rate of firing of A and to $P(\alpha,\beta)$, the probability that B fires when A does. Thus

$$\nu(\alpha,\beta) = \rho p P(\alpha,\beta) \qquad (2)$$

where ρ is a constant for a given cell, but may differ from cell to cell. $P(\alpha,\beta)$ is obviously a monotonic increasing function of α, for each fixed β.

If we assume that, in the absence of the inhibitory factor E, the synaptic area α grows, we must have $a = \lambda - \mu > 0$. Thus

$$\frac{d\alpha}{dt} = \alpha\,(a - \rho p P(\alpha,\beta)) \qquad (3)$$

Provided $\rho p P(0,\beta) < a < \rho p P(\infty,\beta)$, equation (3) shows that α tends towards the stable limiting value which is derived from setting $\frac{d\alpha}{dt} = 0$ in equation (3). (See Fig. 1, in which curve I crosses the horizontal line at height a at a stationary point.) This point is stable because of the monotonic increasing character of the continuous function $P(\alpha,\beta)$. The stable value for α is therefore determined from the equation

$$P(\alpha,\beta) = \frac{a}{\rho p} \qquad (4)$$

We see that α depends both on the area β of the other synaptic contacts and also on the afferent firing rate p. However, the rate at which B fires under the influence of A is $pP(\alpha,\beta) = a\rho^{-1}$ which is constant.

These conclusions only apply if the condition on the range of a is satisfied. If p is very large, then $a < \rho p P(0,\beta)$ and α decreases to zero. In our model, then, if the afferent bombardment is too great, the synapse dwindles and the axon finally drops off. The values of the constants a and ρ may, however, be such that this cannot occur. For p can scarcely be more than, say, 1,000/sec. If p is very small, then $a > \rho p P(\infty,\beta)$ and α increases without limit.

Of course, physically, α cannot actually become infinite and would ultimately be limited by the available area of contact with cell B or by the metabolic availability of F. As the total rate of flow of F across the area α is $\lambda\alpha$, then if the availability of F has an upper bound A, say, we have $\lambda\alpha < F$ and therefore, as $\alpha \to \infty$, so $\lambda \to 0$. Thus the curve for $\lambda-\mu$ must fall off and eventually become negative for large enough values of α. This means that α can never become larger than α_0, the value of α for which $\lambda-\mu = 0$. Two curves, II and III, for small p are shown in Fig. 1. Of course, for the stable points α_{II} and α_{III}, the firing rate of B following A is $(\lambda-\mu)\rho^{-1}$ which is no longer equal to $a\rho^{-1}$, but is less.

Not only can the condition $a > \rho p P(\infty,\beta)$ be satisfied with p small, but it can also be achieved with ρ small. If ρ is zero, then the stable value for α is α_0, independently of the firing rate. Thus in a cell which does not produce the inhibitory growth factor, the total synaptic area will be determined simply by the availability of F and hence by the cell B. As ρ passes from a value which gives a curve, like curve I in Fig. 1, which crosses $\lambda-\mu$ in its horizontal region, down to $\rho = 0$, so does the servomechanism pass between two limiting types. In the first,

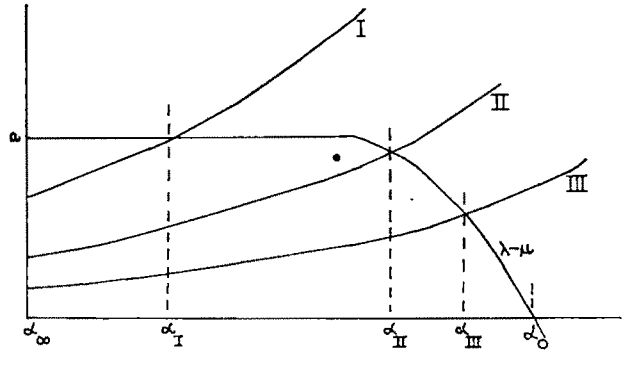


Fig. 1. Plots of $\rho p P(\alpha,\beta)$ against a for medium (I), small (II) and still smaller (III) values of ρp. The intersections with the curve of $\lambda-\mu$ give the stable values for the synaptic area, α_I, α_{II}, and α_{III}, respectively. α_∞ corresponds to $\rho p P(0,\beta) > a$ and α_0 to $\rho p = 0$.

it keeps the various conditional frequencies constant, while in the second it keeps the total synaptic area constant. For intermediate values of ρ, a mixed criterion operates. When the frequency is kept constant, the stable value of α is determined from equation (4) and is inversely related to p: as p decreases so α increases and so, therefore, does the excitability of cell B from that afferent pathway.

So far the discussion has implicitly referred to excitatory synapses. For an inhibitory synapse it is still necessary to suppose the existence of a growth substance F exuded from the cell, which first attracts the axon and then maintains it. To use equation (3) in this case would lead to difficulty, however, because at an inhibitory synapse an increase in α results in a decrease in the probability of firing for B and thus in $P(\alpha,\beta)$. Therefore, $P(\alpha,\beta)$ is a monotonically decreasing function of α and the point $P(\alpha,\beta) = a/\rho p$ is now stationary but metastable. α thus always goes towards 0 or ∞. The simplest way of getting around this is to suppose that, at such synapses, only the growth substance F passes. Thus F leaks across the synaptic cleft at a constant rate as before, but its motion is enhanced in proportion to the rate of joint firing. This idea rests on the entirely reasonable assumption that the two membranes are slightly permeable to F in the resting state, but when depolarized allow it to flow through more freely.

Then, in place of equation (3), we have

$$\frac{d\alpha}{dt} = \alpha(a + \rho p P(\alpha,\beta)) \qquad (5)$$

This has a stationary and stable point at $P(\alpha,\beta) = -a/\rho p$, which shows that we must assume a to be negative. This means that inhibitor axons are only attracted to cells which fire sufficiently often at about the same time as they do (in contrast to the excitatory axons discussed earlier). This seems quite reasonable as it implies that inhibitory interneurones only get attached to cells which "need" inhibiting, and which they can inhibit at the right time. On the other hand, it is again possible at an inhibitory synapse for the synapse to be maintained by a process which is independent of firing rate. This would occur if $\rho = 0$ in equation (5) and a is replaced with $\lambda-\mu$, which reproduces equation (1) with $\nu = 0$, as discussed earlier.

A plausible and simple theory has now been developed which goes far towards explaining both the formation and maintenance of structure and function in the peripheral nervous system. Although it does not give independent support to our theory, it is interesting to mention that the properties assumed for frequency-controlled synapses can explain the re-establishment of reflexes in the period of readjustment from the condition of "spinal shock". When one realizes that the effects of lesions in the brain often show a considerable parallel to the effect of spinal transection, the loss of specific function often being followed by at least a partial ultimate restoration of function, one is led to hypothesize that the mechanisms outlined operate at all levels in the nervous system (see, for example, ref. 22, p. 274). At the cerebral level, however, they are complicated by the presence of quite another mechanism for memory.

Before dealing with that, however, some consideration should be given to the question of what substances E and F are likely to be. It would be attractive to suppose that they are *m*RNA. If they were, however, there would need to be polyribosomes in the axonal tips in order to utilize them. Unfortunately, these have not been observed[23,24], and therefore, on these grounds alone, it seems unlikely that E and F are *m*RNA. Of course, the steady production of E and F must ultimately be under the control of *m*RNA formed in the nucleus of cell B. In this sense *m*RNA is implicated and there may well be some subsidiary control mechanism, relating to this production, which we have neglected to consider.

There is, however, another approach to the nature of F, at least. As F is a nerve growth factor, it is likely to be of the same nature as those factors which have already been found[21]. These appear to be protein and may be enzymes. In that case, E would not necessarily be protein but might either inactivate F or inactivate, or compete with the substrate of, some other quite independent enzyme in the axonal tip.

It should be borne in mind that the phenomenon of supersensitivity of deafferentiated nerve cells may duplicate some of the effects of servomechanism[25], and therefore complicate the interpretation of experimental data. It can scarcely duplicate them all, because it is a mechanism operating on cell B without distinguishing between the various attached cells A.

Cerebral Memory

The need for reinforcement to occur[26,27], the existence of Korsakoff's syndrome[28] and the effect of lesions in the hippocampal region[29], taken together, suggest strongly that, even if memory at the conscious level is mainly mediated by changes in synaptic strength at the cortex, it is nevertheless partially controlled by some quite localized subcortical centre or centres.

The control of synaptic strength now depends on three things—the firing of the cortical cells A and B and also, later, of some "reinforcing" cell C, the axon of which is apposed to the synapse in question. If it is assumed that the previous picture of synaptic control still holds, it is immediately apparent that the most natural way for the new control to be operated is by increasing the flow of F from cell B to cell A. For this to happen, there must still remain evidence, when cell C fires, that cell A and cell B have recently fired jointly. The firing of cell B depolarizes its membrane and so it is postulated that F passes into the synaptic cleft at this time. It would seem likely that this extra package of F, which now passes, is actively transported by a biochemical process requiring the transmitter, T, from cell A as a constituent or, at least, that the extra F can only pass in the presence of T. Finally, the firing of C mediates the passage of F across the second membrane into cell A, possibly by releasing another constituent necessary for an active transport process.

The picture may now seem a little complicated, but it is difficult to see how any essentially simpler process could show the right dependence on the three cells A, B and C. The part which is least clear is the precise mechanism of action of cell C. The model as we have described it would seem to imply that every synapse needs a separate axonal ending—some 10^{13} in all—a figure which is rather large, although perhaps not impossibly so. A way out of this difficulty would be if the cells C did not directly influence the synapses at all, but acted only indirectly through the glial cells. This is quite plausible in view of the fact that we do not require the cells C to carry specific messages to specific synapses, but merely the general instruction of "record". The cells C would then simply form a general glial activating system, whereupon the glia would exude the constituent which "helps" F across the axonal membrane of cell A. In that event, the exudate from C into the glial cells might be *m*RNA.

Burns's point[2] that a theory of memory must explain the classical Pavlovian conditioning experiments can now be discussed. Suppose two cells, A and A^1, both synapse on B and that the firing of A will fire B but of A^1 will not. A, then, is on the path of the unconditioned stimulus (US) and A^1 of the conditioned stimulus (CS). We may fire A^1 as many times as we like, without A, and no C-mediated learning will occur, because B does not fire. Once A and A^1 both fire, however, so does B. So the combination of the depolarization of the membrane of B, due to its firing under the influence of A, with the presence of the transmitter T from A^1, due to its firing, brings F into the synaptic cleft between B and A^1. Thus, providing reinforcement occurs through C, F passes ultimately into the axon of cell A^1.

Thus we can interpret the conditioning experiments in a general way. But note now that F can only cross the membrane of B into the synaptic cleft with A^1 if the transmitter T is already there when B fires. Thus the conditioning can only work if A^1 fires at the same time as B or earlier, providing it is not so much earlier that T has all disappeared. The time by which the firing of A precedes that of B is so small that this makes it possible to say, approximately, that A^1 must precede A for conditioning to occur. The CS must precede the US and thus it would seem that the model very satisfactorily fits the temporal requirements of the conditioning experiments. The ultimate decay of conditioning would be a consequence of the servomechanism described earlier.

Finally, note that this theory of cerebral memory implies the existence of several stages of memory storage, in general agreement with recent experimental work[30–32]. Also, as the strength of the influence of a synapse on the receiving cell depends on two quite independent things, T and the growth of the axonal tip, memory may be regarded as a sum of a short-term part and a long-term part. The time sequence of the two is very different. The short-term part is largest at first and decays monotonically. The long-term part must be zero at first, rise to a maximum and then decay. The sum of the two need not be monotonically decreasing, but might have a minimum at a time when the short-term part has largely decayed, but the long-term part has not yet become fully established. There is evidence that this does occur in the "reminiscence effect"[33], and an explanation of the general kind given previously has been advanced[5,34].

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LETTERS TO THE EDITOR

PLANETARY SCIENCE

Ice-forming Nuclei in the Atmosphere

THERE is considerable meteorological interest in the nature and concentration of ice-forming nuclei in the atmosphere and their variation with time and weather situation[1]. Apart from their significance in natural precipitation processes, improved knowledge of their nature and occurrence is essential to any realistic assessment of weather modification by cloud seeding with artificial ice nuclei.

Most measurements of the concentration of ice nuclei have until now been made with mixing chamber and expansion chamber methods and the results have suggested that the concentration is extremely variable. It is probable, however, that much of this variability can be attributed to limitations in the methods of measurement, largely because the conditions under which the ice crystals are formed are unlike those in natural clouds. In addition, these methods are difficult to use in an aircraft, with the result that apart from those at ground level very few measurements have been made[2,3].

Recently, a much simpler method using 'Millipore' filters has been developed by Bigg[4]. In this method, the sample of air is drawn through the filter which collects all the particulate material down to at least 0·4μ in diameter. To determine the number of ice nuclei which are active at a certain temperature, for example, at $-15°$ C, ice crystals are grown on the nuclei by cooling the filter to $-15°$ C in an atmosphere saturated or supersaturated with respect to water. The use of a 'Millipore' filter has many advantages; it can be used to sample large volumes of air and it can readily be used for sampling from aircraft—the development of the filters can be carried out later in the laboratory. However, Bigg *et al.*[5] found that the results with this method are not wholly satisfactory, because the measured concentration of ice nuclei per unit volume depends on the volume sampled and also critically on the development technique. Also it was found that the background count could be large. Mossop[6] found that for 10 per cent of the filters the background count was more than ten, which was comparable with the number of ice crystals found on many of the exposed filters.

After reviewing the methods available for measuring the concentration of ice nuclei, we decided to investigate further the 'Millipore' filter method. We thought that it might be possible, by means of a different development technique, to obtain better results than those of Bigg *et al.* After 3 years of experiments, our method is now giving promising results and does not appear to suffer from some of the difficulties encountered by Bigg. The main differences between the two techniques are in the means of providing the water vapour and in the method used to observe the ice crystals when they have formed. In Bigg's method, a cloud of water droplets is formed above the cooled filter and allowed to fall on to it. A suitable supercooled solution is then poured over the filter so that the newly formed ice crystals are made large enough to be seen easily. In our method, before the filter is cooled it is placed on molten petroleum jelly, which partly fills the pores, so that water vapour cannot pass through them. The filter is then exposed to air which has been saturated with respect to the ice at the bottom of the chamber and, by cooling the filter further, the air in contact with the filter can be made saturated with respect to water. The filter is kept under these conditions for 30 min. The ice crystals in the central portion of the filter (about 40 per cent of the total area) are observed with a low-powered microscope.

Our preliminary results show that the ice crystal count does not depend on the length of time that the filter is exposed to air at water saturation—the count becomes nearly constant after 15 min—whereas Bigg *et al.* found that the number of ice crystals which formed on filters similarly exposed depended critically on the humidification time. With our apparatus we are able to keep the humidity within 2 per cent of water saturation indefinitely. Only if the air above the filter is made more than 5 per cent supersaturated with respect to water is there a significant increase in the number of ice crystals formed. In contrast to Bigg's results, we find that for samples of less than 150 l. there is no decrease in the measured concentration of ice nuclei per unit volume as the volume of air sampled increases; however, for larger volumes there is a decrease. We have compared the ice crystal count of pairs of filters which had each sampled simultaneously 100 l. and found that more than half the counts were within 5 per cent of the average. This is some improvement on Bigg's experiments in which nearly half were within 10 per cent of the mean. With our method we found that unexposed filters gave a background count of nil to six, with a mean of one, in the observed area, whereas over the whole filter the background count was one to six, with a mean of three. These background counts are insignificant compared with the counts of exposed filters.

From October 1965 to March 1966 more than forty 100 l. samples of air were taken outside the Meteorological Office at Bracknell and these show that the concentration of ice nuclei varied between 0·25 and 3·0/l. with 75 per cent between 0·6 and 1·8/l. at a temperature of about $-18°$ C. The results show none of the great variability from hour to hour and day to day that has previously been reported[2], although as yet the number of samples is not large.

We are continuing our experiments at ground level and hope shortly to obtain samples at higher levels from aircraft.

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Radiocarbon Dated Postglacial Land Uplift in Northern Ungava, Canada

LATE Quaternary glacio-isostatic uplift curves based on radiocarbon dates of material from raised marine formations have been constructed for several parts of the Canadian Arctic[1–3]. So far, none has been published for areas bordering the Hudson Strait and in particular for the north-western part of the Labrador–Ungava Peninsula.

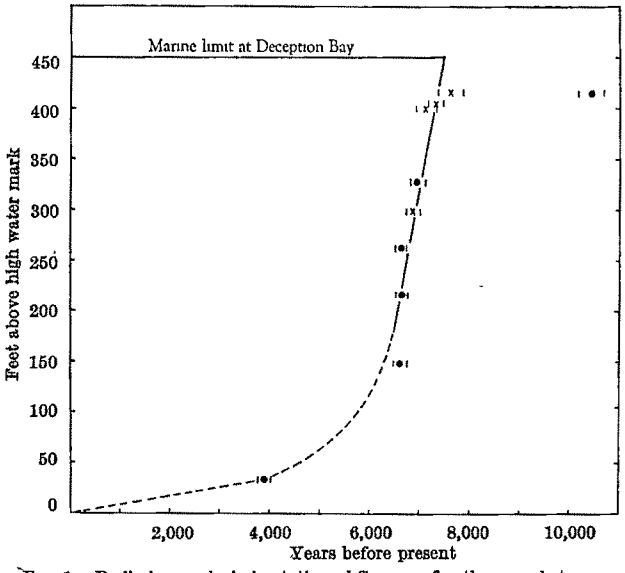


Fig. 1. Preliminary glacio-isostatic uplift curve for the area between Deception Bay–Cape Wolstenholme, Northern Ungava. |●| and |×|, Carbon-14 dates with limits of statistical errors; ●, Deception Bay; ×, other localities (see Table 1). All altitudes have been corrected for eustatic rise of sea-level before 6,000 years B.P. (that is, 3 ft./100 years).

Ten radiocarbon dates[4,5] of molluscs from raised marine terraces between Deception Bay (62° 7′ N., 74° 35′ W.) and Cape Wolstenholme (62° 35′ N., 77° 30′ W.) in Northern Ungava, Quebec, are now available. The dates provide sufficient data for construction of a provisional uplift curve (Fig. 1) in which ages are plotted against altitude.

I collected nine of the samples of dated shells during field work in 1961 and 1962. The tenth sample (*GSC*-327) was obtained by D. A. Nichols of the Department of Mines, Canada, in 1935. The molluscs were dated by Isotopes Incorporated, the National Physical Laboratory and the Geological Survey of Canada. Details of localities, shell identifications and radiocarbon ages are summarized in Table 1.

Before discussing the significance of the curve several points deserve comment:

(1) In constructing the curve it is assumed that the sites, despite the fact that Erik Cove is 96 miles west-north-west of Deception Bay, lie along rather than across the local trend of isobases. This assumption is based on my detailed studies[6–8] of the pattern of glaciation and deglaciation of the area. In support of this theory is the fact that marine shells from approximately the same altitudes (360–365 ft.), but at widely spaced locations

Table 1. RADIOCARBON-DATED SHELLS FROM NORTHERN UNGAVA

Radiocarbon age in years B.P.	Laboratory No.	Locality	Elevation above high water mark (ft.)	Species dated*
10,450 ± 250	*I*–488	Deception Bay	281	*H.a.*; *M.t.*
7,650 ± 250	*I*–729	Sugluk Inlet (62° 15′ N.; 74° 40′ W.)	365	*H.a.*
7,350 ± 150	*GSC*–327	Erik Cove (62° 32′ N.; 77° 25′ W.)	360	*M.t.*
7,160 ± 195	*I*–726	"Baie Oblongue" (62° 25′ N., 76° 20′ W.)	365	*M.t.*; *H.a.*; *M.c.*; *B.b.*
6,970 ± 150	*NPL*–85	Deception Bay	297	*H.a.*
6,900 ± 130	*NPL*–58	Erik Cove	271	*M.t.*; *M.c.*, *M.b.*; *H.a.*
6,760 ± 140	*NPL*–82	Deception Bay (8 miles inland)	148	*M.t.*; *H.a.*: *B.*
6,660 ± 130	*NPL*–84	Deception Bay	242	*M.t.*; *H.a*; *M.c.*, *C.c.*; *B.c.*, *S.g.*
6,660 ± 125	*NPL*–83	Deception Bay	195	*H.a.*; *M.t.*; *C.c.*; *M.c.*
3,900 ± 125	*NPL*–71	Deception Bay	34	*M.t.*; *M.a.*; *C.c.*; *S.g.*

* *H.a.*, *Hiatella arctica* Linné; *M.t.*, *Mya truncata* Linné; *M.a.*, *Mya arenaria* Linné; *M.c.*, *Macoma calcarea* Gmelin; *B.b.*, *Balanus balanus* Linné; *B.*, *Balanus* sp.; *B.c.*, *Balanus crenatus* (Bruguière); *M.b.*, *Macoma balthica* Linné; *C.c.*, *Clinocardium ciliatum* O. Fabricus; *S.g.*, *Serripes groenlandicus* Bruguière.

along the coast, have similar radiocarbon ages, if the statistical errors inherent in dating the samples are taken into account (compare *I*-729, *I*-726 and *GSC*-327, Table 1): this suggests that west-north-west–east-south-east tilting of the land did not occur during uplift.

(2) As with all shell dates there is uncertainty as to the depth at which the molluscs lived or have been transported and the sea level related to any date was higher than the actual level of the molluscs. Because of this, the true uplift curve must always lie above the graphed position of the molluscs. Nevertheless, in the case of samples *I*-729, *I*-726, *NPL*-84 and *NPL*-71, the error will be slight, as the molluscs were obtained from shoreline deposits rather than fiord-bottom material; they were collected from topset beds of deltaic material and were associated with intertidal species of mollusca, such as *Mytilus edulis* Linné, and in one instance (*NPL*-71) thin peat layers and brackish-water ostracods.

(3) It should be noted that the dates *I*-729, *I*-726, *NPL*-84 and *NPL*-71 were obtained for bivalved mollusc shells which were found in living positions with their periostracum and, in some cases, siphons still attached, which suggests that they had not been disturbed since death.

(4) As can be seen from Fig. 1, it was possible to draw an uplift curve near nine of the dates. The oldest date of 10,450 years B.P. cannot be fitted into the scheme and it is possible that the sample dated was a mixture of old shells moved by ice and postglacial shells.

(5) Allowance has been made in Fig. 1 for eustatic rise of sea level of 3 ft. per century before 6,000 years B.P. (ref. 9).

Like all other uplift curves drawn, the pattern of uplift with respect to time is that of a strongly exponential decrease from approximately the time of ice removal to the present day. The graph indicates that the rate of uplift was initially rapid between about 7,700 and 6,700 years ago, that is to say, of the order of about 20 ft. per century: this compares favourably with that of 19·5 ft. per century calculated by Lee[1] for the coast of Hudson Bay.

From about 6,700 years ago to the present, the rate of emergence of the land gradually decreased and for the past 4,000 years would appear to have been just less than 1 ft. per century—a rate slightly less than those of 1–3 ft. suggested for other parts of Arctic Canada[1,3,10]. If, however, a marine transgression took place during the formation of the lowest marine terraces, as indicated by the sequence of thin peat layers and fossiliferous marine sediments in the "*Gemma* beaches"[8], then the rate of uplift would have been much higher and probably between 1·5 ft. and 3 ft. per century—a rate similar to that postulated for the historic rate of uplift of the Hudson Bay area[11,12].

Although the general form of this provisional curve is very similar to those constructed for other parts of Canada and Greenland[1,2,13], it is somewhat displaced in time; deglaciation took place at a later period than in most other areas but earlier than in the Foxe Basin area[3]. It appears that, even if sections of the south coast of Hudson Strait were open to the sea and, therefore, deglaciated in part by 10,450 years ago, as suggested by date *I*-488, the general deglaciation of Northern Ungava was delayed until 8,000 years ago in coastal areas and possibly as late as 6,700 years ago in inland areas, as suggested by date (*NPL*-82) of shells from the highest fossiliferous marine deposit 8 miles inland from Deception Bay. Thus local ice caps, or at least large valley glaciers, seem to have existed in Northern Ungava during the equivalent of the early part of the hypsithermal interval, preventing the incursion of the sea; a theory in keeping with that recently proposed for central and eastern Arctic Canada[14].

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Trace Element Concentrations in the Sea off South Africa

A PRELIMINARY investigation of the distribution of the trace metals copper, iron and manganese, with special emphasis on copper, was recently undertaken in the ocean area south-west of the Cape of Good Hope. Oceanographic information collected concurrently with the samples was used in a provisional interpretation of the results.

Samples of sea-water were taken with a polypropylene sampler and stored frozen (−18° C) in polythene until analysed. Copper and iron were concentrated from the unfiltered samples by solvent extraction; manganese was concentrated by co-precipitation. The concentrated metals were determined spectrophotometrically. The chemical procedures will be described elsewhere[1].

Copper was estimated in seventy samples of surface water taken from an area up to 600 miles south and west of Cape Town (Fig. 1). Both coastal and deep-sea areas were sampled. The results indicate a marked relation between the copper concentrations and the water masses present (determined by analysis of temperature/salinity diagrams).

Copper concentration is at a minimum (2 μg/l.) in the Agulhas current and reaches a maximum (17 μg/l.) in vigorously upwelling water off the Cape of Good Hope. Off-shore water away from upwelling zones, in the south-east Atlantic Ocean, shows moderate concentrations (9–12 μg/l.) of copper as does also the sub-antarctic surface water just south of the sub-tropical convergence. Intrusion of Agulhas current water into the Atlantic Ocean could, in general, be followed by observing the change in copper content.

The low surface concentration of copper observed in the Agulhas current is possibly explained by its tropical origin. In equatorial regions, nutrients and trace metals are taken up by zoo- and phyto-plankton, which later die and sink. The strongly developed thermocline in tropical regions inhibits vertical mixing, and a net loss of trace metals from the surface layer is to be expected. Similarly, Morita[2] found low copper concentrations (0·5–0·7 μg/l.) in the tropical water of the Kuroshio current off Japan.

An apparent seasonal variation of copper content in surface waters off the Cape of Good Hope may be linked with upwelling processes. The maximum concentrations of copper (15–17 μg/l.) coincided with the season of maximum upwelling (summer) while, during the winter season of minimum upwelling, minimum concentrations were found (6–11 μg/l.). Because river discharge is negligible in this region, and indeed the season of maximum run-off is in the winter, it appears that the copper content must be controlled almost entirely by movements of water masses associated with upwelling.

In the mixing region south of Cape Agulhas, some Agulhas current water moves round to the north-west and penetrates some distance up the Cape west coast before its identity is lost by mixing with water of Atlantic Ocean origin in the Benguela current region. A steady variation in copper content could be linked to the extent of penetration of this current.

One set of samples was obtained from sub-surface depths in the upwelling region south-west of the Cape of Good Hope. High concentrations (15–16 μg/l.) of copper were found to a depth of 400 m. This layer consists mainly of South Atlantic Central water welling up to the surface. At greater depths lower concentrations were found (10 and 7 μg/l. at 700 and 1,200 m respectively). It is interesting to note that the Antarctic Intermediate water mass was found to lie at about 800–1,000 m depth in this region.

The Central water has its source in the mixing region just to the north of the sub-tropical convergence and then sinks and moves northwards[3]. Copper concentrations at the source of this water were found to be moderately high (13–16 μg/l.) in good agreement with values (15–16 μg/l.) found in the upwelling water off the Cape of Good Hope.

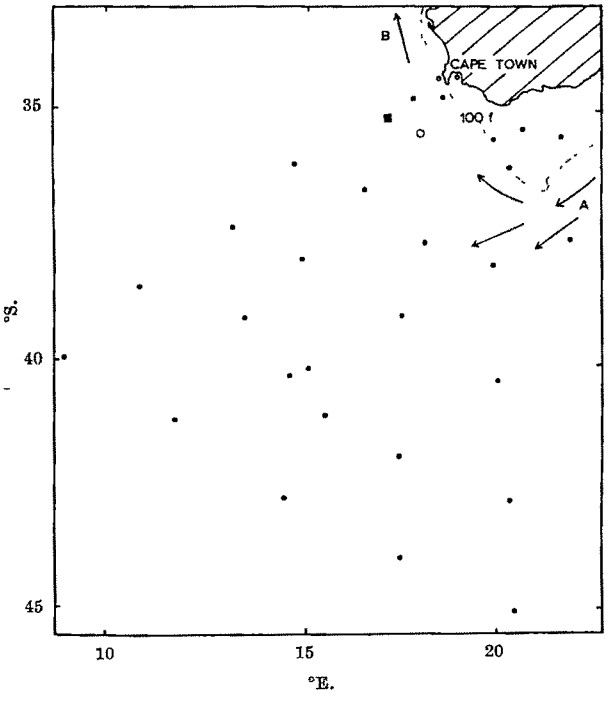


Fig. 1. Positions at which samples were obtained. *A*, Agulhas current; *B*, Benguela current. ●, Copper; ■, copper, iron; ○, copper, iron, manganese.

Ten samples of coastal water were collected for iron determination. It was concluded from the provisional results that upwelled inshore water off the Cape of Good Hope contained relatively high iron concentrations (10–13 μg/l.), whereas 60 miles offshore the iron content was much lower (1 μg/l.). Manganese was estimated in seven samples of coastal water. Little variation was noted and values ranged between 1 and 4 μg/l.

No previous data on these elements in the area studied were available for comparison, but our results are in general agreement with other data on copper, iron and manganese in the oceans[4].

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were collected aboard their research vessel *Africana II*. This support is gratefully acknowledged.

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PHYSICS

Comparison of Electron Temperature with Thermosphere Probe Data

DURING the past few years evidence has been obtained for the existence of thermal non-equilibrium in the *F*-region of the ionosphere. A number of heat sources have been proposed to account for the observed selective heating of electrons in the ionosphere. The solar ultra-violet radiation from the Sun was shown to be the main heat source for the daytime heating of the lower- and mid-latitude ionosphere[1-3]. A comparison between measurements of electron temperature T_e, obtained from the *Dumbbell* rocket probe[4,5] and the atmospheric model values of neutral gas temperature T_g, has provided the first evidence for thermal non-equilibrium of the ionosphere. The simultaneous measurements of T_e and T_g with the modified "thermosphere probe" have now confirmed that the electron temperature in the ionosphere is considerably larger than the gas temperature. With the help of recent measurements of solar ultra-violet flux and absorption cross-section, we have calculated the heat input and electron temperature in the *F*-region of the ionosphere and have compared it with the thermosphere probe data[6].

The atmospheric model, ionizing solar ultra-violet flux and their absorption cross-section for various atmospheric constituents are taken from the values tabulated by Davies[7]. The total rate of ion production and electron density distributions in the ionosphere have been calculated from these data and various coefficients of production and loss rates. (Available coefficients of production and loss rates are adopted with reasonable changes to fit experimental ion and electron density profiles.)

Adopting the heating efficiency given by Hanson, the heat input of solar ultra-violet radiation, which is the product of heating efficiency and rate of ion production, is calculated for the *F*-region of the ionosphere. Fig. 1 shows a comparison of the calculated heat input of solar ultra-violet radiation with the values measured by the thermosphere probe. It is evident from Fig. 1 that the total heat input measured is an order of magnitude greater than the calculated heat input of solar ultra-violet radiation.

If Q is the energy of the solar ultra-violet radiation in ergs cm^{-3} given to the electron gas, N cm^{-3}, then in equilibrium

$$Q = -1{\cdot}30 \times 10^{-4} N \left[\left(\frac{dT_e}{dt}\right)_O + \left(\frac{dT_e}{dt}\right)_{O_2} + \left(\frac{dT_e}{dt}\right)_{N_2} + \left(\frac{dT_e}{dt}\right)_{O^+}\right] \text{ eV cm}^{-3}\,\text{sec}^{-1} \quad (1)$$

The cooling of fast photoelectrons owing to vibrational excitation of nitrogen molecules is ignored in the determination of electron temperature. The cooling rate of equation (1) is rather slow at higher temperatures and thus this omission may be justified in view of numerous uncertainties in the cooling rates and the heat sources.

The difference between electron and gas temperature is not linearly dependent on the heat input Q. The determination of electron temperature from equation (1) involves

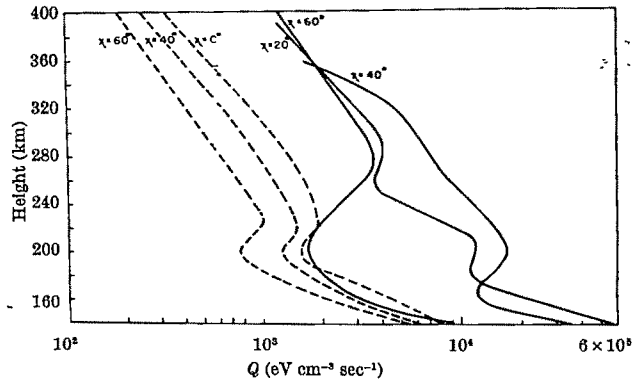


Fig. 1. Heat inputs calculated with data for solar ultra-violet radiation (– – – –) for $X=0$, 40 and 60, and with data from thermosphere probe (——) for $X=20$, 40 and 60.

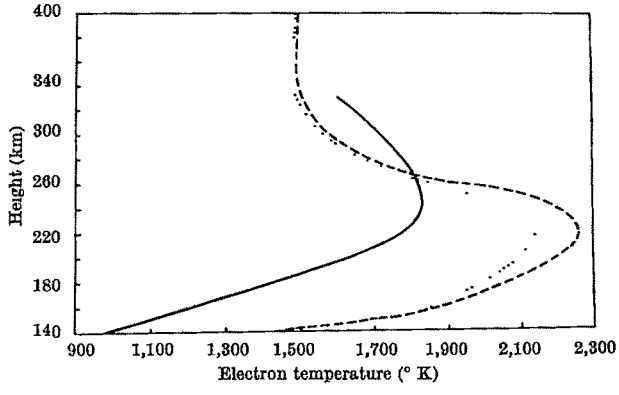


Fig. 2. Calculated electron temperature using solar ultra-violet flux data and collision frequencies for sun-spot maximum (. . . .) and minimum (– – – –), and electron temperature measured by thermosphere probe (—•—). Flight 6·07. Wallops Island, Virginia, April 1963, 1604 E.S.T.

solution of a seventh order equation in square root of T_e and is somewhat complicated. In the present communication the electron temperature corresponding to the heat input profile for solar ultra-violet (dotted curve Fig. 1) is obtained by graphical solution of equation (1). The electron temperature profiles thus obtained are compared with the data from the thermosphere probe as shown in Figs. 2 and 3.

The heat input of solar ultra-violet radiation to the ionosphere for three zenith angles is shown in Fig. 1 together with corresponding total heat input obtained from the thermosphere probe data. The calculated heat input of solar ultra-violet radiation is an order of magnitude less than the total heat input obtained from the thermosphere probe data. The electron temperature in the ionosphere resulting from heat input of solar ultra-violet is calculated using equation (1) and is compared with the electron temperature as measured by the thermosphere probe (Fig. 2). The absolute electron temperature of the solar ultra-violet depends very much on the atmospheric model used for calculation. The difference between electron and gas temperature ($T_e - T_g$) is better suited for comparative studies (Fig. 3). The electron temperature profile for solar ultra-violet shows a remarkable fit to the data obtained from the thermosphere probe. The data from the thermosphere probe gives a peak temperature at 240 km whereas calculated temperature profile gives a peak at 220 km and is about 100° K smaller than the peak temperature obtained by the thermosphere probe.

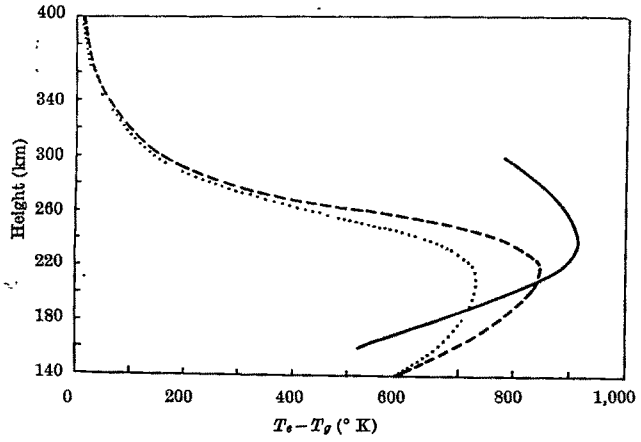


Fig. 3. Difference of electron-gas temperature calculated using solar ultra-violet flux data and collision frequencies for sun-spot maximum (. . . .) and minimum (– – – –), and values measured by thermosphere probe (——). Flight 6·07. Wallops Island, Virginia, April 1963, 1604 E.S.T.

There has been much controversy over the effect of magnetic disturbance on electron temperature. Jacchia and Slowey[8] have recently shown that during less disturbed periods ($k_p \leqslant 5$) there is a heat input which is best correlated with the k_p index. The rocket and satellite measurements of T_e (refs. 4, 5 and 9–11) have shown no increase in T_e correlated with the disturbed conditions. Maynard and Werstiuk[12] and Evans[13] have measured the electron temperature on the ground by means of radar observations of electron back-scatter and have shown that the electron and ion temperature increases at F-region heights during magnetically disturbed conditions. If our values for heat input are compared with the heat input measured on the magnetically disturbed days, the discrepancy in heat input, as already mentioned, is increased. The electron temperature profiles for disturbed days may not show a good fit as obtained here. This could be explained in terms of additional heat sources, namely, hydromagnetic[14] and corpulcular[15].

The calculated electron–gas temperature difference is found to fit well with the values measured with the thermosphere probe. The calculated heat input is, however, an order of magnitude smaller than the values measured with the thermosphere probe. The electron temperature below 200 km depends on Q/N and above this height it is controlled by Q/N^2. The higher values of electron temperature calculated may arise from an overestimate of the electron density, or from uncertainties in the electron cooling processes. It is evident from the present investigation, however, that the solar ultra-violet flux is the main source of selective heating of electrons in the ionosphere.

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Suppression of Halo on Photographic Plates used in Mass Spectrometry

The halo effect is well known in mass spectrometry and consists of a large black spot on the photographic plate in the neighbourhood of high intensity isotope lines. It causes an undesirable rise in the limit of detection of impurities with adjacent isotope masses. It was suggested[1] in 1964 that this halation could be suppressed by applying a conductive layer between the emulsion and the glass of the photographic plate. The ions reflected by the silver bromide gelatin layer have lost a great deal of their energy, for example from 20 keV down to 2–4 keV. Some of these ions are brought back into the magnetic field

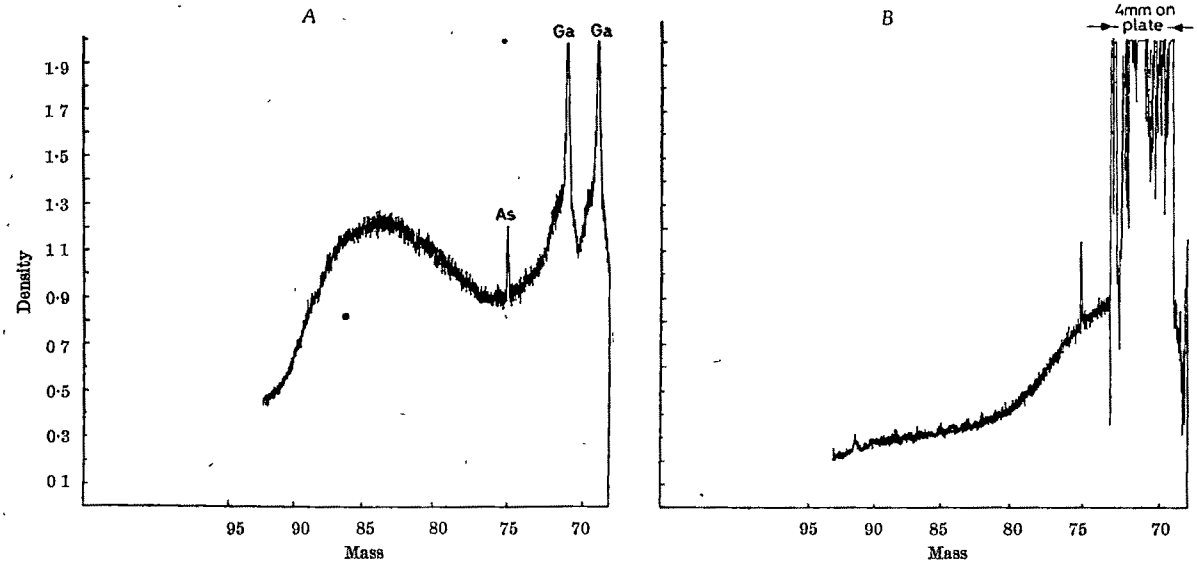


Fig. 1. Joyce Loebl recordings of part of a mass spectrogram of gallium obtained on an A.E.I.–*M.S.*7 mass spectrometer without (*A*) and with (*B*) the use of a small strip of graphite containing paint at the point of impact of the main isotope lines of gallium.

which returns them to regions of the photographic emulsion corresponding to masses higher than those of the main isotopes.

If, however, ions lose their charges more rapidly during passage through the emulsion, fewer of them are reflected. Swift[2] confirmed this view experimentally.

Mai[3] has shown that if the photographic plate is cut into parts in such a way that the ions of the main component do not strike the emulsion at all, no halo is produced.

All these precautions are fairly complicated. An easy and simple method of eliminating halation consists of painting a small conducting strip on the spot at which the main isotope line(s) will appear. This can easily be done in the dark room before the plate is placed in its hold using a rule and a small brush. This painted strip can be earthed by applying a second strip at the bottom of the plate so as to make contact with one of the pins on which the plate rests. If the strip is not earthed the effect is very poor.

The paint consists of a mixture of 300 mg graphite powder, 100 mg cellulose nitrate and polyvinyl acetate, and 180 mg ethyl acetate. After drying, the layer has a thickness of about 0·1 mm. Graphite has been chosen because of its low mass, which gives greater depth of penetration[4] and more chance of the ions losing their charge.

Figs. 1 *A* and *B* show Joyce Loebl densitometer recordings without and with the use of the strip of graphite paint mentioned above. This strip has a width of 4 mm on the plate. The mass spectrogram (obtained with an A.E.I. *M.S.*7 instrument) is of gallium with arsenic present as an impurity.

This work was carried out with the assistance of Miss H. Savenije, Mr. H. Kraay and Mr. A. Witmer.

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THE SOLID STATE

Fast Domain Wall Motion in Double Nickel–Iron Films

Recently, Humphrey and Clow[1] found that flux reversal in multilayer films[2], consisting of 200 Å thick nickel–iron films interleaved with silicon monoxide layers each about 100 Å thick, was much faster than that observed in normal single films. A possible explanation for this fast flux reversal might be that domain wall motion is much faster in multilayer than in single films. To verify this assumption, Patton and Humphrey[3] have measured wall velocities in double films. For small driving fields, the wall velocity obeys the relation

$$V = m(H - H_c)$$

in which H is the applied field, H_c the wall motion coercive force and m the so-called wall mobility. Patton and Humphrey found that—contrary to expectation—the wall mobilities in double and in single films were about the same.

This communication reports the results of wall velocity measurements in double nickel–iron films (81/19) which show that wall mobilities in double films are much larger than those measured in single nickel–iron films. The measuring technique used has already been described[4] and is similar to that used by Patton and Humphrey[3].

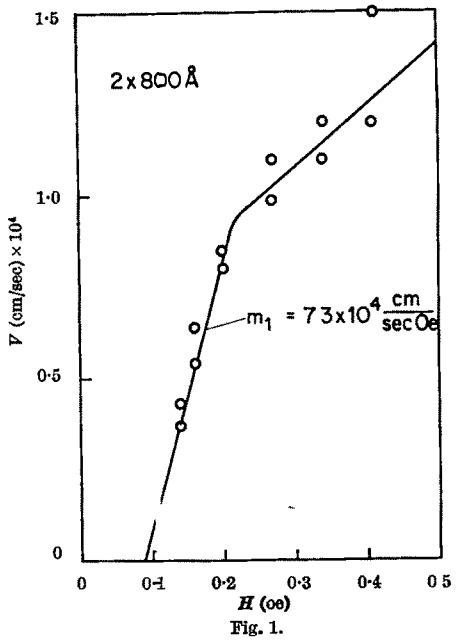


Fig. 1.

Fig. 1 shows the wall velocity measured in a double film (2 × 800 Å nickel-iron, 100 Å SiO, H_c = 0·09 oe, H_k = 5·4 oe) as a function of the applied field. The wall mobility as obtained from the straight line relationship for small driving fields is 7·3 × 10^4 cm/sec oe, which is much larger than that measured both in 800 Å and in 1600 Å single nickel–iron films. It is interesting to note that in double films the wall mobility decreases for higher fields, just as is observed in single films[4].

Table 1

Single films		Double films D_{SiO} = 100 Å	
D (Å)	m_1 (10^4 cm/sec oe)	D (Å)	m_1 (10^4 cm/sec oe)
90[1]	1·39	2 × 135[6]	9·1
260[2]	0·91	2 × 210[7]	5·4
450[3]	0·29	2 × 440[8]	4·1
880[4]	0·71	2 × 870[9]	7·3
1,650[5]	0·96		

Table 2

Film	H_c (oe)	H_k* (oe)
1	1·3	5·0
2	1·9	5·0
3	1·5	4·2
4	3·0	7·0
5	1·8	7·4
6	0·52	4·8
7	0·48	5·0
8	0·43	5·4
9	0·09	5·4

* Anisotropy field.

In Table 1, wall mobilities measured in double and in single films are compared; Table 2 gives the H_c and H_k values of the films. That walls in double films move much faster than those in single films can easily be observed, which is clearly in contradiction to the results of Patton and Humphrey.

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E–X Band System of the Copper Iodide Molecule

THE coarse spectrum of copper iodide in the visible region was investigated by Mulliken[1] in 1925 and Ritschl[2] in 1927. Ritschl recorded five extensive band systems in absorption, all degraded to red in the region 3750–5500 Å. From a study of their vibrational analysis he concluded that all systems have a common lower state which is the ground state of the molecule. This communication reports the results of the rotational analysis of the *E–X* bands of $^{63}Cu^{127}I$.

The molecule was excited in an electrodeless discharge tube by a 2,450 Mc/s 'Raytheon' microwave oscillator and the rotational structure was photographed in the second order of a 10 m concave grating spectrograph[3] (dispersion 0·33 Å/mm). The overlapping structure due to the less abundant species $^{65}Cu^{127}I$ has been avoided by adjusting the time of exposures. Exposures of 1·5 h duration on 'Kodak II-O' plates were found to be suitable for recording the structure of the main bands. The iron arc spectrum was used as the comparison spectrum. The probable error of the measurements is estimated to be 0·04 cm^{-1}.

The seven bands (4,0), (3,0), (2,0), (1,0), (1,1), (0,1) and (0,2) which were found suitable for measurement have been analysed. Each of these bands shows the existence of only two branches *P* and *R*. The *J* numbering was fixed in the usual way by comparing the combination differences and the various rotational constants were determined by the graphical procedures described by Herzberg[4]. Results are given in Tables 1 and 2.

Table 1. ROTATIONAL CONSTANTS

Band	$B_{v'}$ (cm^{-1})	$B_{v''}$ (cm^{-1})	γ_0 (cm^{-1}
4,0	0·0642	0·0732	24,882·06
3,0	0 0645	0 0732	24,660·62
2,0	0 0649	0 0732	24,436·90
1,0	0·0652	0·0732	24,211·30
1,1	0·0652	0·0730	23,948·00
0 ,1	0 0656	0·0730	23,720·50
0,2	0·0656	0 0726	23,458 37

Table 2. MOLECULAR CONSTANTS

State	B_e (cm^{-1})	D_e (cm^{-1})	α_e (cm^{-1})	r_e (Å)	$I_e \times 10^{-40}$ (g cm^2)
E	0·0660	$2·2 \times 10^{-8}$	0·0004	2·463	423·64
X	0 0735	$1·6 \times 10^{-8}$	0·0003	2·334	380·41

From the fact that a single *P* and a single *R* branch are observed, it is concluded that the bands arise from a transition of the type $^1\Sigma - {}^1\Sigma$. A recent rotational analysis of the analogous *E–X* system of copper chloride by Rao, Asundi and Brody[5] lends support to this assignment. Rotational analyses of the other band systems are in progress

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Effect of Temperature on the Triplet–Triplet Annihilation Rate in Anthracene Crystals

Jortner, Rice, Katz and Choi have recently shown that triplet–triplet annihilation leading to delayed fluorescence in crystalline anthracene can be adequately described in terms of a random walk diffusion model when the effects of charge transfer interactions are included[1]. This model is valid if the scattering of the triplet excitation wave by lattice phonons is so strong that the mean free path of the triplet exciton is of the same order of magnitude as the crystal lattice spacing. On the basis of the random walk model the triplet–triplet interaction rate constant (γ) is given by

$$\gamma = 8\pi DR \qquad (1)$$

where *D* is the triplet excitation diffusion constant and *R* is the triplet–triplet interaction distance, taken to be 10^{-7} cm for crystalline anthracene[2]. The value of γ calculated by Jortner *et al.* for anthracene is 4×10^{-11}/cm^3/sec, and is in good agreement with the experimental values[2] which range from 1×10^{-11} to 5×10^{-11}/cm^3/sec. (Moore and Munro[3] have recently reported $\gamma = 2·1 \times 10^{-11}$/cm^3/sec for a carefully purified anthracene crystal.) The value of *D* calculated using equation (1) is 30×10^{-6}/cm^2/sec which shows quite good agreement with the value of 6×10^{-6}/cm^2/sec obtained by King and Voltz[2]. (In calculating the value of *D*, Jortner *et al.*[1] assume a pure anthracene crystal; King and Voltz[2], however, analysed the slow scintillation component obtained from anthracene crystals of commercial grade. They suggest that the ionizing radiation may perturb the crystal lattice, thus giving a value of *D* which may be different from diffusion in the perfect lattice.)

The diffusion length (*L*) of a triplet exciton is given by[1]

$$L = (2D\,T_0)^{\frac{1}{2}} \qquad (2)$$

where $T_0 = 10^{-2}$ sec is the approximate value of the lifetime of the anthracene triplet[4]. This gives $L = 3·5 \times 10^{-4}$ cm which is in quite good agreement with the experimental value of $10 \pm 5 \times 10^{-4}$ cm reported by Avakian and Merrifield[5].

Jortner *et al.*[1] have also considered the effect of temperature on the triplet diffusion constant (*D*) and the bimolecular annihilation rate constant (γ) as predicted by the strong scattering model. Considering the effect of thermal expansion of anthracene crystals, Jortner *et al.* expect the diffusion constant to increase by about 20–50 per cent on cooling the crystal from 300° K to 77° K. They suggest that investigations of the temperature dependence of the triplet–triplet annihilation process will provide an important test of the applicability of their theory[1].

In the present work the triplet–triplet annihilation rate constant (γ) has been determined as a function of temperature for a pure anthracene crystal 4 mm thick. For times short compared with the triplet lifetime (T_0) the fluorescence signal (*F*) is given by[3]

$$F = \tfrac{1}{4}A\gamma\,[N_0/(1+\gamma\,N_0\,t)]^2 \qquad (3)$$

where *A* is an instrumental factor and N_0 is the initial density of triplet states. From a plot of $F^{-1/2}$ against *t* the ratio of the slope/intercept gives the quantity γN_0. For excitation times (t_x) short compared with the triplet lifetime the initial density of triplet states is

$$N_0 \doteq Q_T\,t_x\,\varepsilon\,I_0 \qquad (4)$$

Q_T is the triplet quantum yield, equal to unity minus the prompt fluorescence quantum yield[6]. This gives $Q_T = 0·35$ for thick anthracene crystals[7]. ε is the absorption coefficient of the light and I_0 is the exciting light intensity measured using a thermopile. N_0, and hence γ, can therefore be determined[3].

Moore and Munro have used this technique to determine γ for several crystalline anthracene samples using exciting radiation at 434 nm to produce volume excitation of the crystals. The average value of γ obtained from four commercial grade crystals was $1·4 \times 10^{-11}$/cm^3/sec, this value being correct to within an order of magnitude[3].

Decay curves were obtained at various temperatures for a pure anthracene crystal using 434 nm exciting radiation, and the linear plots of $F^{-\frac{1}{2}}$ against *t* obtained at each temperature are shown in Fig. 1. I_0 and t_x are

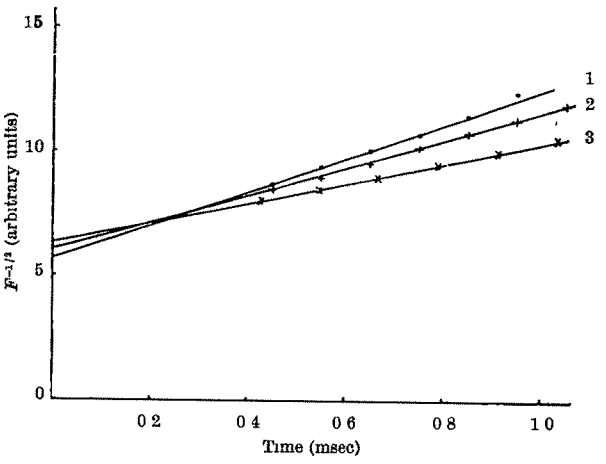


Fig. 1. Plots of $F^{-1/3}$ against t for a pure anthracene crystal at various temperatures. (1) 77° K, (2) 196° K, (3) 293° K.

independent of temperature and Q_T decreases by about 20 per cent when the temperature is reduced to 77° K. Although the variation of ε with temperature is not known it is considered probable that reducing the temperature to 77° K produces no significant change in this parameter. It was therefore assumed that N_0 did not change appreciably with temperature and, from the values of γN_0 estimated graphically, the values of γ at each temperature were calculated. The values of T_0 and γ are listed in Table 1 together with the corresponding values of D and L calculated using equations (1) and (2) respectively.

Table 1

Temperature	T_0 (sec)	γ (cm³/sec)	D (cm²/sec)	L (cm)
293° K	$4\,0\times10^{-3}$	$2\,1\times10^{-11}$	8×10^{-6}	3×10^{-4}
196° K	$5\,4\times10^{-3}$	$3{\cdot}0\times10^{-11}$	11×10^{-6}	4×10^{-4}
77° K	80×10^{-3}	$3{\cdot}7\times10^{-11}$	15×10^{-6}	15×10^{-4}

The results in Table 1 show that as the crystal temperature is reduced from 300° K to 77° K the diffusion constant (D) increases by nearly 100 per cent which shows good agreement with the increase of about 50 per cent calculated by Jortner *et al.* and therefore provides further evidence in support of the random walk diffusion model proposed to explain delayed fluorescence from anthracene and other organic crystals[1].

The rate determining process in the triplet–triplet annihilation mechanism is the formation of adjacent excited molecules. For reasons of spin conservation, however, only one-ninth of the triplet–triplet collisions produce a singlet exciton, and Singh *et al.*[8] suggest that the conclusion of Jortner *et al.* that triplet–triplet annihilation is controlled by diffusion is possibly premature. Kepler and Switerdick report that the diffusion constant calculated by Jortner *et al.* for anthracene may be several orders of magnitude too small and suggest that a band model may be more appropriate to describe triplet exciton motion in anthracene. The band model also predicts an increase in D and γ as the temperature of the crystal is reduced and so the results in Table 1 also may possibly fit into this model.

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CHEMISTRY

Oxidation of Sulphur Dioxide in Air at 950° C: Co-operative Influence of Carbon Monoxide and Nitric Oxide

The oxidation of sulphur compounds in fuel to form sulphur dioxide, and the further oxidation of a small proportion of this to sulphur trioxide, give rise to problems of atmospheric pollution and of corrosion. The severe corrosion frequently experienced in those zones of boilers where the combustion products have become relatively cool is related to the sulphur trioxide of the flue gases, and the formation of sulphur trioxide in boilers has therefore been subjected to extensive investigations. The mechanisms generally held to be responsible for the production of the greater part of the sulphur trioxide detected in boiler flue gases are: (1) oxidation of sulphur dioxide by oxygen atoms in the flame; and (2) catalytic oxidation of sulphur dioxide at surfaces in the post-flame region.

Our recent investigations have directed attention to the possibility that other mechanisms may be important at temperatures below 1,000° C. In particular, attention has been directed to the part played by oxides of nitrogen and carbon, both of which occur in flue gases from normal fuels, and their influence on the oxidation of sulphur dioxide in a clean system has been investigated. A marked effect on sulphur trioxide production was found when nitric oxide and carbon monoxide were present together. The reactions

$$2NO + O_2 \rightarrow 2NO_2 \qquad (1)$$

$$NO_2 + SO_2 \rightarrow NO + SO_3 \qquad (2)$$

might be expected to lead to the production of sulphur trioxide in suitably designed experiments. In a flow system, when a mixture containing 0·2 per cent volume/volume of sulphur dioxide, and about 0·1 per cent v/v of nitric oxide, in air, was made to react at 950° C in a tube of the purest available silica to minimize heterogeneously catalysed reactions, practically no sulphur trioxide could be detected in the quenched products. The residence time at 950° C was about 1·5 sec. Similarly, when the nitric oxide was replaced by carbon monoxide (the combustion of which would give rise to oxygen atoms) no sulphur trioxide could be detected amongst the quenched products. When nitric oxide and carbon monoxide were present together, however, significantly large amounts of sulphur trioxide were found. Typical results are given in Table 1.

Table 1

0·2 per cent v/v of sulphur dioxide in air in the presence of certain additives

Temp. (°C)	Concentration of additives (p.p.m. v/v) NO	CO	CO_2	N_2 (carrier gas for CO)	Concentration of SO_3 found in products (p.p.m. v/v)
960	930	0	0	0	0
1,000	0	1,000	0	10^5	4
975	930	500	0	10^4	14
970	930	250	0	$2{\cdot}5\times10^4$	76
960	930	1,000	0	10^5	100
950	600	500	10^5	10^4	14
945	600	1,000	10^5	10^5	88

As can be seen from the last two lines of Table 1, in comparison with earlier ones, the addition of a considerable quantity of carbon dioxide did not substantially affect the amount of sulphur trioxide detected. The determinations of sulphur trioxide in the products were all made by the barium chloranilate colorimetric method, using the continuous analyser described by Laxton and Jackson[1].

The results are tentatively interpreted as follows. Because the air was not dried, we are concerned with the water-catalysed combustion of carbon monoxide. The reactions involved give rise to oxygen atoms capable of oxidizing nitric oxide, if this is present, to nitrogen dioxide. Nitrogen dioxide, it is known, sensitizes the water-catalysed (and incidentally the dry) combustion of

carbon monoxide[2], thus probably giving rise to an increased supply of oxygen atoms. These may directly oxidize sulphur dioxide, but on the basis of published rate data for the reactions

$$SO_2 + O + M = SO_3 + M \quad (3)$$
$$NO + O + M = NO_2 + M \quad (4)$$

where M denotes a third body it seems more likely that most of the sulphur trioxide detected in the experiments reported here arises through reaction (4), followed by reaction (2); extrapolation to 950° C of the data of Boreshov and Illarionov[3] suggests that reaction (2) is very fast at this temperature[4]. Data on reaction (3) can be found in reports by Kaufman[5], Levy and Merryman[6], and Webster and Walsh[7]; those on reaction (4) can be found in Kaufman's review[8].

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Thermochemistry of Lanthanide Complexes

VALUES are now available for ΔH_1, the heat content change in the reaction

$$Ln^{3+}(aq) + aY^{n-}(aq) \rightarrow LnY^{(an-3)-}(aq) \quad (1)$$

where Ln represents a lanthanide element and Y a complexing agent such as the ethylenediaminetetraacetate (EDTA) or nitrilo-triacetate (NTA) ion. The measurements usually refer to dilute solutions of molar concentrations of ~ 0·01 or less. The dependence of ΔH_1 on the atomic number of the lanthanide is often rather complicated, a plot of one quantity against the other showing both a maximum and a minimum. This is true, for example, of the EDTA[1] and 1 : 1 NTA complexes[2,3] and the 1 : 1 and 1 : 2 diglycollate and dipicolinate complexes[4]. There are, however, grounds for believing that a complicating factor may be a change somewhere in the middle of the series in the effective hydration number of the uncomplexed lanthanide ion, consequent on the lanthanide contraction. This factor can be eliminated by measuring the integral heats of solution of a series of isomorphous lanthanide salts to give suitably dilute solutions. Such series of salts covering all the lanthanides are not numerous, but two well known ones are the bromates and the ethylsulphates, both of which crystallize as enneahydrates. If, for example, ΔH_2 is measured for the process

$$Ln(C_2H_5SO_4)_3 \cdot 9H_2O(c) \rightarrow Ln^{3+}_{\infty}(aq) + 3C_2H_5SO_4^-(aq) + 9H_2O(aq) \quad (2)$$

then $\Delta H_1 + \Delta H_2 = \Delta H_3$, where ΔH_3 is the heat content, increase for the process

$$Ln(C_2H_5SO_4)_3 \cdot 9H_2O(c) + aY^{n-}(aq) \rightarrow LnY^{(an-3)-}(aq) + 3C_2H_5SO_4^-(aq) + 9H_2O(aq) \quad (3)$$

from which the simple hydrated lanthanide ion has been eliminated.

We briefly report here the outcome of an investigation of this kind. We have measured the integral heats of solutions of lanthanide bromates and ethylsulphates in water at 25° C to give solutions of concentration ~ 0·01 M. The differential calorimeter used is capable of a precision of ~ 0·1 per cent in favourable circumstances, but the factor limiting the accuracy of our measurements is not the calorimetry, but departures of the solid hydrated salts from an exact stoichiometric composition. The bromates, which we studied first and which tend to crystallize rather rapidly from their supersaturated solutions, were found on analysis to tend towards a ratio of moles of water to gram ions of lanthanide somewhat more than nine, probably owing to the occlusion of other liquor in the crystals. The ethylsulphates appeared to crystallize rather more favourably, and all of them gave identical powder photographs. Nevertheless, while some of them analysed exactly as the enneahydrate within experimental error, others proved, like the bromates, to have a somewhat higher water content. This could again have resulted from the occlusion of mother liquor, or perhaps to the interstitial trapping in the lattice of individual water molecules, or both. In principle, a correction can be applied for occluded mother liquor by combining the analytical results for the specimen used with determinations of the solubility of the salt at 25° C and of the heat of dilution of the saturated solution. It is less easy to correct for errors due to interstitial water. We shall describe our work more fully elsewhere, but meanwhile we present in Table 1 values of the best estimates we can make at present of the heats of solution of the lanthanide ethylsulphates at 25° C. We do not believe that the main conclusions set out here are falsified by any corrections which we have had to apply.

Table 1. INTEGRAL HEATS OF SOLUTION IN CAL/MOLE OF THE LANTHANIDE ETHYLSULPHATE ENNEAHYDRATES AT 25° C

Concentration of solution ~0·01 M. 1 cal. = 4·184 abs. joules.

La	Ce	Pr	Nd	Sm	Eu	Gd
+11,260	11,620	11,870	11,790	12,490	12,350	12,130
Dy	**Ho**	**Er**	**Tm**	**Yb**	**Lu**	**Y**
10,890	10,520	10,010	9,170	8,720	8,590	9,970

When the values of ΔH_3 ($= \Delta H_1 + \Delta H_2$, ΔH_2 being the heat of solution of the ethylsulphate) are plotted against atomic number of the lanthanide, curves implying that the relation between the two quantities is still complicated are obtained for the following complexes: EDTA, 1 : 1 NTA, 1 : 1 and 1 : 2 diglycollate and dipicolinate. We think this is because these complexes are still partially hydrated and are subject to a change in co-ordination number about halfway along the series. For the EDTA complexes, crystallographic work supports this view[5]. So, although interference from a varying degree of hydration of the simple lanthanide ions has been removed, that associated with partial hydration of the complex remains. Plots of ΔH_3 (incorporating Grenthe's[4] values of ΔH_1) against atomic number for the 1 : 3 diglycollate and 1 : 3 dipicolinate complexes, however, are strikingly and significantly different, as is shown in Fig. 1. It is probable that each of these ligand anions occupies two co-ordination centres, and trials with models suggest that in these 1 : 3 complexes the co-ordination number of the metal is probably six, the shape of the complexes being about octahedral with water molecules sterically excluded from direct contact with the metal ion. Removal of the complications caused by partial hydration of the complex then reveals an essentially monotonic trend in the heat of complex formation, ΔH_3. But of particular interest is the bearing of the plots of Fig. 1 on the question of whether ligand field effects involving the $4f$ electrons contribute significantly to the stability of lanthanide complexes other than those of lanthanum, gadolinium, and lutecium. Such effects have been invoked, for example, by Yatsimirskii and Kostromina[6] to explain observed trends in the properties of lanthanide complexes, where in fact we believe any underlying simplicity is obscured by the hydration of the simple, and usually also

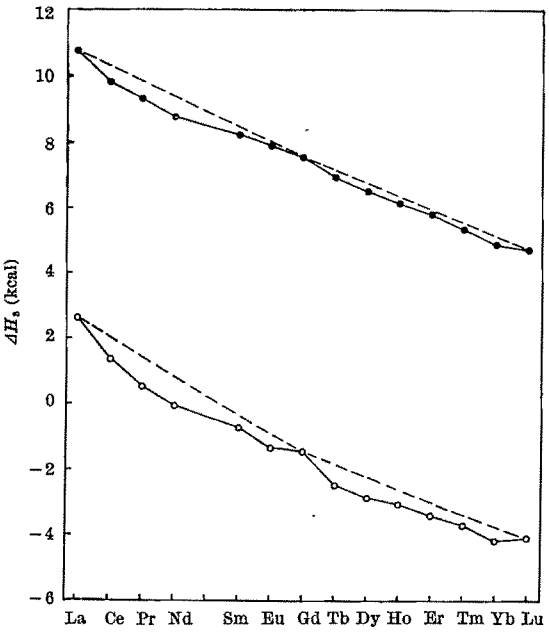


Fig. 1. Plot of ΔH_3 in cal/mole at 25° C against atomic number of the lanthanide for the 1 : 3 diglycollate and dipicolinate complexes. ΔH_3 is the heat content change for the process: $Ln(C_5H_5.SO_4)_3.9H_2O(c) + 3Y^{=}(aq) \rightarrow LnY^{3-}(aq)$. Full circles, diglycollate system; open circles, dipicolinate system.

of the complexed ions. It will be seen from Fig. 1 that the 1 : 3 complexes seem to be stabilized with reference to those of lanthanum, gadolinium and lutecium by an amount of the order of a few hundred calories, and it is significant that this effect is larger for the 1 : 3 dipicolinate than for the 1 : 3 diglycollate complexes, because the dipicolinate anion forms much the more stable complexes and presumably therefore exerts a stronger field on the metal ion. It is known that a plot of the ionic radii of the trivalent lanthanide ions against atomic number is made up of two crescents, and it might be asked how far this is therefore responsible for the shape of the curves in Fig. 1. If ΔH_3 for the two 1 : 3 complexes is plotted against ionic radius, the points, in fact, still tend to lie below a line through those for lanthanum, gadolinium, and lutecium, even though the deviations from this plot are smaller than those of Fig. 1.

The somewhat less complete results which we have obtained for the heats of solution of the bromates are consistent with these conclusions.

We therefore consider that this work shows that on eliminating the factors which normally complicate the thermochemistry of lanthanide complexes (namely, changes in the effective co-ordination numbers of both the simple and complexed ions) there is revealed a regular decrease of heats of formation with atomic number, superimposed on which there are ligand field stabilization effects which may contribute a few hundred calories to the heat of formation.

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Chemical Reactions induced in Gases by means of a Laser

The induction of chemical reactions in condensed phases by means of a laser has been reviewed by Wiley[1]. During the course of studying the heating of gases by laser light pulses, we analysed the residual gases and found in some cases a significant change in composition. These results are preliminary in nature inasmuch as the experiments were not designed primarily for this purpose.

In our experiment, a typical *Q*-switched ruby laser pulse, of wavelength 694 mμ, had an energy of 5 J and a duration of 30 nsec, corresponding to an average power of $1{\cdot}7 \times 10^8$ W. This was focused to a "point" inside the target gas by a simple plano-convex lens, 23 mm in diameter and 37 mm focal length. Measurements of the focal volume showed that in the region of maximum intensity about 50 per cent of the light intensity fell within a circle of 200μ diameter, corresponding to the maximum power density of $\approx 2{\cdot}7 \times 10^{11}$ W/cm². The cone of gas within which the power density was at least 1×10^{11} W/cm² has a volume of about $4{\cdot}2 \times 10^{-5}$ cm³. Complete absorption of the light within this region would correspond to the delivery of about 3×10^4 eV of energy to each molecule, corresponding to a temperature over 10^7 °K. From our other work with hydrogen, however, we believe that the temperatures were below 2×10^6 °K.

The laser output was monitored by reflecting a small portion of the incident beam from a plastic pellicle into a rat's nest calorimeter. The exit beam was similarly monitored for each shot including shots through the evacuated cell. The percentage of absorption was determined by comparing the exit energy from the gas-filled cell to that from the evacuated cell. These measurements were qualitatively substantiated by observations of the energy-time trace of the exit beam, illustrated by Figs. 1*a* and *b*.

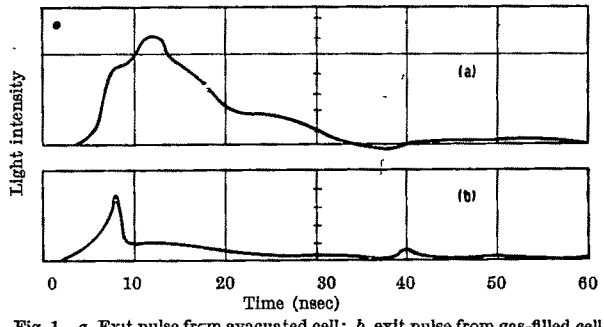


Fig. 1. *a*, Exit pulse from evacuated cell; *b*, exit pulse from gas-filled cell showing breakdown.

The gas mixtures which were irradiated are listed in Table 1. The cell volume was 4·5 cm³ for experiments (1)–(7) and 114 cm³ for experiment (8). The large percentage of light absorbed indicates that breakdown occurred in all cases. The mass spectrographic analyses of the gas samples are listed in Table 2. Some impurities were present in the starting materials and it is evident also that some air was incorporated in the course of filling, irradiating, and sampling.

With methane, the fraction decomposed was calculated from the analyses, taking into consideration the error which results from the presence of air and also the increase

Table 1. Summary of Irradiations

Experiment No.	Composition	No. of shots	Pressure (atm.)	Average percentage of light abs.
(1)	CF_4	1	1 0	50
(2)	CF_4	3	1·34	82
(3)	~10% CH_4, 90% N_2	3	1·34	78
(4)	CO_2	5	1 21	73*
(5)	~20% CO_2, 80% D_2	1	2 0	78
(6)	~73% H_2, 27% N_2	1	2·74	83
(7)	~55% O_2, 45% N_2	3	1 30	81
(8)	Solid graphite in H_2	3	1·03	100

* Seemingly increased on successive shots, that is (per cent) 10, 71, 94, 91 and 100.

Table 2. MASS SPECTROGRAPHIC ANALYSES
(Percentage by moles)

Experiment	N_2	O_2	H_2	D_2	Ar	CO	CO_2	CH_4	C_2H_6	C_2H_2	C_4H_2	C_2H_4
CH_4 (unirrad.)	0 93	0 06	—	—	—	—	—	98 93	0 08	—	—	—
No. 1	0·88	0 04	2 25	—	0·02	—	—	96 21	0 08	0 50	0·02	—
No. 2	1·28	0 13	6 03	—	—	—	—	90 83	0 10	1·49	0 06	0·08
No. 3	90 84	0 33	1·97	—	—	—	—	6·50	—	0 33	0 01	0 02
CO_2 (unirrad.)	0 50	0 12	—	—	—	—	99 38	—	—	—	—	—
No. 4	0 97	1·40	—	—	0 01	2·40	95 18	0·03	—	0·01	—	—
No. 5	0 82	0 17	—	77 74	0 04	0 99	20·20	0 04	—	—	—	—
No. 6	27·30	—	72 68	—	—	—	0 02	—	—	—	—	—
No. 7	45·27	54·57	—	—	0·16	—	—	—	—	—	—	—
H_2 (unirrad.)	1·63	0 35	97·98	—	0 02	—	0 02	—	—	—	—	—
No. 8	4·86	1·10	93 96	—	0·06	—	0 02	—	—	—	—	—

in total volume which results from decomposition of methane, and the energy consumption was found to be 11 eV/molecule decomposed for experiment (1), 14 eV/molecule for experiment (2), and 49 eV/molecule for experiment (3). The ratio of hydrogen formed to methane decomposed was slightly greater than 1·5 which applies to the reaction

$$2CH_4 \rightarrow C_2H_2 + 3H_2$$

For CO_2, in a similar fashion, the energy consumption was found to be 34 eV/molecule in experiment (4) and 11 eV/molecule in experiment (5). The oxygen yield was as expected in experiment (4), but was very low in experiment (5), probably having been converted to deuterium oxide which at these concentrations and sample size was lost by adsorption during analysis.

Search for ammonia, nitrogen oxides and hydrocarbons in experiments (6)–(8) gave negative results.

Although the temperature within the directly irradiated volume was sufficient to cause complete dissociation and ionization, the low overall energy requirements imply that the number of molecules decomposed far exceeded the number originally ionized, and that most of the molecules decomposed received their energy indirectly. The pattern of product formation is what would be expected from high temperature reactions rather than from ionizing radiation. This is clearly shown in the methane results by the small yield of ethane or other derivatives of methyl radicals and, in general, by the lack of any product molecules more complex than the starting materials. Thus there cannot be a large contribution resulting from any mechanism which involves irradiation of the cold gas with ions or hot atoms from the plasma.

Those reactions which did occur proceeded with 10–20 per cent energy efficiency. The yields, expressed in radiation chemistry terms, correspond to G values of 2–9 molecules/100 eV, a value of 9 being considered quite large for endothermic radiolysis. For a reaction such as methane decomposition, wherein there is no appreciable back reaction to form methane again, higher yields might be anticipated if quenching were by the surrounding gas exclusively and not by the walls of the vessel. The nearest wall in our cell was only 3 mm from the focal "point". Further experiments in larger vessels and at various pressures are likely to be of considerable interest. Geometrical factors might also be important in the decomposition of carbon dioxide, and yields of nitrogen–oxygen synthesis.

The present work indicates that the giant pulse laser can be conveniently used to study very high temperature chemical reactions. Studies to establish the dependence of yield on intensity and configuration of focal spot would also determine the usefulness of the method as an absolute dosimeter or calorimeter for laser shots. Such a dosimeter would be useful in laser plasma studies.

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Transition-metal Chelates of Hydrazincarboxylic Acid

THE structural and chemical properties of compounds formed by hydrazincarboxylic acid, H_2N'-NH.COOH, with transition metals are being investigated in this laboratory, and this communication presents a brief account of the present stage of the structural researches. Three sets of compounds have been prepared (hyc = H_2N'-NH.COO)

(*a*) $M(II)(N_2H_4)_2$ hyc_2, where $M(II)$ = Mn, Co, Ni, Zn;

(*b*) $(N_2H_5)[M(II)\,hyc_3].H_2O$, where $M(II)$ = Fe, Co, Ni, Zn;

(*c*) $K[M(II)\,hyc_3]$, where $M(II)$ = Fe, Co, Ni, Zn.

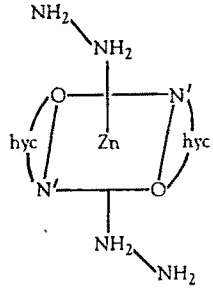
It has been found from X-ray diffraction patterns that the crystals of each set are isostructural. The crystal structure of some of them has been determined, and results indicate that the compounds are chelates of hydrazincarboxylic acid.

Compounds belonging to group (*a*) were prepared[1,2] from aqueous solutions containing a salt of the metal and hydrazine hydrate. When the solutions were left in open vessels, carbon dioxide was slowly absorbed from the air and crystals separated out. While the crystals of the zinc and the manganese compounds are colourless, those of the cobalt compound are orange-red, and those of the nickel compound blue-violet. Crystals are monoclinic and have space groups of the type $P2_1/c$. The crystal data for the zinc compound are

$a = 8{\cdot}412$ $b = 7{\cdot}384$ $c = 8\,270$ Å
$\beta = 109°\ 53'$ $D_m = 1\,936$ $D_c = 1\,926$ g.cm^{-3}

Two formula units are contained in the unit cell.

The crystal structure of the zinc compound, *bis*(hydrazine)–zinc *bis*(hydrazincarboxylato-N′,O), was determined[3] and found to consist of chelates of the type



where the hydrazine molecules, which act as monodentate ligands, and the hydrazincarboxylato anions, which act as bidentate ligands, are bound in the *trans* position. The co-ordination around the metal is of the octahedral type.

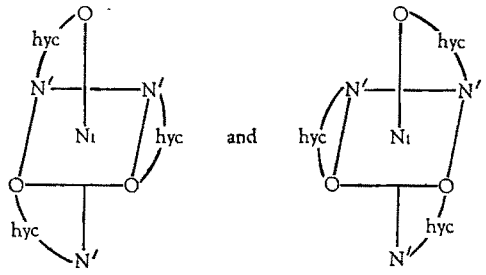
Compounds belonging to group (*b*) were prepared by bubbling carbon dioxide into a solution containing a salt of the metal and hydrazine hydrate. Crystals of the nickel compound are blue, those of the cobalt compound orange-red, those of the iron compound pale green, and

those of the zinc compound colourless. The crystals, which are piezoelectric, are monoclinic. From the systematic absences of the reflexions and from the piezoelectric properties exhibited, the compounds can be uniquely assigned to the space group *Cc*. The unit cell constants of the nickel compound are

$a = 12\,129$ $b = 10\,858$ $c = 10\,255$ Å
$\beta = 121° 06'$ $D_m = 1\,889$ $D_c = 1{\cdot}868$ g.cm^{-3}

Four formula units are contained in the unit cell.

The structure of the nickel compound was determined and found to consist of anionic chelates of the type



with cations $N_2H_5^+$ and water molecules. The co-ordination around the metal is again of the octahedral type. The hydrazincarboxylato groups are bound in the *cis*-position, that is to say, all oxygen atoms lie on the same face of the co-ordination octahedron and N′ atoms on the opposite face. The crystals are racemic, and both enantiomorphous forms coexist in the structure. The compound can thus be called hydrazinium *cis-trans*(hydrazin-carboxylato-N′,O)niccolate hydrate; the other metals form similar compounds. Before structural determinations were undertaken the formula M(II) $hyc_2.(N_2H_5)_2CO_3$ had been assigned to this class of compounds[4–6].

Compounds belonging to group (*c*) were prepared by evaporating mother liquors of type (*b*) compounds and treating them with potassium hydroxide. The colour of the crystals is the same as the corresponding salts of group *b*. The crystals, which are also piezoelectric, are trigonal; from systematic absences of reflexions and from piezoelectric properties it is possible to assign them to the space group $R\bar{3}c$. Crystal data for the zinc compound are (hexagonal cell)

$a = 10\,325$ $c = 17\,024$ Å
$D_m = 2{\cdot}123$ $D_c = 2{\cdot}088$ g.cm^{-3}

Six formula units are contained in the unit cell. The M(II) atoms lie on the trigonal axes. Chelate anions of the type $[M(II)\ hyc_3]^-$ are also present in these compounds and have trigonal symmetry; the ternary axis is normal to both the plane formed by three oxygen atoms and the plane formed by three N′ atoms. These crystals are also racemic.

Transition-metal chelates of hydrazincarboxylic acid exhibit similar properties to those of glycine[7].

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Evidence for the Pyridine.2I_2 Complex in the Gas Phase

Some recent spectroscopic studies[1] have led to the identification of charge-transfer complexes in the gas phase, for the diethyl ether–iodine and the benzene–iodine systems. This was made possible by investigations of the charge-transfer ultra-violet band, and it was deduced, from the shift of the maximum of absorption, compared with that of the liquid-state system, that there is considerable interaction between the complex and the solvent molecules. To our knowledge, no direct evidence has previously been obtained by mass spectrometry of a charge-transfer molecular complex; this communication reports some preliminary results using this technique.

We have investigated the pyridine.2I_2 crystalline complex, prepared according to the method of Prescott and Trowbridge[2]; the structure of this compound, in the solid state, has been established by Hassel and Hope[3]. Crystals were directly introduced on the high vacuum side of the introduction system of an AEI-*MS*9 double focusing mass spectrometer, and the mass spectrum was recorded of the vapour in equilibrium with the crystal at about 35° C.

The mass spectrum consists mainly of the free pyridine and free iodine peaks. Additional peaks[4], however, were observed at many masses. The most important ones are given in Table 1, and it is probable that they correspond to the formulae indicated. The contributions of pyridine are also given for comparison. All figures are for electron energies of 50 V. The contributions of the complex to masses lower than 79 are minimum values; these were obtained by deducting the contribution of pyridine, assuming that all ions at *m/e* 79 result from pyridine itself. The sum of ion abundances below *m/e* 79, ascribed to the complex, represent 15 per cent of the pyridine contribution as evaluated; obviously this cannot be ascribed to possible variation of the cracking pattern of pyridine itself. The masses below *m/e* 79 have very different relative abundances from those of pyridine—the 51 peak is the principal ion, instead of the 52 peak in pyridine. Also noticeable are the abundances of the peaks

Table 1. Mass Spectral Features of the Pyridine.2I_2 Complex
(Abundances in percentages of total ionization)

m/e	Formula	Abundance* (*a*)	Abundance* (*b*)	*m/e*	Formula	Abundance* (*a*)	Abundance* (*b*)	*m/e*	Formula	Abundance* (*a*)	Abundance* (*b*)
26	C_2H_2	0 416	0 965	39	CH_3N, C_3H_3, 78^{++}	0·235	1·086	53	C_3H_3N	0 147	1·020
27	CHN, C_2H_3	0 176	0·341					78	C_5H_4N	0 234	1 640
				49	C_4H	0·198	0 334	79	C_5H_5N	—	14·516
28	CH_2N, C_2H_4	0 278	0 308	50	C_3H_2, C_3N	0·782	1 930	254	I_2	—	43 918
36	C_3	0 113	0 018	51	C_4H_3, C_3HN	0 995	3 339	460	$Py.I_2$	0 005	
37	C_3H	0·190	0 062	52	C_4H_4, C_3H_2N	0 888	8·821	587	$Py.I_4$	0 006	
38	C_3N, C_3H_2, 76^{++}	0 103	0 316								

* (*a*) Contribution of complex; (*b*) contribution of pyridine or iodine.

at masses 26, 28 and 50. A small peak is observed at mass 128, corresponding to HI^+. Most important for our purpose, however, are the peaks observed at mass 460 ($Py.I_3$) and 587—the latter corresponds to the parent molecular ion of the complex $Py.2I_2^+$. As would be expected, its abundance is low (0·006 per cent total ionization); however, it indicates the presence of the charge-transfer complex in the gas phase. It is difficult to estimate the composition of the gas phase; however, a rough calculation, assuming as a first approximation a square root relation between molecular weights and total ionization cross-sections, would lead to about 2 per cent of complex and, assuming a pressure of 10^{-4} mm in the ionization chamber, the stability constant, K_c, could be calculated: $K_c = p_{\text{compl.}} (RT)^2/p^2_{I_2} \times p_{Py}$, which would have a value of the order of $7 \cdot 10^{14}$ mole^2 l.$^{-2}$.

Table 2. APPEARANCE POTENTIALS (A.P.) IN $Py.2I_2$ (IN eV)

m/e	Ion	A.P.	Literature (free molecules)
79	C_5H_5N	10·08	10·04 (electron impact) (ref. 7), 9·23 (photo-ionization) (ref. 6)
127	I^+	(1) 8·81	8·62 (ref. 8) (ion pair formation from I_2) 8·83 (calculated for ion pair formation from I_2)
		(2) 11·28	11·96 (calculated for $I_2 + e \rightarrow I^+ + I + 2e$)
254	I_2^+	9·37	9·35 (ref. 8)
587	$Py.2I_2^+$	8·9–9·1	No values

Appearance potentials have been determined using Warren's method and the vanishing current method of interpreting the data[5]. The results are summarized in Table 2 (mean values are given).

It can be seen from Table 2 that, owing to the high proportion of free pyridine and iodine at equilibrium, the appearance potentials for the $C_5H_5M^+$ and I_2^+ ions are practically the ionization potentials of these molecules, as determined by electron impact. For the same reason, the two distinct appearance potentials measured for I^+ certainly are related to the dissociation processes from a free iodine molecule, the lowest appearance potential corresponding to the ion pair process, the highest appearance potential corresponding to the normal dissociative process, without formation of a negative ion.

As can be seen, a value for the ionization potential of the $Py.2I_2$ molecular complex itself is obtained. It is somewhat lower than the ionization potentials of both iodine and pyridine, but the difference is not important if one takes into account the difficulty of such a measurement for a very low intensity ion. Because the electron impact value for pyridine is considerably higher than the photon impact value, our results, obtained by electron impact, suggest that in the complex the ionization might refer to an iodine $\pi_g 5p$ orbital, as in the iodine molecule itself[8], rather than to the π orbital of the pyridine ring.

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Radiothermoluminescence of 4,4′-Dimethylbenzophenone

THE formation of radiation-induced colour centres in inorganic crystals is a well-known phenomenon[1,2]; however, such colour centres have been previously reported for only a limited number of simple organic compounds[3]. Colour formation in irradiated organic crystals probably results either from the unpairing of electron spins to give free radicals or triplet states or by the transfer of an electron into a lattice defect[4]. The latter would be the more probable explanation for stable colour formation. On heating the irradiated organic crystal to the melting point, emission of light and disappearance of colour would be expected as the electrons become released from lattice defects or free radicals combine.

We have now found that when solid 4,4′-dimethylbenzophenone (melting point 91° C) is irradiated with 2 MeV electrons from a Van de Graaff accelerator, the compound acquires a light pink to a deep red colour depending on both the dose and the particle size of the compound. At 2·0 Mrads the first visible coloration is obtained and the intensity of the colour increases progressively up to 20 Mrads. On melting, the colour of the irradiated 4,4′-dimethylbenzophenone was found to disappear with the emission of light. The amount of light emitted—that is, the "radiothermoluminescence"—was calculated as a function of a number of variables.

The effect of radiation dose on 4,4′-dimethylbenzophenone was tested as follows: 1·00 ± 0·01 g of the compound were placed in a 40 mm diameter aluminium pill box with a cover and was melted at 105° C. It was then allowed to crystallize slowly to give large crystals. Samples were then irradiated with 2 MeV at doses of 5–20 Mrads. To avoid overheating the samples, the dose was delivered in 1·0 Mrad pulses at 3 min intervals. Six hours after irradiation, the samples were placed on a thermostatically controlled hot plate in a dark box. The light emitted was measured by a phototube and the results were printed out directly[5]. The area proportional to the light emission was determined by a planimeter with the chart scaled so that 1 cm in width was equal to 24 sec of recorded time and 1 cm in height was equal to 0·003 μamp from the phototube. A plot of the results for radiothermoluminescence area against the dose of radiation used (Fig. 1) reveals a linear relationship for samples irradiated and stored in the dark.

To determine the effect of storage time between irradiation and the radiothermoluminescence measurements, individual samples were prepared and then irradiated as before. Fig. 2 shows the results of radiothermoluminescence after various periods of time. Substantial decreases were noted during the first few hours of storage after irradiation and a less noticeable decrease after 5 h. The same pattern of radiothermoluminescence decrease was found for both large and small crystals.

To test the effect of storage conditions—in the light as opposed to in the dark—radiothermoluminescence measurements were made both on small crystal samples both irradiated in the dark and stored in the dark and on similar samples irradiated in the dark but stored in the light. As shown in Fig. 3, the radiothermoluminescence decays more rapidly in the light than in the dark. In subsequent experiments, it was also shown that the red colour induced in 4,4′-dimethylbenzophenone by electron irradiation is bleached by light.

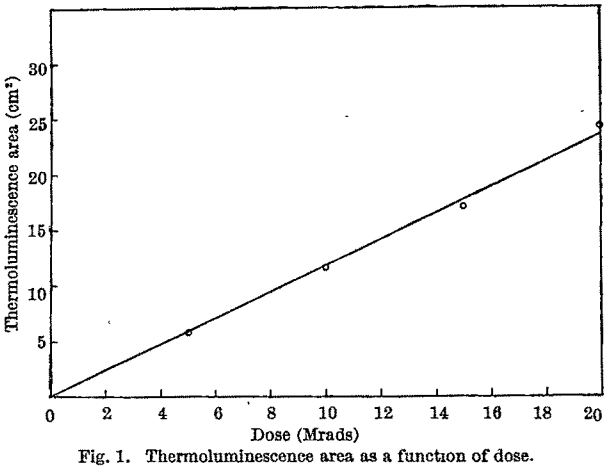


Fig. 1. Thermoluminescence area as a function of dose.

The luminescence after irradiation observed in these experiments could have been caused either by radiothermoluminescence or by oxyluminescence. (The latter phenomenon is the continuing luminescence which arises when an irradiated sample is continuously heated in oxygen[6,7].)

To eliminate the possibility that oxyluminescence was responsible for the luminescence obtained on heating an irradiated sample of 4,4′-dimethylbenzophenone, we melted the irradiated sample, allowed it to solidify, and then observed whether any additional luminescence resulted when it was further heated. The absence of any

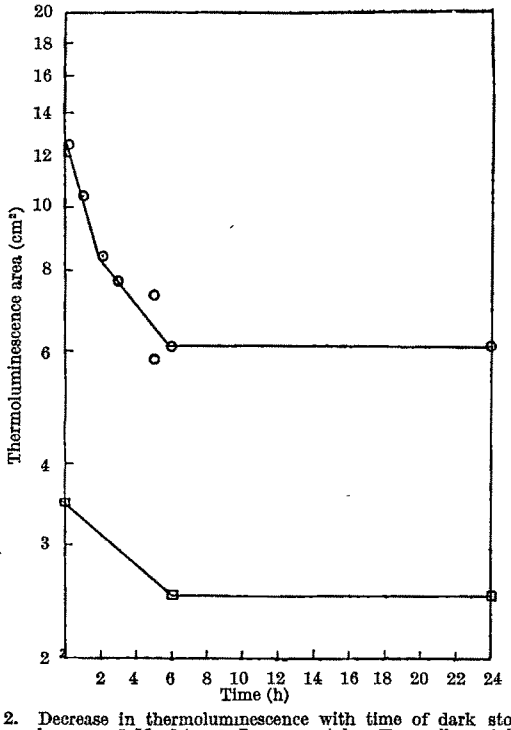


Fig. 2. Decrease in thermoluminescence with time of dark storage (samples given 5 Mrads). ○, Large crystals; □, small crystals.

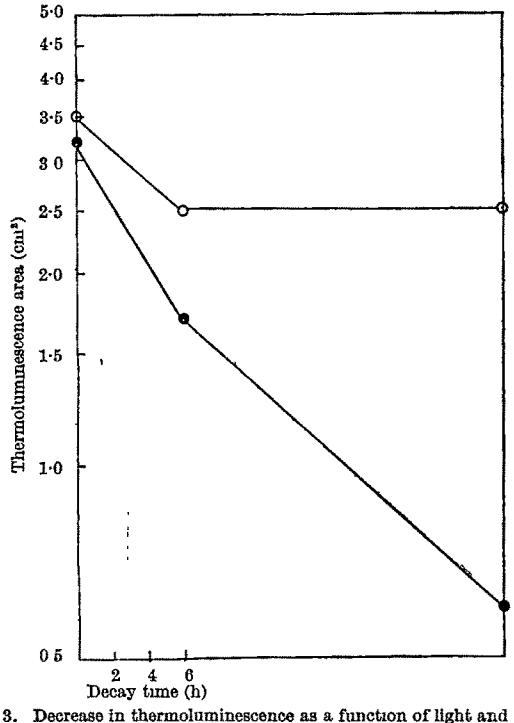


Fig. 3. Decrease in thermoluminescence as a function of light and dark decay time for small crystals. ○, Dark; ●, light.

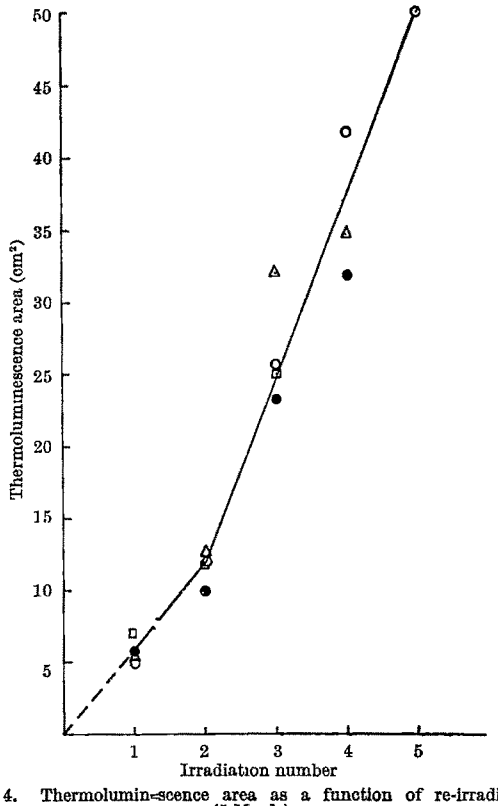


Fig. 4. Thermoluminescence area as a function of re-irradiation (5 Mrads).

such luminescence indicated that oxyluminescence played no part in the present findings.

Although 4,4′-dimethylbenzophenone could be recoloured by taking it through twenty cycles of irradiation, melting, resolidification and re-irradiation, the radiothermoluminescence did not remain constant with each re-irradiation. Instead, as can be seen from Fig. 4, measurement of the radiothermoluminescence for a series of cycles indicated that the response to the same irradiation dose increased as a function of the number of exposures to radiation.

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Determination of the Sodium bound by Poly-acids

The fact is now widely accepted[1–3] that some part of the sodium in animal tissues occurs not in the form of free sodium ion but, rather, in an osmotically inactive form. The structures on which the binding occurs, as well as the amount of the sodium so bound, remain to be determined. A number of substances are capable of binding the sodium ion and some of them, such as the acid mucopolysaccharides and nucleic acids, belong to the group of polyacids—polymers with at least one acidic group in the monomer unit. Sodium as well as other positively charged ions are attracted into the neighbourhood of the fixed

negatively charged acidic groups by the strong electrostatic fields present, and their apparent activity coefficient is thus considerably lowered in comparison with that in corresponding solutions of sodium salts of simple mineral acids. This type of binding is called "site-binding"[4].

The binding of the sodium ion can be estimated by comparing the value of the apparent activity of this ion in the poly-acid solution with that in a standard solution of sodium chloride containing the same concentration of sodium. The apparent activity of the sodium can be determined by means of a glass electrode sensitive to sodium[5]. While there are no special difficulties in using this electrode in solutions of simple electrolytes, there are possible sources of interference with measurements in solutions of macromolecules. The unknown value of the liquid junction potential, which may differ significantly from that in standard solutions of sodium chloride, must be considered one of the most important. In order to prove that the low value of the apparent activity of the sodium ion in solutions of the sodium salts of the poly-acids results entirely from site-binding, an attempt has been made to demonstrate the binding indirectly.

Because the site-binding is purely electrostatic, it might be expected that a polyvalent cation having a much higher charge density would replace that of sodium bound in the neighbourhood of the charged groups of the poly-acid. As all the bound sodium could thus be liberated, it would be possible to infer the amount of it from the difference between the apparent activity of the sodium ion in the original solution and that in a solution in which all the sodium ions had been liberated by the addition of an excess of a polyvalent cation.

The sodium salt of heparin ('Heparin Spofa', twice recrystallized) was used. To determine this concentration of the acidic groups, solutions of the sodium salt were passed through a column of 'Dowex 50' in the hydrogen form. Completeness of the ion exchange was confirmed by flame photometry. Solutions of the free acid thus obtained were titrated with sodium hydroxide solutions free from carbon dioxide. The apparent activity of the sodium ion was measured with a glass semi-microelectrode sensitive to sodium and a saturated calomel electrode. The e.m.f. of the cell thus formed was standardized over the range 0·01–0·2 M with solutions of sodium chloride, and found to be reproducible to within 0·3 mV. The trivalent ion of lanthanum in the form of a 0·4 M chloride solution was used as the polyvalent cation. The glass electrode was insensitive towards the lanthanum ion in the range of concentrations used.

Because the potential of the glass electrode becomes dependent on *p*H at *p*H below 6, care had to be taken that the poly-acid solution was completely neutralized; otherwise, the lanthanum ion would have liberated not only the sodium ion, but also that of hydrogen. For the same reason, an atmosphere of nitrogen was used to guard against contamination by carbon dioxide.

The concentration of free sodium ions in a solution of the sodium salt of heparin is plotted in Fig. 1 as a function of the concentration of lanthanum chloride. The measurements have been corrected for the change of volume and for variations of ionic strength. It will be noted that an excess of lanthanum is necessary to liberate all the bound sodium. An identical curve would have been obtained if the solution of the sodium salt had been prepared by neutralizing the free heparin. The values found for binding of the sodium ion by heparin are comparable with those obtained by Ascoli *et al.*[6] by a different technique.

If the sodium salts of hyaluronic acid and deoxyribonucleic acid are used with the cation exchange resin 'Dowex 50*W*–*X*8' (200–400 mesh) in the sodium form instead of heparin, similar curves are obtained. The resin had to be suspended in a 0·01 M solution of sodium chloride.

Applications of this method are not limited to binding of the sodium ion by the poly-acids. It can also be applied to systems in which binding other than site-binding is involved, as with ethylenediamine tetraacetic acid or adenosine triphosphate. Moreover, because of this high selectivity of the glass electrode, the determination can also be carried out if cations other than sodium are present. Preliminary results indicate that this method, slightly modified, could also be used to analyse this binding of the sodium ion in biological systems.

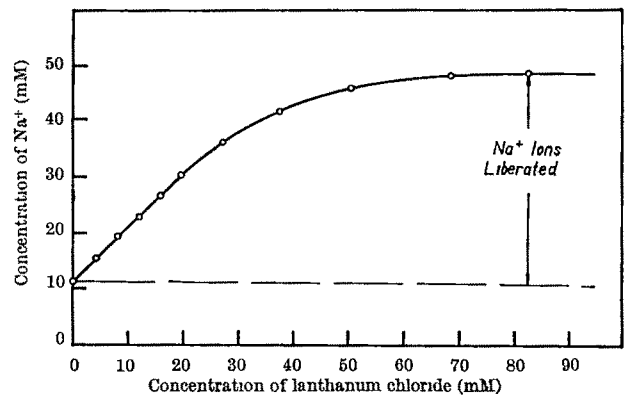


Fig. 1. Concentration of the free sodium ion in a solution of the sodium salt of heparin as a function of the concentration of lanthanum chloride. The concentration of the acidic groups of heparin as well as that of sodium was 48 mM.

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Nomenclature of Elements

WOLFENDEN[1] has suggested changes in the names of the elements so as to ensure that the names of all the metals, but none of the non-metals, end in *ium*. For example, he proposes the new names platinium and helion. If this desirable proposal is to be adopted, a criterion is necessary to distinguish metals from non-metals.

An element can be defined as a metal if, at room temperature and pressure, it has a low electrical resistivity which increases with temperature. By this criterion there are twenty-two non-metals:

H	He					
		B	C	N	O	F	Ne
			Si	P	S	Cl	Ar
				Ge	Se	Br	Kr
					Te	I	Xe
						At	Rn

Changes in the names of elements in addition to those proposed by Wolfenden would be: germanon[2], arsenium[3], selenon[4] and telluron[5]. The references are to resistivity data for pure samples of these elements.

Least satisfactory is the choice of a name for tin, for below 13° C the stable phase is a non-metal[6,7].

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RADIOBIOLOGY

Long-term *in vivo* Retention of Cerium-144 by Beagles

CERIUM-144 is a high yield (~ 5 per cent) product of nuclear fission that can be readily detected in debris from fission reactions. From about the first to the fourth year after fission, cerium-144 contributes most to total radioactivity[1]. Because each nuclear disintegration of cerium-144 and its daughter praseodymium-144 can result in deposition of the effective equivalent of more than 6 MeV in bone[2], cerium-144 ranks among the more hazardous fission products. Although cerium-144 is only poorly absorbed from the gut[2,3], it has been detected at low concentrations in foods, including clams and animal bone[4]. Liebscher *et al.*[5] detected it in both lung tissue and pulmonary lymph nodes from man. Other workers also identified cerium-144 in lung tissue ash[6]. A similar situation was observed for plutonium-239 by Bair[7]. To assess properly radiation protection guides, it is essential also to consider the bodily retention of the element in question. Virtually nothing is known about the retention of cerium-144 by mammals other than rodents. The purpose of this work was to study the long-term *in vivo* retention of cerium-144 following a single dose to adult beagles.

Four adult male beagles with an average weight of 13·4 kg (range 12·0–15·27 kg) were used. Ages ranged from 60 months to 76 months. Because the animals had been maintained in our colony since birth, they were known to be free from disease. Each dog was well acclimated to periods of confinement in metabolism cages. Immediately after a cage conditioning period of 2 weeks, each dog received about 28 μc. of carrier-free cerium-144 as cerium chloride in 1 ml. sterile solution by injection into an antecubital vein. The *p*H was adjusted to 3·5, because Aeberhardt *et al.*[8] have been able to demonstrate complete ionization and lack of colloid formation for *p*H values less than 4·5. Also, it has been demonstrated that both *p*H and carrier affect deposition patterns of lanthanons in the body[9]. Water and commercial dog food were always available. Within 30 min of injection, each dog was placed in a large volume radiation detector designed to measure the γ-ray activity of the entire animal[10]. The almost 4π steradian geometry of the detector virtually eliminates the effect of tracer redistribution. About 1 yr after injection the dogs were measured (whole-body counted) in an improved counting system[11]. Between day 369 and day 425 whole-body counts in both counters were made on each dog on five different occasions; after day 425, measurements were made only in the improved counter; the overlap period allowed for normalization of the counting data. A polyethylene bottle containing about 28 μc. of cerium-144 and tap water to make a total weight of 13 kg was used as a reference standard. Each dog was counted for 200 sec for each measurement, and appropriate corrections were made for pre-experimental γ-ray activities (caesium-137 and potassium-40). The percentage of biological retention (PBR) was calculated from the following relation:

$$(\mathrm{PBR})_n = 100 \cdot A_n/S_n \cdot S_i/A_i$$

where (PBR) is the percentage of biological retention on day n, and A and S are count rates of an animal or standard at the nth or ith (initial) measurement, respectively. All data processing was carried out on an IBM model 7094 computer; the data reduction and analysis techniques used have already been reported in detail[12].

Table 1 gives whole-body retention parameters for each dog. During the 1,050 day experiment each dog was measured 126 times. A simple exponential model was used because of the observed linear reduction of log retention as a function of time after injection. At least two power functions ($y = ax^b$) were needed to describe retention after day 1; one power function described the data reasonably well from days 100 to 1,050. Effective half-lives (T) approach the maximum value of 285 days, which is the physical half-life (T_r) of cerium-144 (that is, if no biological turnover occurred, the serial changes in body activity would result from physical decay of cerium-144 alone). That only two parameters are needed to describe retention over approximately four effective half-lives is of considerable interest, because cerium-144 is known to be deposited in tissues other than bone (for example, kidneys, liver and spleen) when injected into rodents at *p*H 3·5 (ref. 13). Probably the half-life observed is a reflexion of reasonably similar turnover rates from more than one site.

Table 1. COMPUTER-DERIVED WHOLE-BODY RETENTION PARAMETERS FOR CERIUM-144 INJECTED INTRAVENOUSLY INTO MALE BEAGLES

Dog. No.	a* (per cent)	$-k$* (day^{-1})	T_b (days)	T† (days)
19	97·7	$1\,789 \times 10^{-4}$	3,873 ± 623‡	265
21	97·8	$2\,032 \times 10^{-4}$	3,411 ± 263	263
24	99 1	$1\,861 \times 10^{-4}$	3,723 ± 501	265
31	99 3	$2{\cdot}141 \times 10^{-4}$	3,283 ± 378	262

* $R_t = a\,e^{-kt}$, for $t \geqslant 0 \leqslant 1{,}050$ days.

† $T = \frac{T_r \cdot T_b}{T_b + T_r}$, where T_r, the physical half-life for cerium-144, is 285 days.

‡ Best value ± one standard deviation. $T_b = 0{\cdot}693/k$; $\sigma T_b = \frac{0{\cdot}693\ \sigma_k}{(k)^2}$.

Biological turnover is extremely slow with an apparent biological half-life of about 10 yr. The biological half-life (T_b) of 1,500 days currently listed for bone by the International Commission on Radiological Protection[14] would seem to be too small. For cases involving internal deposition of cerium-144 in human beings, it would seem prudent and conservative to assume that physical decay alone would account for subsequent changes in the body burden of cerium-144. Furthermore, it is possible that following previous atmospheric nuclear weapon tests small quantities of fall-out cerium-144 might have been deposited in human bone. To our knowledge this conjecture has not been verified.

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Recovery Effect of Heterologous Deoxyribonucleic Acid from Various Organs on Irradiated Mice

WE have previously shown that native homologous deoxyribonucleic acids (DNAs) can be used as therapeutic agents in irradiated animals. We showed that, for example, native homologous testes DNA significantly increases the percentage of survival of infantile rats and mice (8 and 17 days old), irradiated with a dose of 600 r.[1]. Furthermore, we obtained a positive therapeutic effect in infantile rats of the same age injected with liver DNA after irra-

diation with a dose of 600 r.[2]. Our results showed that other DNAs, such as those from thymus and spleen, can increase the survival from radiation disease[3]. We also showed that these homologous native DNAs from various organs not only increase the proportion surviving radiation disease, but also support the regeneration of function in the reproductive organs of irradiated immature male rats. The percentage of fertile rats is significantly higher (60–70 per cent) in animals treated with DNA (isolated from various organs) after irradiation, than in control animals (25–30 per cent)[3,4].

Histological investigations have shown that there is less damage to the spleen, bone-marrow, digestive tract and liver in the animals treated with DNA after irradiation than in the controls[5,6].

It has also been shown that the effects of lethal irradiation can be modified in different animals by other treatments applied after irradiation, for example, with bone-marrow cells[7,8] or spleen homogenates[9].

In view of the importance of this problem from both the theoretical and the practical point of view, we investigated the possibility of curing lethally irradiated animals with heterologous DNA. A greater effect was achieved if the donors were of the same species. Nucleic acids of rabbits injected into irradiated rats failed to give any protection[10].

Paoletti examined the restorative effect of calf thymus DNA in lethally irradiated mice[11], and found that the percentage of survival was at the limit of statistical significance. When he applied 2-β-aminoethylisothiourea (AET) before irradiation and then injected heterologous DNA, however, eleven animals out of forty survived.

In our experiments we irradiated 2-month-old *C57* male mice with 600 r. X-rays (approximately the 100 per cent lethal dose). The animals were irradiated under the following conditions: 200 kV, 14 m.amp, aluminium 3, distance 46 cm. Immediately after irradiation the animals were injected intraperitoneally with DNA extracted, according to the method devised by Zamenhoff[12], from various organs of rats of comparable age. Standard curves for the extracts are shown in Table 1.

Table 1

Extract	DNA : RNA	E_{260}/E_{230}
Liver	0 51	2 4
Testes	0 52	2 2
Spleen	0·52	2·3
Thymus	0 53	2·1
Kidney	0·50	2·1

The survival rates were determined over a period of 30 days for 288 adult male mice, 236 of which had received post-irradiation injections of DNA (see Table 2).

Table 2. DOSAGE OF DNA EXTRACT AND NO. OF MICE INJECTED AFTER IRRADIATION WITH 600 R. X-RAYS

Extract and dosage (mg)	No. of mice
Liver, 1 6–1 8	49
Testes, 1·3–2·0	48
Spleen, 1·0–1·4	53
Thymus, 2 0	51
Kidney, 1·2–1 4	35
(Controls)	52

The results obtained are shown in Fig. 1. They indicate that 4 per cent of male mice irradiated with a dose of 600 r. were able to survive (controls). Mice irradiated with the same dose and treated with DNAs show increased survival. Thus, liver DNA increases the percentage of survival to 53 per cent, kidney DNA to 54 per cent, thymus DNA to 37 per cent (above), spleen DNA to 37 per cent and testes DNA to 31 per cent (below).

Our results show that DNAs reduce the effects of irradiation by a factor of 9–13. Statistically the differences are highly significant ($P < 0{\cdot}001$).

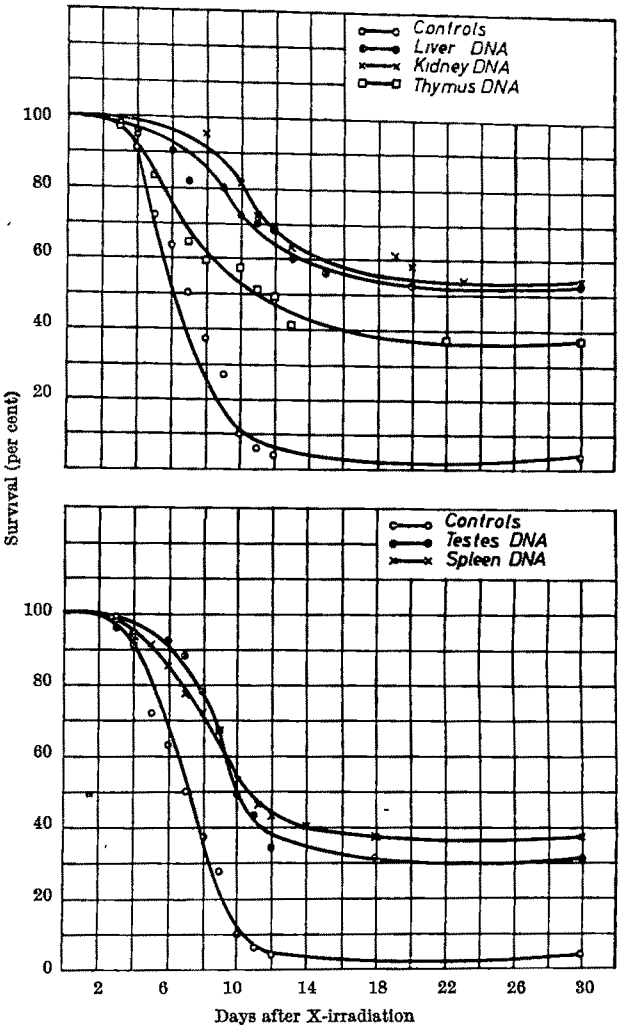


Fig. 1. Restorative effect of DNA isolated from different organs on the survival of adult male mice.

From these data, we conclude that heterologous native DNAs extracted from various organs of adult rats enable a high percentage of mice to survive the effects of irradiation. The exact nature of the mechanism involved has not as yet been established, but it is possible that DNA which has been damaged during irradiation can be replaced or restored by the injection of either homologous or heterologous deoxyribonucleic acids.

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PHYSIOLOGY

Photoreceptive Features of Vesicles associated with the Nervous System of Cephalopods

FROM the results of an investigation using both the light and the electron microscope Nishioka, Hagadorn and Bern[1] concluded that the epistellar body of *Octopus bimaculatus*(?) found off the coast of California was probably a photoreceptive organ. Present within the lumen of the epistellar body are processes equipped with microvilli; these are often organized into rhabdomes as in the eyes of many protostome invertebrates[2]. In this species of *Octopus*, as well as in others studied, the epistellar body is only barely discernible as a pale orange spot at the base of the posterior stellar nerves of the stellate ganglion. In *Eledone*, however, the similarly located epistellar body is much larger and more intensely orange than in *Octopus*. Even with light microscopy the reticular pattern suggestive of rhabdomeric organization was apparent at the periphery of the lumen. The morphology indicative of a photoreceptive function and the large amount of pigment observed provided the basis for the present investigation of the biochemical nature of the pigment of the epistellar body of *Eledone moschata*. The details of this study and related information on vesicles associated with the nervous system of cephalopod molluscs will be reported elsewhere[3].

Sixty-three *E. moschata* were dark-adapted for at least 16 h before killing them. The stellate ganglia and eyes were then removed under red light. Both tissues were immediately frozen in beakers suspended over dry ice in acetone. After thawing and further dissection, the portion of the stellate ganglia which contained the epistellar bodies and the remainder of the stellate ganglia were homogenized separately and centrifuged in a sucrose gradient. The eyes were also homogenized and centrifuged in a similar sucrose gradient after an initial crude separation of cellular material from pigment granules. Material floating in the 0·5 M sucrose concentration was then removed and mixed with 1 M disodium acid phosphate and centrifuged. The precipitate was dissolved in digitonin and transferred to a Beckman cuvette, and the absorption spectrum characteristic of rhodopsin was obtained on the eye material using a Beckman ratio recording spectrophotometer. A similar spectrum was obtained with material from the epistellar body. The *p*H of the solution was then raised slightly above 9·0 with sodium carbonate, and the solutions containing rhodopsin were exposed to room light for about 1 h before a second absorption spectrum was obtained; this recorded the shift to metarhodopsin in both instances (Table 1). The λ_{max} of rhodopsin in the eye[4] was slightly higher than in the epistellar body, whereas the λ_{max} of alkaline metarhodopsin was identical for both organs. These curves were obtained on three separate batches of material, and the data for one run are given in Table 1. No evidence for the presence of rhodopsin–metarhodopsin was obtained from the stellate ganglion (control tissue preparations).

In their earlier paper, Nishioka *et al.*[1] concluded that the epistellar body could not be a neuroendocrine structure[5–7] or the homologue of the third-order giant fibre system of squids[5]. We have suggested that the parolfactory vesicles in decapod cephalopods[7] were more likely homologues of the octopod epistellar body. Ac... factory vesicles of the squid *L.* ... investigated. Histological ...

those of the octopod epistellar body[7]. These vesicles vary in number from four to fifteen on each side and lie superficially in connective tissue adjacent to the olfactory lobes—thus the name "parolfactory". Because of their minute size, we initially expected that they would be extremely difficult to find in fresh material; however, here again the intense orange pigmentation facilitated their collection.

Parolfactory vesicles from ninety-two *Loligo vulgaris* were used. Except for a shorter duration of dark adaptation (about 6 h)—because of the higher mortality of squids in captivity—the experimental procedure was identical with that used on *Eledone* material. Pieces of optic lobe approximately equal in volume to the parolfactory vesicles in their enveloping connective tissue were taken as control tissue, and the eyes[8] were again used for comparison. The λ_{max} of rhodopsin and of metarhodopsin from one of two determinations are given in Table 1. The optic lobes showed no evidence of visual pigment.

To substantiate further the specificity of the biochemical features of these photoreceptive structures, the antimony chloride method for determination of vitamin A_1 (see refs. 8 and 9) was used on extracts. Vitamin A_1 (ref. 9) was detected in both organs, but none was found in the control tissues (the remainder of the stellate ganglion in the octopus and parts of the optic lobe in the squid).

Because of the small size of both epistellar and parolfactory structures, no weights were taken. Nevertheless, we are impressed by the appreciable amounts of visual pigment isolated from these organs. Gross comparison with the eye leads us to believe that the concentration of visual pigment in the epistellar body and parolfactory vesicles is probably greater than in the eye.

We conclude from the biochemical information presented here that the epistellar body and the parolfactory vesicles contain considerable amounts of visual pigments very similar to those present in the eyes of the same animals. Our initial belief that the epistellar body was a vestigial organ in the adult organism[1] (conceivably important only in larval life) may now be untenable. It seems more likely that these organs are of importance in photoperiodic regulation in the adult, despite their cryptic locations. The vesicles are not to be viewed as "3rd and 4th eyes"[10], inasmuch as they would scarcely seem responsible for vision (that is to say, image perception). Photoreceptive function can extend to physiological regulations and include much more than vision *per se*. It is to this more general aspect that comparative physiologists need now address themselves.

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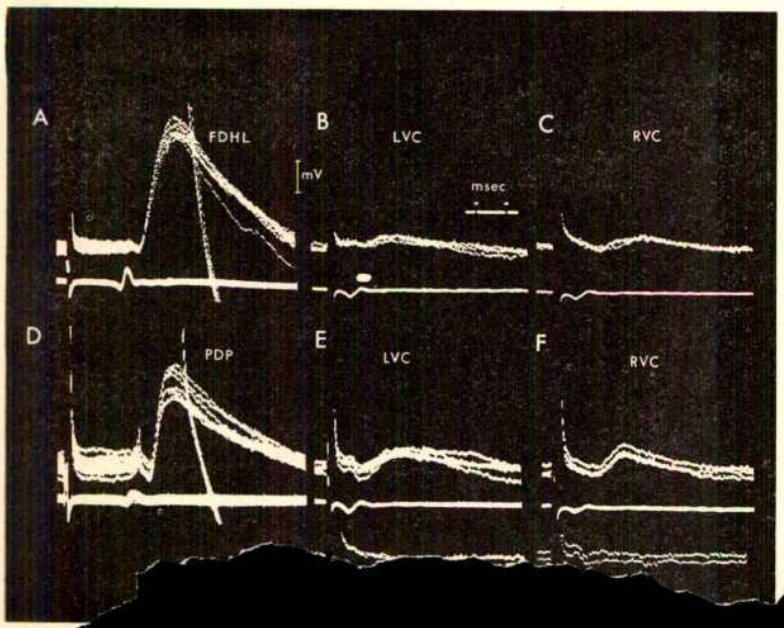
A Descending Pathway which is Monosynaptically Excitatory to Motoneurones

NEARLY twenty-five years ago, Lloyd[1] described a pathway descending in the ventral part of the spinal cord of the cat which ended directly on lumbosacral motoneurones and which facilitated the monosynaptic reflex. The pathway was shown by Lloyd to originate from neurones of the brain stem and from spinal proprioneurones. He named the pathway the "bulbospinal correlation system". Since Lloyd's original description, there has been some physiological work which suggests that the brain stem neurones from which this pathway originates may belong either to the reticular formation[2] or to the vestibular nuclei[3] or to both. Recent studies of the mode of termination of the reticulospinal and vestibulospinal tracts, however, offer little support for the presence of more than a few monosynaptic endings on lumbosacral motoneurones from fibres descending in these tracts[4]. The experiments to be described confirm the presence of long descending axons in the ventral part of the spinal cord which monosynaptically excite motoneurones.

Activity in what is presumed to be the bulbospinal correlation system of Lloyd has been investigated in the following manner. Cats were anaesthetized with pentobarbital sodium and their cords transected at the first lumbar level. The dorsal columns were removed for about a segment below the transection, and the dorsal halves of the lateral columns severed. The ventral part of the cord was placed on a pair of stimulating electrodes. Motoneurones of the lumbosacral enlargement were impaled by glass microelectrodes filled with 2·7 M potassium chloride or 4 M sodium chloride (resistances of 3–10 megohms). Because the roots were generally left intact, a motoneurone could be identified by the antidromic action potential that resulted from stimulation of the appropriate muscle nerve[5].

Motoneurones belonging both to extensor and to flexor muscles were frequently observed to receive short latency excitatory postsynaptic potentials from volleys descending in the ventral part of the spinal cord. Furthermore, when the ventral cord was separated into left and right halves, motoneurones often received comparable excitatory potentials from the two sides of the cord. For example, Fig. 1 illustrates the effects of stimulation of the left and right ventral cord quadrants on an extensor and a flexor motoneurone. The neurones were identified by their receiving antidromic action potentials from the flexor digitorum and hallucis longus (Fig. 1*A*) and the peronei-deep peroneal (Fig. 1*D*) nerves. Both these action potentials are shown at threshold stimulation of the motor axon; when no action potential was produced, the monosynaptic excitatory potential produced by the afferent volley in the same nerve could be seen. Each of the motoneurones received a small excitatory potential from volleys descending in either of the two halves of the ventral cord (Fig. 1*B–C* and *E–F*). The descending volley was recorded from the cord dorsum by a ball-tipped electrode placed near the entry point of the microelectrode. The interval between the arrival of the fastest component of the descending volley and the excitatory postsynaptic potential was less than 1 msec in each case, and so the potentials were monosynaptic.

The fact that the monosynaptic excitatory potentials which result from descending ventral cord volleys are rather small explains Lloyd's observation[1] that such volleys facilitate reflex transmission but do not discharge motoneurones. Lloyd also noted that repetitive stimulation of the bulbospinal correlation system greatly enhanced the facilitation. Fig. 2 illustrates the effect of brief repetitive stimulation of the ventral cord on the excitatory potentials recorded from motoneurones. The antidromic action potentials in Fig. 2*A* and *D* identify the motoneurones as belonging to the gastrocnemius-soleus and the posterior biceps-semitendinosus muscles, respectively. The monosynaptic excitatory postsynaptic potentials that were produced by single stimuli applied to the ventral cord are shown in Fig. 2*B* and *E*. The records in Fig. 2*C* and *F* were the result of brief tetanic stimulation of the ventral cord (four volleys at about 300/sec). The individual excitatory potentials not only merge with the residual depolarization of the previous potentials, but in addition they grow larger. The potential of Fig. 2*C* resembles the augmenting excitatory potentials produced by repetitive stimulation of the corticospinal and corticobulbar tracts and recorded from cervical motoneurones[6] or reticular formation neurones[7]. A difference may be seen, however, especially in Fig. 2*F*, in that the rising phases of the excitatory potentials from the ventral cord pathway are often double. The second component of the excitatory potentials evoked by repetitive stimulation begins too late to be monosynaptically evoked by the earliest component of the appropriate descending volley. Lloyd[1] pointed out that the ding volley has two peaks, which he to a directly conducted volley relayed through a set of



ation begins within 1 msec of the second component of the preceding descending volley. Thus, the simplest hypothesis to explain the augmentation of the excitatory potential with repetitive stimulation would be the adding together, with time, of the descending excitation of ventral horn interneurones, increasing their contribution to the relayed descending volley as more interneurones discharge. This would increase the second excitatory potential, adding to the peak of that produced monosynaptically by the direct volley. There may be in addition a growth of the initial excitatory potential, however, owing to a form of postactivation potentiation.

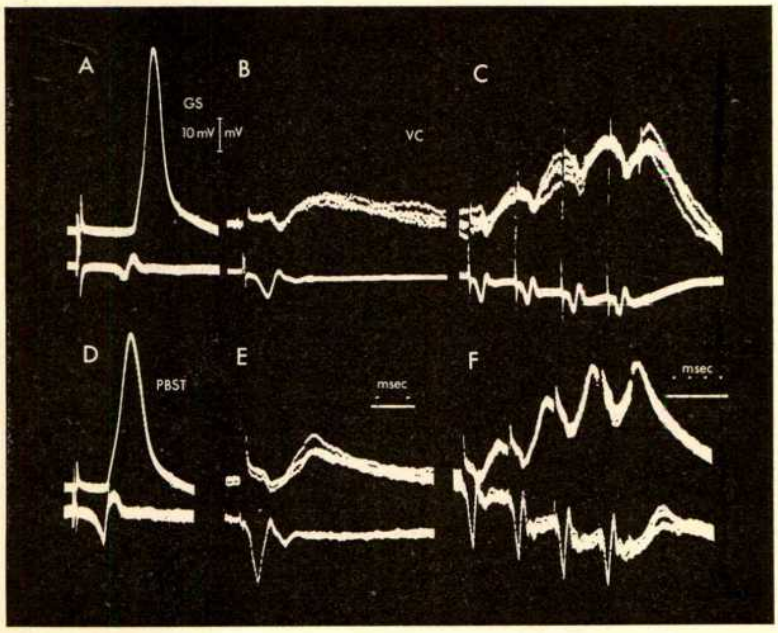


Fig. 2. *A–C* are potentials recorded from a gastrocnemius-soleus motoneurone. *A* is the antidromic action potential. *B* is the monosynaptic EPSP that was produced by stimulation of the ventral half of the spinal cord at the first lumbar level. *C* is also from ventral cord stimulation, but from four volleys at 300/sec. *D–F* are similar records from a posterior biceps-semitendinosus motoneurone. The potential scale is 10 mV for *A* and *D*; it is 1 mV for the other records. The time scale above *E* is for *A*, *B*, *D* and *E*; that above *F* is for *C* and *F*.

Further work is in progress to determine the site of origin of the descending ventral cord pathway. Assuming that it is identical to Lloyd's bulbospinal correlation system, this pathway should be of considerable importance both as a mechanism used by supraspinal control systems and as a part of the system for interlimb reflexes.

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Note added in proof. Since this report was submitted for publication, two similar findings have been reported of descending monosynaptic excitatory fibres ending on spinal motoneurones (Lund, S., and Pompeiano, O., *Experientia*, **21**, 602; 1965; and Grillner, S., and Lund, S., *Experientia*, **22**, 390; 1966).

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Sensory Discharges from Lactating Mammary Glands

Afferent nervous impulses from mammary glands are known to play a part in the reflex release of oxytocin during suckling[1]. They may also be implicated in the subjective sensation accompanying milk-ejection in women[2], in nervous reflex arcs controlling intramammary muscle tone[3] and milk secretion[4] postulated by Russian workers, and in the control of secretion from the anterior pituitary[5]. This communication describes preliminary findings from electrophysiological investigations of action potential discharges in mammary afferent nerves.

Lactating female rabbits were anaesthetized with urethane. A teat duct was cannulated for recording intramammary pressure changes. The abdominal segmental nerves were exposed, transected dorsally and filaments of the peripheral end were placed on silver–silver chloride recording electrodes under a pool of warmed liquid paraffin. Nerve fibre activity was recorded by means of a push-pull input to a high-gain oscilloscope and also monitored on a loudspeaker. Permanent records were made by photographing the stationary cathode-ray oscilloscope spot on a moving film. Selected nerve filaments were stained in osmic acid and sectioned transversely at 5μ.

The effects of various mammary stimuli were examined. These included: (*a*) pressure applied with a finger or a Von Frey hair; (*b*) the teat grasped with blunt forceps and light pressure applied; (*c*) suckling by a pup from the doe's litter; (*d*) cold, produced by spraying the area with ethyl chloride; (*e*) oxytocin injected intravenously to elicit milk-ejection; (*f*) distension of a mammary lobule by introduction of saline into the cannulated teat duct; (*g*) traction on an interlobular septum. Stimuli *a*, *b*, *e*, *f* and *g* were used after the skin over the gland under observation had been removed except over the teat itself, and the exposed surface sprayed with 2 per cent lignocaine to prevent injury discharges from cut or damaged nerve fibres.

With the skin over the gland intact, receptors have been detected which respond to pressure, including many of the low threshold, slowly adapting, type described by Frankenhauser[6]. Many teat pressure receptors adapting rapidly and a few adapting slowly were demonstrated, and these were activated in a characteristically rhythmic and vigorous manner by suckling (Fig. 1*A*). Most fibres activated by mechanical stimuli were also excited by cold. Injection of oxytocin caused excitation of many receptors coincident with the rise in intramammary pressure, though the pressure rise considerably outlasted the receptor discharge. Similar adaptation of pressure receptor discharge was seen when saline was introduced into the teat duct.

After removal of the skin, the number of units participating in a response recorded from any given nerve filament was greatly reduced. The response of remaining active fibres to finger pressure, distension of a mammary lobule and oxytocin injection (Fig. 1*B*) was retained. The number of responding fibres was reduced by infiltrating lignocaine around the base of the teat. Active fibres still present (Fig. 1*C*) were most sensitive to finger pressure when this was applied in an area at some distance from the nipple, indicating that they were situated within the parenchyma of the gland; such fibres were often excited by traction on the interlobular septum in this area.

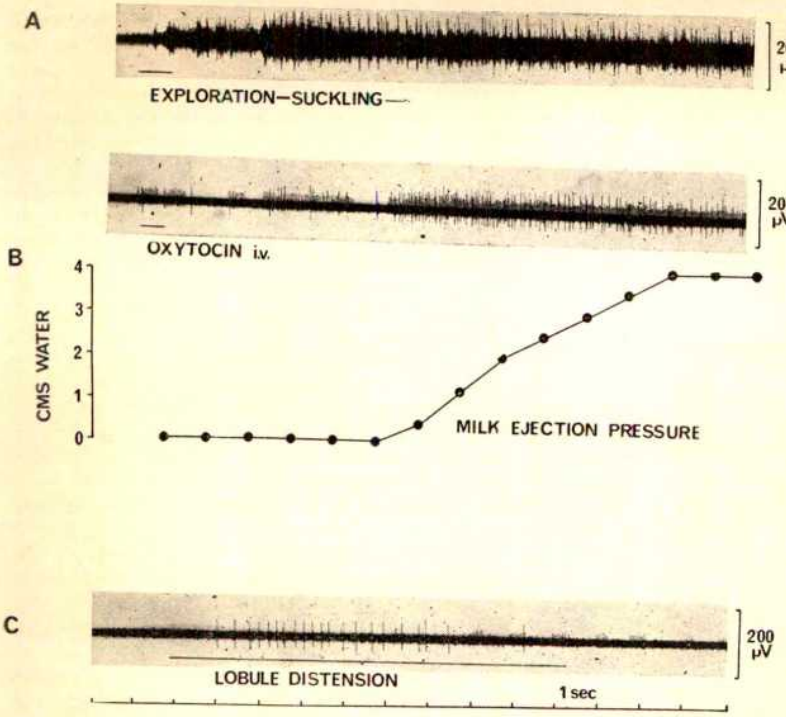


Fig. 1. Action potentials from mammary nerves. The bursts of small spikes in *B* and *C* can be correlated with respiratory rhythm. *A*, Skin intact. Short exploratory period followed by rhythmic suckling. *B*, Skin removed. Effect of intravenous oxytocin (250 mU) on milk-ejection pressure and nerve activity. *C*, Skin removed. Anaesthesia of base of teat. Effect of lobule distension.

Histological examination of the nerve filaments revealed the presence of both myelinated and unmyelinated fibres; it has not yet proved possible to assign modalities of sensation to particular fibre groups.

The data so far obtained confirm histological evidence of the profusion of teat receptors and provide physiological evidence for the existence of receptors within the gland itself. An understanding of their role in normal sensory and reflex phenomena must await a more detailed study of their thresholds and adaptation properties.

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Influence of Sex Hormones on Frequency of Micturition

THE frequency of micturition during pregnancy is regarded as a normal reaction to this physiological state. The mechanism which is responsible for this action, however, remains a matter of conjecture. It was once assumed that, during pregnancy, frequent micturition resulted from the impingement of the enlarging uterus on the urinary bladder. It has since been recognized that this is not the complete answer to the problem[1,2].

Although a neural component has been suggested[3], other factors in addition to neural stimuli should be considered as influencing vesicle activity. It is possible that the vesicle muscle is influenced not only by nerve supply but also by chemical substances carried in the blood, urine, or both. There is no evidence, however, to substantiate the suggestion that sex hormones have a direct influence on bladder motor activity[4]. Furthermore, studies have alluded to the fact that, in the pregnant state, the vesicle muscle is attended by a marked increase of hormones not only in the blood but also in the urine. The effective action site of these agents in influencing bladder motility to stimuli has not been ascertained. In an attempt to obtain information on this, *in vitro* studies were undertaken with rat urinary bladder according to the classical tissue-bath technique.

Thirty-two female Wistar rats were followed through three consecutive oestral cycles by the vaginal smear technique. During the fourth cycle, they were killed in the oestrus and dioestrus stages. These stages were selected because of their relative dominance of oestrogen or progesterone. Additional bladder preparations were used from thirteen full term pregnant rats.

The entire bladder was placed in an organ bath of 250 c.c. capacity containing oxygenated Tyrode's solution which was kept at constant temperature and *p*H. Following normal suspension, the bladder was everted, re-suspended in the bath of Tyrode's solution, and subjected to similar procedures. The following drugs were added directly to the bath: acetylcholine chloride, 5-hydroxytryptamine creatinine sulphate (serotonin), histamine dihydrochloride, pilocarpine hydrochloride, progesterone aqueous suspension, and polyoestradiolphosphate ('Premarin').

From our results, *in vitro* bladder muscle showed no spontaneous activity nor could it be activated by increased tension alone. In all preparations, acetylcholine always increased the tonus and force of contractions. This response occurred after a very brief latent period and was sustained for several minutes. No evidence of tachyphylaxis was obtained. In all cases, the dioestrus bladder responded to acetylcholine with a marked increase in tone, and contractions of greater magnitude and duration than those exhibited in the oestrus stage. Contractions of the pregnant bladder were similar to those of a bladder from an animal in dioestrus. No significant effects were obtained with pilocarpine, histamine, or serotonin.

The reactivity of the bladder was also followed after oestrogen and/or progesterone had been added to the bath. Irrespective of the stage, these hormones did not affect bladder motility. There was a distinct influence, however, on the responsiveness to acetylcholine which was the same whether oestradiol and progesterone were used alone or in combination. We found that pretreatment of the *in vitro* bladder with relatively large doses of oestrogen and/or progesterone (2 μg/ml.) made the muscle sensitive to acetylcholine (Fig. 1). This increased responsiveness to acetylcholine was not seen when the respective bladder was everted. It is interesting to note that administration of these hormones to serosal or mucosal preparations did not affect the bladder responsiveness to other parasympathomimetic agents.

Earlier investigations suggested a hormonal factor in the modification of bladder responses observed during pregnancy[5,6]. In the present investigation, *in vitro* evidence has been obtained suggesting (1) that hormones in the urine have no apparent effect on bladder contractility; (2) that circulating oestrogen and progesterone may exert a permissive effect on bladder contractility to cholinergic stimuli. It is, of course, recognized that the

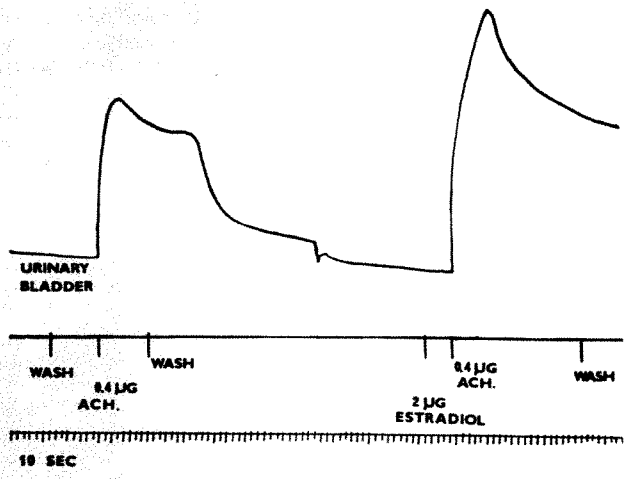


Fig. 1. Record showing the increased response of the excised dioestrus bladder to acetylcholine after treatment with oestradiol.

oestrogen and progesterone substances used may have exerted actions as chemical agents rather than as hormones. This is all the more true in view of the relatively high concentrations used in our *in vitro* studies. Experiments with doses of these substances in a lower concentration range are now in progress.

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Anaphylactic Shock and the Secretion of Adrenal 17-Hydroxycorticosteroid in the Dog

IN earlier investigations we evaluated directly the adrenal 17-hydroxycorticosteroid (17-OHCS) secreted in response to histamine by unanaesthetized dogs[1]. Marked increase in 17-OHCS secretion was observed after injection of histamine. It has been suggested by some authors that histamine is released from mammalian cells in anaphylactic shock. Very little experimental information is available, however, on the effect of anaphylactic shock on the adrenal cortical secretion. In the present investigations we attempted to evaluate directly the adrenal 17-OHCS secretion in response to anaphylactic shock in unanaesthetized and anaesthetized dogs.

Mongrel dogs weighing 10–17 kg were used. In unanaesthetized dogs the adrenal venous blood was collected by a modification of the method of Satake, Sugawara and Watanabe[2] just as in our previous studies[1,3–6]. The dorsal spinal roots of T_{11}–L_3 were cut bilaterally under sodium pentobarbital anaesthesia. The experiments were performed more than 3 weeks after the spinal cord sections. The lumboadrenal vein was exposed by way of the lumbar route and its small branches were doubly ligated and cut. A small glass cannula, connected with a short rubber tube, was inserted into the vein on the side of the adrenal gland. The cannula and the rubber tube were filled with heparin–saline solution. A loop of silk thread was placed around the adrenal vein between the posterior vena cava and the adrenal gland. The actual experiments were performed without anaesthetizing the animals about 18 h after the cannulation of the adrenal vein. Adrenal venous blood samples were taken 30 and 10 min before anaphylactic shock was induced. At the time of collecting the adrenal venous blood the silk thread around the adrenal vein between the adrenal gland and the posterior vena cava was gently tightened so as to direct adrenal venous blood flow to the exterior through the cannula and the rubber tube.

In anaesthetized dogs the adrenal venous blood samples were withdrawn by almost the same procedure as in unanaesthetized dogs. No dorsal spinal root sections, however, were performed in this group. On the day before the experiments were performed the adrenal vein cannulation was performed under sodium pentobarbital anaesthesia. Actual experiments were started about 18 h after the cannulation. One hour before the start of observation the animals were anaesthetized with an intravenous injection of sodium pentobarbital (25 mg/kg).

Anaphylactic shock was induced in both groups of animals by the method used by Sato, Ohguri and Wada[7]. About 4 weeks before the experiments were performed 0·5 ml. horse serum/kg body-weight was injected subcutaneously. On the following day the same amount of serum was injected intravenously. On the day of the actual experiments, horse serum diluted 1 : 1 with isotonic saline solution was injected intravenously at a speed of 2ml./min until the femoral artery pulse became feeble.

In advance of the experiments described here, some experiments were undertaken on anaesthetized dogs in which the measurements of blood pressure were made by a mercury manometer connected with the femoral artery; horse serum was injected intravenously until the blood pressure began to fall. In those experiments, before injection of horse serum the blood pressure was 90–94 mm mercury. It fell to 20–34 mm mercury within 2 min after the end of injection. This low blood pressure level continued for a few minutes and then slow recovery occurred. About 40 min after injection the blood pressure resumed the pre-anaphylactic level. In those experiments, however, the 17-OHCS secretion before anaphylactic shock was induced was sometimes so high that it was far outside the range of physiological variations of 17-OHCS secretion rate. Thus in the experiments described here the blood pressure was not registered. In actual experiments horse serum was injected in volumes of 0·18–0·46 ml./kg. The adrenal venous blood samples were also taken at 10, 20, 40 60, 90 and 120 min after the horse serum was injected. The volume of adrenal venous blood collected at each period before and after inducing anaphylactic shock was 2·5–4 ml. Isotonic saline solution was injected intravenously to replace all blood loss. The blood samples were immediately centrifuged and adrenal venous plasma was analysed for 17-OHCS according to the method devised by Nelson and Samuels[8].

Experiments were performed on four unanaesthetized and three anaesthetized dogs. In all cases the femoral artery pulse became definitely feeble after the injection of horse serum. In addition to this hypotension, a variety of symptoms of anaphylactic shock was observed in unanaesthetized dogs. Just before the end of the period during which the horse serum was injected, the dogs became restless. Salivation, dilatation of the pupils and retraction of the nictitating membranes were observed. Respiration became irregular and laboured, and its frequency exhibited a definite increase. Within a few minutes a marked paralysis developed. The animals were unable to stand, and lay on their side. In this condition they did not respond to various painful stimulations, and myasthenia was observed. These symptoms lasted for about 20 min, after which the animals slowly recovered. In four dogs (dogs 4, 5, 6 and 7) defaecation occurred about 10 min after the injection of serum. Table 1 shows the results of the adrenal 17-OHCS secretion rate before and after anaphylactic shock was induced. The 17-OHCS secretion rate markedly increased after anaphylactic

Table 1. EFFECT OF ANAPHYLACTIC SHOCK ON ADRENAL 17-HYDROXYCORTICOSTEROID SECRETION RATE IN UNANAESTHETIZED AND ANAESTHETIZED DOGS

Dog No.	Body wt. (kg) and sex	Adrenal 17-OHCS secretion-rate (μg/kg/min) Min before 30	10	Min after inducing anaphylactic shock 10	20	40	60	90	120
		(Experiments in unanaesthetized dogs)							
1	15·3 ♂	0·01	0·03	0·66	1·00	0·14	0·08	0·04	0·12
2	17·0 ♀	0·03	0·02	0·47	0·96	1·17	1·04	1·22	1·36
3	12·6 ♀	0·09	0·21	0·15	0·43	0·53	0·78	0·66	1·06
4	15·6 ♂	0·09	0·16	1·29	0·77	0·73	0·76	0·23	0·27
		(Experiments in dogs anaesthetized with sodium pentobarbital)							
5	9·8 ♂	0·11	0·23	1·26	1·61	1·11	0·20	0·02	0·06
6	10·7 ♂	0·24	0·23	1·37	1·34	1·55	1·38	1·35	0·81
7	10·8 ♀	0·00	0·04	0·56	0·84	0·41	0·06	0·08	0·20

shock was induced in anaesthetized as well as in unanaesthetized dogs. There seemed to be a possibility that in unanaesthetized dogs psychical excitation as a result of anaphylactic shock could be responsible for the increase in adrenal cortical secretion. We were able to show that this was not the case, however, because a marked response of adrenal cortical activity to anaphylactic shock was also observed in anaesthetized dogs.

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Antagonistic and Synergistic Actions of Thyrocalcitonin and Parathyroid Hormone on the Levels of Calcium and Phosphate in the Rat

THE discovery of the existence of a hypocalcaemic hormone in the thyroid gland[1] raises the question of the respective roles of thyrocalcitonin and parathyroid hormone on plasma calcium and phosphate homeostasis. As the kidney is a target organ for parathyroid hormone and is involved in plasma phosphate homeostasis, it was thought important to investigate the effects of thyrocalcitonin on calcium and phosphate levels in plasma, and on their excretion in the urine, both in the presence and absence of parathyroid hormone.

Male Wistar *CF* rats, 60 days old and weighing 150 g, were thyroparathyroidectomized. One day later, animals showing signs of incomplete operation (plasma calcium level above 8·0 mg/100 ml.) were discarded. At the "beginning" of the experiment (time 0), forty fasted rats received 1 ml. of isotonic glucose intravenously; the urethra was then ligated. The animals were divided into four equal groups: controls; animals treated with thyrocalcitonin; animals treated with parathyroid hormone; and animals treated with both thyrocalcitonin and parathyroid hormone. The hormones were given as follows: 20 U of purified thyrocalcitonin[2,3] was injected intravenously at time 0, and 100 IU of parathyroid hormone (Lilly) subcutaneously at time "−5 h" (that is, 5 h before the "beginning" of the experiment). At +1 h, blood was collected, the rats were killed and the contents of the bladder quantitatively collected. Calcium was estimated by flame photometry and phosphate by the technique of Fiske and Subbarow[4]. Creatinine was measured colorimetrically[5].

The results presented in Fig. 1 are expressed as differences between the rats treated with hormone and the control rats. The expected changes in plasma calcium levels that are produced by parathyroid hormone and thyrocalcitonin are abolished by the combined administration of the hormones; 20 U of thyrocalcitonin can counteract the effect of 100 IU of parathyroid hormone. This antagonism is consistent with a dual hormonal control to ensure blood calcium homeostasis. Both thyrocalcitonin and parathyroid hormone lower plasma phosphate, and their action is additive when administered simultaneously. This finding raises the question of plasma phosphate homeostasis since the interplay of thyrocalcitonin and parathyroid hormone cannot possibly explain the maintenance of a constant phosphate plasma level, as they are synergistic in lowering phosphate.

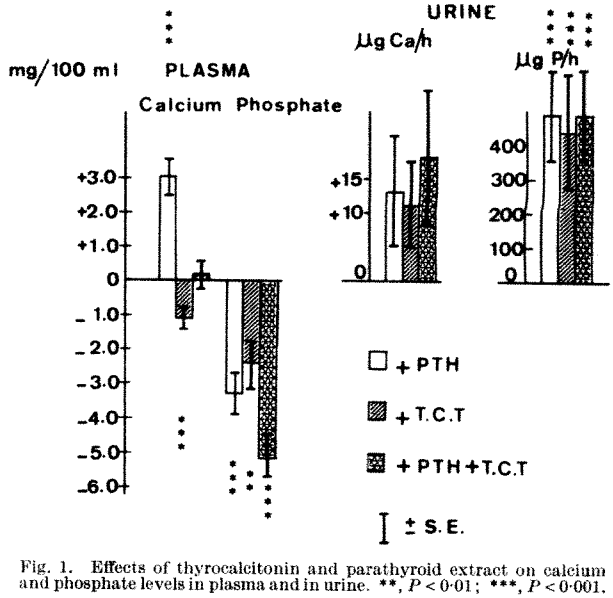


Fig. 1. Effects of thyrocalcitonin and parathyroid extract on calcium and phosphate levels in plasma and in urine. **, $P < 0.01$; ***, $P < 0.001$.

As to the urine composition (Fig. 1), parathyroid hormone elicits the expected phosphaturic response. Thyrocalcitonin has the same phosphaturic effect[6]. The combined administration of both hormones does not increase the phosphate excreted, which would indicate that the maximal response was obtained for each hormone at the dose used. That the phosphaturic effect is present with unchanged glomerular filtration rate is obvious because creatinine excretion rates do not vary significantly in any groups: control = 231·4 ± 46·4 μg/h; parathyroid hormone-treated animals, 261·0 ± 22·55 μg/h; thyrocalcitonin-treated animals, 252·0 ± 26·15 μg/h; and animals treated with both hormones, 262·0 ± 37·76 μg/h. This is consistent with a diminished tubular reabsorption for phosphate, even though the experimental condition used cannot distinguish between a competitive and a non-competitive mechanism for phosphate excretion or reabsorption.

Phosphate clearance should not be used to demonstrate hypersecretion of parathyroid hormone without adequate controls because thyrocalcitonin has the same effect, which cannot be mediated in our experiments by a secondary response of parathyroid hormone in thyroparathyroidectomized animals. In the normal rat, a phosphaturic effect was observed after injection of a crude thyroid extract[7]. Urinary calcium is increased in the three treated groups, but this increase is not statistically significant. For this reason, renal excretion of calcium is not likely to contribute to the effect of lowering the calcium in plasma brought about by thyrocalcitonin.

In summary, thyrocalcitonin and parathyroid extract are effective in the control of the calcium level of plasma and act antagonistically. The two hormones lower plasma phosphate synergistically; thus it is not possible to account for the mechanism responsible for raising the phosphate level in plasma. Thyrocalcitonin possesses an important phosphaturic effect.

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Aorticopulmonary Glomus Tissue in the Cat

HUGHES has recently reported a portal blood supply to aorticopulmonary glomus tissue in the cat[1]. The pulmonary arterial vasa vasorum, arising from the coronary arteries, form an intramural capillary network in which it is suggested the blood may come into gaseous equilibrium with that in the lumen of the pulmonary artery itself. This network is said to drain into venae vasorum which supply the aortic bodies.

Although a schematic representation of the hypothetical portal system is depicted, there is nothing in the histological figures or elsewhere to suggest that this is more than a speculation. In fact, we have recently re-examined the blood supply of the aorticopulmonary bodies, and careful scrutiny of serial sections of cat pulmonary artery with its associated glomus tissue has failed to reveal any such portal vessels. Hughes also states that the large arteries—said by previous workers to supply the aortic bodies—are not in fact end arteries but pass straight through the glomus tissue, the glomus being supplied instead by the portal system he has described. In our experience this is not the case. In several of our experiments we found an aortic body which had a single (relatively large) artery which was traced in continuity from a systemic source. On histological examination, the vessel could be traced through many sections as it divided into the small vessels of the body.

In a series of new-born kittens the systemic and pulmonary arteries of which were injected with masses of contrasting colour, some aortic bodies were supplied by a single branch of the pulmonary artery. The vasculature of these bodies contained the pulmonary injection mass but none of the systemic injection material that would have been present if the portal system described by Hughes had existed. As post-natal development proceeds, anastomoses develop between this pulmonary vessel and certain systemic arteries; finally, the pulmonary connexion is obliterated, leaving the glomus supplied solely by the systemic vessels. All these arterial connexions could be traced in continuity under the dissecting microscope or through serial histological sections. At no stage in development was there any sign of the intramural vasculature described by Hughes. Our investigation into the post-natal changes in the blood supply of aorticopulmonary bodies confirms and extends the findings of several earlier workers (for review, see Comroe[2]).

Physiological evidence for the existence of pulmonary arterial chemoreceptors was obtained by Duke *et al.*[3]. With the help of his portal system, Hughes avers, it is possible to reconcile the findings of Duke *et al.* with the absence of a direct blood supply from the pulmonary artery to glomus tissue in the adult animal. On the contrary, this could not possibly explain the results of Duke *et al.* In one set of experiments these investigators recorded action potentials while perfusing the isolated pulmonary artery alone. Because there was no circulation of blood in the systemic vessels, there can have been no flow through the coronary arteries nor through the vasa vasorum of the pulmonary artery. It is true that Duke *et al.* did perfuse the systemic circulation in a second series of experiments in which reflex effects were examined, but, as the blood was usually pumped into the rostral end of the brachiocephalic artery, there can again have been no flow through the coronary arteries, nor through the hypothetical portal system.

A number of other points in Hughes's article merit some comment.

(1) Contrary to Hughes's interpretation, the existence of a pulmonary arterial blood supply to a particular group of aortic body tissue in the foetus, while interesting, is not evidence of its function as a chemoreceptor.

(2) No evidence is advanced to substantiate the report of chemoreceptors in the retroperitoneal tissues.

(3) The analogy drawn between aortic bodies (vascular chemoreceptors) and adrenal medullary (endocrine) tissue is erroneous. The term "glomus", which is generally well understood in the context of chemoreceptor tissues, should not be used for the endocrine system and such usage only serves to reintroduce confusion into a nomenclature which had been reasonably clarified[4,5].

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Reflex Regulation of Gill Water Flow (Ventilation) in *Squalus acanthias*

THE factors regulating the volume flow of water through fish gills are obscure. This communication describes a mechanism which serves this function in the elasmobranch.

These studies were performed in the dogfish shark, *Squalus acanthias*. Three animals were studied. The animals were removed from water and plastic cannulae were inserted into their spiracles. These cannulae were connected to a sea water reservoir so that the rate of gill flow could be varied by appropriate manipulation of a valve controlling the flow from the reservoir. Effluent water from the gills was collected by means of a funnel and the volume measured directly in a graduated cylinder. Opercular rate was determined by direct observation.

The data are summarized in Fig. 1. The opercular rate (15–44/min) varied more or less directly with the rate of flow of water through the gills (0·09–2·00 l./min) over a wide range. This mechanism operates so that when a given volume (or pressure) is attained inside the respiratory chamber of the fish it results in a respiratory

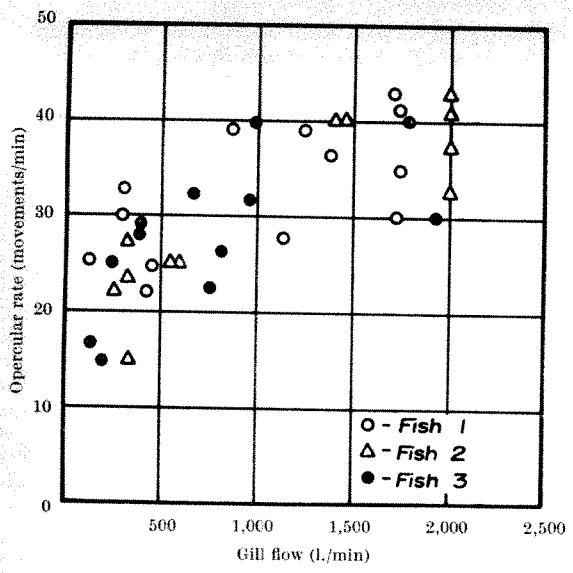


Fig. 1.

effort as shown by opercular movement. This suggests that the major adjustment of gill ventilation occurs by modification of the respiratory rate rather than by the volume of water flowing through. The nature of the stimulus for this reflex (pressure as compared with volume), the receptors and central connexions are not known. Its function, however, appears to be analogous to that of the Hering-Breuer reflex in mammals.

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BIOCHEMISTRY

Chondromucoprotein-degrading Enzymes

THE chondroitin sulphate-protein complex of cartilage, chondromucoprotein[1], is normally unable to diffuse out of the tissue, but can be released in a soluble form by severe mechanical disruption of the tissue[1] or *in vivo* by chemical degradation, for example, by papain[2]. Other enzymes capable of degrading chondromucoprotein so that it may be lost from the intercellular matrix are present in lysosomes[3], and these enzymes may play an important part in the pathological deterioration of cartilage. It seems probable that the lysosomal enzyme activity involved is of the acid protease type[3,4], but hyaluronidase[5,6] or xylosidase[7] may also be implicated.

In the past the most widely used method for detecting and estimating the degradation of chondromucoprotein by enzymes has been viscometry[8], but this is unsatisfactory for crude protein mixtures owing to precipitation of chondromucoprotein by oppositely charged protein molecules at acid *p*H[9], and also because of spontaneous alterations in the viscosity of chondromucoprotein solutions[1]. Viscometry is also ill-suited to problems such as the localization of small quantities of enzyme after disk electrophoresis. A new method is described here.

A polyacrylamide gel containing 7 per cent (weight/volume) of polymer[10] was modified by the inclusion, before polymerization, of chondromucoprotein (0·05 per cent of light protein-polysaccharide—prepared from bovine nasal septum[11]). Gel columns 5 mm in diameter were cast in glass tubes and sliced transversely with a razor blade to form disks about 1·5 mm thick. The disks were stored under sodium acetate buffer (*p*H 4·0, 0·1 M containing 1 per cent *n*-butanol) at 4° C until required.

A preparation of particle-bound enzymes was made as follows: livers from freshly killed rabbits were passed through a cold mincer and mixed with iced 0·25 M sucrose solution. The tissue was then homogenized and a pellet of mitochondria and lysosomes was prepared by differential centrifugation[3]. The pellet was treated with a large volume of acetone at 4° C and the solid material was then filtered off and dried under reduced pressure. The acetone powder was stored at 4° C.

A crude enzyme preparation was obtained by extraction of the powder (10 mg/ml.) with acetate buffer (*p*H 4·0).

Groups of disks were shaken for 16 h at 37° C with acetate buffer (0·25 ml.; *p*H 4·0, 0·2 M) and enzyme preparation (0·25 ml.). Control groups were incubated with buffer in place of enzyme. The gels were then placed in the destaining tubes of a disk electrophoresis apparatus, and electrophoresis was carried out at 8 m.amp per tube (30 min) with acetic acid (7 per cent volume/volume) as electrolyte. The disks were then stained in alcian blue (1 per cent in 7 per cent volume/volume acetic acid) for 15 min, rinsed with water, and returned to the destaining tubes of the disk electrophoresis apparatus. Unbound dye was removed electrophoretically with acetic acid electrolyte.

Undegraded chondromucoprotein was completely immobile when trapped in the polyacrylamide gel, and intense staining was obtained in the absence of degrading enzymes (Fig. 1*a*). On the other hand, gels that had been incubated in the presence of the enzyme preparation showed only the faintest staining (Fig. 1*b*); this is attributed to the fact that free chondroitin sulphate is mobile in this gel.

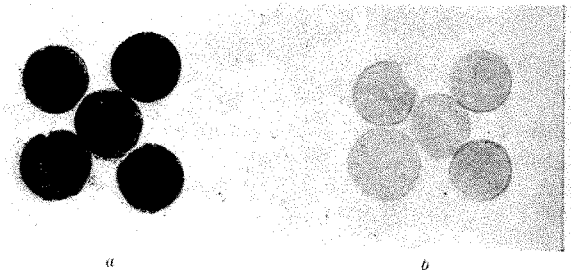


Fig. 1. Disks of polyacrylamide gel, containing chondromucoprotein, stained with alcian blue after incubation at *p*H 4·0 with (*a*) buffer alone, or (*b*) a buffered extract of particle-bound enzymes (see text).

The stained disks could be stored, at least for several months, without deterioration. The method has been used qualitatively to locate enzymes capable of degrading chondromucoprotein in effluent fractions from chromatographic columns, but experiments are in progress to modify the method for quantitation by spectrophotometry.

An adaptation of this method has made it possible to locate enzymes which are responsible for the degradation of chondromucoprotein after disk electrophoresis[10], in a manner analogous to that which has been applied to deoxyribonucleases[12]. Disk electrophoresis gels were prepared in the usual way[10], but with the inclusion of chondromucoprotein (0·05 per cent) in the small-pore gel. The enzyme sample (E 1 cm/280 mμ = 20) was obtained by concentration of the crude preparation described earlier, by means of ultrafiltration, followed by dialysis against a *tris* chloride buffer (*p*H 8·6, 0·01 M). A portion (0·04 ml.) of this preparation was applied to each gel.

After electrophoresis the gels were removed from the tubes and each was halved by a longitudinal cut (with an apparatus based on that of Fairbanks *et al.*[13]). Half of each gel was stained with naphthalene black to show the distribution of protein (Fig. 2*a*), while the other was placed in an ice-cold acetate buffer (*p*H 4·0, 0·1 M) for 30 min, and then in a fresh portion of the same buffer for 30 min at 45° C. After incubation the gels were equilibrated with ice-cold stock *tris*-glycine buffer[10] diluted 1 : 20 and then returned to the destaining tubes for electrophoresis at 8 m.amp per tube (30 min) with the buffer system of Boyd *et al.*[12]. They were then stained with alcian blue, and destained electrophoretically. Enzyme activity was visible as a rather broad, clear zone in the otherwise blue gel (Fig. 2*b*). The mobility of this zone was approximately equal to that of the transferrin band in a standard run simultaneously.

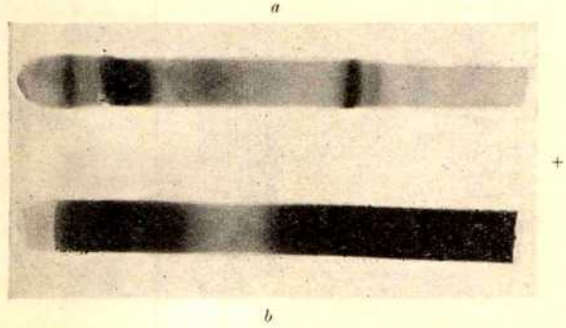


Fig. 2. Half gels showing the result of disk electrophoresis of a preparation of mitochondrial-lysosomal proteins. *a*, Stained with naphthalene black; *b*, stained with alcian blue, as described in the text, to demonstrate chondromucoprotein degrading activity.

Apart from its application to the study of enzymes responsible for the breakdown of chondromucoprotein, the method described here is of interest as a possible model for the way in which chondromucoprotein is held in cartilage. The large molecules of the complex may simply be trapped in a three dimensional network of collagen fibrils so that they cannot be released intact except by destruction of the network.

Previously work[3,4], indicating that chondromucoprotein-degrading activity is present in lysosomes, is supported by the results described here, and it is hoped that, by use of the new methods described, it will be possible to gain further knowledge of the enzymes involved.

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Collagen Levels in Tendon of Wounded and Unwounded Animals

IT was reported by Peacock[1] that the skin of rats bearing a 6 cm skin incision shows a small increase of collagen soluble in salt at sites distant from the lesion. Houck *et al.*[2] found, apparently in contrast, that skin distant from an area of necrosis mediated by croton oil lost salt-soluble collagen. Nevertheless, here the fall in dermal collagen was accompanied by a loss of collagen from the lesion itself, while in the former experiment both wound and distal skin collagen increased during the period of observation. Smith and Wogensen[3] could find no significant decrease of total collagen in the skin of rats distant from a croton oil injury. Adamsons *et al.*[4] found a sustained rise in skin collagen in rats distant from a large paramedian incision. Recently, Houck[5] has postulated that a previously observed rise in acid-soluble collagen in skin distant to a croton oil injury results from labilization of insoluble collagen by a collagenase, which itself shows a fall in concentration[6].

In connexion with a programme of research concerning the dynamics of chemical composition of various tissues in animals carrying lesions of connective tissue, we have measured the quantity of hydroxyproline in tail tendon of rats with a dorsal skin wound. The results are now reported.

Three experiments were performed. In each case, actively growing rats weighing 130–140 g were arranged in groups of four. The groups of four were matched in pairs having as nearly as possible the same average weight, one group of four in each pair being set aside for testing with the lesion, and the other as a control. A separate group of four rats was killed on the same day as the infliction of the wound on the test animals. (This was a 6 cm incision through the dorsal skin and panniculus along the line of the vertebral column. As an extra irritant, a polythene tube 3 cm long (external diameter 6 mm, internal diameter 4 mm) was buried in the cavity, which was then closed with five or six Michel clips.) One group of wounded animals and one group of controls were killed on selected days. In the second experiment only female rats were used, but in the first and third experiments, because of supply difficulties, each group of four rats was composed of two males and two females in the 130–140 g range. Growth rates were significantly lower in the wounded rats up to the fourth day after wounding. Thereafter no difference was apparent between test animals and the controls. After the animals had been killed, 0·2–0·3 g tendon was removed from the tails. Hydroxyproline was assayed by the Leach modification[7] of the Neumann–Logan method.

Table 1. HYDROXYPROLINE CONTENTS OF TAIL TENDON FROM WOUNDED AND CONTROL RATS*

Days after wounding	Experiment	Control animals	Wounded animals
0	1	28·41 ± 3·55	—
0	2	36·18 ± 2·79	—
0	3	99·5 ± 4·5	—
4	1	33·2 ± 1·6	35·2 ± 2·22
4	2	116·0 ± 4·3	122·2 ± 4·4
5	1	25·35 ± 3·6	31·3 ± 1·85†
7	1	37·7 ± 1·72	44·2 ± 1·94†
7	2	104·4 ± 3·1	120·0 ± 2·9†
10	1	28·1 ± 0·65	33·25 ± 1·75†
10	2	41·0 ± 1·63	39·0 ± 2·85
10	3	95·4 ± 3·9	108·3 ± 3·5†
15	1	31·5 ± 1·63	38·1 ± 3·07
15	2	43·5 ± 1·89	38·5 ± 2·02
15	3	106·1 ± 3·3	113·8 ± 1·2

* Results from three experiments, expressed as mean value ± standard error of the mean. For experiments 1 and 2, values are mg per g fresh tissue, and for experiment 3 mg per g dry weight.
† Significantly different from control ($P < 0·05$).

The results are presented in Table 1. There is a transitory but significant rise in tendon collagen in the wounded animals, apparent between the fifth and tenth days after wounding. The results for tendon therefore parallel those of Peacock[1] and Adamsons *et al.*[4] for skin.

The implications of a rise in collagen in connective tissues distinct from those bearing the actual lesion are of great interest. It appears that in the early stages of response to injury, the body proteins may be classified into three groups: (*a*) those which do not maintain their normal tissue concentration and are depleted; (*b*) those which maintain their normal concentration; and (*c*) those which exhibit an elevated concentration. In category (*a*) will fall muscle proteins[8], in (*b*) proteins from liver[8], and in (*c*) we can at the present moment place only collagen. The humoral or other factors controlling this multiple response must be quite complex.

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Effect of Dipicolinate on the Heat Denaturation of Proteins

DIPICOLINIC acid (DPA, pyridine-2,6-dicarboxylic acid) is synthesized during sporogenesis in spore-forming bacteria and excreted during germination as the calcium salt. Heat resistance of spores has long been thought to be associated with the presence of DPA. Church and Halvorson[1] reported a quantitative relationship between heat resistance and DPA content in spores. Black *et al.*[2] found that at a concentration of DPA greater than 50 μg/mg dry spores the heat susceptibility appears to remain constant and minimal.

This communication describes the results of experiments on the heat treatment of proteins in the presence of the sodium or calcium salts of DPA. Human serum albumin solution becomes turbid when heated at 60°–95° C. However, the occurrence of turbidity in 0·05 per cent solution is completely prevented by 10^{-3} M of sodium DPA. A similar effect of relatively small amounts of sodium DPA can also be seen in solutions of bovine serum albumin, ovalbumin and human serum γ-globulin. The following procedure was used for the precipitation test at the isoelectric point. Two ml. of 3×10^{-1} M sodium DPA was mixed with the same volume of 1 per cent bovine serum albumin. The mixture was heated at 65° C. Then 4 ml. of 0·2 M acetate buffer, *p*H 4·8, stored at the same temperature, was added and the solution was cooled for 5 min in water. After centrifugation (3,000 r.p.m., 15 min), the supernatant was filtered. Two ml. of the filtrate was mixed with 8 ml. of the biuret reagent. The mixture was maintained at 37° C for 30 min, and the optical density was measured at 530 mμ. The results are shown in Fig. 1.

Further experiments were carried out with the sulphydryl group of proteins. One ml. of 3×10^{-1} M sodium DPA was mixed with 1 ml. of 1 per cent bovine serum albumin, and then 0·5 ml. of 0·5 M phosphate buffer (*p*H 6·8) and 0·5 ml. of 0·01 M potassium ferricyanide were added. After heating at 90° C, the solution was cooled for 5 min in water, and mixed again with 5 ml. of the phosphate buffer. The optical density of the filtrate was measured at 420 mμ. The results are shown in Fig. 2.

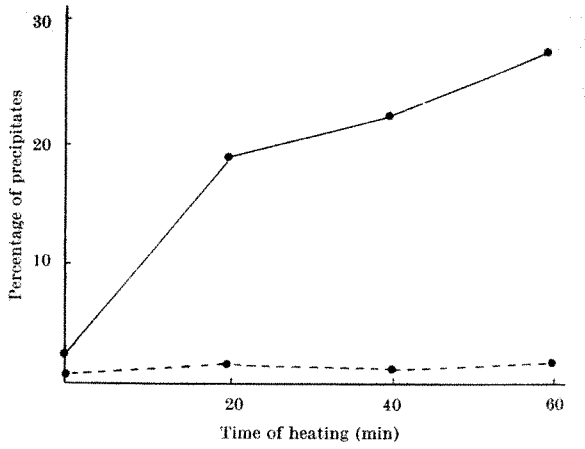


Fig. 1. Precipitation test. The ordinate indicates the percentage of the total amount of protein precipitated. Broken line, material with DPA; full line, control without DPA.

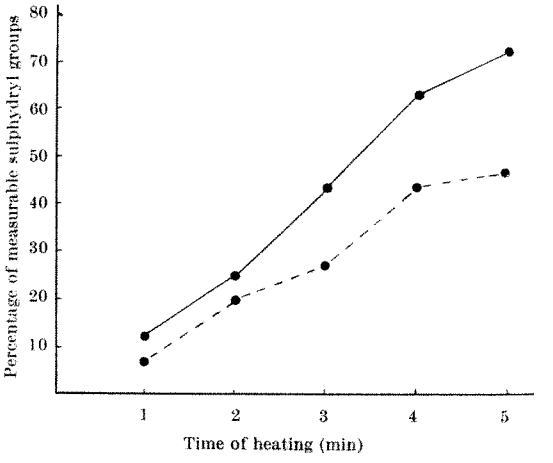


Fig. 2. Appearance of sulphydryl groups. The ordinate indicates the percentage of the total amount of sulphydryl revealed. Broken line, material with DPA; full line, control without DPA.

As shown in Figs. 1 and 2, both the precipitation of protein and the numbers of sulphydryl groups measured are decreased by the addition of sodium DPA. Similar results are obtained when calcium DPA is used. These experiments indicate that DPA has a preventive action on the heat denaturation of proteins. This effect seems likely to be connected with the heat resistance of spores.

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Inhibition of Protein Synthesis by *Cyclo*heximide (Actidione) in *Chlorella*

*Cyclo*heximide is an antibiotic produced by *Streptomyces griseus*. It inhibits the growth of fungi[1,2], algae[3], protozoa[4] and higher plants[5], but has no effect on bacterial growth[1]. In yeast cells[6,7] and mammalian cells[8] *cyclo*heximide inhibits both protein and DNA synthesis simultaneously, whereas the inhibition of RNA synthesis is delayed. With intact cells, it was not possible to resolve the controversy over whether the primary effect of *cyclo*heximide was on the synthesis of deoxyribonucleic acid or on the synthesis of protein. Investigations with extracts free of cells[9,10],

however, showed that the primary effect of *cyclo*heximide was to inhibit synthesis of protein, and that it did this by preventing the transfer of the amino-acids from the *s*RNA–amino-acid complex to the polypeptide chain.

Although growth of algae is also sensitive to *cyclo*heximide, so far as I am aware there have been no investigations of the mechanism of its action in these organisms. Features of *cyclo*heximide inhibition in the green alga *Chlorella pyrenoidosa* have therefore been examined and the results show that the primary effect is an inhibition of protein synthesis and that inhibition of both DNA and RNA synthesis is delayed.

Chlorella pyrenoidosa, strain 211/8 *p* (the name of this organism has recently been changed from *C. vulgaris*[11]), was grown under conditions described previously[12] with ammonium sulphate as the source of nitrogen. After 4 days growth (by which time the cultures had reached the stationary phase of growth) the cells were collected by centrifugation, washed once, resuspended in medium free of nitrogen and the cell density adjusted to 5 mg dry wt. per ml.

The experiments were carried out in darkness at 25° C with ammonium sulphate (0·003 M) as the source of nitrogen and glucose (1 per cent) as the source of carbon. Protein synthesis was measured by following the incorporation of ^{14}C-phenylalanine into the protein fraction, and nucleic acid synthesis was measured by following incorporation of ^{14}C-adenine into the DNA and RNA fractions.

Fractionation of the cells in experiments which involved phenylalanine was achieved by suspending the cells successively in 75 per cent ethanol, absolute ethanol (100° C for 2 min), trichloroacetic acid, ether, and then hydrolysing with N sulphuric acid at 100° C for 3 h. After centrifugation, the residue (that is, the protein fraction) was resuspended in 50 per cent ethanol, 0·1 ml. was plated on to a ground glass disk and the radioactivity counted. Fractionation of the cells in those experiments utilizing radioactive adenine was carried out by the method of Smillie and Krotkov[13]. Preliminary experiments confirmed the validity of this method. Thus when ^{14}C-phenylalanine was supplied and the cells fractionated by the method of Smillie and Krotkov, less than 3 per cent of the radioactivity incorporated could be detected in the nucleic acids, which indicated that there was little contamination between the protein and nucleic acid fractions. Also, when ^{14}C-uracil was incorporated, no radioactivity could be detected in the DNA fraction.

Growth of *Chlorella* is inhibited by *cyclo*heximide at a concentration of 0·25 μg/ml. Protein synthesis in resting cell suspensions (as measured by the incorporation of ^{14}C-phenylalanine) is inhibited by about 70–80 per cent at a concentration of 2·5 μg per ml. (Fig. 1). Increasing the concentration of antibiotic up to 20 μg/ml. has no significant effect on the degree of inhibition. The inhibition of protein synthesis seems to be a primary effect because it can be detected within 15 min of adding the inhibitor, and there is no significant increase in the degree of inhibition with time.

Comparable inhibition of protein synthesis has been observed when $^{14}CO_2$ (under photosynthetic conditions) or ^{14}C-(U)-glucose is assimilated. These experiments also show that *cyclo*heximide has no inhibitory effect on the synthesis of polysaccharide or on the respiration of glucose.

Nucleic acid synthesis as measured by the incorporation of ^{14}C-adenine is also inhibited by *cyclo*heximide (there is no inhibition of incorporation into the cold-acid-soluble fraction). The inhibition is not immediate, however, and inhibition of RNA synthesis is more delayed than is that of DNA (Fig. 2). Thus if incorporation is measured after 30 min, *cyclo*heximide at a concentration of 3 μg/ml. does not inhibit RNA synthesis, whereas DNA synthesis is inhibited by about 30 per cent. The corresponding figures after 90 min are 20 and 50 per cent, and after 2 h RNA synthesis is inhibited by about 35 per cent and DNA synthesis by about 70 per cent. Inhibition of nucleic acid synthesis is also delayed when incorporation of ^{14}C-glycine is measured. In such experiments, however, the delay is shorter than when adenine incorporation is measured. This shorter delay presumably arises because purine biosynthesis is also sensitive to *cyclo*heximide. Although no direct evidence for this has yet been obtained with *Chlorella*, the idea of indirect inhibition of purine biosynthesis by *cyclo*heximide agrees with the results which Widuczynski and Stoppani[14] obtained with yeast cells.

Thus, the inhibitory effect of *cyclo*heximide in *Chlorella* appears to resemble that in yeast and mammalian cells. That is, the primary effect is an inhibition of protein

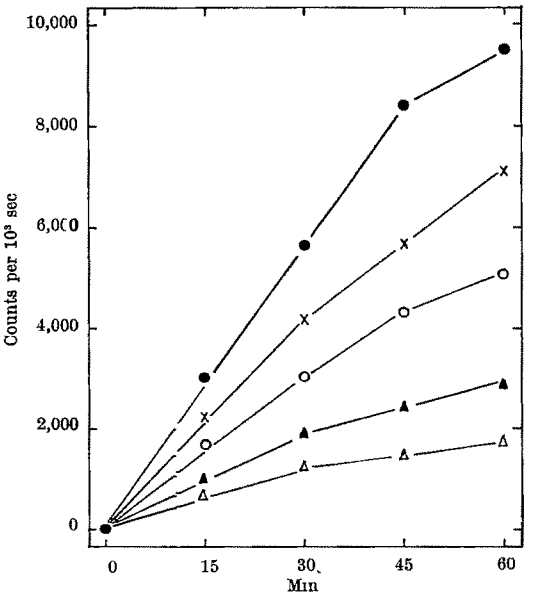


Fig 1. The effect of *cyclo*heximide on the incorporation of ^{14}C-phenylalanine into the protein fraction of *Chlorella* The cultures were shaken in the dark in the absence of *cyclo*heximide (●) or in the presence of the antibiotic at final concentrations of 0·5 (×), 1 0 (○), 1 5 (▲) and 2 5 (△) μg/ml ^{14}C-Phenylalanine (a final concentration of 4 0 mM and a specific activity of 0 3 μc./mmoles) was added at zero time and 3 ml. samples withdrawn at suitable time intervals.

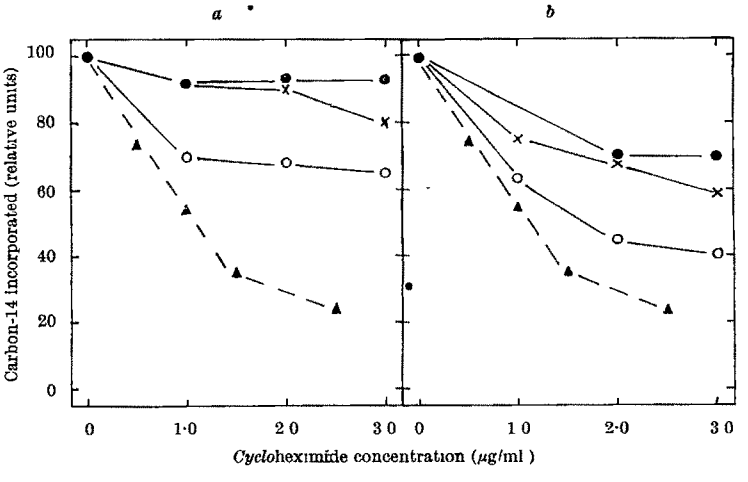


Fig. 2. The effect of *cyclo*heximide on the incorporation of ^{14}C-adenine into (*a*) the RNA and (*b*) the DNA fractions The cultures were shaken in the dark in the presence of various concentrations of *cyclo*heximide, ^{14}C-adenine (a final concentration of 10^{-7} M and a specific activity of 30 mc. mmole) added at zero time and 3 ml. samples were withdrawn after 30 (●), 90 (×) and 120 (○) min. – – ▲ – – , ^{14}C-Phenylalanine incorporation into protein after 30 min (for details see legend to Fig. 1).

synthesis, whereas inhibition of nucleic acid synthesis is indirect.

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Role of Glucose 6-phosphate in the Synthesis of Glycogen by the Rat Liver *in vivo*

SINCE Leloir and Cardini[1] showed that glycogen was synthesized from uridine diphosphate–glucose (UDPG) by liver extracts, it has become commonly accepted[2,3] that the normal mechanism of glycogen synthesis in liver and muscle does not involve a reversal of the phosphorylase reaction but the following sequence of reactions:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{Glucose 6-PO}_4 + \text{ADP}$$

$$\text{Glucose 6-PO}_4 \xrightarrow{\text{phosphoglucomutase}} \text{Glucose 1-PO}_4$$

$$\text{UTP} + \text{Glucose 1-PO}_4 \xrightarrow{\text{UDPG pyrophosphorylase}} \text{UDPG} + \text{pyrophosphate}$$

$$\text{UDPG} + \text{primer} \xrightarrow[\text{synthetase}]{\text{glycogen}} \text{Glycogen} + \text{UDP}$$

Figueroa *et al.*[4] (and others referred to in ref. 4) working with rat liver preparations have suggested that the hexokinase reaction is not an obligatory step in the synthesis of glycogen from glucose. Petrova[5] working with liver slices and Beloff-Chain *et al.*[6] studying the uptake of glucose by the rat diaphragm have reached similar conclusions. Experiments *in vivo* reported here lend support to these latter suggestions.

^{14}C-glucose was injected intravenously into rats and the livers rapidly removed into liquid nitrogen 10 min later. Fractionation of a perchloric acid extract of the liver on 'Dow 1'—formate resin[7] followed by paper chromatography enabled the specific activities of liver glucose, glucose 6-phosphate, UDPG glycerophosphate and lactic acid to be determined (Table 1).

Table 1. RATIO OF SPECIFIC ACTIVITY PER CARBON ATOM IN LIVER METABOLITES TO THAT IN LIVER GLUCOSE

	UDPG	Glucose 6-PO$_4$	Glycerophosphate	Lactic acid
Normal fed rats (5)†	0·646 ± 0·032*	0·291 ± 0·017	0·224 ± 0·012	0·244 ± 0·032
Fasted	0·597	0·392	—	—
Anaesthetized	0·770	0·371	—	—

* Results are means ± *S.E.*
† Number of rats in parenthesis.

If glucose 6-phosphate occupies the position in the sequence glucose→glycogen indicated above, then its specific activity should at least be equal to that of UDPG at this time. A conclusion in support of the claim made by the workers already mentioned that glucose 5-phosphate is not a direct intermediate in the synthetic route glucose to glycogen could be drawn from these data.

Other possibilities for consideration are:

(1) Glucose 6-phosphate compartmentation, that is, the glucose 6-phosphate initiating glycolysis, is separated from that involved in glycogen synthesis. This is pertinent because it has recently been shown[8] that two distinct enzymes are involved in the initial phosphorylation of glucose. The specific activities of α-glycerophosphate and lactic acid are only slightly lower than that of glucose 6-phosphate and it can be calculated from the data that 14 per cent of the glucose 6-phosphate pool should have a specific activity equal to that of UDPG and the remainder equal to that of the glycolytic intermediates, α-glycerophosphate and lactic acid. If phosphorylation of unlabelled glycerol from triglyceride degradation by liver glycerokinase were to occur, then the specific activity of α-glycerophosphate would be lowered relative to glucose 6-phosphate and the size of the glucose 6-phosphate pool involved in UDPG synthesis calculated here would be even lower.

(2) Breakdown of liver glycogen, when the animals are being killed, by phosphorylase to provide an unlabelled source of glucose 6-phosphate from glucose 1-phosphate by phosphoglucomutase. This possibility has been investigated in a rat fasted for 24 h the glycogen reserves of which are much lower than those in fed rats and by taking a liver sample from a rat in which the injection of ^{14}C-glucose and subsequent operations were performed under ether anaesthesia. The ratios of the specific activities of glucose 6-phosphate to UDPG were not grossly different in these rats from those in the fed unanaesthetized rats. Neither of these experiments suggested that glycogen provides unlabelled glucose 1-phosphate.

These results obtained from *in vivo* experiments lend some support to the suggestions based on evidence obtained *in vitro* that glucose 6-phosphate is not an obligatory intermediate in the direct pathway from glucose to UDPG in rat liver, and also that glucose 1-phosphate derived by the action of phosphoglucomutase on this glucose 6-phosphate is similarly not a precursor of UDPG. Because glucose 1-phosphate is the only known precursor of UDPG, at least two pools of glucose 1-phosphate should exist, the average specific activity of which is unpredictable because the contributions from the different sources are unknown.

The lability of glucose 1-phosphate prevented its isolation by the present technique; however, the relationship of the specific activity of glucose-1-phosphate to that of glucose 6-phosphate and UDPG requires investigation in order to resolve the problem of the initial reactions of glucose which occur before metabolic conversion to glycogen and carbon dioxide.

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Use of Sudan Black as an Indicator of Vitamin D on Thin-layer Chromatograms

THE chief problems in the accurate analysis of vitamin D in natural products by physico-chemical methods are: (*a*) the elimination of large quantities of interfering sterols, vitamin A and other carotenoids; (*b*) the need to

measure minute amounts of vitamin D by a reproducible and specific technique.

The structural differences between vitamin D and other related sterols, such as cholesterol, 7-dehydrocholesterol or ergosterol consist only in the presence of one or two methyl groups in the side chain and an extra double-bond in the open ring B of the penteno-phenanthrene moiety. The resolution of these sterol mixtures by column adsorption chromatography is thus not easily effected[1]. Paper chromatography has been investigated extensively as a means of separating sterols and carotenoids present in animal tissues from minute quantities of vitamin D. Because conventional partition technique is generally unsuitable for these strongly lipophilic substances, a reverse-phase system using paraffin impregnated paper and an aqueous organic solvent is preferred[2]. The use of acetylated paper with benzene–methanol mixtures has not been fully explored[3]. Mayer *et al.*[4] have shown that the instability of vitamin D is accentuated on exposure to high alcoholic dilutions or in spots on paper impregnated with paraffin.

Our own recent attempts at the gas-chromatographic separation of vitamin D and cholesterol have not been successful. However, thin-layer chromatography (TLC) using 'Silicagel G' (Merck) and chloroform as solvent was found suitable. Vitamin D was found to be unstable if left in the dry state on the active layers. It was therefore necessary to place the "chromatoplate" in the solvent tank immediately after applying the solution containing vitamin D, and also to extract the vitamin from the plate immediately after chromatography. When this was done, quantitative recovery of 30 μg of vitamins D_2 and D_3 was obtained.

Table 1. MOVEMENT OF SUDAN BLACK, VITAMIN D AND CHOLESTEROL ON THIN-LAYER CHROMATOGRAMS AT DIFFERENT TEMPERATURES

Temperature in °C	Vitamin D R_F value	Vitamin D R_s value (mean)	Vitamin D Standard error of mean	Cholesterol R_F value	Cholesterol R_s value (mean)	Cholesterol Standard error of mean
4	0·45	0·94	0·0003	0 32	0·68	0 001
23	0·40	0 97	0·002	0·30	0 77	0 003
27	0·41	0 98	0·002	0·30	0·76	0·005

R_s value is distance run in relation to that of Sudan black.

During chromatography of colourless substances, such as vitamin D and other sterols on thin layers, we considered using reference dyes which have R_F values similar to that of vitamin D. A fat-soluble dye, Sudan black, was found to have several components, one of which behaved like vitamin D when run on TLC as shown in Table 1. Thus, by running a dye spot alongside the vitamin D, it was possible to predict its position on the plate without the application of locating reagent. The adsorbent on the plate containing vitamin D was collected in a "zone extractor"[5] constructed from a glass column (10 × 1 cm) incorporating a sintered glass filter disk (porosity 3). The vitamin was eluted with chloroform, and a modification of the method of Nield, Russell and Zimmerli[6] was used to detect it. It proved impossible by this procedure to locate less than 0·5 μg of the vitamin.

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Tritiated Angiotensin : Preparation and Purification

THE preparation of tritiated angiotensin with a specific activity of 300 μc./mg in 40 per cent yield was reported earlier[1]. This peptide was purified by chromatography on carboxymethyl cellulose followed by electrophoresis on paper at *p*H 2·1. While electrophoresis on paper gave a pure product, the procedure is tedious and time consuming, especially for the preparation of larger amounts. An improved method of purifying tritiated angiotensin substituting partition chromatography on 'Sephadex G–25' for paper electrophoresis permitting larger samples to be readily purified has been elaborated. A similar chromatographic procedure has been used by Yamashiro[2] for purification of oxytocin. The procedure is illustrated with angiotensin tritiated by the Wilzbach technique[3] rather than the spark discharge procedure used earlier[1].

The starting material, 50 mg of asparagine[1]–valine[5]–angiotensin II (supplied by Dr. R. Schwyzer), was placed under 3 c. of tritium gas at a pressure of 250 mm of mercury at 27° C. After exposure for 2 weeks, the peptide was dissolved in a few millilitres of 0·1 N acetic acid and evaporated to dryness under a stream of nitrogen. This was repeated three times to remove exchangeable tritium. The residue was then dissolved in 5 ml. of the upper phase of *n*-butanol–acetic acid–water (4 : 1 : 5) solvent system and applied to the 'Sephadex' column.

The column was prepared as follows: 'Sephadex G–25' (coarse) was mixed with the lower phase of the solvent system and was allowed to stand for about 4 h. To remove entrapped air, the flask was evacuated to about 20 mm of mercury pressure for 20 min. The slurry of adsorbent was poured into a glass chromatograph tube 22 mm in diameter partially filled with the upper phase of the solvent system. After the particles settled by gravity, the upper phase of the solvent was passed through the column until the excess water (lower phase) had been removed. The finished column was 700 mm high.

The column was developed with upper phase solvent and the effluent was collected in 6·3 ml. fractions. Fractions 85–115 contained the angiotensin and gave a single radioactive peak on paper chromatography using the same solvent system. It was shown to be heterogeneous, however, by paper electrophoresis at *p*H 2·1 (ref. 4). The peptides of these combined fractions were further separated on carboxymethyl cellulose as described previously[1] to yield 7·85 mg (15·7 per cent) of homogenous angiotensin with a specific biological activity of 12 pressor units (ref. 5) for each nanogram and a specific radioactivity of 104 mc./mg.

This partition column has been applied to samples tritiated by the spark discharge procedure used earlier[1], and the yield of peptide, biological activity and radioactivity of the final product was similar to results previously reported. A modified spark discharge procedure, in which the gas phase consisted of 3 c. of tritium at 10 mm of mercury pressure and 4 c.c. helium, gave a crude peptide sample containing less than 5 per cent of the radioactivity of a similar sample run without the helium (these tritiations were carried out by Dr. T. Sullivan). Thus, the spark discharge procedure previously reported remains the preferred method of introducing tritium into angiotensin II.

Radiolysis of tritiated angiotensin into several peptides has been observed when the peptide was stored in 0·1 N acetic acid at 3° C for more than a year. This destruction of peptide can be prevented by addition of 0·1 per cent ascorbic acid to a 0·1 M acetic acid solution of peptide. After 1·5 years of storage of such solutions only 5 per cent of the radioactivity was not contained in angiotensin as demonstrated by paper electrophoresis.

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Glass Microelectrodes: the Origin and Elimination of Tip Potentials

ONE of the most important artefacts in electrophysiology is the microelectrode tip potential[1,2]. Its existence precludes accurate determination of cell membrane resting potentials, particularly with extracellular solutions of low ionic strength where the error may be as large as 50 mV. Adrian[1] found that the tip potential of electrodes filled with 3 M potassium chloride was logarithmically related to the external concentration of sodium chloride or potassium chloride (in an inverse way) and correlated with electrode resistance (correlation coefficient $\approx 0{\cdot}7$). It was concluded that tip potentials are junction potentials produced by some substance blocking the tip of the electrode, reducing the mobility of the chloride ion and exaggerating the difference of mobility of the sodium and potassium ions. This communication is primarily concerned with a simple method for eliminating tip potentials and with an extensive series of observations on the tip potential phenomenon.

The major group of observations was made with 3 M potassium chloride electrodes pulled from 'Pyrex 7740' tubing and filled by the alcohol–distilled-water–potassium chloride method. Measurements of tip potentials were made with a silver–silver chloride reference electrode system and either a Keithley 610*A* electrometer or a Grass *P6 DC* preamplifier. We define the tip potential as the difference between the total circuit electromotive force before and after breaking the tip of the microelectrode, with the sign of the tip potential taken as that of the inside of the electrode relative to that of the outside solution. The results for 558 electrodes are shown in Table 1. Because of the correlation of electrode resistance with tip potential, only electrodes of resistance 1–10 megohms in 100 mM external solution are included.

In the first place it is seen that the experiments of Adrian are confirmed, and in the second it is seen that appropriate trace concentrations of thorium chloride can eliminate the tip potential and that physiological concentrations of calcium chloride significantly reduce it. It is also seen that at certain concentrations of thorium chloride the sign of the tip potential may be reversed. We have observed that frog sartorius muscle fibres can be soaked for 1 h in Ringer's solution containing 100 μM thorium chloride without any observable effect on either resting or action potentials. This is about ten times the concentration needed to eliminate tip potentials.

Several other observations are pertinent to the interpretation of the results in Table 1. (1) If electrodes are filled with 10 mM salt solution and exposed to an identical solution, substantial tip potentials (for example, 10 mV) both positive and negative can be measured. The high electrode resistances (10^9 ohms or greater) prevented a quantitative analysis. (2) If electrodes are filled with 3 M potassium chloride containing 10 μM thorium chloride and no thorium chloride is present in the outside solution, the tip potentials are not affected. (3) If 'Pyrex' glass membranes approximately 0·1μ thick (as evidenced by the presence of interference colours) are exposed to water, they rapidly change their shape and become soft. We believe this is an indication of extensive hydration and swelling. It is known that 'Pyrex' can absorb 1·8 mg of water per c.c. of glass[3,4]. It is probable that the electrical resistance of such thin membranes falls by five to ten orders of magnitude on exposure to water.

Table 1. MEAN AND STANDARD ERROR OF MICROELECTRODE TIP POTENTIALS (IN mV) WITH OUTSIDE SOLUTIONS OF VARIOUS ELECTROLYTE COMPOSITIONS

Composition	Sodium or potassium concentration of outside solution: 1 mM	10 mM	100 mM	1 M
NaCl	−33·9±3·3	−22·2±2·0	−11·9±0·9	−5·5±0·5
NaCl+2 mM $CaCl_2$	−12·6±1·2	−13·8±1·1	−10·0±0·9	−6·0±0·5
NaCl+10 μM $ThCl_4$	+5·0±0·9	+2·3±1·0	+0·8±0·9	−5·4±0·4
KCl	−30·0±2·0	−20·3±1·8	−10·5±0·7	−5·0±0·3
KCl+2 mM $CaCl_2$	−15·3±1·1	−15·2±1·4	−10·3±0·8	−5·1±0·3
KCl+10 μM $ThCl_4$	+9·1±1·0	+6·4±0·9	−0·3±0·9	−4·9±0·2
NaCl+1 μM $ThCl_4$		−8·6±0·9		
NaCl+50 μM $ThCl_4$		+1·2±0·9		
NaCl+100 μM $ThCl_4$		−3·8±0·6		

Although there are several ways to interpret all these observations, we will here discuss only one of them. We believe that these results are most simply explained by considering the tip potential to have originated from an interfacial potential between glass and electrolyte solution and that the system can be approximately described by the equivalent circuit shown in Fig. 1. It is well known that glass-electrolyte interfacial potentials are greatly reduced in solutions of high ionic strength[5]. If electrodes contain 3 M potassium chloride, the internal interfacial potential is practically non-existent. The outside interfacial potential, however, can be substantial and will depend on the ionic strength and species of ions of the outside solution. With symmetrical solutions, differences between the inner and outer surfaces will cause a net potential difference in a manner similar to that presumed for asymmetry potentials in glass *p*H electrodes[6,7]. Near the tip of the electrode the glass wall should be about 0·1μ thick. The critical point in this interpretation is the magnitude of change in specific resistivity when glass this thin is exposed to water. As has already been indicated, our observations suggest to us that extensive hydration and swelling occur. The specific resistivity of 'Pyrex 7740' glass is 10^{14}–10^{15} ohm cm. Where the glass is 0·1μ thick the areal resistivity is 10^9–10^{10} ohm cm^2. If the wall is this thin over 25μ^2, the dry resistance of the wall near the tip would be of the order of 10^{16}–10^{17} ohm. If hydration, swelling and perhaps dissolution cause the resistance to decrease by ten orders of magnitude, the amplitude of the tip potential would be related to a voltage-divider network and the observed correlation of tip potential with electrode resistance would follow from

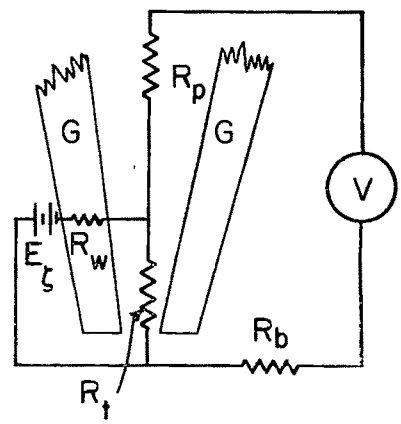


Fig. 1. Equivalent circuit illustrating origin of microelectrode tip potentials. *V*, Electrometer; *G*, wall of micropipette; R_w, resistance of wall near tip, R_p, resistance of bulk electrolyte; R_b, resistance of outside solution; R_t, resistance at tip; E_ζ, outside interfacial potential.

this. That the correlation is far from perfect is an indication that the equivalent circuit in Fig. 1 is at best an approximation.

So far as the action of thorium is concerned, it is well known that glass-electrolyte interfacial potentials can be eliminated by trace concentrations of heavy metal ions while electrochemical junction potential differences are not affected[5]. It should be noted in Table 1 that thorium chloride produces a reversal of sign.

We therefore suggest that tip potentials are related to interfacial potentials, perhaps according to the scheme shown in Fig. 1, but that in any case they can be controlled by appropriate concentrations of thorium chloride in the extracellular solution. A constant error still remains because of the tip potential associated with the intracellular solution, but for experiments involving variation of extracellular ion concentrations, this error is eliminated by treating differences rather than absolute magnitudes.

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3,4-Dimethoxyphenylethylamine in Schizophrenia

Reports of the occurrence of 3,4-dimethoxyphenylethylamine (DMPE) in the urine of schizophrenic patients are attracting increasing attention[1,2], although some of the chromatographic procedures used to detect this amine have been criticized as being insufficiently definitive. This communication reports an investigation in which, using two-way thin-layer chromatography, we have failed to detect urinary DMPE in any of twenty-two schizophrenic patients. In seventeen of the patients the illness was of recent acute onset, the remaining five having been ill for several years. Of the seventeen acute cases, fourteen had received no previous medication at all for their condition, while the other three had been treated with barbiturates only. None of the five chronically ill patients had received drug therapy for at least 3 years. No dietary restrictions were imposed.

Twenty-four hour urine collections were made from each patient and a volume of 400–500 ml. was adjusted to *p*H 9 with sodium hydroxide solution and extracted with chloroform (2 × 200 ml.). The dried extract was shaken thoroughly with N/1 hydrochloric acid (2 × 10 ml.), which was then evaporated to dryness *in vacuo*. The solid residue was dissolved in a small volume of aqueous methanol and chromatographed in two dimensions (15 cm each way) on silica-gel plates, using the solvents described by Kuehl *et al.*[3]. Primary amines were located with ninhydrin reagent. As little as 3 μg of DMPE in each 400 ml. of urine could be detected by this method, as shown by adding appropriate amounts of authentic amine to urine.

Initially, in sixteen cases the presence of a purple spot in very close proximity to the position indicated for DMPE was interpreted as resulting from the latter. Subsequent work, in which two chromatograms were run (one with added DMPE) for each patient, however, indicated that this spot was not exactly coincident with DMPE, and in no case were we able to locate a pink spot corresponding to the DMPE on the duplicate chromatograms. Furthermore, overspraying the chromatograms with Ehrlich's reagent, as described by Friedhoff and Van Winkle[4], failed to demonstrate an orange spot in the DMPE position, except when this amine had been deliberately added. It would thus seem that, in the cases we have studied, either excretion of DMPE did not occur or that the amounts excreted were below the limit of detection of 10–15 μg/24 h.

This result is surprising in view of the large proportion of acute subjects in our sample, a fact which other workers have indicated as being favourable for detection of DMPE. We would tentatively suggest that our use of two-dimensional thin-layer chromatography, with its excellent resolution and thus reduced probability of detecting false positives, plus the definite absence of drug metabolites which are known to interfere with detection of DMPE, might be important factors in the results obtained in this study

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Recoveries of Iocine-131-labelled Iodo-amino-acids in the Plasma Protein-bound Iodine Assay

It is the orthodox view that circulating thyroid hormone consists mainly of thyroxine (T_4), together with small amounts of triiodothyronine (T_3), while the iodotyrosines account for only 0–4 per cent of the total organic iodine[1–4]. There have been reports[5–8], however, that the iodotyrosines account for 30–50 per cent of the iodine in normal human blood. In the condition of thyrotoxicosis, quite large amounts of diiodotyrosine (DIT) are detected in the plasma, although the amount has not yet been precisely quantitated[3,4,9].

Most of the reports on the recovery of organic iodine compounds by means of the various protein-bound iodine (PBI) procedures refer only to the recovery of iodothyronines. However, there are two reports[10,11] which suggest that there are marked differences in the extents of recovery of the iodotyrosines by the two commonly used protein precipitation procedures, namely, the zinc sulphate-sodium hydroxide (Somogyi) system and the trichloroacetic acid (TCA) system.

In view of the increased attention which has been given to the possibility of finding greater amounts of iodotyrosines in the blood of normal people than were previously estimated, we decided to investigate the recovery of iodine-131-labelled monoiodotyrosine (MIT) and diiodotyrosine using both the Somogyi and trichloroacetic acid systems throughout the PBI procedure. At the same time, the opportunity was taken to re-investigate the recovery of iodine-131-labelled T_3 and T_4. The recovery of each iodo-amino-acid was studied after addition to plasma samples of diverse ability to bind thyroid hormone.

Blood was withdrawn from a healthy volunteer, heparinized and the plasma separated under conditions free of iodine. Plasma containing increased amounts of thyroid binding proteins (TBP) was obtained by pooling specimens from four euthyroid, pregnant patients.

Plasma containing a decreased amount of TBP was obtained from two patients with nephrosis and hypoalbuminaemia. The PBI values for the three plasma specimens—namely, for normal binding, for decreased binding and for increased binding—were, respectively, 4·7, 3·2 and 7·9 μg per 100 ml. of plasma (normal range 3·8–7·0 μg per 100 ml.). The values for resin uptake of T_3 were: for normal binding 100, for decreased binding 132, and for increased binding 55 per cent (normal range 70–112 per cent).

Iodine-131-labelled T_3 and T_4 were obtained from Abbott Laboratories at specific activities of approximately 40 μc./μg on receipt. Each was purified by chromatography on filter paper in *n*-butanol : ethanol : 2N ammonia (4 : 2 : 2). After the purified T_3 and T_4 had been located by autoradiography, each was eluted from the filter paper in 10 per cent (v/v) acetic acid, immediately before the plasma samples were enriched with the iodine-131-labelled amino-acids.

Iodine-131-labelled MIT and DIT were manufactured by the iodination of tyrosine with Na ^{131}I. Each was purified by two chromatographic separations on paper in *n*-butanol saturated with 2N acetic acid and eluted as for T_3 and T_4.

About 0·07 μc. in 10 μl. of each iodine-131-labelled amino-acid was then added separately to duplicate 1·0 ml. portions of each of the three samples of plasma, and the enriched specimens were equilibrated overnight at room temperature. The PBI was assayed using two methods for precipitation of protein, namely, the Somogyi method, and with trichloroacetic acid[12]. After precipitation and washing, the protein bound iodine was fired according to the alkaline incineration procedure of Acland[13].

Initial counts were made following precipitation and centrifugation of the protein but before washing the precipitate. Subsequent counts were made after each wash and after firing. Where necessary, counts were adjusted with the appropriate correction factors, including background, decay, dead time and for variation in scaler performance. All counts were made in a volume of 3 ml. of fluid in $\frac{5}{8}$ in. test-tubes, using a scintillator detector fitted with a well-type crystal with the scaler set to 30,000 counts.

The percentages of recovery of the iodoamino acids obtained using the two procedures are given in Tables 1 and 2.

The conclusions are that in the Somogyi system, an average of 94·0 per cent of T_4, 99·1 per cent of T_3, 93·3 per cent of DIT and 87·8 per cent of MIT can be precipitated from plasma of normal thyroid binding capacity (that is, are recoverable following precipitation and washing of the precipitate). There appears to be no alteration in the recovery of T_3 and T_4 when they are added to plasma samples of decreased or increased binding capacity. For both MIT and DIT there appears to be a reduced precipitability in the Somogyi system with plasma of increased T_4 binding capacity, but the number of observations are insufficient to allow statistical analysis.

In the TCA system, the recoveries of pooled results of T_4 and T_3, 93·8 per cent and 94·6 per cent respectively, are of the same order as in the Somogyi system. There are, however, marked reductions in the recoveries of DIT and MIT (8·2 and 3·7 per cent respectively). There are probably no differences in the TCA system between the recoveries with plasmas of differing binding capacity. Losses in firing were tested only for the iodothyronines. For both T_3 and T_4 the losses were negligible (Table 2), and amounted to less than 2 per cent of the activity after the final wash before firing.

The excellent recovery of T_4 and T_3 by Somogyi precipitation agrees with previous reports on this subject[12,13]. This investigation has made the useful observation that the precipitability of iodothyronines is not markedly affected by substituting a TCA for the Somogyi precipitating system. However, Malkin's observation[14] that T_3 is slightly more readily precipitated than T_4 when using the Somogyi system has been confirmed.

The absence of losses on firing, as noted in the present investigation, confirms the findings of Acland[12]. Malkin, however, encountered losses of iodine up to 50 per cent of the total amount during firing. These losses must, in some way, be associated with the ultramicro system used. Possibly the use of a carrier such as the glycine, recommended for the butanol extractable iodine (BEI) procedure[15], may minimize losses in an ultramicro system.

A further observation of practical importance is that the precipitability of the iodothyronines is not altered when studies are performed using plasmas of differing capacity to bind T_4. Conditions such as nephrosis and hypoalbuminaemia cause a decrease in binding of T_4 by the plasma and are associated with a low PBI, while the patient remains in euthyroid status[16]. Conversely, pregnancy and oestrogen treatment cause increased T_4 binding and an associated increase in PBI[17,18]. Our findings eliminate any possibility that the abnormal PBI values have a chemical basis.

A further interesting finding of this investigation was the striking discrepancy between the precipitability of the iodotyrosines by the TCA system and that by the Somogyi system. This discrepancy is more marked than those noted by Tong *et al.*[10] and Blizzard and Mosier[11]. These observations are relevant when considering the controversial claim of Werner *et al.*[6] and of Russell Fraser *et al.*[8] that the normal circulating thyroid hormone in man contains up to 2 or 3 μg per 100 ml. of iodotyrosines in addition to iodothyronines. In that event there should be a marked discrepancy between the PBI values obtained for normal plasma after the two types of precipitation; and this does not seem to occur.

Assuming that there is no iodotyrosine in normal plasma[1–4] it would seem justifiable to replace the widespread use of the TCA precipitation system with the Somogyi system, accounting as it does for at least 95 per cent of the iodothyronines and 85 per cent of the

Table 1. Percentage of Recovery of Iodine-131-labelled Iodotyrosines in Protein-bound Iodine Procedure

	Monoiodotyrosine				Diiodotyrosine			
	TCA		Somogyi		TCA		Somogyi	
	After first wash	After final wash	After first wash	After final wash	After first wash	After final wash	After first wash	After final wash
Decreased binding	84·4	9·2	97·3	85·9	19·8	3·9	96 6	95·3
	34·3	8·7	97·2	86·2	19·0	3 4	94·7	89·9
Normal	35·9	9 0	98·0	85·9	18·6	3·5	96·3	93·8
	31·6	6·9	97·1	89·8	18·9	3 3	97·7	92·8
Increased binding	32·2	7·9	96·8	80 3	19·0	4·2	90·2	78·5
	32·3	7·3	97·5	80 8	19·2	3 7	92·5	87·7

Table 2. Percentage Recoveries of Iodine-131-labelled Iodothyronines in Protein-bound Iodine Procedure

	Triiodothyronine						Thyroxine					
	TCA			Somogyi			TCA			Somogyi		
	First wash	Final wash	After firing	First wash	Final wash	After firing	First wash	Final wash	After firing	First wash	Final wash	After firing
Decreased binding	95·3	94·3	93·6	100·7	98·4	96·7	96·6	93 5	92·4	97·6	95·9	94·3
	91·1	91·0	92·0	99·9	97·7	96·4	97·9	92·8	92·1	95·2	93·5	93 1
Normal	94·1	93·7	93·6	100·1	100·3	98·3	95·9	93·4	92·7	95·8	95·2	93·8
	94·8	96·4	95·4	98·4	97 8	97·2	97 0	95·1	94 3	96·6	95 8	94·2
Increased binding	97·2	96·7	93·3	98·4	96·3	94·1	95 1	94·4	92·1	96·8	91·6	91·3
	97·0	95 3	94 8	100·2	98·4	96·3	95·7	93·6	92·7	99·1	96·1	94 7

iodotyrosines. This should give a sharper differentiation in the border line region of the hyperthyroid state.

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Light-stimulated Oxygen Uptake and Glycolic Acid Oxidase in *Typha latifolia* L. Leaf Disks

The relationship between light and green plant respiration has been the subject of a considerable debate[1] which has been carried over into recent literature[2]. Investigations using simultaneous monitoring of water and oxygen-17 indicated that light-stimulated oxygen may occur in some species but not in others[3–6]. Similar results were obtained in experiments monitoring carbon dioxide absorption and evolution[7].

We have investigated the temperature dependence of photosynthesis and respiration in the broad-leaved cattail (*Typha latifolia* L.). We found light-stimulated oxygen uptake in leaf disks at high temperatures. Zelitch[2] has related such light-stimulated respiration to glycolic acid oxidase activity. The activity of this enzyme is resistant to inhibition by potassium cyanide[8] but is sensitive to inhibition by α-hydroxysulphonates[9]. Glycolic acid synthesis in *Chlorella* is inhibited by carbon dioxide concentrations exceeding 0·1–0·2 per cent[10,11].

In leaf disks of *T. latifolia*, the rate of oxygen consumption at 52° C in the light was more than 150 per cent of the rate in the dark (Table 1). This stimulation was not inhibited by potassium cyanide. Although the rates of oxygen consumption are higher in the lowest concentration of carbon dioxide, the proportionate stimulation by light is similar in the presence and absence of potassium cyanide. This shows that the same system is probably operating at both concentrations of carbon dioxide.

Table 1. Effect of Light and Potassium Cyanide on Oxygen Consumption by Leaf Disks of *T. latifolia* at Two Concentrations of Carbon Dioxide

Treatment	Oxygen consumption (μl./g/h)	
Carbon dioxide concentration:	0·2%	1·0%
Light	184	136
Dark	116	88
Dark + potassium cyanide	108	82
Light + potassium cyanide	198	139

Three to five leaf disks 10 mm in diameter weighing 200–300 mg were floated on 2·5 ml. 0·067 M phosphate buffer, *p*H 7·8, in each of ten Warburg flasks with 0·5 ml. diethanolamine-thiourea solution in centre well adjusted to maintain constant carbon dioxide concentration. A control flask lacking leaf disks was run at each treatment. Flasks were equilibrated with shaking in the dark before manometric determination of oxygen changes on a 'Gilson *GRP*-14' constant-pressure differential respirometer. ($T = 52°$ C.) Illumination from below at 900 ft.-candles. Five min equilibration periods allowed between treatments. Potassium cyanide tipped in from sidearm to make 0·02 M.

Table 2. Effect of a Glycolic Acid Oxidase Inhibitor (2-Pyridyl-hydroxymethanesulphonic Acid) on Light-stimulated Oxygen Uptake by *T. latifolia* Leaf Disks at Different Concentrations of Carbon Dioxide

Treatment	Oxygen consumption (μl./g/h)	
Carbon dioxide concentration:	0·2%	1·0%
Light	191	146
Dark	75	61
Light + inhibitor	134	97
Dark + inhibitor	52	36
Treatment $A \div$ treatment $B \times 100$		
Light ÷ dark	254	241
Light inhibitor ÷ light	70	66
Dark inhibitor ÷ dark	69	60
Light inhibitor ÷ dark inhibitor	256	266

Experimental technique identical to that for Table 1 except *tris*-buffered (*p*H 7·8) 2-pyridylhydroxymethanesulphonic acid added from sidearm to make 0·04 M.

Although insensitivity to potassium cyanide constitutes presumptive evidence that glycolic acid oxidase is the active component, more definitive evidence was provided by experiments with 2-pyridylhydroxymethanesulphonic acid (Aldrich Chemical Co., Milwaukee, Wisconsin), a specific inhibitor of glycolic acid oxidase[9]. Table 2 shows that the oxygen consumption by leaf disks of *T. latifolia* was inhibited 30–40 per cent by this inhibitor and the inhibition was similar in light and dark and at high or low carbon dioxide concentrations. Light and dark rates were equally inhibited, resulting in similar light stimulation in the presence and absence of inhibitor, indicating that glycolic acid oxidase is responsible for oxygen consumption in both illuminated and unilluminated leaf disks. As in the previous experiment, rates were greater in lower concentrations of carbon dioxide, but the proportionate values were similar.

Table 3. Effect of Alternating Light and Dark Periods on Photostimulated Oxygen Uptake in *T. latifolia*

Time (min)	Treatment	Oxygen consumption (μl./g/h)
0–5	Light	191
5–10	Light	176
10–15	Dark	143
15–20	Dark	88
20–25	Light (0·5 min) + dark (4·5 min)	110
25–30	Dark (4·5 min) + light (0·5 min)	77

Experimental technique identical to Table 1 except no equilibration period allowed between treatments. Carbon dioxide concentration was 0·2 per cent.

These data provide support for Zelitch's contention that glycolic acid is the substrate of photorespiration[2], but they also indicate that the same pathway is functional at high concentrations of carbon dioxide and in the dark. In an experiment in which no period was allowed for equilibration between treatments, the rate of oxygen consumption in the light was found to be higher if the light period followed a dark period and higher in the dark if the dark period followed a light period (Table 3). Although the effect on dark rates is undoubtedly due in part to equilibration overshoot, the light pattern cannot be explained on this basis. Instead, it shows a dependence of light reactions on substrates that can be generated in the dark. The ability of a 30-sec light flash to restore 25 per cent of the difference in activity between illuminated and unilluminated treatments substantiates this conclusion. It seems probable, on the basis of these data, that photostimulated respiration at high temperatures in *T. latifolia* leaves results from the oxidation of light-generated substrates in a reaction linked to glycolic acid oxidase.

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PHARMACOLOGY

Alanosine, a New Antiviral and Antitumour Agent isolated from a Streptomyces

DURING the course of a systematic screening for antibiotics with antiviral activity, *Streptomyces alanosinicus* nov. sp. (ATCC 15710) was isolated from a Brazilian soil sample. This culture produced, under submerged conditions, a new antibiotic with antiviral and antitumour activity to which the name "alanosine" was given.

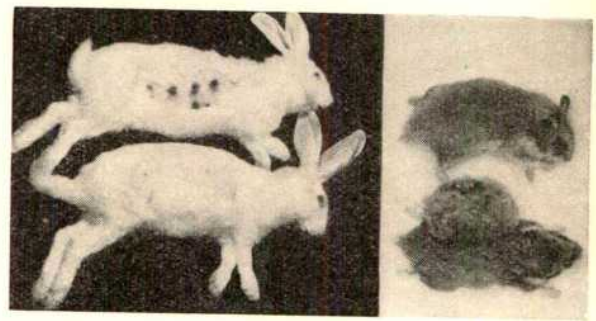
The organism grew well on the standard culture media, forming a light brown to brown vegetative mycelium with a diffusible brown to dark brown pigment. The spores (oval to ellipsoidal) appear in monopodially branched sporophores with tightly coiled spirals. Cultures for antibiotic production were made in a medium containing (per cent w/v) 5·0 glucose, 1·0 dried whale meat, 0·5 calcium carbonate, 0·1 ammonium sulphate, 0·1 Epsom salt, and copper, iron, zinc and manganese in trace amounts. The fermentations were carried out in small fermentors containing 4 l. of this medium. After 72–96 h incubation at 28° C with constant aeration and agitation, spectrophotometric titres of 1,200 μg/ml. were obtained.

After centrifugation the fermentation broth was purified with carbon; the filtrate was concentrated under vacuum at 45° C, acidified with sulphuric acid to separate calcium sulphate and precipitated with an excess of methanol. The purification of the crude product was carried out by dissolving the active substance in acid methanol and reprecipitating by neutralization. The product obtained, with an ultra-violet titre of 40–45 per cent, can be crystallized from an aqueous concentrated solution containing acetic or sulphuric acid. Recrystallization from distilled water yielded a white crystalline powder with an ultra-violet titre of 100 per cent.

Alanosine is insoluble in organic solvents, slightly soluble in water at neutral *p*H, and very soluble in water at *p*Hs lower than 3·5 and higher than 7·0. It gave a positive ninhydrin reaction. An aqueous solution heated with sulphanilic acid and alphanaphthylamine developed a red-violet colour characteristic of a nitroso group. The ultra-violet absorption spectrum showed a maximum at 250 mμ ($E^{1\%}_{1cm} = 630$) at neutral *p*H and at 228 mμ at acidic *p*H. $[\alpha]^{25}_{D} = -37{\cdot}8°$ ($c = 0{\cdot}5$ per cent in water). Microanalytical data: carbon, 24·07; hydrogen, 4·86; nitrogen, 28·15; oxygen, by difference, 42·92. These results correspond to an empirical formula $C_3H_7N_3O_4$ giving a molecular weight of 149·1. The molecular weight determined by potentiometric titration was 155. The structural formula of alanosine as elucidated by ultra-violet, infrared, nuclear magnetic resonance and catalytic reduction studies proved to be L(−)2-amino, 3-nitroso hydroxylamino propionic acid. The antibiotic is very stable at neutral and unstable at acidic *p*H.

The *in vitro* antiviral activity was investigated using a human embryo epithelial cell line. Tubes containing a cell layer 72 h old were incubated for 24 h with various non-toxic dilutions of the compound and then inoculated with 100 $TCID_{50}$ of the virus suspension. When the control tubes showed 100 per cent cytopathic effect (CPE), the protection afforded by alanosine was estimated. Under these conditions CPE was inhibited at 8 γ/ml. for three strains of polio and Coe, and at 2 γ/ml. for a neurovaccinia, a sheep pox and two strains of cow pox viruses.

In the case of neurovaccinia, a significant protection was afforded even when treatment started as late as 24 h after virus infection. Rabbit skin infection through scarification using a neurovaccinia strain was used for the estimation of *in vivo* activity. Topical treatment with a 0·5 per cent ointment administered 18 h after infection, twice daily for 5 days, gave a significant protection. In the control animals the "take" was positive up to 10^{-5} dilution of the stock virus suspension and in the treated ones it was never more than 10^{-2} dilution. Administration of alanosine for 6 days *per os* at 5 mg/kg/day and intramuscularly at 0·5 mg/kg/day gave complete inhibition, even at the site where undiluted virus suspension was inoculated (Fig. 1*a*).



a *b*

Fig. 1. *a*, Antivaccinia activity: upper animal treated with buffer and lower one treated with alanosine *per os* at 5 mg/kg/day. *b*, Antitumour activity: lower animal treated with buffer and upper one with 20 mg/kg/day subcutaneously.

Alanosine has demonstrated a marked antitumour activity against a transplanted fibrosarcoma induced by *SV*-40 virus in newborn hamsters, although the antibiotic did not show any *in vitro* antiviral activity against the virus itself. Six- to eight-week old hamsters were transplanted with the tumour tissue and 24 h later alanosine at 20 mg/kg/day was administered subcutaneously at a site far removed from the zone of transplantation. Final results were taken 21 days after the appearance of a palpable tumour in the majority of the control animals. Although the transplantation efficiency checked over a 2-year period has always been 100 per cent, no tumour developed in any of the treated animals (Fig. 1*b*). A second transplantation was carried out using the treated animals from the previous experiment, and it was observed that the pre-treatment with alanosine did not affect either the period taken for the appearance of a palpable tumour or the further development of it. In another experiment, animals with a well developed tumour were treated subcutaneously once daily with 30 mg/kg, and the controls having tumours of similar size were treated with buffer solution. Weekly measurements of the tumours were taken for 7 weeks. In the control animals, the tumour size increased 400–500 per cent, and was occasionally of the same order as that of the host animal. In the treated group, however, any further development of the tumour was completely arrested. In some cases the size was reduced to such an extent that an accurate measurement was impossible.

Detailed reports on the chemical and biological properties of alanosine will be published elsewhere.

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PATHOLOGY

Trauma and Melanoma Production

IT has long been held that malignant melanoma of man can arise as a result of trauma to a mole (naevus or benign melanoma) or even normal skin[1-4]. Other workers[5-8], however, do not accept this thesis. They believe that as yet there is no authenticated case of a mole having become malignant as a result of trauma and that the local appearance of a malignant melanoma after incomplete removal of a supposed benign naevus indicates that the lesion was already malignant before removal.

Hitherto, only one attempt has been made to investigate this problem in the experimental animal. My colleagues and I[9] traumatized about eighty carcinogen-induced benign melanotic tumours in the Syrian hamster but failed to induce malignant change or even a modest growth stimulation in any of these tumours. In fact many of the lesions regressed as a result of trauma.

This problem has now been investigated in another experimental animal, and forms the subject of this report.

It is now well established[10,11] that deeply infiltrating and destructive melanomata can be produced in various viviparous fishes by hybridization and that these tumours arise as a result of the genetic imbalance produced, which permits the proliferation of macromelanophores and melanocytes. In most instances this proliferation affects large areas of the skin which are thickened (a state referred to by Reed and Gordon[10] as melanosis) and it is from such genetically predisposed sites that the malignant melanoma ultimately arises.

In this respect the piscine model is similar to the human, for most human melanomata arise from pre-existing moles which are considered to be areas of melanocyte proliferation produced as the result of a developmental defect[4]. Therefore, it appeared worth while to investigate the effect of trauma to the skin of *Xiphophorus maculatus* × *X. helleri* hybrids showing various grades of melanosis to see if frank melanomata could be precipitated by this procedure.

With this in view 112 hybrids of both sexes, aged between 3 and 5 months, were divided into two equal groups. The skin of each experimental animal was traumatized in one of the three following ways: (1) Pricking with a needle over a selected area once a week for 3 months. (2) Excising a strip of skin measuring about 5 mm × 1 mm. (3) Excising portions of dorsal or caudal fin through hyperpigmented areas. The experimental and control animals were observed until they died, or for a period of 12 months. During this period three melanomata developed in the control group and four in the experimental group, but in no case did a melanoma arise from, or near, a traumatized site.

These observations indicate that single or repeated mechanical trauma does not induce melanomata to develop in the skin of these hybrid fishes genetically predisposed to melanoma formation. These observations are therefore in keeping with the observation that trauma does not induce malignant change in the benign melanomata of hamsters and also with current opinion that trauma does not precipitate malignant change in human moles.

It is extremely difficult to evaluate the role of trauma in tumour production or in the transformation of benign tumours into malignant ones. There is, however, fairly conclusive evidence that mechanical trauma can produce epithelial tumours from skin that has been pretreated with carcinogens. This has been shown to occur both in the rabbit and man[12]. The situation regarding melanocytes and melanomata, however, seems to be different, for neither recent clinical opinion nor experimental investigation supports the idea that melanomata arise as a result of trauma.

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IMMUNOLOGY

Transfer of Experimental Nephritis in Rats by means of Lymphatic Duct Lymphocytes

THE successful transfer of nephrotoxic nephritis (Masugi's method) in rats to essentially healthy animals of the same inbred strain by parabiosis was described earlier[1,2]. In these experiments, transfer of rabbit antibody from the first into the second rat was excluded by the fluorescent antibody technique[3]. Recently, experiments were reported in which the so-called auto-immune nephrosis was also induced by parabiotic transfer[4]. Furthermore, this disease has been transferred by means of lymph node cells[5]. Nephrotoxic nephritis was "transferred" by peripheral white blood cells of nephritic donors[6]. It was assumed that blood lymphocytes were the immunologically responsible cells and caused the renal lesions in the recipient. It was considered that further investigations of the transfer of circulating lymphocytes obtained from a lymphatic vessel would prove whether or not this assumption was correct.

Sprague-Dawley rats of an inbred strain were injected with 0·5 ml. of nephrotoxic rabbit serum per 100 g body weight. Between twelve and sixteen days later, when the nephritic lesions were fully established, the lymphatic vessel accompanying the superior mesenteric artery was cannulated with a *PE* 50 polyethylene catheter using the Bollmann technique[7]. The animals so treated were kept in a special cage throughout the experiment which allowed forward and backward movement, but prevented the animals from turning around. The rats were fed an ordinary diet and received water *ad libitum*. The outflowing lymph was collected in tubes containing Hanks's solution with 100 units of heparin. Up to 15 ml. of lymph fluid was obtained from a single donor. The recipients of lymphatic material were divided into three groups. The first group of twenty-seven animals received the whole lymph containing 100–500 × 10^6 lymphocytes in 10–12 ml. lymphatic fluid. The material was injected intravenously in from four to thirteen portions. In the second group, twenty-two rats were given isolated lymphocytes in a total of 70–300 × 10^6 cells injected in between two and nine portions. When preparing the cell suspension, the whole lymph was centrifuged for 20 min at 1,000 r.p.m. The supernatant was discarded and the cells were resuspended in Hanks's solution. This procedure was

repeated three times. Microscopic observation using Schreck's method of eosin staining revealed that 80–90 per cent of the cells were alive after this washing procedure. The third group of twenty rats received 7–16 ml. of lymph fluid free of cells by way of between four and twelve injections. The lymph fluid was obtained by high speed centrifugation for 10–15 min. No cells were found on examination under the microscope.

All rats of the three groups were killed 10 days after the final injection. Their kidneys were removed and after decapsulation fixed in 10 per cent formol. After a careful imbedding procedure which lasted 6 days, 5μ paraffin sections were stained with haematoxylin–eosin and periodic acid-Schiff (PAS). Controls were performed by injection of lymphatic material from healthy donors prepared in the same way as in the experimental groups.

The results are summarized in Table 1. Six out of twenty-seven animals which received whole lymph developed nephritic lesions. Positive results were obtained only if lymphatic fluid containing more than a total of 200×10^6 lymphocytes was given. Similar relations were found in the second group. Four animals out of twelve developed renal disease when injected with more than 200×10^6 pure cells, whereas all ten rats receiving only $70–200 \times 10^6$ cells showed no lesions. In the third group only one rat out of twenty developed nephritis. This animal had been injected with a total amount of 16 ml. of lymph free of cells.

When investigated histologically, it was found that the kidney lesions were mainly confined to the glomerula. They consisted of enlargements of the tufts, thickening of the basement membrane, adhesions between the loops, and increases in the endothelial and epithelial cells both in number and in size. Occasionally, adhesions occurred between loops and the Bowman's capsule. Crescent formation was never observed. Epithelial cells of the proximal convoluted tubules contained hyalin droplets, but casts were seldom found. In the interstitial tissue of the external cortex some small perivascular infiltrates of lymphocytes were found. The alterations were the same as those found earlier during successful transfers of nephrotoxic nephritis by parabiosis or by peripheral white blood cells[8]. The severity of the kidney lesions is less than in nephrotoxic nephritis and can be compared with the membranous type of human glomerulonephritis despite certain typical differences. Sections stained with PAS showed PAS-positive material in the thickened basement membrane and around the cytoplasm of the mesangial cells. Studies with the fluorescent antibody technique showed that the lymphatic cells of the donors, whether suspended in the original lymph or washed with Hanks's solution, did not contain traces of the rabbit anti-rat kidney serum which might have been responsible for the nephritis induced in the recipients.

From their histological appearance the renal lesions observed in the recipients would be classified as nephritis. The morphological pattern is the same as that of renal changes produced by parabiotic transfer. There is considerable evidence for the existence of a secondary nephritis inducing factor, which is possibly part of an auto-immune mechanism. Attempts have failed to induce renal lesions by serum alone from donors with nephrotoxic nephritis or with auto-immune nephrosis. Likewise, in other experimental auto-immune diseases such as allergic encephalomyelitis[9], thyroiditis[10], and aspermatogenesis[11], serum, even if circulating antibodies were present, did not lead to successful transfers. On the other hand, it was possible to induce experimental thyroiditis[12] and experimental auto-immune nephrosis[5] with lymph node cells in an essentially healthy recipient. Reactions arising from histo-incompatibility were excluded by inducing immunological tolerance in the recipients or by using inbred strains. It should be emphasized that our experiments—in contrast to those of other authors working in this field—were performed without Freund's adjuvant. This seems to be of special importance so far as the induced renal lesions are concerned. In our experiments the possibility of alterations in the glomerula caused by Freund's adjuvant alone certainly can be excluded.

Table 1. Transfer of Renal Disease by Lymphocytes

Material injected	No. of recipients	Amount of cells (lymph) injected	No. of injections	Survival time after the final injection	Histological examination: Normal	Nephritis
Whole lymph	6	$100–200 \times 10^6$	4 to 13 ×	10 days	6	0
	12	$200–300 \times 10^6$		10 days	9	3
	9	$300–500 \times 10^6$		10 days	6	3
Washed lymphocytes	10	$70–200 \times 10^6$	2 to 9 ×	10 days	10	0
	12	$200–300 \times 10^6$		10 days	8	4
Cell-free lymph	7	7–10 ml.	4 to 12 ×	10 days	7	0
	13	10–16 ml.	4 to 12 ×	10 days	12	1

Further investigations should elucidate the part played by the injected cells and also their fate. With the fluorescent antibody technique no traces of the nephrotoxic rabbit antibody were found on the surface of the lymphocytes. Nevertheless, the transport of antigen-antibody complexes by the injected cells cannot be completely excluded because of the possibility that they were masked in the interior of the cells. It is, however, unlikely that those small amounts of transferred nephrotoxin would be sufficient to cause renal disease. It is more likely that the lymphocytes are effective by synthesizing antibodies systemically or locally in the target organ. On the other hand, they might only give information to the immunologically competent system of the recipient; in this case the mechanism responsible for kidney injury would represent a second step.

There is some evidence for the latter hypothesis. Experiments (as yet unpublished) with lymphocytes labelled with tritiated thymidine, which belong to the same series as those described here, indicate that the transferred cells do not adhere to the glomerular capillaries. Autoradiographs showed no special avidity for the lymphocytes for the renal tissue.

These findings support the suggestion that a transfer factor similar to the findings of Lawrence[13] in tuberculin hypersensitivity causes nephritis in the recipient animals.

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Antigenic Constituents of Human Cortical Grey Matter

Recent work in this laboratory has been directed towards the development of improved methods for the extraction of proteins of human brain, and their chemical and immunological characterization. Thus Bogoch, Rajam

and Belval[1] have demonstrated a large number of protein entities in an extract of low ionic strength of human cortical grey matter. This extract (*A*) was prepared by cold homogenization in 0·005 M phosphate buffer, *p*H 7, and ultracentrifugation at 80,000*g* to yield a clear supernate. Chromatography of concentrated *A* on DEAE cellulose, using a gradient increasing in ionic strength and decreasing in *p*H, indicated the presence of at least fifteen main protein groups; that these chromatographic groups were heterogeneous was suggested by the results of assays for hexose, hexosamine, neuraminic acid and protein in each tube of chromatographic effluent as well as by electrophoretic findings. The total number of distinct or partially related proteins appeared to exceed a hundred. Supporting evidence, obtained in work on animal brain material, has been adduced by others[2].

This communication is concerned with a preliminary assessment of the minimum number of distinct major antigens present in *A*. Rabbit antiserum against total extract *A* was prepared using complete Freund's adjuvant emulsified with 10–12 mg quantities of total *A* protein for each dose. Nine such doses were administered by the intramuscular route, over a 9 week period, and the rabbits were bled 14 days after the final dose. Serum was absorbed with whole human serum (anti-*A*/HUS) to minimize reactions with serum proteins present as contaminants in *A*, when the following experiments were run.

Macro double-diffusion reactions were performed in 1 per cent Noble agar, using saline–borate buffer *p*H 8 (ionic strength 0·16) as the equilibration medium. *A* was made to react against anti-*A*/HUS and, for comparison, with unabsorbed *A*. Original wet preparations were photographed, washed, dried and stained with amido-schwartz 10*B*. Samples of both types of preparation are presented in Fig. 1. Results indicate that *A* contains a minimum of nine distinct antigenic components, three of which are either identical with human serum proteins or are contaminants of serum protein. The *A* : anti-*A*/HUS interaction apparently indicates that the major precipitin arc was identical with serum protein on the basis of deflexion of that end of the arc approaching the human serum reservoir. That this was a physical deflexion which resulted from the length of the arc in relation to the periphery of the reservoir is suggested by the absence of deflexion of the same arc in the *A* : anti-*A* (unabsorbed) reaction, where it suffered no deflexion in the area of diffusing serum proteins. The wet preparation suggested that the arc nearest the *A* reservoir in the *A* : anti-*A* (unabsorbed) reaction could be identified with a major serum protein arc; however, the stained preparation clearly indicated that no such reaction occurred.

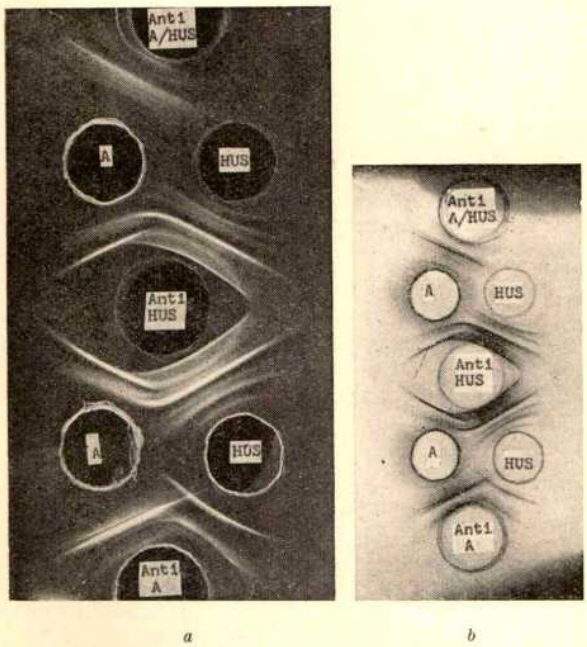
These results suggest that the antigenic heterogeneity of the component proteins of *A* does not parallel the molecular heterogeneity which we reported earlier; indeed, a unit relationship between variants of a given molecular type and individual antigenic species need not necessarily be expected. A similar situation was encountered in analogous experiments on chemical and antigenic properties of lens proteins[3]. In additional work, however, *A* has been chromatographically fractionated into two distinct complexes of basic (*B*) and progressively acidic (post-basic, *PB*) proteins. Immunoelectrophoretic examination of the separated, concentrated *B* and *PB* fractions indicated a minimum content of four and seven antigenic components respectively, one of which is a protein resistant to 100° C for 1 h, which can be precipitated with ethanol at a final concentration of 75 per cent. The total number of antigenic components so far detected is thirteen. Thus, further sub-fractionation and concentration of sub-fractions may result in the detection of a considerably larger number of antigenic components in dilute salt extracts of human grey matter.

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a *b*

Fig. 1. Reactions of human grey matter extract with homologous antiserum. HUS, Human serum 1 : 20 dilution; *A*, extract *A*, 5 mg/ml. total protein; anti-HUS, rabbit anti-human serum; anti-*A*, rabbit antiserum against extract *A*, unabsorbed; anti-*A*/HUS, anti-*A* absorbed with human serum. *a*, Original wet preparation; *b*, preparation after washing, drying and staining with amido-schwartz 10*B*.

Organ Specificity of Heat-stable Antigens from Human Brain

A PRELIMINARY assessment of a neutral aqueous extract of low ionic strength of human grey matter (extract *A*) has indicated that there are nine distinct major antigens, of which three are either human serum protein contaminants or species-specific antigens shared with serum[1].

This communication reports the presence in *A* of five or six tissue antigens of the class known as "*BE*" (ref. 2)—this nomenclature denotes that they are resistant to boiling for 1 h and can be precipitated at an ethanol concentration of 70–75 per cent. Milgrom *et al.*[2] demonstrated earlier that such an antigen can be extracted from human grey matter by methods somewhat different from those reported here.

Scheme I outlines the method of extraction of normal human cortical grey matter to yield extract *A*, and the preparation of both crude and purified heat-stable antigens (*HT*) from *A*. Heat-stable antigens were prepared in the same manner from whole normal human liver and kidney. Rabbit antiserum against purified brain *HT* (anti-*HT*) was prepared using complete Freund's adjuvant emulsified with 0·64 mg protein for each dose. Between three and five doses were administered to rabbits by the intramuscular route at intervals of 10 days, and the animals bled 10–15 days after the final dose.

Fig. 1 shows the antigenic inter-relationships of *HT* from human liver, kidney and brain when they were made to react in double-diffusion tests against anti-*HT*.

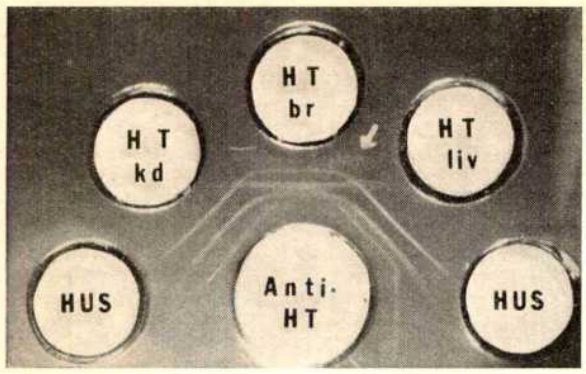


Fig. 1. Antigenic inter-relations of heat-stable antigens from human brain, liver and kidney as revealed by double-diffusion in agarose. *HT*, Heat-stable antigens. Source is indicated by *br* (brain), *liv* (liver), *kd* (kidney). Approximate antigen concentration 1 to 2 mg/ml. Anti-*HT*, Rabbit antiserum against brain *HT*; HUS, human serum, 1 : 10.

HUMAN GREY MATTER

Blender 3 min, 5° C
0·005 M phosphate, *p*H 7

Centrifuge 13,200*g*, 1 hour, 5° C

Residue — Supernate

Re-extract — Pooled supernates

Ultracentrifuge, 80,000 *g*, 20 min, 5° C

Supernate — Residue

Extract A

Dialyse against 0·005 M phosphate, *p*H 7, 5° C

Heat, 100° C, 1 hour

Centrifuge 40,000*g*, 30 min

Residue — Supernate
Crude heat-stable antigens

Ethanol, 5° C, 75 per cent final concentration

Supernate — Precipitate, soluble in 0·15 M sodium chloride

Reprecipitate from 0·15 M sodium chloride

Purified heat-stable antigens

Scheme I. Outline of preparative method.

Two species-specific antigens were common to *HT* from all three sources; with the antiserum used, brain *HT* exhibited three immunoprecipitin arcs not shared by liver or kidney *HT*. The three preparations contained a species-specific component which was also present in human serum.

The fact that we were unable to detect three of the heat-stable antigens from the aqueous extracts of human brain in comparable extracts of tissues usually rich in species-specific antigens suggests that they are organ-specific. Our investigations are being extended to a larger number of representative human tissues in order to evaluate these preliminary findings.

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MICROBIOLOGY

Microbial Decomposition of Various Fractions of Ureaformaldehyde

CONSIDERABLE interest has been shown in the mineralization of ureaformaldehyde, a compound with "slow release" properties of value in fertilizer technology. It has been suggested that the release of ammonium–nitrogen from this polymer is biological[1], although Winsor and Lang[2] proposed that the first stage of decomposition may be non-biological. Commercial ureaformaldehyde is composed of various chain length polymers, which can be arbitrarily grouped into the cold water soluble (CWS), hot water soluble (HWS) and hot water insoluble (HWI) fractions.

In these investigations, release of ammonium–nitrogen was followed using the soil perfusion technique and the accumulation of nitrate–nitrogen (nitrification) was used as an index of mineralization. Fifty grams of Guelph loam (2 to 5 mm mesh, *p*H 7·0–7·3) were perfused with 200 ml. of distilled water. The soils were treated with urea, ammonium sulphate, CWS and HWS fractions at concentrations equivalent to 0·4 mg of nitrogen/g of soil. Nitrate–nitrogen in perfusates was measured using the phenoldisulphonic acid procedure and was recorded as μg nitrogen/ml. of perfusate.

Both urea and ammonium sulphate were nitrified after lag periods of 1–2 days, following which nitrate–nitrogen accumulated at about the same rate from both nitrogen sources. The CWS fraction showed a slightly longer lag of from 4 to 5 days, and a lesser slope of accumulation; the HWS had a lag of from 6 to 8 days and a greatly reduced slope of accumulation of nitrate–nitrogen (Fig. 1*A*). It was obvious that the mineralization of the CWS and HWS fractions was limiting the rate at which nitrate–nitrogen accumulated in perfusates containing these materials. If this process of release of ammonium–nitrogen is biological, then it is logical that microbial "enrichment" should occur after the logarithmic phase of mineralization of either CWS or HWS. Moreover, in this particular system a double "enrichment" should occur, first, by the population of organisms capable of splitting or mineralizing the particular ureaformaldehyde polymer, and second, by the population of nitrifiers developing on the released ammonium–nitrogen.

Soils were therefore treated with the CWS fraction and perfused to enrich the soil with both groups; the soils were then washed free of nitrate–nitrogen and replicated units were treated with ammonium sulphate, CWS and HWS fractions and re-perfused with distilled water. In this experiment (Fig. 1*B*) ammonium sulphate was nitrified without any lag indicating that the soils were enriched with nitrifying bacteria in the sense of Lees and Quastel[3]. Both the CWS and HWS fractions were nitrified without a lag, indicating that the initial treatment with CWS enriched the soil with mineralizing organisms as well as nitrifying bacteria. Moreover, the organisms associated with the decomposition of the CWS fraction were also involved in the decomposition of the HWS fraction.

Because the decomposition of the fractions used was obviously microbial, an attempt was made to isolate the soil organisms responsible. This was carried out using a basal mineral salts–glucose liquid medium in tubes, supplemented with CWS–nitrogen at a concentration of 50 mg nitrogen per 100 ml. of medium. The enriched soils were diluted and portions transferred to tubes. After incubation, streak transfers were made from the highest dilutions showing turbidity to a solidified CWS medium. After 7 days at 27° C, representative colonies were selected, purified and maintained on the CWS medium.

One of the isolates (strain *C*2) obtained in this manner was representative of the majority of colonies appearing on plates, and was a Gram-negative rod showing the

peculiar morphology described by Jensen[4] for a species of *Agrobacterium*. Growth was abundant in most routine media; nitrates were reduced. Acid and gas were produced from glucose, fructose, mannose and maltose but not from arabinose, rhamnose, xylose, galactose, lactose and sucrose. Fats were not hydrolysed; urease activity was only slight and gelatine was hydrolysed.

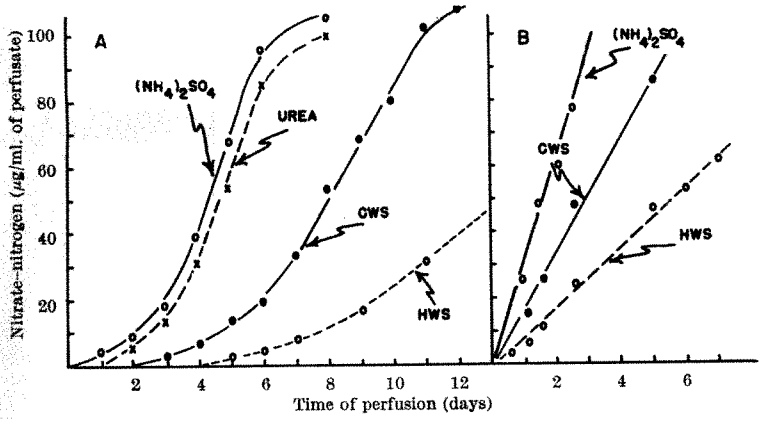


Fig. 1. *A*, Nitrification of ammonium sulphate, urea, CWS and HWS fractions of ureaformaldehyde in perfused Guelph loam. *B*, Nitrification of ammonium sulphate, CWS and HWS fractions of ureaformaldehyde in perfused Guelph loam previously perfused with CWS fraction.

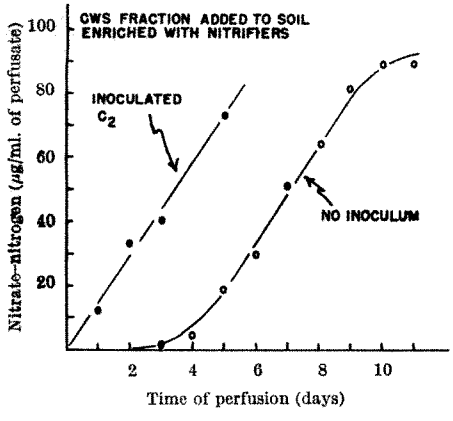


Fig. 2. Nitrification of CWS fraction of ureaformaldehyde in nitrifier-enriched, perfused Guelph loam, uninoculated and inoculated with CWS decomposing bacterium (strain *C*2).

To test the ability of this bacterium to mineralize the CWS fraction of ureaformaldehyde in soil, soils were first "enriched" with nitrifying bacteria by perfusing with N/100 ammonium sulphate solution and then washed free of nitrate–nitrogen. All units received a further CWS treatment and were then perfused again, one set not inoculated, the other set inoculated with $1{\cdot}5 \times 10^7$ washed cells (strain *C*2) per gram of soil. The results obtained are shown in Fig. 2. They indicate that this bacterium was one of those responsible for the decomposition of the CWS fraction, and provide further evidence for the biological nature of ureaformaldehyde mineralization in soil.

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Distinctive Behaviour of *Eperythrozoon coccoides* in Plasma and Spleen of Infected Mice

Eperythrozoon coccoides is a minute organism of somewhat uncertain taxonomy, at present classified among the Protophyta[1] (order, Rickettsiales; family, Bartonellaceae). It is indigenous to mice and induces in them a mild disease characterized by splenomegaly and general lymphadenopathy[2]. It is frequently present in transplantable tumours[3] and it is possible that it is, in part, responsible for the splenomegaly which follows transplantation of such tumours. The pronounced synergism of *E. coccoides* with a variety of other infectious agents is now well known.

It was previously reported[4] that, on the fourth day after infection, the plasma of splenectomized mice was about one hundred times as infective as the plasma of intact mice; it appeared, therefore, that the spleen, though itself infective, was a deterrent to the activity of the plasma. The reason for this was not apparent, but it has served to direct attention to the distinctive behaviour of *E. coccoides* in the plasma and spleen as, for example, in thermal stability, and in the apparent association of *E. coccoides* with an inhibitor present in plasma but not in spleen.

Infectious material from mice was prepared[5] and two procedures used to determine biological activity according to methods previously described[4]. Both procedures used to determine biological activity depend on the increase in spleen weight of mice 7 days after intraperitoneal infection. One method expresses results as spleen weight per gram body weight; the other is more elaborate and expresses them in "50 per cent spleen enlarging dose" (SED_{50}) units. The SED_{50} is the infectious unit and is defined as the highest dilution of material which will produce a spleen weight of at least 10 mg/g body weight in 7 days in 50 per cent of the mice when inoculated intraperitoneally. In each method an experimental point represents an average result from five mice.

The thermal lability of *E. coccoides* in spleen extract and its stability in plasma are shown in Table 1. It can be seen that after incubation at 37° C for 30 min the titre of a spleen extract was reduced by more than 99 per cent, whereas that of plasma was unchanged even after 60 min at this temperature.

Table 1. Relative Stability of *E. coccoides* at 37° C in Spleen Extract and Plasma

Time at 37° C (min)	Log SED_{50}/0·1 ml. Spleen extract	Plasma
0	6·0	6·7
30	3·2	6·7
60	—	6·7

Fig. 1 suggests that *E. coccoides* is associated with an inhibitor present in infectious plasma. It illustrates a phenomenon repeatedly observed, namely, a marked increase in response of mice to successive dilutions of infectious plasma. The response reaches a maximum at a dilution of 1×10^{-3}, and decreases thereafter. Fig. 1 also shows that *E. coccoides* does not exhibit this behaviour in spleen extracts. These results are interpreted as indicating the presence in plasma of an inhibitor of *E. coccoides* loosely associated with the organism. Frozen plasma did not exhibit the increase in response on dilution that occurred with the same lot of fresh plasma; its SED_{50} units were, however, unchanged.

It has been reported[6] that *E. coccoides* was "entirely" dissolved by trypsin. This suggests that proteolytic enzyme activity of the spleen may be responsible for the

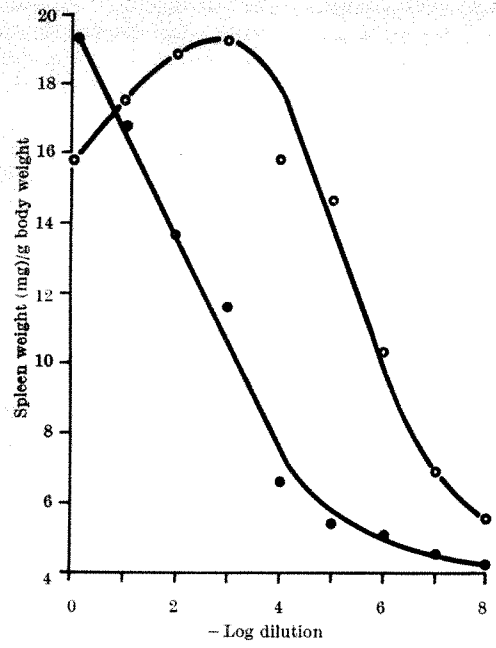


Fig. 1. Comparison of response of mice to dilutions of infectious plasma (○) and spleen extract (●).

lability of the organism in spleen extracts, and for the markedly lower infectivity of plasma of intact in relation to splenectomized mice. The stability of *E. coccoides* in plasma could be explained by the protection afforded it by the property of plasma to inhibit proteolytic enzyme activity[7].

The nature of the inhibitor of the pathological expression of *E. coccoides* that is responsible for the increase in spleen weight, and its site of action, are not known. It seems to be intimately associated with the organism and could, therefore, exert its action at this locus; on the other hand, it could affect the reactivity of the spleen *per se*, inasmuch as increase in spleen weight was the only index of infection used in these experiments. The inhibitor is unlikely to be a conventional antibody because, in contrast to antibody, it was no longer evident after the organism had been stored in the frozen state.

Evidence for such an inhibitor was not found in extracts of spleen, possibly for the same reason that *E. coccoides* is sensitive to incubation at 37° C in this milieu, that is, the inhibitor may be destroyed by the enzyme activity of the spleen.

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VIROLOGY

Interfering Component of Vesicular Stomatitis Virus

INTERFERENCE during the replication of vesicular stomatitis virus (VSV) was first described by Cooper and Bellett[1], who suggested that the interfering activity which was present in viruses collected after several successive undiluted passages resulted from a transmissible component. Although they did not identify the component, evidence was presented that it was not the virion. Cantell *et al.*[2] obtained interference whenever a high multiplicity of infection was used, irrespective of the passage history of the virus. Studies in this laboratory[3] have shown that viruses collected from both high and low multiplicities of infection possess interfering activity, although repeated undiluted passage of virus is more likely to give a product with marked interference effects. These studies also showed that the interference does not result from interferon but is probably caused by an incomplete form of the virus.

Cells infected with VSV produce several complement fixing components in addition to the infective particle[4,5]. Among these is a particle which closely resembles the rounded end or "cap" of the virion. Hackett[6] suggested that this particle might be the interfering agent, and Huang and Wagner[7] reported that virus cultures of low titre produced by several successive undiluted passages consisted almost entirely of these smaller particles. The work described here shows that interference, in fact, arises from this small particle.

Virus of the Indiana sub-type was produced by infecting monolayers of baby hamster kidney cells (*BHK* 21—clone 13, ref. 8) at a low virus-cell ratio. The virus thus cultured was separated from cell debris by centrifugation at 2,000 r.p.m. and then fractionated by centrifuging in an *SW* 25 rotor for 2 h at 20,000 r.p.m. through a 15–45 per cent sucrose gradient. This procedure fractionated the virus suspension into four well defined peaks of complement fixing activity[5]. Peak *A* (fractions 7–11) contained the virion, peak *B* (fractions 12–16) consisted mainly of rosettes and helices together with the cap referred to above, and peak *C* (fractions 17–20) contained small rosettes. The antigens in peak *D* (fractions 21–23) were too small to identify in the electron microscope. These individual peaks were assayed for their interfering activity by measuring the depression of virus yield when monolayers of *BHK* cells were incubated with dilutions of the test material and then challenged with a small inoculum of VSV at a virus : cell ratio of $\leqslant 1:100$. The yield of infective virus 24–48 h after challenge was measured.

The results in Table 1 show that peak *B* contained the greater part of the interfering activity, although the virion fraction, peak *A*, was also slightly active. The high activity of peak *B* compared with the virion was confirmed by the observation that incubation with a 100-fold dilution of *B* depressed the yield of infective virus as much as undiluted peak *A*. It is of interest that

Table 1. INTERFERING ACTIVITY OF FRACTIONS OBTAINED BY SUCROSE GRADIENT CENTRIFUGATION OF VESICULAR STOMATITIS VIRUS

Sample centrifuged	Fractions from gradient	Infectivity (P.F.U./ml.)*	Complement fixing activity (ml. 1/30 *C'* fixed by 1 ml.)	Infectivity of culture in interference test (P.F.U./ml.)
Unfractionated virus	7–11 (*A*)	$3{\cdot}4\times10^{7}$	21	$5{\cdot}0\times10^{8}$
	12–16 (*B*)	$2{\cdot}1\times10^{6}$	70	$1{\cdot}1\times10^{8}$
	17–20 (*C*)	$1{\cdot}7\times10^{6}$	86	$1{\cdot}4\times10^{7}$
	21–23 (*D*)	$6{\cdot}6\times10^{6}$	26	$9{\cdot}0\times10^{7}$
	Control for interference test	—	—	$7{\cdot}8\times10^{8}$
Peak *A* from first gradient	7–11	$3{\cdot}0\times10^{5}$	—	$2{\cdot}0\times10^{7}$
	12–16	$7{\cdot}0\times10^{5}$	—	$6{\cdot}7\times10^{7}$
Peak *B* from first gradient	12–16	$1{\cdot}0\times10^{4}$	—	$8{\cdot}0\times10^{8}$
	17–19	$2{\cdot}0\times10^{5}$	—	$1{\cdot}0\times10^{8}$
	Control for interference test	—	—	$4{\cdot}7\times10^{8}$

* P.F.U., plaque forming units.

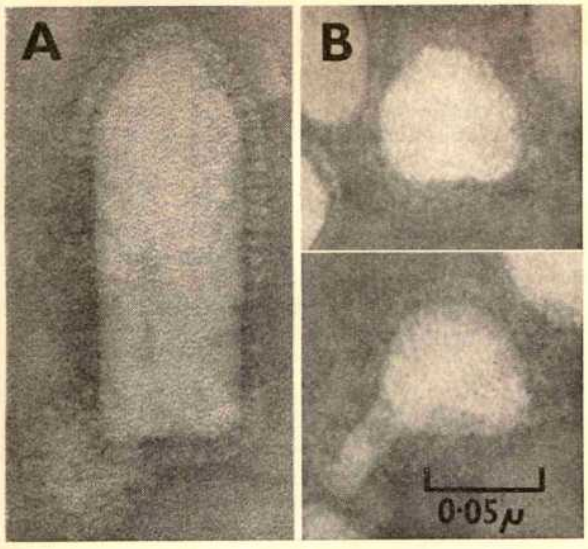


Fig. 1. Electron micrographs of (*A*) the virion and (*B*) the interfering component of VSV. The virion was isolated from a sucrose gradient and the cap from a tartrate gradient. Each sample was dialysed against 0·1 M ammonium acetate, stained with 3 per cent phosphotungstic acid, *p*H 6·0, and examined in a Siemens Elmiskop I electron microscope.

fractions containing considerable interfering activity delay the onset of cytopathic effects so that the sheets remain on the glass. This observation in itself can serve as a rough measure of interfering activity.

To ascertain whether the small activity of peak *A* was a result of contamination with components from peak *B*, portions of each peak were dialysed and then centrifuged through two separate sucrose gradients. The results of this experiment are also shown in Table 1 and provide evidence that the virion itself contains a small amount of interfering activity.

As the major interfering fraction is known to contain at least three components[5], it was fractionated further by centrifuging in a potassium tartrate gradient. A portion of peak *B* from a sucrose gradient was first dialysed to remove the sucrose and 0·5 ml. layered on a preformed gradient prepared from 2·4 ml. of tartrate, density 1·02 g/ml., and 2·0 ml. of tartrate, density 1·30 g/ml. After centrifuging for 2 h at 35,000 r.p.m. in an *SW* 39 rotor of the Spinco ultracentrifuge, fractions each of five drops were collected from the bottom of the tube. The refractive index and complement fixing activity of each fraction was determined. One sharp peak of complement fixing activity was found in fractions 9–13 (density 1·18–1·12 g/ml.) and a second more diffuse area in fractions 2–7 (density 1·27–1·19 g/ml.), which appeared to consist of two poorly separated peaks. On the basis of these determinations the fractions were combined into four groups and these were tested for interference. The results in Table 2 show that the entire activity was present in fractions 9–13. The density of the individual fraction

Table 2. INTERFERING ACTIVITY OF FRACTIONS OBTAINED BY TARTRATE DENSITY GRADIENT CENTRIFUGATION OF PEAK *B* FROM SUCROSE GRADIENTS

Fractions from gradient	Density (g/ml.)	Infectivity (P.F.U./ml.)	Complement fixing activity (ml. 1/30 *C′* fixed by 1 ml.)	Infectivity of culture in interference test (P.F.U./ml.)
1–4	1·29–1·24	11	1·0	$2·2 \times 10^8$
5–8	1·23–1·19	40	2·8	$1·8 \times 10^8$
9–13	1·18–1·12	$1·0 \times 10^4$	2·5	$3·3 \times 10^3$
14–21	1·10–1·02	$4·1 \times 10^3$	—	$2·3 \times 10^8$
Unfractionated peak *B*	—	$1·0 \times 10^5$	—	$2·3 \times 10^4$
Control for interference test minus tartrate	—	—	—	$2·0 \times 10^9$
Control for interference test plus tartrate	—	—	—	$1·3 \times 10^8$

containing the greatest interfering activity ranged from 1·13 to 1·15 g/ml. in five separate tartrate gradients. Fractions 9–13 were dialysed against 0·1 M ammonium acetate for examination in the electron microscope. The only component seen in this portion was the cap already referred to. This component bears a strong structural resemblance to the round end of the virion (Fig. 1); it is probably this similarity which accounts for its interfering activity.

We thank Mr. C. Smale for the electron micrographs.

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Inhibition of Neuraminidase by Polyanions

THE function of viral neuraminidase (*N*-acetylneuraminate glycohydrolase, *EC* 3.2.1.18) during the reproductive cycle of myxoviruses is not yet clearly defined. It was therefore considered that potent inhibitors might be utilized to gain further insight into the role of this enzyme. In experiments involving the interaction of myxoviruses with phosphotungstic acid it has been found that this compound is a potent inhibitor of neuraminidases at extremely low concentrations. A closer examination of this phenomenon revealed that the anionic character of this substance is responsible for the effect. Different polyanions were tested and some of them were found to be even more effective inhibitors of neuraminidase.

The following commercial products with oligo- or polyanionic characteristics, respectively, were tested: phosphotungstic acid (Merck, Germany), silicotungstic acid (Merck), yeast RNA (Boehringer), heparin[1], mucin from porcine submaxillary glands (Sigma), sodium dextran sulphate (molecular weights of 2×10^6 and 4×10^4, Pharmacia).

To test the effect of these substances on the activity of neuraminidase, virus preparations obtained by differential centrifugation[2] of infected allantoic fluids of the following virus strains were used: Newcastle disease virus strains 'Italien' and 'Beaudette'; Parainfluenza 1/Sendai; fowl plague strain 'Rostock'; influenza *A* strain A_2/Singapore/57 (Asian)*, influenza *B* strain 'Lee'. In addition, experiments were done with bacterial neuraminidase from *Vibrio cholerae* (*RDE*, Behringwerke, crystallized) and *Clostridium perfringens* (Sigma, purified). The activity of the neuraminidases was measured by estimating free sialic acid after liberation from *N*-acetylneuraminic acid lactose ('Sialolactose' General Biochemicals, Chagrin Falls, Ohio, U.S.A.)[3]. The assay was performed with 0·1–0·3 ml. neuraminidase or virus (giving an optical density of 0·3–0·5), 0·1 ml. of phosphotungstic acid and 0·1 ml. (100 μg) of 'Sialolactose'[4]. All solutions contained either 0·1 M phosphate buffer (*p*H 7) or 0·1 M acetate buffer (*p*H 5·0).

As shown in Tables 1 and 2, polyanions were capable of inhibiting neuraminidase exclusively at acid *p*H. There was a 50 per cent inhibition of all viral neuraminidases tested at *p*H 5·0 by all the substances used at the concentrations indicated. Differences among polyanions were observed. Higher concentrations suppressed the en-

* The influenza concentrates were a gift of Behringwerke *AG*, Marburg, Germany.

zyme activity completely. The neuraminidase of *Cl. perfringens* was inhibited by all polyanions at *p*H 5·0. The 50 per cent inhibitory concentrations were, however, 10–1,000 times greater than for the viral enzymes. 2 μg/ml. of the polyanions inhibited *V. cholerae* neuraminidase to a limited extent (30 per cent) except for the silicotungstic acid, which gave 50 per cent inhibition. Higher concentrations of polyanions did not cause a greater inhibition: only 200 μg/ml. of phosphotungstic acid gave a 50 per cent reduction of the enzyme activity. The mucin, which is known to contain sialic acid, had a pronounced inhibitory effect at *p*H 5·0; however, no significant liberation of sialic acid from the mucin occurred under these experimental conditions.

Table 1. INHIBITION OF NEURAMINIDASE ACTIVITY AT *p*H 5·0 BY POLYANIONS

Virus or neuraminidase	Concentration (μg/ml.)* of polyanions for 50 per cent inhibition: RNA	Dextran sulphate 2×10^6 mol. wt.	Dextran sulphate 4×10^4 mol. wt.	Mucin (PSM)	Heparin	PTA	STA
NDV, 'Italien'	0·1	0·02	0·02	0·2	0·005	0·4	0·5
NDV, 'Beaudette'	0·1	0·05	0·05	0·2	0·008	0·9	—†
Sendai	0·1	0·06	0·06	0·1	0·008	—	—
Fowl plague	0·5	0·07	0·07	0·5	0·06	2·0	—
Asian	0·3	0·04	0·04	0·6	0·05	0·9	—
Lee	0·2	0·04	0·04	1	0·02	—	—
Cl. perfringens	6	5	5	8	3	6	—
V. cholerae	> 200	> 200	> 200	> 200	200	200	2

* Final concentration of inhibitor in the reaction mixture; mean value of 2–4 estimations.

† Not tested.

To determine the activity of the neuraminidase the amount of sialic acid liberation from 100 μg of bovine 'Sialolactose' dissolved in 0·1 ml. of buffer was measured. The concentration of enzyme chosen was that which gave extinction values of 0·3–0·5 (representing from 15 to 25 units/ml.) (ref. 4). The total volume was 0·5 ml. in the reaction mixture. All reagents were dissolved in 0·1 M acetate buffer (*p*H 5·0) or 0·1 M phosphate buffer (*p*H 6·0 or *p*H 7·0). The enzyme was mixed with buffer and inhibitor at room temperature, placed in a water bath at 37° C for 5 min, and the reaction was started by the addition of 0·1 ml. of 'Sialolactose'. The enzyme reaction was carried out for 15 min and stopped by the addition of sodium periodate. The free sialic acid was determined by Aminoff's method (ref. 3). The concentration of inhibitor corrected for a final volume of 0·5 ml. of the reaction mixture which caused 50 per cent reduction of the enzyme activity was taken as the end point. PTA: phosphotungstic acid. STA: silicotungstic acid; NDV: Newcastle disease virus; PSM: from porcine submaxillary glands.

Table 2. INHIBITION OF NEURAMINIDASE ACTIVITY AT *p*H 6·0

Virus or neuraminidase	Concentration (μg/ml.) of polyanions for 50 per cent inhibition: RNA	Dextran sulphate 2×10^6 mol. wt.	Dextran sulphate 4×10^4 mol. wt.	Mucin (PSM)	Heparin	PTA	STA
NDV, 'Italien'	20	0·02	3	> 200	20	2	2
NDV, 'Beaudette'	20	0·02	3	> 200	20	2	2
Sendai	> 200	> 200	> 200	> 200	> 200	—	—
Fowl plague	> 200	> 200	> 200	> 200	> 200	2	2
Asian	> 200	> 200	> 200	> 200	> 200	2	2
Lee	> 200	20	200	200	> 200	—	—

Table 3. REVERSIBILITY OF THE PHOSPHOTUNGSTIC ACID-NEURAMINIDASE NEWCASTLE DISEASE VIRUS

Procedure or added substances	*p*H	PTA-concentration (μg/ml.)	Percentage of inhibition by PTA: Before*	After†
Dialysis against 0·1 M phosphate buffer	7·0	3,000	100	20
Dilution with 0·1 M acetate buffer (1 : 10)	5·0	0·6	100	20
0·4 per cent egg albumen	5·0	1·2	97	0
0·4 per cent raffinose	5·0	1·2	97	95
0·4 per cent starch	5·0	1·2	97	97
0·4 per cent tributyrin	5·0	1·2	97	100
'Sialolactose'; concentration 25 μg/0·5 ml.	5·0	0·06	39	
'Sialolactose'; concentration 100 μg/0·5 ml.	5·0	0·06	23	
'Sialolactose'; concentration 300 μg/0·5 ml.	5·0	0·06	19	

* Represents the enzyme inhibition by phosphotungstic acid before the designated procedure or added substances.

† Represents the enzyme inhibition after the designated procedure or added substances.

At *p*H 6·0 higher concentrations (0·02–200 μg/ml.) of polyanions were required to inhibit the neuraminidases of the Newcastle disease viruses; strains 'Italien' and 'Beaudette' and influenza B/Lee in contrast to Sendai, fowl plague and Asian influenza viruses, which were not influenced (Table 2). Phosphotungstic and silicotungstic acids (2 μg/ml.), respectively, however, inhibited all enzymes tested. Further experiments are in progress to determine whether the differences found are caused by the characteristic properties of the neuraminidases or to virus structure.

There is no correlation between the *p*H optimum of neuraminidases and the inhibition at *p*H 6·0 by certain polyanions. Newcastle disease virus neuraminidase is strongly inhibited by sodium dextran sulphate, whereas the enzyme of fowl plague virus is not affected, even though their optimal activity is at *p*H 6·0. On the other hand, the inhibitory effect of the anions shows a dependence on their molecular weight. A concentration of 0·02 μg/ml. of sodium dextran sulphate with a molecular weight of 2×10^6 has the same inhibitory effect as 3 μg/ml. of sodium dextran sulphate with a molecular weight of 4×10^4 (Table 2). The smaller one of the two polymers required a concentration 150 times as great as that of the larger one.

The only substance active at *p*H 7·0 was phosphotungstic acid (silicotungstic acid was not tested), which inhibited the neuraminidases of Newcastle disease virus, fowl plague virus and Asian influenza virus to 50 per cent, at a concentration of 150, 180 and 240 μg/ml., respectively. About 6,000 μg/ml. were needed to obtain a 50 per cent inhibition of *V. cholerae* neuraminidase at this *p*H value.

Phosphotungstic acid is known to precipitate proteins. Nevertheless, the inhibition of neuraminidases by this compound did not seem to be a simple denaturation of the enzyme, because it could be reversed by dialysis against 0·1 M phosphate at *p*H 7 (Table 3). Dilution with 0·1 M acetate at *p*H 5·0 also causes an almost complete reactivation of the enzyme activity (80 per cent). Inactivation was completely reversed by the addition of 0·5 per cent egg albumen (grade *V*, Sigma) into the enzyme–phosphotungstic acid mixture. Higher substrate concentrations also markedly reduced the inhibitory effect. An evaluation of these data by Dixon's method[5] indicated that a non-competitive inhibition was involved. On the other hand, reactivation did not occur on addition of raffinose, starch or tributyrin to the phosphotungstic acid-inactivated enzyme. The inhibition of neuraminidases by the other polyanions, however, was only partially reversed on addition of 0·5 per cent ovalbumin. An explanation for this different behaviour cannot yet be given.

Polyanions have been found capable of inhibiting neuraminidases at *p*H 5·0 and in some instances at *p*H 6·0 and *p*H 7·0. The concentration needed for 50 per cent inhibition of viral neuraminidases depends on the strain of the virus and on the type of polyanion and its molecular weight. Experiments are in progress to investigate the mechanism of the inhibition.

The data presented indicate clearly that there is an interaction between the myxovirus neuraminidases and phosphotungstic acid at *p*H 7·0. At this *p*H value, phosphotungstic acid is used for "negative staining" in electron microscopy. It is therefore suggested that the dark areas observed in electron micrographs of myxoviruses are not "holes", but may represent components which have bound phosphotungstic acid. This could mean that the surface of myxoviruses may not necessarily have "spikes", but may possess a lamellar-like structure made up of phosphotungstic acid-binding protein alternating with substances such as lipids and carbohydrates which do not bind phosphotungstic acid.

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BIOLOGY

Distribution of Fat Body around the Air Sacs and Muscle Fibres in the Imago of *Oryctes rhinoceros*

In general, the fat bodies of insects are distributed in two anatomical positions: they exist as a peripheral layer beneath the epidermis and a perivisceral layer surrounding the alimentary canal. The lobes lie freely in the haemocoel; in most insects they are held in position by means of the tracheal branches, but in some the individual cells of the fat body seem to float freely in the haemocoel. In many insects the fat body fills all the available space within the body cavity, and can be seen to surround the visceral organs such as the gonads. They are never directly attached, however, to the organs, but are connected to them by means of tracheae. Recently, we have observed a unique distribution of the fat bodies in the imago of *Oryctes rhinoceros*.

The abdominal cavity is filled with innumerable air sacs of more or less uniform size. A casual observation does not reveal free fat body lobes in the haemocoel as in other insects. It can be seen from Fig. 1 that the fat body surrounds the air sacs in the form of a thin coat. Only seldom are air sacs found which are not surrounded by the fat body. The large tracheae are also invested by a layer of fat body (Fig. 2). Frequently, small independent lobes of fat body occur adjacent to the air sacs or large tracheae and are supplied with small branches of tracheae. Few such lobes, however, are present, and for the most part the fat bodies are distributed around the air sacs and tracheae.

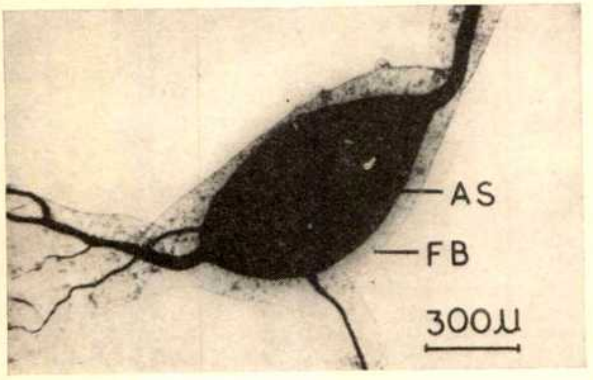


Fig. 1. Photomicrograph showing the distribution of fat body around the air sac in the imago of *O. rhinoceros*. *AS*, Air sac; *FB*, fat body.

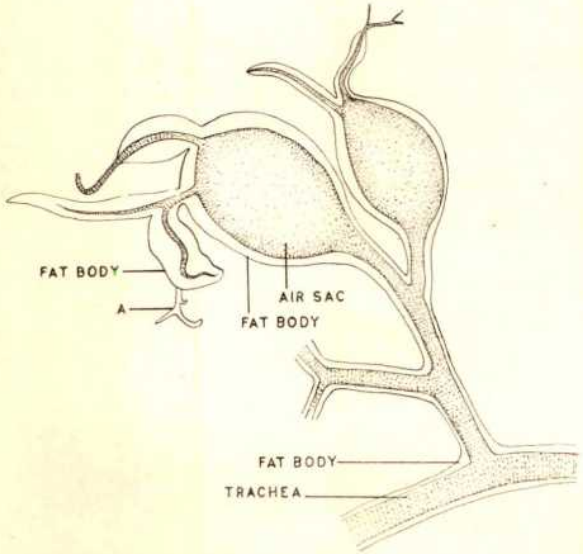


Fig. 2. Diagram showing the distribution of fat body around the air sacs and tracheae. *A*, Explanation in the text.

The fat body of the imago consists of much smaller cells than those of the fat bodies of the larva. The cell boundaries are not visible in paraffin sections of the fat body fixed in Bouin, but owing to a non-specific reduction of nitro-*BT* become clearly demarcated when a fresh fat body is incubated in media for succinic dehydrogenase activity. Attached to the fat bodies are small ribbon-like strands which consist of a double row of cells, arranged side by side, with large nuclei and homogenous cytoplasm (Fig. 2*A*).

In another beetle, *Heliocopris bucephalus*, which we examined, the air sacs do not possess a covering of fat body, and this would seem to apply to many other insects known to possess air sacs. The significance of the peculiar feature in *Oryctes* is not clearly understood. Lazarenko[1] demonstrated that the fat body of *O. nasicornis* is formed continually during larval development. He also showed that when a celloidine tube is introduced into the haemocoel between the lobes of fat body, a layer of connective tissue is first formed around the tube by the transformation of spindle-shaped haemocytes and then a large number of round blood cells is deposited on the surface of the connective tissue layer which later become transformed into fat body cells. In view of this, it can be suggested that the extensive surface area afforded by the tracheae and air sacs in the adult provides a suitable substratum for the formation of the fat body and that, concurrent with the development of the tracheal tubes and sacs, the adult fat body is also formed on their surface. Moreover, the existence of a thin layer of connective tissue around the trachea has also been demonstrated in the larva of *O. nasicornis*[1]. Because the distribution and structure of the adult fat body are different from that of the larva, it is evident that there is a total reconstruction of the fat body in the adult.

The distribution of the fat body around the air sacs may also have some functional significance. It seems possible that an insulation of the thin walled air sacs with a fatty layer would prevent an excessive loss of body heat from their extensive surface which is exposed through spiracular openings. Such an insulation would be of advantage in large flying insects which have to maintain a high body temperature during flight. A fatty interface between the body fluid and the tracheal surface would also safeguard against excessive evaporation of the body fluid.

Fig. 3 shows a muscle fibre from the metathoracic flight muscle invested by small thin lobes of fat body. These lobes are wound around individual muscle fibres leaving

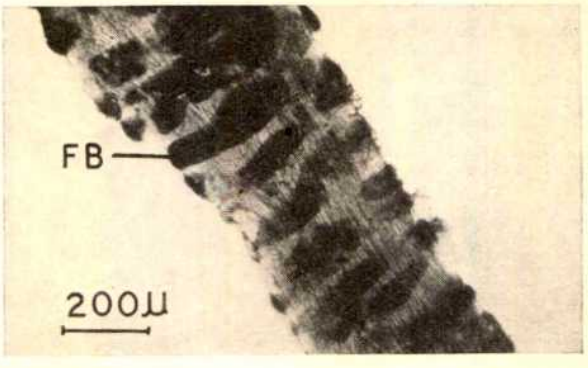


Fig. 3. Photomicrograph of a muscle fibre from the metathoracic flight muscle of *O. rhinoceros* showing the perifibral distribution of fat body. *FB*, A lobe of fat body.

narrow gaps in between. The fat body here has a structure similar to that around the air sacs and tracheae.

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In vitro Formation of Adventitious Organs by *Pharbitis* Embryos

WHEN young *Pharbitis* embryos were cultured on an artificial medium, abnormal organs formed which had not previously been reported for embryo cultures of any other plants[1]. This communication presents some additional observations on the formation of the abnormal organs.

Young embryos of *Pharbitis nil* Chois, strain 'Tendan', were isolated aseptically from seeds by the same procedure as was reported previously[1]. The embryos were cultured either in a liquid medium consisting of a modified White's solution[2], 40 g/l. sucrose and 1,000 mg/l. casein hydrolysate, or on a medium which contained 9 g/l. agar in addition to the same components as the liquid medium. The media were steam-sterilized at 100° C for 1 h. Test-tubes, each with 10 ml. of the medium and one embryo, were maintained at 26° C under fluorescent light, and the embryos were transferred to the fresh medium every 2 or 3 weeks.

In the first series of experiments, approximately 10-day-old embryos isolated from the young seeds were placed in the liquid media. At this stage they were 1–4 mm long and were green. Although they were submerged in the media they grew well, and after a week or so were 1·5 cm long (Fig. 1). When embryos were kept in the liquid medium for more than about 1 week, the green cotyledons became discoloured in places and several grey spots appeared; furthermore, the root and plumule did not develop. Several days later, tiny protuberances, which could not be identified as callus or root, appeared circularly and exogenously around the hypocotyl near the radicle in all cultured embryos (Fig. 2). Such protuberances expanded gradually and sometimes appeared as an opening umbrella (Fig. 3). In the meantime parts of these outgrowths frequently stretched downwards into the medium and mostly resulted in petiolate outgrowths (see Figs. 4 and 5). Some petiolate outgrowths which were partially expanded at their "spearhead" had the appearance of an ascidial leaf. Microscopic observation revealed that such outgrowths possessed some poorly developed vascular bundles and minute sacs, which resembled oil sacs—normally only found in the cotyledon of this plant. This suggests that such adventitious outgrowths are of a cotyledonary nature. In prolonged liquid culture, the rate of development of these adventitious outgrowths usually declined, and the embryos did not develop either root or plumule; however, the green cotyledons with grey spots continued to expand and the hypocotyls to thicken.

In another experiment, embryos which had been cultured in the liquid medium for 1 week were transplanted on the agar medium. They were kept partially submerged in the agar medium—that is, the cotyledons were maintained above the surface of the medium and the hypocotyl in the medium. The cotyledons in the air lost

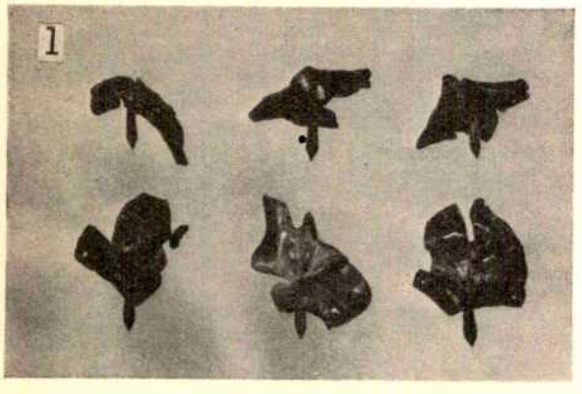


Fig. 1. *Pharbitis* embryos cultured in the liquid medium for a week after the isolation. (× *c.* 1.)

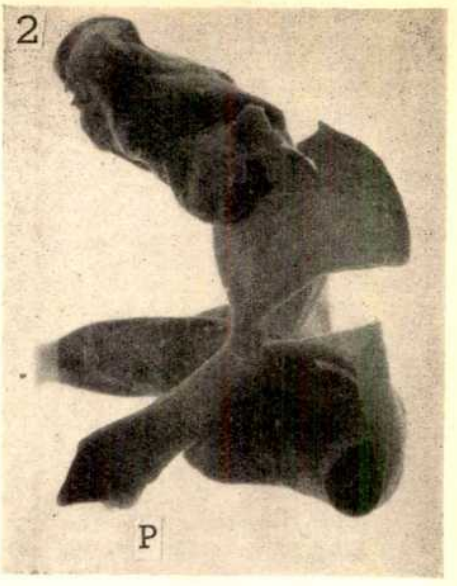


Fig. 2. *Pharbitis* embryo cultured in the liquid medium for about 3 weeks (× *c.* 2·5). Protuberances (*P*) occur circularly around the hypocotyl near the radicle.

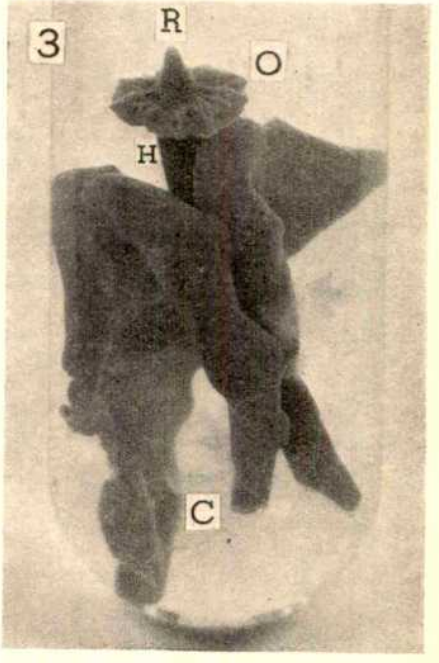


Fig. 3. *Pharbitis* embryo with an adventitious outgrowth similar to an opening umbrella (× *c.* 2·5). The embryo was cultured in the liquid medium for about 4 weeks. *O*, Outgrowth; *H*, hypocotyl; *C*, cotyledon; *R*, radicle.

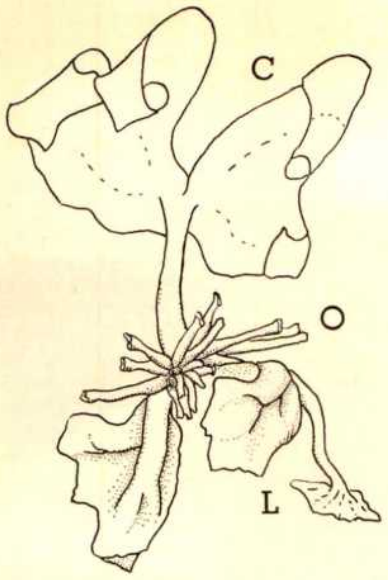


Fig. 4. Drawing of *Pharbitis* embryo cultured on the agar medium for about 1·5 months (×*c.* 2·5). The embryo developed the petiolate outgrowths like ascidial leaf and the cotyledonary "leaf blade" from the hypocotyl. *O*, Petiolate outgrowth; *L*, "leaf blade"; *C*, discoloured cotyledon.

chlorophyll and became pale one week after the transfer, while the hypocotyl in contact with the culture medium remained green in colour. After a further 2 weeks, the embryos attained 3 cm in height. Root and plumule did not develop, but the protuberances appeared from the hypocotyl and developed into the petiolate outgrowths. In the agar medium some of the petiolate outgrowths with partially expanding spearheads developed further into a "leaf blade" with veins in about 1·5 months (Fig. 4). Such a "leaf blade" possessed a number of sacs. This also suggests that the adventitious outgrowths from the hypocotyl are of a cotyledonary nature. In prolonged culture, some embryos with adventitious cotyledonary outgrowths developed a root and first leaf, followed by normal growth of the seedling.

In the second series of experiments, discoloration of the green cotyledons always occurred before initiation of the adventitious outgrowth (as was observed in the previous experiment). This suggests that the green cotyledons play an important part in the initiation of the outgrowths. In order to investigate this influence, three groups of cultures were prepared in the liquid media. In the first group the intact embryos were cultured. In the second group cotyledons were aseptically removed from the embryo 1 week after the start of the culture and only the remaining "hypocotyl" involving the radicle was cultured. In the third group "hypocotyl" and isolated cotyledons were cultured together in the test-tube.

The "hypocotyls" without cotyledons initiated the adventitious outgrowths most rapidly and vigorously, resulting in dishevelled outgrowths with irregular external forms (Fig. 5*a*). The intact embryos in the first group began to exhibit outgrowths later—after the grey spots had developed in the cotyledons—however, the "hypocotyls" cultured with the isolated cotyledons (third group) did not. This indicated that, whether or not the green cotyledon was isolated from the hypocotyl, its presence strongly suppressed the development of outgrowths. It is probable that the green cotyledons produce some diffusible substance which inhibits the development of outgrowths: the nature of this substance is as yet unknown. In prolonged culture, the "hypocotyl" of seedlings in the second group often developed a disfigured first leaf which possessed many sacs. This is believed to result from its cotyledonary nature (Fig. 5*b*).

In young seeds *Pharbitis* embryos are green, but on maturing they yellow. In the third series of experiments, embryos at various stages of development were isolated from the seeds and cultured in the liquid medium. All young embryos which had been isolated when they were green produced adventitious outgrowths within 3 weeks. On the other hand, when old yellowing embryos were cultured, they did not initiate any adventitious outgrowths but grew regularly and developed a root and normal first leaf; however, there were no sacs in the latter.

Thus, in summary, only young green embryos produced the adventitious cotyledonary outgrowths. Nevertheless, green cotyledons do have some inhibitory effect on the development of outgrowths. In other words, to initiate the outgrowth the embryo must be green at the beginning of the culture, but the cotyledon must be partially discoloured before the development of the outgrowths is initiated. It is possible that green hypocotyl without green cotyledon may be able to develop adventitious outgrowths.

Embryos cultured on the agar medium developed their normal first leaf later, and this did not have any sacs, whereas submerged "hypocotyls" of which the plumules were in contact with liquid medium developed the cotyledonary first leaf possessing a number of sacs. A component in the medium would therefore seem to be playing a part in causing such abnormal growth of the embryo.

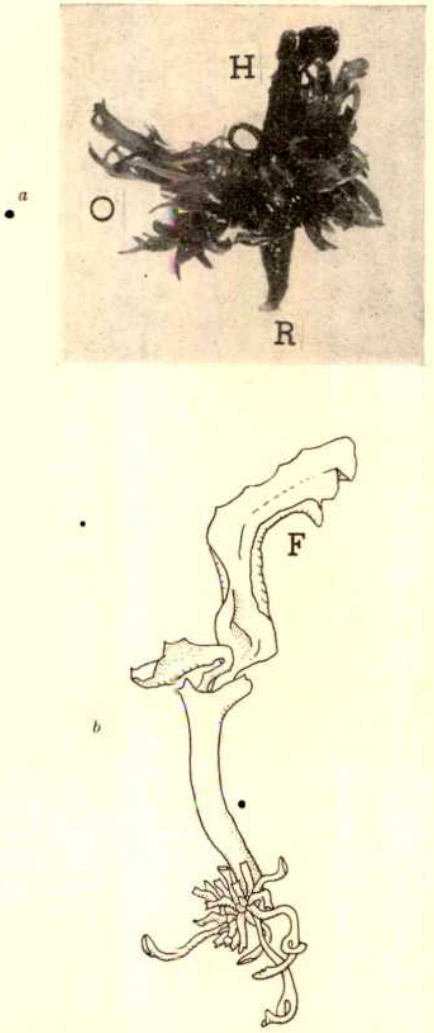


Fig. 5. "Hypocotyl" without cotyledons, developing dishevelled outgrowths (*a*) (×*c.* 1·5) and the cotyledonary first leaf (*b*) (×*c.* 2·5). *F*, Cotyledonary first leaf *H*, hypocotyl; *O*, petiolate outgrowth; *R*, radicle.

In the cultures the young embryos often germinated precociously without full differentiation during the early stage[3,4]. A high concentration of casein hydrolysate is known to retard such precocious germination and to prolong the embryonic stage[5]. The media used in our experiments contained 1,000 mg/l. casein hydrolysate and this might have suppressed normal germination and also caused the excessive cotyledonary outgrowth.

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Respiration in Euphausiids

RESPIRATORY rates provide estimates of catabolism in organisms. These rates, together with estimates of anabolism (such as growth rates), are used to assess gross production (assimilation) rates, which themselves are necessary to an understanding of energy transfer in food webs. This communication reports measurements of the respiration of euphausiids (class Crustacea) collected off the Oregon coast in winter. The effect of temperature on respiration was evaluated, and length–weight relationships were determined in order to study the effect of body size on respiration.

The study was confined to the winter months because of the known seasonal differences in respiratory metabolism in certain species of *Calanus*[1,2]. Environmental conditions in our areas of collection are reasonably uniform between November and March. In the upper 200 m of water, temperatures range from 7° C to 10° C, and salinities vary from 32·50 to 33·75 parts per thousand.

The species of euphausiids used in all experiments were *Euphausia pacifica* Hansen and *Thysanoessa spinifera* Holmes, with *E. pacifica* predominating. We collected these animals with either half-metre or metre nets (mesh size 0·239 mm) and placed them immediately in wide-mouth jars containing water of similar temperature and salinity as that from which they were collected. We then placed these jars in black plastic bags and transported them in insulated coolers to a laboratory cold room maintained at 10° C. In the cold room the animals were once again transferred, this time to wide-mouth jars containing filtered sea water. No more than three euphausiids occupied any one jar, with a maximum of one animal per 300 ml. sea water. These jars were then returned to the black plastic bags and held in the cold room. The animals were fed mixed phytoplankton cultures sporadically, and those not used immediately in experiments were transferred to clean water every few days. With this treatment most of the euphausiids, particularly the larger animals, remained healthy for many weeks.

Measurements of respiration were made in a Gilson differential respirometer[3] in the dark. The theory behind this instrument and its application to respiration studies has been discussed by Umbreit *et al.*[4]. Rates determined in the respirometer compared favourably with those found by the Winkler oxygen technique. Using this latter method, we determined oxygen utilization by subtracting the oxygen concentration in bottles in which euphausiids had been kept for 3–6 h from the oxygen concentration in control bottles containing no animals.

Length–weight relationships were determined for the euphausiids using total length measurements and dry weights obtained by desiccation to constant weight with silica gel. Dry weights by desiccation did not differ significantly from those determined at 60° C in an oven.

The dry weight of *E. pacifica* used in these experiments varied as the total length raised to the 3·059 power, which indicates that this species in winter obeys the so-called "cube law". Dry weight of fourteen specimens of *T. spinifera*, on the other hand, varied as total length to the 3·731 power. This latter relationship supports visual evidence that in winter *T. spinifera* is fuller bodied for its length than *E. pacifica*.

Even though the specimens of *T. spinifera* used in our experiments were usually heavier than *E. pacifica*, their weight-specific respiratory rates were not significantly different. Thus, data for both species were dealt with together. The average weight-specific respiratory rate for winter euphausiids at 5° C was 0·75 ± 0·04 μl. oxygen/mg dry weight/h (thirty-three animals), where ± 0·04 sets the limits of the 95 per cent confidence interval. The average rate at 10° C was 1·28 ± 0·07 μl. oxygen/mg/h (thirty-eight animals), and at 15° C 1·61 ± 0·13 μl. oxygen/mg/h (twenty-four animals).

Conover[5] collected three unidentified euphausiids in August 1958 in slope water off the east coast of the United States at about 1,000 metres depth. The rate for these animals averaged 0·67 μl. oxygen/mg dry weight/h at 5·1° C. One of these animals, however, was reported to be in poor condition. A fourth animal respired at 1·29 μl. oxygen/mg/h at 5·1° C. From these meagre data we conclude that respiratory metabolism of euphausiids collected in deep water off the east coast of the United States in late summer is approximately similar to that of winter euphausiids off Oregon. Lasker[6] obtained an average of 1·4 μl. oxygen/mg/h at 10° C for *E. pacifica* collected off southern California.

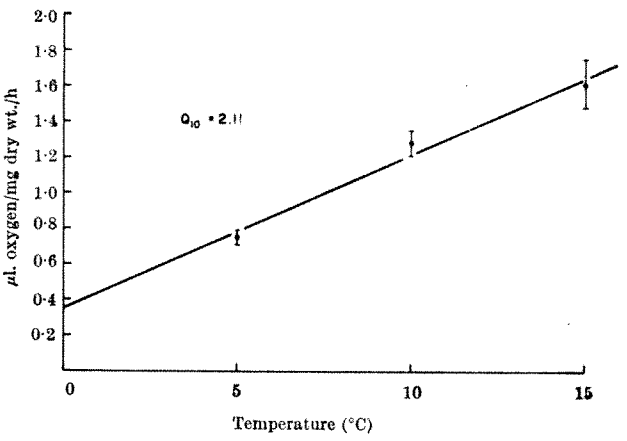


Fig. 1. Average weight-specific respiratory rates, with 95 per cent confidence intervals, of euphausiids from Oregon waters in winter, measured at 5°, 10°, and 15° C.

The Q_{10} for our animals was 2·11, and this was determined from the relationship between mean weight-specific respiratory rates and temperature (Fig. 1). This value clearly indicates non-acclimated respiration at 5° C and 15° C. We removed the euphausiids from the 10° C holding temperature to the experimental temperature 17–24 h before the beginning of each experiment. A Q_{10} of 2·11 under these conditions may indicate that euphausiids, often subjected to temperatures ranging from 5° C to 10° C in diel vertical migrations off Oregon in winter, do not acclimate to these temperature changes during their migrations. These results, in substance, support the hypothesis that a zooplankton that feeds in warm surface waters and "rests" in cooler waters gains an energy bonus that possibly can be put into fecundity[7].

Acclimation may occur with longer residence times in water of uniform temperature, but this remains to be tested with our animals. McWhinney[8] has demonstrated strong temperature acclimation in the Antarctic krill,

Euphausia superba, and Conover[2] has shown that the deep water copepod *Calanus hyperboreus* will regulate its respiratory rate within the known temperature range of the species. The temperature ranges for both these species are relatively narrow.

Respiratory metabolism (R), not corrected for weight and expressed as oxygen consumption per unit time, can be related to body weight (W) as:

$$R = a\,W^b$$

where a and b are constants. In a log–log plot of R and W, b is the slope of the line fitted by the method of least squares, and a the intercept on the y axis. The b values for our data were 0·962 at 5° C, 0·935 at 10° C, and 1·141 at 15° C (Fig. 2). Covariance analysis showed that the differences between these values were not significant at $P = 0{\cdot}05$. This indicated that temperature did not affect the proportionality of respiratory rate to dry weight; that is, the Q_{10} did not vary with size of the animal. For crustaceans, b generally is between 0·667 and 1·000, with such factors as physiological activity, sex, season, etc., affecting the actual value. A b value of 0·667 indicates proportionality of respiration to body surface area, and a b of 1·000 indicates proportionality of respiration to body-weight. In our studies, factors other than body size were controlled or minimized by experimental design, and respiration became a function of body-weight.

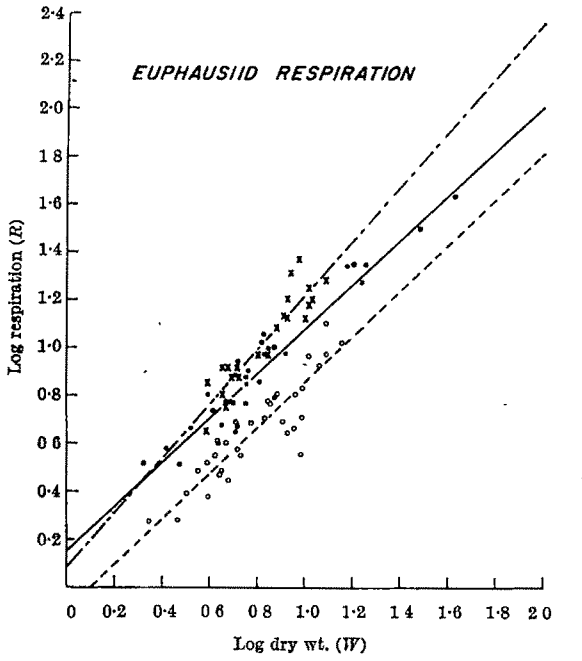


Fig. 2. Scatter diagram and regression lines showing the relationship between log μl. oxygen/h and log mg dry weight, at three temperatures. O - - - O, 5° C, $\log R = 0{\cdot}962 \log W - 0\,102$, $r = 0{\cdot}943$. ●——●, 10° C, $\log R = 0{\cdot}152 + 0{\cdot}935 \log W$, $r = 0\,960$. ×—·—×, 15° C, $\log R = 0{\cdot}083 + 1{\cdot}141 \log W$, $r = 0\,921$.

Our experiments led us to the conclusion that, in general, water temperature and body size are the two factors most significantly affecting the respiration of euphausiids during winter off the Oregon coast. Furthermore, over the weight range of animals used, neither Q_{10} nor weight-specific respiration varies with size, and no short-term temperature acclimation occurs. Whether these phenomena occur through all seasons remains to be determined. Differences might be expected in the spring and summer off Oregon, when food is abundant, surface waters are warmer, and reproduction occurs.

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Spermagglutination and Spermadsorption due to Myxoviruses

WHILE studying the interactions between germ cells and viruses, it was observed that spermatozoa of some mammalian and avian species were agglutinated by members of the myxovirus group. This phenomenon was designated as spermagglutination in analogy with haemagglutination. It was further observed that spermatozoa adsorbed to cells which were producing virus *in vitro*; this was called "spermadsorption". This communication describes these two phenomena as well as the conditions under which they occurred.

The bulk of the investigation was performed using bull and rooster semen. Bull semen was received as fresh and undiluted, individual or pooled specimens from an artificial insemination centre; rooster semen was collected from White Leghorn roosters between 6 months and 1 year of age, by the technique of abdominal massage[1]. Altogether, individual semen samples from ten bulls and five roosters were tested.

Semen samples were used up to 1 h after ejaculation unless they were kept for a longer time on purpose. Care was taken to avoid drastic changes in temperature while the semen was handled before the test proper. Before interfacting with the virus all semen samples were sedimented by centrifugation at 2,500 r.p.m. for 20 min, washed once in phosphate buffer (pH 6·0 for bull spermatozoa, pH 7·2 for rooster spermatozoa) and resuspended at a concentration of one per cent in the same buffer.

The viruses used were: one field and two attenuated vaccine strains[2] of Newcastle disease virus (NDV); the Melbourne and Formosa strains of influenza; local field strains of foot and mouth disease virus (types *O*, *A*, *Sat* 1); infectious bovine rhino-tracheitis (1 BR) virus; West Nile and Sindbis viruses. Anti-NDV and influenza hyperimmune sera were prepared in rabbits.

For the spermagglutination test, equal volumes of 1 per cent suspensions of spermatozoa and an appropriate virus dilution in the standard buffer were mixed. In the spermagglutination experiments for Sindbis and West Nile viruses, the procedure for haemagglutination of Clarke and Casals[3] was followed. Spermagglutination-inhibition as well as haemagglutination-inhibition tests were performed by titrating the immune sera against ten spermagglutinating or haemagglutinating units.

Spermagglutination was observed with bull, rooster, human and ram spermatozoa, and there were no qualitative differences between the individual samples. Under

the conditions tested, spermagglutination was observed only with the myxoviruses, NDV and influenza.

The phenomenon of spermagglutination manifested itself by the appearance of clearly visible macroscopic floccules which contrasted sharply with the homogeneous cloudy appearance of the non-agglutinated control suspension of spermatozoa. The time required for spermagglutination to occur was inversely proportional to the concentration of the virus and started in about 5–10 min. The final reading could be made after 60 min although after 30 min there was already a good indication of the titre. The floccules finally sedimented to form a typical dentated pellet, leaving a clear supernatant. Under the microscope, these floccules were observed to consist of aggregates of spermatozoa agglutinated by the mutual apposition of their heads with their tails vibrating at the periphery.

The following physico-chemical requirements for spermagglutination were determined: (1) *p*H: for bull spermatozoa the optimal *p*H range was between 6·0 and 6·6. At higher *p*H values, spontaneous agglutination was liable to occur. For rooster spermatozoa the optimum ranged from 6·5 to 7·2. The optimal *p*H for man and ram spermatozoa has not been studied, but spermagglutination occurred at *p*H 6·6. (2) Temperature: at 37° C, spermagglutination appeared sooner and to a higher titre than at room temperature. At 4° C there was practically no spermagglutination but when such samples were transferred within 1 h to 37° C, they showed the same titre as samples initially incubated at 37° C. (3) Electrolytes: spermagglutination was observed in the standard phosphate buffer, but it did not occur in isotonic 5 per cent glucose solution or when potassium ions were omitted from the standard buffer. The absence of potassium ions resulted in a reduction or loss of motility of the spermatozoa and because of this their agglutinability was diminished (see below). (4) Motility of the spermatozoa: a relationship was found between the motility and the agglutinability of the spermatozoa. Whenever the motility was impaired, it was reflected in the results of spermagglutination tests. Impairment of the motility to different degrees was provoked by heating to 45° C for 20 min or to 56° C for 10 min, by keeping the spermatozoa overnight at 4° C or by omission of potassium ions from the standard buffer. Of these, only the last was found to be reversible, as was cooling for 1 h only at 4° C. Although motility and its impairment could be evaluated by semi-quantitative means only (observing the fraction of motile spermatozoa and the magnitude of their motility), the results were nevertheless rather convincing. Gradual loss of motility coincided with gradual drop of virus titre in the spermagglutination test; immotile spermatozoa gave no spermagglutination. The specificity of spermagglutination produced by NDV and influenza viruses was shown by the spermagglutination-inhibition test with specific hyperimmune sera. Anti-NDV serum inhibited spermagglutination by NDV but not by influenza viruses, and the converse was found in reciprocal tests. The titre of the antiserum for each virus had similar values when measured by haemagglutination-inhibition or spermagglutination-inhibition. It was observed throughout the many trials that the titres of NDV and influenza strains were practically the same in the spermagglutination and haemagglutination systems (Table 1). It was further shown by adsorbing NDV to chicken red blood cells that the drastic fall of virus titre in the supernatant was reflected in spermagglutination and haemagglutination to the same degree. Recovery of the adsorbed virus by eluting it from the red blood cells restored its titre equally for spermagglutination and haemagglutination. Reduction of virus titre in the supernatant after adsorption to spermatozoa could be seen only when tested by spermagglutination and not by haemagglutination because it was found that spermatozoa and their washing fluids are themselves capable of agglutinating chicken red blood cells.

Spermadsorption, by analogy with haemadsorption, implies the attachment of spermatozoa to virus-infected monolayer of cell cultures. Bull and rooster spermatozoa showed this phenomenon only with NDV and influenza virus-infected cells. When infected monolayers of chicken embryo cells were allowed to interact with a suspension of spermatozoa for 10 min at 37° C, a massive adsorption of the spermatozoa to the monolayer could be seen under the microscope. 0·5 ml. of a 1 per cent suspension of spermatozoa for each culture tube was required for a clear picture. The firm attachment thus obtained could not be broken mechanically by washing or shaking (in contrast spermatozoa were floating and actively moving on the uninfected monolayers). NDV and influenza viruses could be titrated in this way (Table 1). The virus specificity of the spermadsorption was shown by inhibiting it; this was achieved by pretreating the infected cells with specific antiserum.

Bull seminal plasma was found to be a potent inhibitor of spermagglutination—sometimes active up to dilution of 1 : 1,000—while rooster seminal plasma showed a weak inhibitory activity. Bull semen, therefore, required to be washed before it was tested with the virus whereas rooster semen could be used directly once diluted to form a 1 per cent suspension. The seminal plasma exerted a similar inhibitory effect on haemagglutination having the same quantitative values. This inhibition could be completely abolished by treating the seminal plasma with potassium periodate and/or trypsin, but only partially by heating to 56° C for 30 min. It should be noted that the seminal plasma of different animal species is known to contain high levels of sialo-mucoproteins[4].

Table 1. COMPARATIVE TITRES OF MYXOVIRUSES BY SPERMAGGLUTINATION SPERMADSORPTION, HAEMAGGLUTINATION AND HAEMADSORPTION*

	CPE (TCD_{50}/ml.)	SAD (TCD_{50}/ml.)	HAD (TCD_{50}/ml.)	SA	HA
NDV (field strain)	$10^{6.5}$	$10^{6.0}$	$10^{6.2}$	1 : 2,048†	1 : 2,048†
NDV (vaccine strain)	0	$10^{7.8}$	$10^{7.5}$	1 : 1,024	1 : 1,024
Influenza (Formosa)	0	$10^{6.5}$	$10^{6.2}$	1 : 320	1 · 640

* Abbreviations: CPE, cytopathic effect in culture of chicken embryo cells; SAD, spermadsorption; HAD, haemadsorption; SA, spermagglutination; HA, haemagglutination.
† Highest dilution showing clear spermagglutination or haemagglutination.

The agglutinability of spermatozoa from different species by myxoviruses points to the existence of viral receptors on the surface of these cells. This finding may not only provide an additional system for the rapid detection and titration of myxoviruses but may itself be of fundamental biological interest. The study should be extended to an investigation of other viruses and should be carried out under various experimental conditions. In addition to the ability to agglutinate, other parameters which might be influenced by viruses—namely, the motility and the metabolic activity of the spermatozoa—should be taken into account. Another point of interest is the possible part played by male-haploid cells as carriers of virus in congenitally transmitted viral diseases[5].

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Recovery of Unengorged Larval Trombiculid Mites (Acarina) from Ground Holes

PREVIOUSLY it was found possible to identify the natural habitats of a limited number of species of larval trombiculid mites (chiggers) in Malaysia by recovering them from the ground on black 'Formica' plates[1]. In such habitats as were found, it was possible to observe with the naked eye chiggers both singly and in clusters. Only two species of chiggers have so far been subjected to intensive investigation using this recovery technique and both were vectors of scrub typhus, *Leptotrombidium* (*Leptotrombidium*) *deliense* (Walch, 1922) and *L.* (*L.*) *akamushi* (Brumpt, 1910). In the few localities studied by this means, the former was found in lalang grassland (*Imperata cylindrica*) and the latter on ground leaf-litter of secondary forest galleries[2]. In the course of current investigations of scrub typhus in the primary and secondary forests of Malaysia, we have collected a number of species of larval trombiculids—previously found only on small forest mammals—by insertion of a flat strip of black 'Formica' into ground holes.

The ground holes were probably formed as a result of activities of large animals, although some may have resulted from weathering. They are frequently to be found on the sides of escarpments or small mounds, among the roots of trees, and often in natural rock and earth fissures. In general, however, the holes which are infested occur in shaded areas; their interiors are cool, damp and usually free of litter or debris. Chiggers have been recovered from wide, low crevices on sloping ground and from holes which are as shallow as 4 in., and which vary in diameter from 2 in. to 6 in. Although the mites have generally been recovered from within 1–14 in. of the mouth of the hole, the maximum depth at which they are found has not yet been determined.

About half the holes which we have examined have yielded larval mites. The number of larvae recovered from a single hole may vary from one to more than two hundred. It was found that chiggers could best be recovered by simply inserting the 'Formica' plate into the hole for a second or so; its presence somehow stimulated the mites to drop on to it from the roof of the hole (where they were most frequently situated). (An investigation of the stimulus involved here is at present being carried out; so also is a search for nymphal and adult forms and the identity of their usual hosts.)

The specimens of *L.* (*L.*) *deliense* recovered from holes are white whereas those of *L.* (*L.*) *deliense* collected in abundance from established ground surface habitats are orange in colour. Indeed all but two of the twenty-five collected from holes were whitish in colour; the exceptions were *W. oudemansi* and *E. wichmanni*. The relationship between colour and habitat is also the subject of active investigations.

So far, twenty-five larval species, including several which have not previously been described, have been recovered from ground holes (Table 1). Thirteen of the twenty-five belong to the genus *Gahrliepia*.

Previous attempts to grow nine species of *Gahrliepia* under ambient laboratory conditions were not successful. Fourteen species of chiggers collected have now been successfully bred under conditions simulating the natural micro-environment[3]; seven of these had been recovered from open fields and plantations or from rodents trapped there, while the others were from more heterogeneous habitats. A knowledge of temperature, humidity and food requirements such as could be derived from further studies of these newly recognized habitats should enable us to breed gahrliepiines and other forest chiggers in the laboratory. Because of the relative ease with which these mites can be recovered, investigations of the bionomics of a large number of Malaysian mites and their possible role in the transmission of scrub typhus and other enzootic infections can now be put into operation.

Table 1. SPECIES RECOVERED FROM GROUND HOLES

Species	General location of holes*			
	1	2	3	4
Leptotrombidium (*L.*) *deliense*			×	
Leptotrombidium (*L.*) sp.		×	×	
Leptotrombidium (*L.*) sp.		×		
L. (*Trombiculindus*) *hastata*			×	
Eutrombicula wichmanni				×
Eltonella eltoni			×	
Schoengastia vieta				×
Neoschoengastia sp.			×	
Schoutedenichia bisetosa			×	
Walchiella oudemansi			×	×
Walchiella impar			×	
Walchiella sp.			×	
Gahrliepia (*G.*) *darta*	×			
Gahrliepia (*G.*) *neterella*	×			
Gahrliepia (*G.*) *cetrata*		×		
Gahrliepia (*G.*) *fletcheri*		×	×	
Gahrliepia (*G.*) *insigne*		×	×	
Gahrliepia (*G.*) *picta*		×	×	
Gahrliepia (*G.*) *rutila*		×	×	
Gahrliepia (*G.*) *marshi*				×
Gahrliepia (*G.*) sp	×			
G. (*Schoengastiella*) *argalea*		×	×	
Gahrliepia (*Walchia*) *rustica*	×	×	×	
Gahrliepia (*W.*) *turmalis*			×	
Gahrliepia (*W.*) *cuspidata*			×	

* (1) Primary forest, >5,000 ft.; twenty holes examined; (2) primary forest, <3,000 ft.; sixty holes examined; (3) secondary forest, <3,000 ft.; one hundred holes examined; (4) palm island in rice field; twenty holes examined.

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Antagonistic Interaction between *Schistosoma mansoni* Sporocysts and Echinostome Rediae in the Snail *Australorbis glabratus*

IT has recently been shown that antagonistic interaction takes place when schistosome, strigeid or plagiorchiid sporocysts occur together with certain echinostome rediae in the same snail[1]. This interaction results in the total elimination of the first three groups of parasites. This communication discusses the antagonistic interaction between sporocysts of *Schistosoma mansoni* Sambon and rediae of an unidentified *Echinostoma* species in the snail *Australorbis glabratus* (Say).

The *Echinostoma* species was isolated from an *A. glabratus* collected near Recife, Brazil. It is morphologically close to *E. audyi*, but its exact identity is still under study. Cercariae of this echinostome encyst in various freshwater snails, including *A. glabratus*, and these cysts develop into adults when fed to chicks and ducklings. The strain of *S. mansoni* and the experimental snails were obtained from the National Institutes of Health, Bethesda, Maryland. Only albino snails were used because it is then easier to observe rediae and sporocysts and their interaction through the shells.

The *Echinostoma* sporocysts develop in the ventricle cavity of the snail, rediae mainly in the ovotestis but also in the liver, lung, kidney and mantle edge. Daughter sporocysts of *S. mansoni* migrate from the anterior of the snail, where the mother sporocyst develops, to the visceral mass. Development of both species of parasites is apparently normal so long as they occupy different areas in the snail. Observations made on thirty-six snails with double infection have shown that wherever schistosome daughter sporocysts and echinostome rediae are

in direct contact with each other interaction occurs. The sporocysts die and are consumed by the rediae. The mechanism by which sporocysts are killed is unknown, but this is presumably the result of direct mechanical action of the rediae since parasites not attacked develop normally and produce cercariae.

The rate at which sporocysts are eliminated depends largely on relative numbers of both species of parasites in the snail. *S. mansoni* sporocysts are usually numerous, while the rediae of *Echinostoma* species are relatively few, varying from 46 to 432 in snails dissected 19 to 81 days after exposure. Of these, 10–14 per cent are usually located in areas not occupied by the schistosome—that is, in the lung, kidney and mantle region of the snail—and do not participate in such interaction. Because of this, relatively few rediae are usually confronted with numerous sporocysts, which are gradually eliminated. Moreover, rediae of this *Echinostoma* species are active for only 4–5 weeks, after which they become smaller and sluggish and contain few or no larvae. These inactive rediae probably play no part in the interaction and ultimately die. New rediae continue to be produced (as there are many redial generations in this species), but the rate of interaction decreases steadily after the appearance of inactive rediae. Significant interaction is therefore confined to a limited period.

When schistosome sporocysts are already widely distributed in the visceral mass of the snail, newly produced rediae readily interact with them. The rate of interaction increases with the number of vigorous rediae, but declines rapidly as they become inactive. If echinostome rediae are already established in the snail, the rate varies with the stage of redial development at the time schistosome sporocysts invade the visceral mass and is negligible when many rediae have become inactive. Even in the presence of active rediae, however, many schistosome sporocysts may escape immediate destruction and produce cercariae. Sporocysts are first cleared from the ovotestis where rediae are most numerous, then from the distal part of the liver, regardless of which parasite first appears in the visceral mass. Sporocysts persist in the anterior of the viscera where rediae are usually few and sometimes absent.

Table 1 shows the results of interaction. The experiments were carried out at 26–29° C. Double infection was achieved in nine snails by simultaneous exposure to both species of miracidia and in these animals echinostome rediae arrived first in the viscera. In eighteen snails, *Echinostoma* infection was superimposed on *S. mansoni* infection with an exposure interval varying from 9–19 days. In nine other snails the sequence of exposures was reversed, with intervals varying from 7–19 days. Each snail was exposed to ten miracidia of *S. mansoni* and ten and fifteen echinostome miracidia. Nine snails were killed, twenty-six were kept until they died, and all were dissected.

In eleven snails clearance of sporocysts was slight (group 1 in the table) due to one or more of the following factors: too few rediae, early death of the snails, or arrival of *S. mansoni* sporocysts in the viscera after development of many inactive rediae. In fourteen snails (group 2) clearance was achieved in the ovotestis, in eight snails (group 3) also in the posterior part of the liver and in three snails (group 4) in more than half the liver. In one snail of group 3 and one of group 4, clearance occurred in large areas of the liver within a relatively short period because only a few schistosome sporocysts had migrated into the viscera, the majority probably still remaining within the mother sporocyst. When numerous sporocysts invade the visceral mass, clearance of large areas of the snail cannot be achieved quickly. Significant clearance was observed when *Echinostoma* infection was superimposed on *S. mansoni* infection and in snails with simultaneous exposure. Snails with *S. mansoni* superimposed on *Echinostoma* infection showed limited clearance.

Interaction between *S. mansoni* sporocysts and echinostome rediae in *A. glabratus* is similar to that between various trematode sporocysts and echinostome rediae in *Lymnaea rubiginosa* but with a different outcome. In the latter, interaction occurs rapidly; for example, when *Echinostoma audyi* Lie and Umathevy or *Echinoparyphium dunni* Lie and Umathevy is superimposed on *Trichobilharzia* infection, complete elimination of sporocysts is usually achieved before echinostome cercariae are produced, and the snails rarely shed both cercariae at the same time[1,2]. In the former, interaction is slow and only partial elimination of sporocysts is achieved; the snails always shed both species of cercariae. *Lymnaea* snails infected with these echinostomes always have abundant rediae. The difference in interaction is probably due to the larger number of rediae produced by *E. audyi* or *E. dunni* and their longer period of activity. Heyneman[3] introduced twenty-five to fifty *E. dunni* mother rediae into a clean snail and counted about 2,000 rediae 7 days later. Number and activity of rediae are probably related to suitability of the snail host. *Lymnaea rubiginosa* is easily infected with *E. audyi* or *E. dunni* showing high infection rates[2,4]. *A. glabratus* is less easy to infect with the *Echinostoma* species. *Lymnaea* snails are a better host for *E. audyi* and *E. dunni* than *A. glabratus* for the *Echinostoma* species.

Although interaction is direct between rediae and sporocysts, the relative degree of adaptation of both parasites to the snail host is probably of great importance. *E. audyi* and *E. dunni* are at least as well adapted to the snail host as the *Trichobilharzia* species, and *A. glabratus* is undoubtedly a better host for *S. mansoni* than for the *Echinostoma* species. *A. glabratus* is more easily infected with the schistosome (more than 90 per cent infected when exposed to ten miracidia each as compared to 30 per cent on exposure to ten to fifteen miracidia for the *Echinostoma* species). An echinostome species as well adapted to *A. glabratus* as *S. mansoni*, however, might give an efficient interaction.

Antagonistic interaction between rediae and sporocysts may be a feature of all echinostomes. It has been shown to occur in two species of Malayan echinostomes and is now demonstrated in a Brazilian species. The findings of Cort *et al.*[5] in Michigan, and of Martin[6] in Los Angeles, that echinostomes in the freshwater snail *Stagnicola emarginata angulata* (Sowerby) were not found in double infections with other trematodes and in the marine snail *Cerithidea californica* Haldeman only rarely, support this assumption. It would be interesting to know whether rediae of trematodes other than echinostomes are also capable of destroying sporocysts in the snail.

Table 1. EXTENT OF CLEARANCE OF *Schistosoma mansoni* SPOROCYSTS BY *Echinostoma* REDIAE IN THE SNAIL

Sporocysts distribution in snails	Duration of interaction	Number of snails: Simultaneous exposure	*Echinostoma* superimposed on *S. mansoni*	*S. mansoni* superimposed on *Echinostoma*	Total
1. Sporocysts in ovotestis and liver	4–35	2	5	4	11
2. Clearance of sporocysts in ovotestis	5–54	4	5	5	14
3. Clearance of sporocysts in ovotestis and ¼–½ liver	8–40	2	6	—	8
4. Clearance of sporocysts in ovotestis and more than ½ liver	9–36	1	2	—	3
Total		9	18	9	36

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Colour Change in Flowers of *Lathyrus hirsutus* during Senescence

ALTHOUGH there has been some work on the nature of changes in flower colour during development[1–3], little is known about changes during senescence. The anthocyanin-pigmented flowers of *Lathyrus hirsutus* L. exhibit as they age a well marked colour change immediately before the petals show obvious loss of turgor. The mature standard petal is bluish red and it fades through blue to greenish blue; the wing petals are pale blue and fade to greenish blue. It was suggested in earlier work that blueness in mature flowers of *Lathyrus* species is promoted by flavonol glycoside co-pigments[3–5]. Thus it was found that the blue wing petals of *L. hirsutus* contain greater quantities of co-pigment relative to anthocyanin than the standard petals[4]. In this species the *p*H of the expressed sap of the wings was found to be rather higher than that of the standard petal, and it was suggested that this *p*H difference might contribute to the bluer appearance of the wing petal.

Three anthocyanins were found in the standard petal—namely, glycosides of delphinidin, petunidin and malvidin[4]. In the wing petals only the malvidin glycoside was detected, and this behaved chromatographically like the 3-rhamnoside, 5-glucoside identified in *Lathyrus* by Harborne[6]. It should be noted that the malvidin 3-rhamnoside attributed by Harborne[2] to *L. hirsutus* was, in fact, from the species *L. sativus* L.[7].

The present work was carried out to determine whether the factors believed to be involved in the determination of the colour of the mature petals are also involved in determining the colour fade.

Hydrolysed extracts of mature and faded flowers were prepared and then examined by filter-paper chromatography[4]. Determinations of the *p*H of expressed sap from other samples of petals were made with a micro-electrode[4]. Small but consistent differences of *p*H were found.

Table 1. CONSTITUENTS OF HYDROLYSED EXTRACTS AND *p*H OF EXPRESSED SAP IN RELATION TO PETAL COLOUR

Petal	Colour	*p*H	Anthocyanidins*			Flavonols	
			D	*P*	*M*	*Q*	*K*
Mature standard	Bluish red	5·6	+	+	+ + †	+	+
Faded standard	Greenish blue	6·1	–	+	+ +	+	+ + +
Mature wings	Pale blue	6·1	–	–	+	+ +	+ +
Faded wings	Greenish blue	6·7	–	–	+	+ + +	+ + +

* *D*, Delphinidin; *P*, petunidin, *M*, malvidin; *Q*, quercetin; *K*, kaempferol.
† + + +, + +, +, Relative quantities of constituents; –, not detected on chromatograms at loading used.

The results (Table 1) confirm that the mature wing petals differ from the mature standard petals in having a higher *p*H and a greater quantity of flavonol glycoside relative to anthocyanin. The data also show that as the standard and wing petals age, the *p*H of the expressed sap increases. The differences are not large but are likely to be important[8]. The chromatographic data (Table 1) clearly indicate that the faded petals, standard and wing alike, contain greater quantities of flavonol glycosides relative to anthocyanin than the mature petals. These changes could arise from a synthesis of flavonol glycoside, from a destruction of anthocyanin, or from a combination of both processes. To determine which of these processes is involved, quantitative estimations of total anthocyanin and flavonol glycoside were carried out. Samples of ten mature and ten faded petals were extracted with 1 per cent methanolic hydrochloric acid. The optical density of the extracts was determined at 535 mμ as an estimate of anthocyanin and at 260 mμ as an estimate of flavonol glycoside[3]. The results indicated that there is no significant change in the quantity of flavonol glycoside during senescence of either the standard or wing petals. The anthrocyanin content of the standard petals decreases by about 37 per cent as the tissue fades, however, and that of the wings falls by about 23 per cent.

It seems very probable that the fading of the petals of *L. hirsutus* results from an increase in the *p*H of the cell sap and to a greater influence of co-pigment, which results from a decrease in the quantity of anthocyanin present. Thus the same factors, co-pigmentation and *p*H, which were suggested as being responsible for the control of the colour of the mature flowers, appear also to operate in the control of the colour change during senescence.

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Effect of Cycocel and its Analogues on Growth and Soluble Protein Content of Young Barley Seedlings

CYCOCEL, (2-chloroethyl)trimethylammonium chloride, and its analogues have a profound effect on plant growth[1–3]. The same cycocel analogues which retard stem growth[1,2,4] also inhibit gibberellin biosynthesis in *Fusarium moniliforme*[4], reverse auxin[5,6] and coumarin[5,7] inhibition of lettuce seed germination and root growth and accelerate abscission of petiole in cotton[8]. This communication describes an attempt to find a metabolic basis to explain the action of this class of compounds.

Barley (*Hordeum vulgare*, var. Besbar, 1965 crop) seeds were sterilized for 15 min with clorox (1 per cent) and washed thoroughly with running water. Twenty seeds were germinated in 9 cm deep Petri plates lined with one layer of Whatman No. 1 filter paper and moistened with 8 ml. of test solutions. Seeds were incubated in the dark at 25° C for 4 days. After this, coleoptile (with primary leaves) and roots (all seminal roots) were severed from each seed with a sharp razor blade, weighed and their length measured. In the case of root the length of the longest seminal root was recorded. Dry weight was obtained by drying the tissues at 65° C for 24 h. Coleoptile and root tissues, each weighing 250 mg, were homogenized in cold phosphate buffer[9] (*p*H 6·1) and centrifuged in the cold at 5,000 r.p.m. for 10 min. The supernatants were used for assay. Soluble protein was determined by the method of Lowry *et al.*[10]. Cycocel and choline were obtained from Eastman Organic Chemicals and Merck and Co., Inc., respectively. Other analogues used in this experiment were synthesized by Dr. N. E. Tolbert[1] and were kindly supplied by him.

The effects of various cycocel analogues on growth of coleoptile and soluble protein content at two concentrations are given in Table 1. Cycocel and (2-bromoethyl)-trimethylammonium bromide decreased coleoptile growth

Table 1. EFFECT OF ANALOGUES OF (2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE ON GROWTH AND SOLUBLE PROTEIN CONTENT OF BARLEY COLEOPTILE

Ten randomly selected coleoptiles were used for fresh weight determination. Length is the mean length of ten coleoptiles.

Treatment	Dry* weight (per cent of fresh wt.)	Fresh* weight (mg)	Length ± S.E. (mm)	Protein (mg/g fresh wt.)
		5×10^{-3} M		
$(CH_3)_3N^+—CH_2—CH_2Cl\ Cl^-$	11·0	360	22 ± 3·8	13·6
$(CH_3)_3N^+—CH_2—CH_2Br\ Br^-$	12·5	270	20 ± 5·7	13·2
$(CH_3)_3N^+—CH_2—CH_2OH\ Cl^-$	9·5	610	42 ± 9·0	9·5
$(CH_3)_2N—CH_2—CH=CH_2$	9·3	750	52 ± 7·2	7 2
$H_2N—CH_2—CH_2Br$	8·7	720	52 ± 7·4	8·3
Water	8 9	750	54 ± 5 2	9 3
		10^{-2} M		
$(CH_3)_3N^+—CH_2—CH_2Cl.Cl^-$	12·6	230	16 ± 2 1	15·0
$(CH_3)_3N^+—CH_2—CH_2Br.Br^-$	13·3	260	19 ± 2 2	15·0
$(CH_3)_3N^+—CH_2—CH_2OH.Cl^-$	9·1	580	40 ± 3 3	11·0
$(CH_3)_2N—CH_2\ CH=CH_2$	8 3	710	50 ± 5·5	10·0
$H_2N—CH_2—CH_2Br$	8 4	690	46 ± 3·2	—
Water	8 9	630	50 ± 8 8	12 0

* Weight represents weight of coleoptile plus primary leaves.

(length and fresh weight) but increased the soluble protein content of the tissue and dry weight (percentage of the fresh weight). With other analogues where growth was not significantly affected, neither protein content nor dry weight showed any significant difference from the control.

Table 2. EFFECT OF ANALOGUES OF (2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE ON GROWTH AND SOLUBLE PROTEIN CONTENT OF BARLEY ROOTS

Seminal roots from ten randomly selected seedlings were used for weight determinations. The length of the longest seminal root from each of the ten seedlings was measured and length entered as mean of ten such roots

Treatment	Dry weight (per cent of fresh wt. (mg))	Fresh weight (mg)	Length ± S.E. (mm)	Protein (mg/g fresh wt.)
		5×10^{-3} M		
$(CH_3)_3N^+—CH_2—CH_2Cl\ Cl^-$	10 6	150	30 ± 6 1	15·1
$(CH_3)_3N^+—CH_2—CH_2Br.Br^-$	9·4	110	21 ± 5·0	17·7
$(CH_3)_3N^+—CH_2—CH_2OH\ Cl^-$	6 1	550	91 ± 10·6	11·1
$(CH_3)_2N—CH_2—CH=CH_2$	8·8	880	94 ± 15·0	10·2
$H_2N—CH_2—CH_2Br$	9 0	320	69 ± 9·5	12·4
Water	6·4	530	98 ± 13·7	11·5
		10^{-2} M		
$(CH_3)_3N^+—CH_2—CH_2Cl.Cl^-$	8 8	60	19 ± 3·0	17·0
$(CH_3)_3N^+—CH_2—CH_2Br\ Br^-$	10·8	90	16 ± 3 5	17·0
$(CH_3)_3N^+—CH_2—CH_2OH.Cl^-$	7 0	320	51 ± 12 0	13 0
$(CH_3)_2N—CH_2\ CH=CH$	5·4	670	90 ± 9 2	10·0
$H_2N—CH_2—CH_2Br$	6 0	510	84 ± 8·0	9 0
Water	4·2	790	103 ± 18·1	10 0

Similar results were obtained in the case of roots (Table 2). Cycocel and (2-bromoethyl)trimethylammonium bromide reduced the length of root from one-third to one-fourth of the control at 5×10^{-3} M and nearly one-sixth at 10^{-2} M. Choline inhibited root growth by about 50 per cent at 10^{-2} M and this was accompanied by a considerable rise in soluble protein content and dry weight. Choline also had some inhibitory effect on the growth of coleoptile (Table 1).

The results show that the structural requirement for activity of the cycocel analogues is the same as reported earlier[1,2,4–8]. A trimethyl amine moiety plus a -chloride or a -bromide substitution in the carbon chain, as in cycocel and (2-bromoethyl)trimethylammonium bromide, are essential for maximum activity[1]. The naturally occurring analogue choline, with a hydroxyl instead of the halogen group, which has been shown to act as an anti-auxin in germination and root growth[6], was also significantly active in inhibiting coleoptile and root growth in the present work.

The mechanism of action of this class of compounds remains vague. It has been shown that there is a considerable rise in the oxidase and peroxidase activities of indolyl-3-acetic acid following growth retardation by cycocel and other growth retardants[11–13]. A decrease in diffusible auxin content has been shown following pretreatment with cycocel[14]. Furthermore, cycocel and its active analogues have been shown to antagonize auxin effects[6,14,15] in a variety of plant systems. The role of auxin in the growth of coleoptile and root tissues is fairly well known. Thus, it would seem that cycocel and its active analogues act by increasing the level of soluble protein or enzymes which are either unavailable or are destructive to the growth promoting system(s) in the coleoptile and root tissues.

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Effect of Oxindoles on the Growth of Tobacco Tissue Cultures

IT has been suggested that oxindoles are products of the enzyme oxidation of indolyl-3-acetic acid (IAA)[1,2], but they have generally been found to be inactive as auxins in standard auxin bioassays[3]. Galston and Chen[4], however, recently reported that isatin (indole-2,3-dione) and oxindole-3-acetic acid promote the elongation of etiolated and green pea stem sections. Isatin was also found to be active in stimulating the *in vitro* growth of *Pelargonium* pith tissue—a tissue which requires an auxin for growth[5]. In light of these findings, and the fact that oxindoles do not conform to generally accepted structural requirements for auxins, it was decided to test the effect of oxindoles as auxins on another system which requires an auxin, namely, the growth of tobacco pith tissue.

The tissue used in this investigation was derived from pith cells isolated from the stems of *Nicotiana tabacum* var. Wisconsin No. 38. The control tissues were grown on a basal medium[6] supplemented with $5{\cdot}7 \times 10^{-6}$ M IAA and $2{\cdot}3 \times 10^{-6}$ M kinetin (6-furfurylaminopurine). The test tissues were grown on the basal medium in which varied concentrations of oxindoles were substituted for the auxin or cytokinin additive. All tissues were grown in the dark at room temperature and at the end of the experimental period the fresh and dry weights were determined.

The results in Table 1 show that of the oxindoles tested for auxin activity only isatin, in the presence of kinetin, promoted growth of the tobacco tissues. This growth promotion was noted to increase as the isatin concentration increased. The maximum concentration of isatin used, 10^{-3} M, produced a growth approaching 50 per cent of that of the IAA control. The remainder of the oxindoles tested were either ineffective as auxins or inhibitory. The results of experiments shown in Table 2 indicate that the oxindoles tested are inactive as cytokinins, because in all cases none acted as substitute for kinetin.

The ability of isatin to substitute, at least partly, for the auxin requirement of tobacco pith callus tissue confirms the results found in *Pelargonium* tissues[4]. The possibility was considered that growth attributed to isatin resulted from a contaminant in the sample such as IAA. The melting-point of the isatin used in this

Table 1. SUBSTITUTION OF OXINDOLES FOR IAA IN THE GROWTH OF TOBACCO PITH CALLUS TISSUE

Test No.	Additive*	Molar conc.	Final† fresh wt. per piece (mg)	Final dry wt. per piece (mg)
(1)	0	—	49	4
(2)	IAA	5.7×10^{-6}	1,230	67
(3)	Isatin	1×10^{-3}	594	39
(4)	,,	1×10^{-4}	115	10
(5)	,,	1×10^{-5}	98	8
(6)	,,	1×10^{-6}	89	7
(7)	,,	1×10^{-7}	86	7
(8)	Oxindole	1×10^{-4}	16	3
(9)	,,	1×10^{-5}	35	4
(10)	,,	1×10^{-6}	49	6
(11)	,,	1×10^{-7}	25	4
(12)	Oxindole-3-acetic acid	1×10^{-4}	15	3
(13)	,, ,,	1×10^{-5}	23	3
(14)	,, ,,	1×10^{-6}	31	4
(15)	,, ,,	1×10^{-7}	16	2
(16)	Oxindole-3-propionic acid	1×10^{-4}	16	2
(17)	,, ,,	1×10^{-5}	19	2
(18)	,, ,,	1×10^{-6}	22	3
(19)	,, ,,	1×10^{-7}	18	2
(20)	Oxindole-3-butyric acid	1×10^{-4}	31	6
(21)	,, ,,	1×10^{-5}	26	5
(22)	,, ,,	1×10^{-6}	56	7
(23)	,, ,,	1×10^{-7}	42	6
(24)	3-methyl-oxindole	1×10^{-4}	24	3
(25)	,, ,,	1×10^{-5}	24	3
(26)	,, ,,	1×10^{-6}	24	3
(27)	,, ,,	1×10^{-7}	34	5

* All media contained 2.3×10^{-6} molar kinetin in addition to the test compounds.

† The data given represent the average of nine pieces of callus; each test was run in triplicate. The tissue was collected 45 days after transplanting.

Table 2. SUBSTITUTION OF OXINDOLES FOR KINETIN IN THE GROWTH OF TOBACCO PITH CALLUS TISSUE

Test No.	Additive*	Molar conc.	Final† fresh wt. per piece (mg)	Final dry wt. per piece (mg)
(1)	0	—	49	5
(2)	Kinetin	2.3×10^{-6}	1,230	67
(3)	Isatin	1×10^{-3}	58	5
(4)	Oxindole	1×10^{-4}	43	5
(5)	Oxindole-3-acetic acid	1×10^{-5}	26	3
(6)	Oxindole-3-propionic acid	1×10^{-5}	42	5
(7)	Oxindole-3-butyric acid	1×10^{-5}	81	10
(8)	3-Methyl-oxindole	1×10^{-5}	35	4

* All media contained 5.7×10^{-6} M IAA in addition to the test compounds.

† The data given represent the average of nine pieces of callus; each test was run in triplicate. The tissue was collected 45 days after transplanting.

investigation, 201°–202° C, compared with that given for isatin in the *Merck Index*[7], however, and analysis of the sample by thin layer chromatography in two developing solvents showed but a single spot.

The relationship between structure and activity for auxins has been under investigation for many years. Many criteria hypothesized as necessary for auxin activity have been nullified by the finding of new active compounds. The longest standing structural requirement, to possess a carboxyl group[8], seemingly does not hold true for isatin. The auxin activity exhibited by isatin suggests a possible re-evaluation of the whole concept of structural requirement of auxins. Before such a modification is considered, however, more information must be obtained on the metabolic fate of isatin *in vivo*.

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Effect of Seed Dressing with 'Granosan' on the Prevalence of Fungi in the Root Zone of Cotton Plants

VARIOUS investigators have reported that various environmental factors, such as temperature, moisture and light, influence micro-organisms in the vicinity of plant roots in two ways: directly, by affecting the rate of growth and multiplication of these organisms and influencing the availability of nutrients in the soil; and indirectly, by controlling plant growth and thereby affecting the nature and amount of root excretions[1]. Various factors affecting the interaction of micro-organisms and plant roots, such as the nature and age of the plant, the nature and treatment of the soil, and moisture content, have been studied by a number of workers[2,3]. Because seed treatment with fungicides is likely to influence the development of fungi in the vicinity of the roots of plants raised from treated seeds and because the consequences of any changes in the balance of micro-organisms in the soil could be harmful[4] or beneficial[5] to the plant, it was considered worthwhile to carry out a preliminary investigation of the effect of seed dressing with 'Granosan' on the prevalence of fungi in the vicinity of the roots of cotton plants.

This investigation was carried out in parallel with one on the effects of 'Granosan' in the control of damping disease of cotton under various sowing conditions in the United Arab Republic on the growth, yield and fibre characteristics of cotton plants. Soil samples were collected from the root zones of plants raised from untreated seeds or from seeds treated with 'Granosan' at the rate of 0·25 per cent by weight of the dry seed under the various experimental conditions. Using sterilized spoons, samples were collected from the root zone of cotton plants (usually at a distance of 7–10 cm from the root and at a depth of 5–10 cm in the soil); these were thoroughly mixed and kept in sterilized containers for further use. Five samples were taken at random from each replica of each treatment. The five samples of each replica were amassed in one composite sample which was thoroughly mixed. Finally, 10 g (calculated on an oven dry basis) of the mixed composite sample were used for determining the frequency of occurrence of the various genera of fungi by the dilution plate method.

We are fully aware that the soil dilution plate method of sampling soil microflora has been criticized by many workers. It was reported to be of little use in estimating the activity of fungi in soil, it favours fungi which sporulate heavily in soil, and it records only a certain number of the species present in the soil, because not all fungal segments and spores will enter into suspension or grow on plates. Warcup[7] considered the soil dilution method to be thoroughly justified in the case of cultivated soils, and Maciejowska and Williams[8] believed that this method of sampling was adequate for the determination of succession of soil microflora. The soil sample (10 g) was suspended into 100 ml. of sterile tap water in a sterile conical flask of capacity 0·5 l. The flask was shaken, using a mechanical shaker, for about 20 min after which dilution series were made under aseptic conditions (to 1/56,100) in sterile containers using sterile water. One-millilitre portions of the diluted suspension were transferred to each of a series of four sterile Petri dishes. Thus, sixteen Petri dishes were used for each treatment. A tube of melted agar (containing approximately 10 ml.) was cooled to 50° C and then added to each Petri dish. The dish was gently shaken to spread the agar medium evenly. All dishes were incubated at 25° C. The number of colonies developing in each dish was counted after incubation for 2–3 days. A record of the relative abundance of the main types of fungi occurring on the plates was made. The culture medium used for growing the inocula was peptone dextrose agar[9,10]. It had the following composition: 20 g agar, 1·0 g potassium dihydrogen *ortho*phosphate; 0·5 g

$MgSO_4.7H_2O$; 5·0 g peptone; 10·0 g dextrose; 1 l. distilled water; rose bengal 1: 30,000; 30 μg streptomycin/ml. Table 1 gives a representative set of the percentage of isolation of some of the more significant fungi which developed on the isolation plates, at 2-week intervals from April 5 to July 3, 1963. The results presented in Table 1 give the average values of the percentages of fungi isolated from soil samples in the root zones of plants raised under the various experimental conditions. The only variable recorded is that some of the seeds were treated with 'Granosan' at the rate of 0·25 per cent by weight of dry seed.

The general observation revealed by the results of this investigation is that the frequencies of occurrence were highest for the genera *Aspergillus*, *Fusarium* and *Penicillium* followed by *Mycelia sterilia* and the genus *Pythium*. Other fungi showed much lower values for frequency of occurrence. This may be explained by the fact that a relatively high temperature was prevalent in the United Arab Republic when the isolations were made (Fig. 1). It may be recalled here that Waksman[11], Werkenthin[12] and Jensen[13] reported that *Aspergillus* was more common in hot than in cold climates. Sabet[14] found that *Aspergillus* was fairly common in Egyptian soils and attributed this to the hot climate. Waksman[15] stated that the most common genera of fungi found in the soil, both in number of species and in frequency of occurrence, are *Zygorhynchus*, *Mucor*, *Rhizopus*, *Penicillium*, *Aspergillus*, *Fusarium* and *Cephalosporium*. Garrett[16] claimed that the delimitation of an ecological group of soil fungi is based primarily on the ability of such a group to function as pioneer colonizers of a particular substrate. Thus the "sugar fungi", represented mainly by Phycomycetes, are typically found as the first colonizers of injured or dead plant tissues in the soil. Fungi having the ability to decompose cellulose usually follow and most frequently comprise Ascomycetes and fungi imperfecti. This might explain the lower frequency of *Rhizopus* and *Mucor* in the soil used in the present investigation, since it had been cultivated for several years.

Statistical analyses of the results of this investigation revealed the following: (1) Seed dressing with 'Granosan' did not influence the frequency of occurrence of the great majority of fungal genera isolated. This was most noticeable for those genera which revealed the highest frequencies of occurrence. It should be mentioned here, however, that the failure of seed dressing with 'Granosan' to influence the prevalence of these fungal genera does not indicate that such seed treatment has no effect on the microbial population of the soil. The soil samples collected were from soil exposed only once to such seed dressing, and it is quite possible that repeated exposure of the soil fungal flora to the fungicide would affect the balance of such flora. (2) The percentage of isolations recovered on the isolation plates was found to be affected by the date of isolation. Generally speaking, the variations observed in the frequencies of occurrence of the fungal flora at the different dates of isolations fell into four groups: (*a*) Fungi the numbers of which increased on the isolation plates with time. This group included the genus *Cryptomella*. (*b*) Fungi the numbers of which decreased with time and this group included the two genera *Penicillium* and *Pythium*. (*c*) A third group of fungal genera which behaved irregularly with respect to the time of isolation and this included the genus *Sepedonium*. (*d*) Another group which revealed no appreciable change as regards frequency of occurrence at the various dates of isolation. This group included most of the genera, namely, *Aspergillus*, *Cephalosporium*, *Fusarium*, *Humicola*, *Mucor*, *Pullularia*, *Rhizopus*, *Scopulariopsis*, *Stachybotrys*, *Trichoderma* and *Verticillium*. The difference in behaviour of the various fungal genera can be attributed to various factors including soil temperature, and moisture, age of plant, and availability of nutrient material in the soil. No definite explanation of such behaviour can be given at this time, and this point will be further investigated.

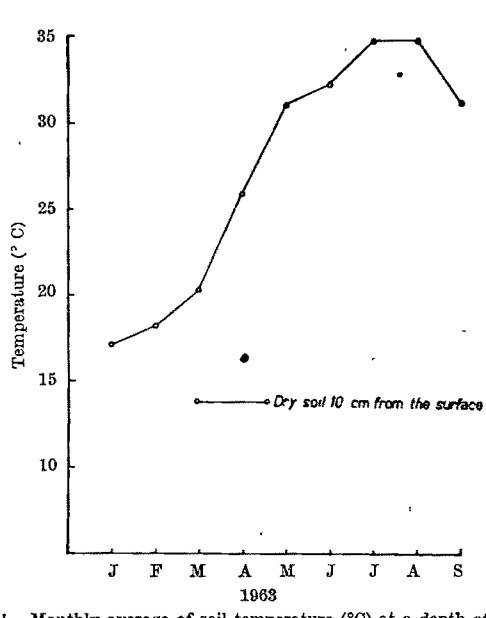


Fig 1. Monthly average of soil temperature (°C) at a depth of 10 cm from surface during the period of fungal isolations in the year 1963.

Table 1. REPRESENTATIVE SET OF PERCENTAGE ISOLATION OF FUNGAL GENERA SHOWING SIGNIFICANT FREQUENCY OF OCCURRENCE DURING THE PERIOD APRIL 5–JULY 3, 1963, FROM SOILS IN THE ROOT ZONES OF PLANTS RAISED FROM UNTREATED SEEDS OR SEEDS TREATED WITH 'GRANOSAN' AT THE RATE OF 0 25 PER CENT BY WEIGHT OF THE DRY SEED

Genera	Percentage isolation at 5/4	22/4	9/5	26/5	15/6	3/7	Mean
Fusarium							
−Gr.*	29 3	13·8	18 3	17 4	19·8	12 8	18 5 ± 5·16
+Gr.	28 0	21·1	24·0	14 5	12 2	14 3	19 1 ± 6 30
Mean	28·7	17·5	21·1	16 0	16 0	13 2	
Penicillium							
−Gr.	19 1	16·1	13 4	13·7	11·1	9 9	14 0 ± 3 31
+Gr.	14 1	20 1	10 1	12 6	11 3	6 2	12 4 ± 4·58
Mean	16·6	18·1	11 8	13 2	11·2	8 1	
Pythium							
−Gr.	13·8	1 7	3 2	2 0	6 6	4·5	5 3 ± 4 50
+Gr.	8·6	4·2	3 9	1 7	3 5	2·3	4 1 ± 2·44
Mean	11 2	3 0	3 6	1·9	5 1	3 4	
Aspergillus							
−Gr.	18·2	11·1	27 9	12 3	27 7	20 8	19 7 ± 7·21
+Gr.	23 1	22 3	16 0	20 7	17 6	22·2	20 3 ± 2 88
Mean	20 7	16·7	21 9	16·5	22 7	21 5	
Mycelia sterilia							
−Gr.	7 2	2 5	4 4	6·2	7 3	8 3	6 0 ± 2 12
+Gr.	9·4	0·9	5·1	2 9	6 4	7 6	5·4 ± 3·08
Mean	8 3	1 7	4 8	4·8	6 9	8·0	

* − Gr., Isolations from soil in the root zones of plants raised from untreated seeds. + Gr, Isolations from soil in the root zones of plants raised from seeds treated with 'Granosan' at the rate of 0 25 per cent by weight of the dry seed.

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[1] Rouatt, J. W., Peterson, E. A., Katznelson, H., and Henderson, V. E., *Canad. J. Microbiol.*, **9**, 227 (1963).
[2] Katznelson, H., Lochhead, A. G., and Timonin, M. I., *Botan. Rev.*, **14**, 543 (1948).
[3] Starkey, R. L., *Bacteriol. Rev.*, **22**, 154 (1958).
[4] Bowen, G. D., and Rovira, A. D., *Plant Soil*, **15**, 166 (1961).
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[6] Tolba, M. K., Salama, A. M., and Ali, M. I., *U.A.R. J. Bot* (in the press).
[7] Warcup, J. H., in *International Symposium*, 3 (Liverpool University Press, 1960).
[8] Maciejowska, Z., and Williams, E. B., *Canad. J. Microbiol.*, **9**, 555 (1963).
[9] Martin, J. P., *Soil Sci.*, **69**, 215 (1950).
[10] Johnson, L. F., *Phytopath.*, **47**, 631 (1957).
[11] Waksman, S. A., *Soil Sci.*, **3**, 565 (1917).
[12] Werkenthin, F. C., *Phytopath.*; **6**, 241 (1916).
[13] Jensen, H. L., *Soil Sci.*, **31**, 123 (1931).
[14] Sabet, Y. S., *Bull. Faculty Sci.; Egyptian Univ.*, **5**, 1 (1935)
[15] Waksman, S. A., in *Soil Microbiology* (John Wiley and Sons, Inc., New York, 1952).
[16] Garrett, S. D., *New Phytologist*, **50**, 149 (1951).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Wednesday, September 14

SOCIETY FOR ANALYTICAL CHEMISTRY, THIN-LAYER CHROMATOGRAPHY GROUP (at the Enfield College of Technology, Queensway, Enfield), at 2 p.m. —Meeting on "Some Applications of Thin-Layer Chromatography in Pharmacy".

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W 1), at 5.30 p.m.—Mr. J. Thomson and Mr. P W. Harrison: "The Development of a New HD Engine Oil for Marine Service".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (in the Physics Department, Imperial College, London, S.W.7), at 6.30 p m.— Mr. D. A. Bayliss: "Problems of Paint Users".

Thursday, September 15—Friday, September 16

BIOCHEMICAL SOCIETY (at University College, Aberystwyth) 463rd MEETING. Programme will include Colloquia on "Training of Biochemists" and "Biochemistry of Herbicides".

Friday, September 16

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. W. T Underhill "A Four Tube Colour Camera".

Monday, September 19

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at Shell Centre, London, S.E.1), at 6.30 p.m.—Dr. V. E. Yarsley. "Hermann Staudinger, His Life and Work".

Monday, September 19—Wednesday, September 21

CHARLES SALT RESEARCH CENTRE (at the Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire)—Symposium on "The Biological Effects of Phytohaemagglutinin".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS (2) (with a good honours degree in chemical engineering and industrial/research experience) IN THE DEPARTMENT OF CHEMICAL ENGINEERING—The Staff Officer, The University of Aston in Birmingham, Gosta Green, Birmingham, 4, quoting Ref. 493/2 (September 14).

SCIENTIFIC OFFICER, BASIC GRADE (with a science degree) in the Serological Laboratories of the South London Transfusion Centre—The Director, South London Transfusion Centre, Stanley Road, Sutton, Surrey (September 14).

MEDICAL OFFICER (highly qualified in medicine and with extensive experience in cardiovascular research as well as in the organization of cardiovascular disease control programmes) IN THE HEADQUARTERS UNIT OF CARDIOVASCULAR DISEASES, GENEVA. Responsibilities will also involve planning, initiation and international co-ordination of research and control programmes in cerebrovascular diseases—Chief, Personnel, World Health Organization, Geneva, Switzerland (September 15).

SENIOR LECTURER IN SOCIOLOGY—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (September 15).

EXPERIMENTAL OFFICER (at least 26 on 1st January, 1966, with a degree, Dip. Tech., H N.C. or equivalent qualification in a scientific subject) in London, to provide support for the scientific staff whose task it is to implement the Council's policy over the whole range of its interests in the environmental sciences (geology, geophysics, hydrology, oceanography, marine biology, ecology and forestry)—The Natural Environment Research Council (E), Room 1221A, State House, High Holborn, London, W.C.1 (September 16).

LECTURER IN CLINICAL CHEMISTRY in the recently expanded DEPARTMENT OF CLINICAL CHEMISTRY, Queen's College, Dundee, and the Royal Infirmary, Dundee—The Secretary, University of St. Andrews, Queen's College, Dundee (September 17).

LECTURER or ASSISTANT LECTURER (mathematician with research interests in any branch of pure mathematics or mathematical statistics) IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Swansea, Singleton Park, Swansea, South Wales (September 17).

SENIOR LECTURER IN THE DEPARTMENT OF CHEMICAL PATHOLOGY—Prof F. T. G. Prunty, Department of Chemical Pathology, St. Thomas's Hospital Medical School, London, S E.1 (September 19).

SUPERVISOR OF PROJECTS IN THE DEPARTMENT OF SOCIOLOGY, to supervise and arrange research projects for graduate and undergraduate students— The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (September 23)

ASSISTANT EXPERIMENTAL OFFICER or EXPERIMENTAL OFFICER to work on selective herbicides in vegetable crops—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (September 24).

ASSISTANT LECTURER (preferably candidate who has worked in the field of insect physiology) IN ZOOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (September 26).

SENIOR LECTURER and a LECTURER IN MICROBIOLOGY at the University of the West Indies—The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W.C.1 (September 26).

SENIOR LECTURER or LECTURER IN (*a*) the DEPARTMENT OF MEDICINE, and (*b*) the DEPARTMENT OF RADIOLOGY, University of Ibadan, Nigeria— The Secretary, Senate Committee on Higher Education Overseas, University of London, Senate House, London, W C.1 (September 26).

LECTURER IN BIOCHEMISTRY IN RELATION TO VETERINARY STUDIES— The Secretary of the University Court, The University, Glasgow (September 28).

LECTURER/SENIOR LECTURER (with a distinguished academic record and able to produce evidence of strong research interests and experience) IN THE DEPARTMENT OF STATISTICS, School of Mathematics, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 28).

LECTURER/SENIOR TUTOR IN MATHEMATICS at the Broken Hill Division, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 28).

TEMPORARY LECTURER or DEMONSTRATOR IN ANATOMY—The Registrar, University of Bristol, Senate House, Tyndall Avenue, Bristol, 2 (September 28).

LECTURER IN MEDICAL CARDIOLOGY—The Secretary of the University Court, The University, Glasgow (September 30).

LECTURER or ASSISTANT LECTURER (with experience in, and a good theoretical understanding of, public health engineering and hydrology) IN THE DEPARTMENT OF CIVIL ENGINEERING—The Registrar, The University, Leeds, 2 (September 30)

LECTURER (or possibly, initially, a Departmental Demonstrator) IN PALAEONTOLOGY IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY— The Prof of Geology, Department of Geology and Mineralogy, University of Oxford, South Parks Road, Oxford (September 30).

LECTURER (with a distinguished academic record) IN CHEMISTRY at the Broken Hill Division, University of New South Wales—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

PATHOLOGIST (Forensic Medicine) (with appropriate professional qualifications and relevant experience) IN THE MINISTRY OF HEALTH, Republic of the Sudan—Appointments Section, Sudan Embassy, 3 Cleveland Row, London, S.W.1, quoting Ref. 4/1200 (September 30).

SENIOR LECTURER (with full training in psychology and special experience in clinical psychology and preferably a higher degree Ph.D. or equivalent) IN CLINICAL PSYCHOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (September 30).

TUTORS or SENIOR TUTORS IN THE DEPARTMENT OF MATHEMATICS—The Registrar (Appointment E. 74), The University of Melbourne, Parkville, N.2, Victoria, Australia (September 30).

JUNIOR RESEARCH FELLOW IN GEOGRAPHY; and a CLIMATOLOGIST (preferably honours graduate in geography and/or physics, mathematics or meteorology) to work in a research unit in the DEPARTMENT OF GEOGRAPHY— The Registrar, The University, Sheffield (October 1).

RESEARCH ASSISTANT (with an honours degree in botany, or an equivalent qualification, and preferably with some field knowledge of bryophytes) IN THE DEPARTMENT OF BOTANY to assist in an investigation of the environmental control of reproduction in bryophytes—The Assistant Registrar (Science and Engineering), The University of Birmingham, P.O. Box 363, Birmingham, 15 (October 1).

SUB-LIBRARIAN (graduate, with a professional qualification in librarianship, and appropriate senior experience)—The Secretary, Royal College of Advanced Technology, Salford, 5, Lancashire, quoting Ref. LY/19 (October 2).

SENIOR LECTURER (with a good degree, preferably honours, extensive experience in teaching, advisory or research work in agriculture, and preferably an interest in possible changes in agricultural education) IN AGRICULTURE—The Secretary, The West of Scotland College of Agriculture, 6 Blythswood Square, Glasgow, C.2 (October 5).

PROFESSOR OF MATHEMATICS at the University of Queensland, Australia— The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 14).

PROFESSOR (with a medical qualification registrable in the State of Queensland, extensive experience in some branch of pathology, evidence of substantial achievements in research, and preferably some teaching experience) OF PATHOLOGY at the University of Queensland, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (London and Brisbane, October 14).

SENIOR LECTURER (Pure Mathematics) at the University of Melbourne, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 15).

CHAIR OF AGRICULTURE at Makerere University College, Uganda (The University of East Africa)—The Inter-University Council, 33 Bedford Place, London, W C.1 (October 22).

CHAIR OF MICROBIOLOGY at the University of Sydney, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, October 25).

PROFESSOR OF PHYSICS (Theoretical) at Flinders University of South Australia, Bedford Park, South Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 31).

SENIOR LECTURER (with an honours degree in electrical engineering or science, and preferably a higher degree; and research or design experience in fields related to digital computers or digital data-processing and instrumentation systems) IN ELECTRICAL ENGINEERING at the University of Adelaide, South Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 31.)

READER IN HISTORY AND PHILOSOPHY OF SCIENCE—The General Board Office, University of Cambridge, The Old Schools, Cambridge (November 16).

ASSISTANT EXPERIMENTAL OFFICERS for (*a*) work on identification and estimation of volatile substances which inhibit the sprouting of potatoes: and (*b*) experiments on the metabolism and storage of soft fruits and vegetables—The Secretary, A. R. C. Ditton Laboratory, near Maidstone, Kent.

BIOCHEMIST (with a degree, or equivalent, in biochemistry) IN THE DEPARTMENT OF ANAESTHETIC RESEARCH, to join a team engaged in work on isolated liver perfusion experiments and on intracellular *p*H measurements—The House Governor, The London Hospital, Whitechapel, London, E 1.

DEMONSTRATOR (graduate in biochemistry or chemistry) IN BIOCHEMISTRY —The Acting Registrar, University College of Wales, Aberystwyth.

PHYSICIST to work on the physics of radiotherapy, clinical applications of radioactive isotopes, and radiation protection—The House Governor, St. Mary's Hospital, London, W.2.

POSTDOCTORAL FELLOW IN BIOCHEMISTRY for work which will involve studies on the control of metabolism by the thyroid hormones—Prof J. R. Bronk, Department of Biology, University of York, Heslington, York.

RESEARCH WORKER (with an interest in the physiological basis of animal behaviour)—The Secretary, Institute of Psychiatry (University of London), The Maudsley Hospital, Denmark Hill, London, S.E.5.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Concrete Block Walls. Fourth (revised) edition. Pp. 32. (London: Cement and Concrete Association, 1966.) 7*s*. 6*d*. [77

The Association of Clinical Biochemists. Scientific Report No. 2: Colorimeters—a Critical Assessment of Five Commercial Instruments. Pp. 62. (Liverpool: Mr. J. T. Ireland, Biochemistry Laboratory, Alder Hey Children's Hospital, 1966.) 15*s*. (Members of the Association, 10*s*.) [77

Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. No. 766, Vol. 250 (7 July 1966): The Fine Structure of the Centriolar Apparatus and Associated Structures in the Complex Flagellates *Trichonympha* and *Pseudotrichonympha*. By A. V. Grimstone and I. R. Gibbons. Pp. 215–242+plates 22–48. 50*s*.; 7·50 dollars. No. 767, Vol. 250 (7 July, 1966): Investigations Into the Causes of the Rise in Aldosterone Secretion During Haemorrhage, Parts 1 and 2. By Magarethe Holzbauer and Marthe Vogt. Pp. 243–310. 25*s*. 6*d*.; 3.80 dollars. (London: The Royal Society, 1966.) [77

The Overseas Development Institute. French Aid. By Teresa Hayter. Pp. 230. (London: The Overseas Development Institute, 1966.) 20*s*. [87

The Scottish Horticultural Research Institute. Twelfth Annual Report for the years 1964 and 1965. Pp. 78. (Invergowrie, by Dundee: The Scottish Horticultural Research Institute, 1966.) [87

Noble Gases and Scientific Opportunism. By Prof. R. D. Peacock. (An Inaugural Lecture delivered in the University of Leicester, 20 January, 1966.) Pp. 28. (Leicester: Leicester University Press, 1966.) 6*s*. [87

General Register Office. Studies on Medical and Population Subjects, No. 19: Regional and Social Factors in Infant Mortality. By C. C. Spicer and L. Lipworth. Pp. iv+40. (London. H.M. Stationery Office, 1966.) 7*s*. 6*d*. net. [87

National Lending Library for Science and Technology. British Research and Development Reports, No. 6, June 1966. Pp. ii+27. (Boston Spa: National Lending Library for Science and Technology, 1966.) [87

Republic of Ireland. Department of Transport and Power—Meteorological Service. Degree-Days Below Various Base Temperatures in Ireland Over the Five Years, 1958–1962. By W. A. Morgan. Pp. 51. (Dublin: Department of Transport and Power, 1966.) 1*s*. 6*d*. [117

Chromosome Studies on Adults. By W. M. Court Brown, Karin E. Buckton, Patricia A. Jacobs, Ishbel M. Tough, E. V. Kuenssverg and J. D. E. Knox. (University of London Galton Laboratory, University College, Eugenics Laboratory Memoirs XLII.) Pp. v+91. (London: Cambridge University Press, 1966. Published for the Galton Laboratory, University College, London.) 32*s*. 6*d*. net.; 6 U.S. dollars. [117

The Radiochemical Centre. The Use of Radioactive Materials in the Study of Lung Function. By John B. West. (Medical Monograph, No. 1.) Pp. 19. (Amersham: The Radiochemical Centre, 1966.) [127

White Fish Authority. Research and Development Progress Report 1966. Pp. 12. (London: White Fish Authority, 1966.) [127

The Royal Observatory, Edinburgh. Descriptive Brochure. Pp. 15. Report of the Astronomer Royal for Scotland for the Year Ending 31st March, 1966. Pp. 19. (Edinburgh: The Royal Observatory, 1966.) [127

Natural Environmental Research Council—The Nature Conservancy. Monks Wood Experimental Station—Report for 1960–1965. Pp. v+93+7 photographs. (London: The Nature Conservancy, 1966.) 8*s*. 6*d*. [127

Northern Ireland: Ministry of Agriculture. Leaflet No. 85: The Propagation of Trees. Pp. 14. (Belfast: Ministry of Agriculture, 1966.) [137

Timber Research and Development Association. Maintaining Timber Exposed to the Weather. Pp. 4. (High Wycombe: Timber Research and Development Association, 1966.) [137

General Register Office. The Registrar General's Statistical Review of England and Wales for the Year 1964. Part 2: Tables, Population. Pp. xlx+176. (London: H.M. Stationery Office, 1966.) 18*s*. net. [137

Agricultural Research Council. Memoirs of the Soil Survey of Great Britain—England and Wales. The Soils and Land Use of the District North of Derby (Sheet 125). By E. M. Bridges. Pp. vii+112+5 plates. (Harpenden: Rothamsted Experimental Station, 1966.) 25*s*. net. [137

Transactions of the Royal Society of Edinburgh. Vol. 66, No. 12 (1964–65): The Structure and Form of the Granophyric Quartz-Dolerite Intrusion, Centre II, Ardnamurchan, Argyllshire. By R. R. Skelhorn and R. W. D. Elwell. With Chemical Analyses by R. C. Tyler. Pp. 285–306+2 plates. 10*s*. 6*d*. Vol. 66, No. 13 (1964–65): Granites and Associated Rocks of the Eastern Part of the Western Redhills Complex, Isle of Skye. By J. D. Bell. Pp. 307–343+1 plate. 15*s*. Vol. 66, No. 14 (1964–65): Some Lower Carboniferous Fructifications from Berwickshire, Together with a Theoretical Account of the Evolution of Ovules, Cupules, and Carpels. By Albert G. Long. Pp. 345–376+4 plates. 12*s*. 6*d*. Vol. 66, No. 15 (1964–65): The Placodern Fish *Rhachiosteus pterygiatus* Gross and Its Relationships. By Roger S. Miles. Pp. 377–392+1 plate. 8*s*. 6*d*. (Edinburgh: The Royal Society of Edinburgh, 1966.) [137

Other Countries

Records of the Australian Museum, Vol. 26, No. 16 (25th May, 1966): Generic and Specific Diagnoses in the Gigantic Macropodid Genus *Procoptodon*. By R. A. Stirton and Leslie F. Marcus. Pp. 349–359. (Sydney: The Australian Museum, 1966.) 38 cents. [77

Handling of Toxicological Information. (A Report of the President's Science Advisory Committee.) Pp. iii+21. (Washington, D.C.: Government Printing Office, 1966.) 15 cents. [77

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IS SCIENCE POLICY POSSIBLE?

THE most remarkable feature of the OECD report on science policy in France, briefly reported on page 1226 of this issue, is that it should ever have been produced. Governments are notoriously but understandably reluctant to give outsiders bent on publication an opportunity to discover not merely what policy consists of but how it has come to be constructed. No doubt the French Government was emboldened by the knowledge that the same kind of examination and inquisition would be conducted in other nations belonging to the OECD. (By the same token, readers of the report on France will have their appetites whetted for what there is to come from elsewhere.) A more delicate means of knocking heads together could hardly have been devised.

The report on France is also remarkable for showing that many of the problems which preoccupy the French Government and its machinery for administering science policy are the same as those which appear as almost exclusive national concerns elsewhere. In France, for example, as in Britain and in the United States, the recruitment of teachers of science and mathematics cannot keep pace with the demand for them. In France as elsewhere, the arrangements for higher education, based as they are on long and cherished tradition, are found to be less than perfect means of training young people in the skills of research and then putting them to work where they are most needed. Then France is not the only country in which major decisions, affecting the whole balance of effort within science and technology, are often made without reference to the committees appointed ostensibly to give advice on scientific matters; if chauvinism has influenced some of the decisions on the use of scientific resources in France, so too must it be held at least partly responsible for the space programmes of the United States and the Soviet Union, and for the over-ambitious aircraft projects which from time to time hang themselves like albatrosses around the neck of the British Treasury. Finally, though French administrators are as much at a loss as their counterparts elsewhere to know what criteria to use in the deployment of scientific resources, they are at least manfully trying to find out.

Not all the problems identified in the report are common problems, however. The problem of the *Grandes Écoles* is, for example, uniquely that of France. The OECD report likens the pyramidal educational system to a distillation column, and says that in France there are really two distillation columns in parallel—the *Grandes Écoles* and the ordinary universities. The prestige of the engineering diplomas awarded by the *écoles* goes back to Napoleon, but the traditions which were once a great advantage now seem to be impediments to progress. Obviously it is a serious matter that the *écoles* cream off the best potential scientists and engineers and then somehow assure that they finish up in industry or public service but only rarely in research. This state of affairs may be the envy of those who regret that academic research often has too much pull in countries such as Britain, and it is probable that the flow of graduates from the *écoles* into French industry has something to do with the economic successes of recent years, but in the long run there can be no doubt that the separatism built into French higher education must be bad for science—in industry as well as in the academic laboratories. The OECD report points out that the *École Polytechnique* has taken the lead in showing how vigorous schools of research can be grafted on to the usual intensive *polytechnique* course, and how there are moves to increase the liaison between the *écoles* and the universities, partly by making diplomas and degrees interchangeable and partly by seeing that entrants to the science faculties at the universities should have had some reasonable preparation for the courses they wish to follow. It is plain, however, in spite of the reforms of higher education announced earlier this year (and after the OECD examiners had finished most of their work) that there is still a long way to go. In France, the strictures of the OECD committee may seem insensitive to the great (and undoubted) virtues of the *écoles*, but in the long run should have some of the advantages of the comment from the small boy who first said that the emperor had no clothes.

What the report has to say about the balance of industrial research in France—and which it might well have been saying about most other countries in Western Europe—is less convincing, though this is tricky ground. The essential question is to know what benefits are to be derived from the investment of effort in industrial research and development, and the OECD inspectors are right to say that there should eventually be great value in the careful and comprehensive studies now being made in France of the relation between research and economic benefit in all kinds of industries. This said, it is unfortunate that the report wanders off, somewhat aimlessly, into a discussion of what is called the "technological balance of payments"—the difference between the royalties which a country pays on foreign patents and the licence revenue which it earns from abroad. The implication seems to be that the ideal state of affairs should be one in which these two sums are in almost exact balance, and that effort spent on research should be increased until one side of the balance sheet cancels out the other. But is this necessarily the case? Earning patent royalties is not, after all, the only way of making money.

The examiners are much more sure of themselves on the machinery of the administration of science. From what they have to say, there are obviously great advantages in the existence of the Delegation-General for scientific research—a permanent secretariat serving both the ministerial committee responsible for major policy decisions (and for the final allocation of the budget) and the advisory committee of scientists responsible for making recommendations on these matters. The size and competence of the secretariat are obviously the chief reasons why it has been possible to sustain such a rapid rate of growth of science in France in recent years without too much absurdity. Yet even so, it has not been possible to iron out all the awkward ups and downs in the scale of financial support. Worse still, the consultative machinery seems to have worked less effectively than the examiners would have wished, so that major decisions have been made on grounds which are too exclusively political.

The essential difficulty is the conflict of interest there is almost certain to be between a nation and the scientific community embedded within it. The objectives of the two entities do not necessarily coincide. The extent to which the United States and the United Kingdom were committed to defence research and development in the decade after the war, inevitable though it may have been, often created a fearful unbalance of the pattern of scientific work. (It is to be hoped that the Government of France will not now feel it necessary to mimic the full extent of this pattern; the OECD examiners were alarmed by some of the signs they thought they could discern.) More recently, the space programme in the United States has not been precisely what scientists themselves, with the interests of their craft in mind, would have dictated. But to ask that the efforts of scientists should be deployed only in ways that suit scientists themselves is to ask too much. It is, of course, entirely respectable that the efforts of scientists should be consciously directed at practical objectives. But is it, then, that the relationship between a nation and its scientists should be like its relationship with its armed services? Is it for society to decide what science should attempt, and for scientists to do society's bidding? This thin analogy seems to lie behind some of the machinery for making decisions in France—and elsewhere. But this, too, is an untenable extreme. One difficulty is that objectives defined from outside may prove to be unattainable. More serious, there is the real danger that objectives which are too rigid or too unreal may rob science of its own self-generating intellectual excitement which, in the last resort, is its only stock in trade. Finally, this same excitement is itself a help in defining realizable and valid objectives. All of this argues in favour of a much better balance—in France and elsewhere—between the interests of governments and scientists in the definition of objectives. Most probably, no system of committees will ever be sufficient. The real need is for some much more vigorous involvement of working scientists in the definition of what their work is for.

NEWS AND VIEWS

Home for Microscopes

THE Royal Microscopical Society is one of the most vigorous of the learned societies in Britain. Far from being a kind of club for those concerned with the technology of building microscopes, it has a catholic interest in all those fields of science in which the micron and the ångström are in common usage. It is therefore sad to see the society having to hunt around for a permanent headquarters outside London. The rent of the present offices in Tavistock Square is being enormously increased according to Dr. John Baker, chairman of the society's council, who feels that the society cannot afford to remain in London. He would like to see the society move to another town, probably close to a university, where it could house its offices, microscope collection and laboratories for its courses in optical microscopy. The monthly meetings would still be held in London.

While the council can make no definite plans until a decision is made by the members, they are investigating various sites. One possible site in Oxford was turned down by the City Council because the city wanted the site, but Dr. Baker stated that Oxford is still very much a possibility. Wherever the society decides to go they will certainly be welcomed, but this hunt for living space may be regarded by some as yet another sign of the need for a more generous provision of board and lodging for British societies.

Atomic Agriculture

THE International Atomic Energy Agency of the United Nations is giving Yugoslavia more than 600,000 dollars worth of equipment for research at the Zemun Institute for the Application of Nuclear Energy in Agriculture, Veterinary Medicine and Forestry. The Yugoslav government has contributed about two million dollars worth of buildings, equipment, training and general expenses to make the institute the centre of agricultural research in the country. During the last three years the United Nations Development Programme, with the IAEA acting as the executive agency, has cooperated with the Yugoslav Federal Nuclear Energy Commission in one of the programme's biggest single projects for applied research and training. With this combined support, the institute has undertaken extensive studies of soil fertility and plant nutrition, use of ionizing radiations to produce mutants for plant breeding, and animal nutrition and health protection.

American Rock Names

THAT much has flowed down the geological rivers of North America since 1936 is plainly obvious from the latest milestone in publications from the U.S. Geological Survey. The three volumed *Lexicon of Geological Names of the United States for 1936–1960* (*U.S.G.S. Bulletin 1200*, Washington, D.C., 1966. $13) is the sixth such compilation to come from the Survey since F. B. Weeks's *North American Geologic Formation Names* was published in 1902.

The present 4,340 page work is an admirable successor to Wilmarth's monumental lexicon of 1938. As before, the area covered includes the United States, its possessions, the Trust Territory of the Pacific Islands and the Panama Canal Zone.

In all, 14,634 names are included, together with summaries of stratigraphical papers which were published between January 1, 1936, and December 31, 1960. The number of such abstracts and "original references" now exceeds 35,000. The lexicon differs from Wilmarth's in that it omits all palaeontological and descriptive terms, trade names, moraines, orogenies and miner's terms.

Obviously there has been erosion of many geological names and also deposition of numerous new ones in the literature. Enthusiasts of the "Western" cult will be disappointed to learn that the U.S. Geological Survey has abandoned "Silver Creek Shales" and the "Panhandle Beds". They can take heart, however, that their heroes will still be able to ride high over the "Death Valley Formation" and "Diamond Creek Sandstone".

Nuclear Fuel for Space

THE establishment of an artificial atmosphere on Jupiter's moons, communications from beyond Pluto, water extracted from the surface of the Moon—science fiction turned to fact, with the help of nuclear energy; these and more immediate problems confronting space scientists will be solved with nuclear power according to Dr. Glenn T. Seaborg, chairman of the United States Atomic Energy Commission.

Speaking at the Western Electric Show and Convention in Los Angeles a few weeks ago, Dr. Seaborg outlined the AEC's present joint programme with the National Aeronautics and Space Administration, and the Department of Defense, on atomic energy in space. The two areas of development are the Rover Programme to develop systems for nuclear rocket propulsion and the SNAP Programme to generate electric power. The nuclear rocket is a single engine that would satisfy a number of upper stage and spacecraft applications—a 5,000 MW NERVA engine producing 250,000 lb. of thrust. So far, 1,000 MW rocket reactors have been successfully operated, and Dr. Seaborg stated that this included restarting and operating them at full power through multiple cycles. He added that the AEC had achieved on a single reactor a running time of 30 min at full power—which is approximately the same running time required for a single engine to boost a manned mission to Mars.

Research is also being carried out on a nuclear-electric rocket for use during long voyages. In this system, a highly efficient low-thrust engine is envisaged which will produce a high specific impulse and use electrical energy to accelerate the propellant. Because such a system could be used continually throughout a long voyage, the rocket could eventually build up to a far greater velocity than a chemical or nuclear rocket. NASA and the Air Force are developing the necessary thrust components; the AEC is developing the power source under its SNAP programme. SNAP's broad aim is to provide long-lived electrical power systems for all the scientific and communications equipment in satellites and to provide the energy needed to keep men alive in space. Dr. Seaborg explained that of the two general categories of SNAP systems, the first was based on the generation of electricity from the heat created by decaying radioisotopes while the second used the heat from fission reactors to produce electricity. The first isotopic power system to be used was powered by plutonium-238; this went into orbit on a U.S. Navy navigational satellite in 1961 and its signals are still being monitored. Dr. Seaborg stated that a SNAP unit will power the *Apollo* lunar surface package left on the Moon by the *Apollo* astronauts.

Looking further into the future, Dr. Seaborg visualized the place of nuclear rockets in lunar and planetary exploration. He sees the more efficient nuclear rockets transporting enough supplies to a lunar base camp to last several months. Later nuclear rockets would be used as lunar ferries, shuttling between re-usable chemical rockets travelling from the Earth or Moon to a rendezvous in orbit with the nuclear rocket. A similar system could be used for voyages to Mars and beyond. As Dr. Seaborg said, "The combination of high-thrust nuclear rockets for escape from planetary gravity fields and low-thrust, low-weight, nuclear-electric systems for interplanetary propulsion promises to do great things for solar system exploration".

Vetlesen Prize

JAN HENDRIK OORT, the Dutch astronomer, has won the 1966 Vetlesen Prize for distinguished achievement in the earth sciences, President Grayson Kirk of Columbia University announced last week. Dr. Oort received the Vetlesen Gold Medal and $25,000 for "outstanding achievement in the sciences resulting in a clearer understanding of the Earth, its history or its relation to the universe". The Vetlesen Prize was established at Columbia University in 1960 by the G. Unger Vetlesen Foundation.

Dr. Oort, 66, is a professor at the University of Leiden and is director of the Leiden Observatory. He has achieved world-wide recognition for his studies of the rotation of our galaxy and of radio astronomy. In 1927, Dr. Oort showed that the galaxy rotates about its centre and determined the speed of rotation and the direction of the centre of rotation. Since the early fifties Dr. Oort has worked in radio astronomy. The mapping and detailed study of the spiral structure of the galaxy, during the fifties, was made possible through his research and other research under his leadership.

Food Research

THE first annual report (1965) of the Food Protection and Toxicology Center, University of California at Davis, describes the foundation, objectives and progress of the centre. The centre is carrying out research, has established an information and documentation service in chemical and microbiological toxicity, and has started new undergraduate and postgraduate courses in agricultural toxicology, fundamentals of radiation biology and environmental engineering. Research at the centre covers a diversity of topics—rapid tissue culture methods for detection of chemical residues and toxins in foods and feed stuffs; the use of isolated organs for detecting the precise location of toxic effects and the nature of the effects; methods to increase mobilization and excretion of DDT in cows; and isolation of breakdown products and determination of the mode of decomposition of agricultural chemicals.

Biochemical mechanisms are being studied to determine how cell division is inhibited by radiometric compounds and an investigation is being undertaken into the biochemical composition and detoxifying function of lysozymes. A wide study of the movement of pesticides and agricultural chemicals in and over soil is being made to show how pollution of surface and ground waters is effected and how the movement may affect plants and animals in their uptake of such polluted waters. A three phase study has been completed of food-borne *Salmonella* infections of avian origin, and an investigation of botulism in foods has been started.

Four more projects have been proposed but still need money. One of these is the isolation of natural plant toxins and assessment of their toxicity—these toxins are mainly of academic interest, but it is surprising that no proposal has been made for work on mycotoxins, which are of some economic importance in the United States.

New Zealand Science

THE New Zealand Department of Scientific and Industrial Research continues to increase the scale of its activity. According to the Annual Report for the year to March 31, 1966, just published (Government Printer, Wellington, 3*s*.), the scientific staff of the department increased by 42 to a total of 553. The total expenditure during the year amounted to £3·477 million, more than 10 per cent of which was spent on grants to various research institutes and research associations.

In its research policy the department is hoping to give increasing attention to the problems of particular concern to the economy of New Zealand, in particular a more efficient use of labour and materials. The Institute of Nuclear Sciences, which supplies radioactive isotopes to users in New Zealand, is particularly pleased with the profit being derived in industry from the use of its materials. Prospecting for minerals continues and there has been some success in research for ilmenite, and there is a possibility that "the commercial climate" will become more favourable for the use of titanium ores in New Zealand in the years immediately ahead. Attention is also being given to the possible extension of the present use of geothermal steam at Wairakei and elsewhere. Preliminary studies for underground reservoirs have suggested that each of these may be big enough to support a geothermal power station similar to that operating at Wairakei. There, during the past ten years, the pressure of the hot water at the bottom of the bore holes has dropped, presumably because of the increasing rate at which steam is being used at the power station. Although the causes of this decline in pressure are not properly understood, it is expected that it will be 70 years before the present reservoir has shrunk by as much as 10 per cent.

The department is making what use it can of parasitic insects in pest control. In the past it has proved possible to control the oak leaf miner and Mexican devil weed by such means. In the current growing season, record numbers of insects are being released to control pests. In the South Island female thynnid parasites are being released in the hope that they will control grass grub, and similar methods are being used against the ryegrass stem weevil, the potato tuber moth and the passion vine hopper.

University News:

British Columbia

PROF. J. H. QUASTEL has been appointed professor of neurochemistry, in the Faculty of Medicine, and honorary professor of biochemistry.

London

THE title of professor emeritus has been conferred on the following: Prof. F. Bergel (chemistry, in respect of his former post as professor of chemistry in the Institute of Cancer Research : Royal Cancer Hospital); Prof. J. C. Buckley (helminthology, in respect of his former post as William Julien Courtauld professor of helminthology in the London School of Hygiene and Tropical Medicine); Prof. J. A. V. Butler (physical chemistry, in respect of his former post as professor of physical chemistry in the Institute of Cancer Research : Royal Cancer Hospital); Prof. G. A. H. Buttle (pharmacology, in respect of his former post as Wellcome professor of pharmacology in the School of Pharmacy); Sir Aubrey Lewis (psychiatry, in respect of his former post as professor of psychiatry in the Institute of Psychiatry); Sir John McMichael (medicine, in respect of his former post as professor of medicine in the Postgraduate Medical School of London); Prof. C. M. White (fluid mechanics and hydraulic engineering, in respect of his former post as professor of fluid mechanics and hydraulic engineering at the Imperial College of Science and Technology); Prof. D. Williams (mining geology, in respect of his former post as professor of mining geology in the Imperial College of Science and Technology).

Pennsylvania

DR. D. WHITE, professor of chemistry at Ohio State University, has been appointed chairman of the Department of Chemistry. Dr. J. Donohue, chairman of the Department of Chemistry in the University of Southern California, has been appointed professor of chemistry.

Appointments

MR. J. A. RATCLIFFE, director of the Radio and Space Research Station of the Science Research Council until his retirement this year, has been appointed president of the Institution of Electrical Engineers for the session 1966–67. He will take office as president as from October 1.

DR. H. KING, scientific director of the London Planetarium, has been appointed first curator of the McLaughlin Planetarium of the Royal Ontario Museum of the University of Toronto. Dr. King is also secretary—and a former president—of the British Astronomical Association.

MR. M. H. LOVELL has been appointed director of the British Society for International Understanding on the retirement of Mr. J. Eppstein. Mr. Eppstein, who for the past 27 years has been director, remains editor of the Society's publications.

Announcements

A COMMITTEE of chemists closely associated with the activities of Prof. T. P. Hilditch, who died on August 9, 1965, have been considering a suitable permanent tribute to his memory in the form of memorial lectures to be delivered at a frequency determined by the sum realized. All those interested are invited to contribute to an appeal fund. A trust fund will be set up. Donations should be sent to Martins Bank, Ltd., Water Street, Liverpool 1, and made payable to the "Hilditch Memorial Fund".

THE Deutsche Akademie der Naturforscher Leopoldina recently elected the following new members in the sections indicated: *Mathematics*, Prof. M. Kneser (Göttingen);

Prof. W. Rinow (Greifswald); *Astronomy*, Prof. C. Fehrenbach (Marseilles); *Physics*, Prof. O. Haxel (Heidelberg); *Geophysics and Meteorology*, Prof. T. Nagata (Tokyo); *Physical Chemistry*, Prof. G-M Schwab (Munich); *Botany*, Prof. H. Mohr (Freiburg/Br.); *General Biology*, Prof. W. Beermann (Tübingen); Prof. M. W. Nirenberg (Bethesda); *Anatomy*, Prof. A. Schabadasch (Moscow); *Physiology*, Prof. A. Gutmann (Prague); *Internal Medicine*, Prof. F. Grosse-Brockhoff (Düsseldorf); Prof. F. Lenoch (Prague); Prof. S. Milcu (Bucharest); Prof. K. Oberdisse (Düsseldorf); *Paediatrics*, Prof. K. Betke (Tübingen); Prof. J. Svejoár (Prague); *Psychiatry*, Prof. T. Bilikiewicz (Gdansk); *Surgery*, Prof. W. Bross (Wroclaw); Prof. D. Juzbašić (Zagreb); *Oto-Rhinolarnygology*, Prof. J. Berendes (Marburg); *Orthopaedics*, Prof. M. R. Francillon (Zürich).

PROF. A. H. MASLOW, Philip Meyers professor of psychology at Brandeis University, has been elected president-elect for 1966–67 of the American Psychological Association. G. Lindzey, of the University of Texas, has now taken office as president of the Association for 1966–67. The Association has also awarded three distinguished scientific contribution awards to: N. Bayley, Institute of Human Development, University of California; Prof. C. H. Graham, professor of psychology in the University of Columbia; Prof. R. L. Solomon, professor of psychology in the University of Pennsylvania. These awards, which were established in 1956, are given annually for "outstanding contributions to the science of psychology". The American Psychological Foundation also presents annually one gold medal for a distinguished lifetime career in psychological research, and Prof. K. M. Dallenbach, professor emeritus at the University of Texas, has been awarded the 1966 gold medal.

A MEETING of the Association of Clinical Biochemists (Southern Region) is being held at Bristol on September 17. Further information can be obtained from Dr. V. Marks, The Area Laboratory at West Park Hospital, Epsom, Surrey.

THE one hundred and thirty-third meeting of the American Association for the Advancement of Science is being held in Washington, D.C., during December 26–31. Further information can be obtained from Mrs. T. C. Heatwole, 5110 West Franklin Street, Richmond, Virginia.

A SYMPOSIUM, arranged by the British Scientific Instrument Research Association, is to be held at the Royal Aeronautical Society on October 5. Further information can be obtained from Mr. I. M. Watt, SIRA, South Hill, Chislehurst, Kent.

AN evening course in photoelasticity is to be held at the City University. The course, which begins on October 11, is intended for engineers holding a degree or diploma in technology, or an equivalent qualification. Further information can be obtained from The City University, St. John Street, London, E.C.1.

A NATIONAL conference on "Profiting by Quality and Reliability", organized by the British Productivity Council on behalf of the National Council for Quality and Reliability, will be held at Blackpool during November 8–10. Further information can be obtained from the National Council for Quality and Reliability, Vintry House, Queen Street Place, London, E.C.4.

A NEW certificate in field biology, replacing the certificate of proficiency in natural history, is now being awarded by the University of London. It is available to all persons interested in the study of living things in their natural habitats. Further information can be obtained from the Assistant Director (Room 14), Department of Extra-Mural Studies, University of London, 7 Ridgmount Street, London, W.C.1.

ERRATUM. In the review by Dr. R. F. Ruth entitled "Tissues and Organs" (*Nature*, **211**, 348; 1966), the second sentence of the fifth paragraph should read, in part, "the presumptive heart cells then migrate anteriad over the surface of the endoderm which itself shifts caudad and mediad as both tissues swing into the midline during the formation of the foregut;".

THE NIGHT SKY IN OCTOBER

All times are in Universal Time

MOON

New Moon	14d 04h
Full Moon	29d 10h

CONJUNCTIONS WITH THE MOON

Venus	—
Mars	10d 15h, 4° S.
Jupiter	8d 18h, 5° S.
Saturn	25d 21h, 2° N.

PLANETS

Times of rising (*R*) and setting (*S*) during the month

Name	*R*/*S*	Beginning	Middle	End	Mag.	D_σ (10^6 miles)	Zodiacal position
Mercury	—	Unfavourable for observation			—	112	Libra
Venus	—	Unfavourable for observation			—	158	Virgo
Mars	*R*	1h 40m	1h 35m	1h 25m	+1·8	195	Leo
Jupiter	*R*	23h 30m	22h 45m	22h 00m	−1·7	495	Cancer
Saturn	*S*	4h 40m	3h 40m	2h 40m	+1·0	805	Aquarius

D_σ is the distance of planet from the Earth on the 15th of the month.

OCCULTATIONS OF STARS BRIGHTER THAN MAGNITUDE +6 AT GREENWICH

Star	*R*/*D*	Time	Mag.
13 Tau	*R*	3d 22h 09·0m	+5·5
125 Tau	*R*	6d 02h 35·8m	+5·0
76 Gem	*R*	8d 04h 59·2m	+5·4

(*D*, disappearance; *R*, reappearance)

METEORS

Name	Active period	Date of maximum	Radiant	Remarks
Giacobinids	9d–10d	10d	262° R.A., +54° Dec.	Favourable
Orionids	15d–25d	21d	96° R.A., +15° Dec.	Favourable

OTHER PHENOMENA

11d 04h, Mars 1° N. of Regulus

29 d, Penumbral Eclipse of Moon: Moon enters Penumbra 07h 53m—beginning visible in N. and S. America, W. Atlantic Ocean, Pacific Ocean, Mid-Eclipse 10h 12m New Zealand, N.E. Asia; end visible in N. America, Pacific Ocean, Australia, Moon leaves Penumbra 12h 32m New Zealand, Indonesia, East Asia.

SCIENCE IN FRANCE—A REPORT BY OECD

FRANCE'S scientific achievements are falling short of her intentions, according to a study which has been carried out by the Organisation for Economic Co-operation and Development (*OECD, Reviews of National Science Policy: France, 1966*). The report is one of a series of studies of science policy in member nations of the OECD. The examiners were Dr. A. H. Haley of the University of Oxford, Professor H. Johnson of the University of Chicago, Professor G. B. Kistiakowsky of Harvard University, and Dr. L. Morandi, vice-president of the Montecatini Company. The survey holds that France, where science planning since 1958 has worked towards an overall concept of science policy and coordinated national options, may be losing her courage. Its authors point to the fact that the Committee of Ministers, which is responsible for coordinating science policy, has met on an average only once a year since 1961 and that it has had a progressively weakening influence on the budget "since the number of decisions in the scientific sphere left to the decision of the Prime Minister is steadily increasing from year to year". As further evidence, they note that the major options such as the construction of the *Concorde* aircraft, the development of the Pierelatte programme for gaseous diffusion and the nuclear developments at Chinon were undertaken without consulting the central agencies responsible for advising the government on science policy, and, finally, that even where definite targets had been put forward in the IVth Plan, it has been reported that only 79·7 per cent of these targets have been reached. The report concludes that, while this may just be a temporary lull between conception and implementation of a unified policy, there is some fear that the government is afraid to take the plunge and commit itself completely.

The report traces science policy in France from tentative beginnings in the late thirties, when the Under-secretariat of State for Scientific Research and the National Centre for Scientific Research were established, through the post-war expansion with new agencies for atomic research, aeronautics, health and agriculture. Until the early fifties France was content to remain dependent on foreign technology. Since then France has moved ahead rapidly on research expenditure—the government alone spent 1,073 million francs in 1965 against 249 million in 1959 and the gross expenditure on research and development in all sectors came to 6,248 million francs, or 1·6 per cent of the gross national product, in 1963. This is still below the corresponding GNP figures for Britain and, of course, the United States (where the percentages of GNP were 2·2 and 3·1, respectively, in 1962), but France is spending far less on defence research; in 1962 only 37·5 per cent of government research expenditure went to the armed forces compared with more than 60 per cent in the two other countries.

France is breaking into space and atomic energy in a big way. She has allocated 33 per cent of her scientific expenditure to these two fields in which the United States is investing only 29 per cent in atomic and space research and the National Science Foundation. Another interesting aspect of the French budget for research is the 19·5 per cent spent on national education, primarily for research in the universities and other higher scientific establishments such as the *École Normale Supérieure*. Of course, in the United States many of the grants for university research are made by the National Science Foundation, the National Institutes of Health and the various branches of the Department of Defense, but the universities still account for only 12 per cent of the basic research in the United States, with 74 per cent originating in private firms (over 40 per cent of the costs are met by the government, however). In France, 47 per cent of the research was carried out by private firms, with the government meeting half the costs.

The first formal attempt to integrate scientific and political objectives took place in France when the IVth Economic and Social Development Plan was being prepared (1962–1965). Since 1965 the ministry in charge of scientific research and responsible to the joint secretariat of the two science committees—the General Delegation for Scientific and Technical Research—has been replaced on the political side by a Secretariat of State for Scientific Research and Atomic and Space Questions and on the scientific side by a Delegate-General for Scientific and Technical Research to work in liaison with the Commissaire Général du Plan. This increasing emphasis on central control by the General Delegation and its Secretariat and various committees, coupled with a recognition of the importance of science to all economic and political questions, is still unique to France.

The examiners, like all those concerned with the role of science, welcome the development, but also express the fear that some scientific decisions are being made for political rather than economic reasons. They applaud the desire to increase contributions to international research efforts, but do not feel that France can afford a policy of "going it alone" in prestige areas of science; they are afraid that in practice there is not yet a realistic dialogue between statesmen and scientists, in which case "there is a fear that considerable sums may be spent without research deriving the full benefit".

The examiners found educational policy to be also more indicative than active—the government has not yet worked out a general policy for training senior scientific staff nor has it developed a concerted plan for technical training. As for the training of engineers and scientists, the examiners question the early specialization required by the baccalaureat and the dual stream of university-level education offered by the universities and the *Grandes Écoles*. When the *Grandes Écoles*—such as the *École Normale Supérieure* and the *École Polytechnique*—were founded about 1900, they filled the need for engineering training that was not met by the universities. But though they are still the most influential science schools in the country, the study does not feel that they have changed sufficiently to meet the increasing need for researchers and teachers in France. About 20 engineering schools are regarded as *Grandes Écoles* and take roughly 1,500 first-year students or about 20 per cent of the intake of all engineering schools. But more than twice as many as this have studied for two or three years to gain a place, thereby forgoing admission to university. Another complaint of the examiners is the emphasis in the *Grandes Écoles* on engineering and administration rather than research.

In contrast to the continuing pressure for admission to the *Grandes Écoles*, the universities are finding it more and more difficult to attract able young scientists; many places are left vacant for lack of candidates. The examiners would like to see closer cooperation between the universities and the *Grandes Écoles* to make it easier to transfer from one type of institution to the other and to enable engineering graduates to do postgraduate research at university.

In all areas of science policy, the examiners feel that the gap must be filled between "the remarkable effort being made to rationalize the preparation of decisions affecting both science and the economy, and the universal tendency to choose certain scientific objectives for political reasons without always realizing their economic implications".

SEA WATER DESALINATION — IN PERSPECTIVE

By Dr. E. GLUECKAUF
Atomic Energy Research Establishment, Harwell, Berkshire

Largely as the result of the initiative of the Office of Saline Water (OSW) of the United States Government, research into new methods of providing additional sources of fresh water has become front page news.

The idea of obtaining water from the sea is not new[1], and indeed most of the modern flash distillation plants of the world are of British design and manufacture. But the change in outlook during the past few years brought about by the American initiative is nevertheless fundamental. It means that the development of new techniques which might have been achieved in the normal way in half a century is suddenly telescoped into a decade—assisted by the prodigious contribution of a single government.

It is most important for others to realize that this is the pattern of the world of to-morrow, and that to reach the forefront in any major field of technology in the future will require a punch backed by a nine figure number. In Britain this has been done in the field of atomic energy. We may need to do it again in those fields which are vital for us.

It is relevant to ask the question whether Britain could have afforded an expenditure similar to that in the United States in the field of desalination, where Britain had a good start. Financially, the answer is an unqualified "yes". Britain is at present using about $6{\cdot}5 \times 10^9$ gal. of water each day, about 15 per cent for domestic requirements, about 8 per cent for agricultural uses and the rest for industrial consumption. A levy of 1*d.*/1,000 gal. of water, which would pass unnoticed in the cost of living, would provide £10 million per annum, a figure which is roughly the annual OSW expenditure. Whether Britain could also divert the thousand or so scientists and engineers without serious consequences is another matter. The main point, however, is that viewed from Britain's angle, desalination is an interesting technological field but not a vital one, at least—according to the Water Board—not before the end of this century. Atomic energy, on which Britain spends about one hundred times as much as on desalination, is vital to us.

In the United States, where vast areas of California and other southern regions are short of water, the point of view is a different one. There, the availability of fresh water transcends the technological interests and affects the pattern of development of the country as a whole. The same political motivation exists also in Israel, where manpower expenditure on desalination research greatly exceeds that normal to a population of 2·5 million.

In Britain the theoretically available rainfall is about 48×10^9 gal./day, of which the present consumption represents about 14 per cent. Unfortunately most of the rainfall is in the north and west and most of the water consumption is in the midlands and the south-east, so that even now there are regions where dry summers regularly result in water shortages. But this means only that in the future, new industries requiring much water will have to move to where the water is plentiful, and that in the industrial regions short of water, better water management will have to be introduced. (The manufacture of plastics, for example, may require more than 2,000 times their weight in water.) At present London uses and then throws away about 500 million gal. daily. Recovery of 75 per cent of this water by conventional sewage treatment followed by partial desalination (for example, by electrodialysis) and recycling would make locally available an extra 1,500 million gal. daily. As the water consumption is doubling every 15 years, this action alone would provide the needed increase in water supply almost up to the end of this century. The technology of these recycling processes is now being seriously developed. Naturally, even though Britain will not need desalination plants much before the end of the century, her industries will want to remain in the export market for such plants and so a modest effort in research and development must continue.

The processes for obtaining water from saline water can be conveniently divided into:

(1) Processes involving a phase change: (*a*) evaporation, (*b*) freezing. (2) Processes involving semipermeable membranes: (*c*) electro-dialysis, (*d*) hyperfiltration (reverse osmosis). (3) Processes involving chemical equilibria: (*e*) ion exchange.

(*a*) *Evaporation.* (i) *Flash distillation.* The best known process to obtain water from saline solutions is evaporation. Indeed, if a large sea water desalination plant—say, between 10^5 and 10^8 gal./day—is required to-day, then this is the only process for which commercial plants can be readily obtained. Invariably a multistage system is used (Fig. 1), where the steam flashed off from each stage is condensed to pure water by preheating the incoming sea water. Each stage operates at a temperature and pressure slightly lower than the previous stage. The water condensed in each stage thus has a temperature slightly above that of the water in the following stages, and this energy too can be extracted by flashing through the successive stages.

Plant units of 12 million gal./day of purified water designed for delivery at present use between twenty and forty such stages, so that allowing for heat losses, as much as 8–12 lb. of water can be produced by 1 lb. of steam. For 1,000 gal. of water produced, this reduces the heat consumption to 350 kWh using low grade steam of 260° F which is no longer useful for power production. An additional 18 kWh of power are required for pumping. The unit capital cost is \$0·69/gal./day (ref. 2). The cost of product water for a 36 million gal./day (MGD) plant using three twelve MGD units is \$0·50/1,000 U.S. gal. (\$1 per 1,000 U.S. gal. = 105*d.*/1,000 Imp. gal.) For the still larger units which may be produced in the more distant future, there could be a further reduction of steam consumption and cost. It has been estimated[3] that by using 3,300 MW reactors providing 675 MW of electricity and 250 MGD of water in units of 50 MGD, a unit capital cost for the flash distillation plant of \$0·41/gal./day and a water cost of \$0.16/1,000 gal. can be achieved.

Comparing the two sets of figures, a unit capital cost reduction from \$0.69 to \$0.41/gal./day may be reasonable for the envisaged scale increase by a factor of seven. The water costs cannot be compared as they depend predominantly on the rate of interest charged. Reactors up to 10,000 MW are now being studied in the United States, but it has to be borne in mind that plants of that size are only useful for densely populated areas.

(ii) *Distillation with vapour compression.* If saturated steam at a temperature (t_1) is adiabatically compressed, its temperature rises to (t_2). If this steam is then condensed at a temperature t_3 (between t_1 and t_2) slightly more than the original heat of evaporation is recovered

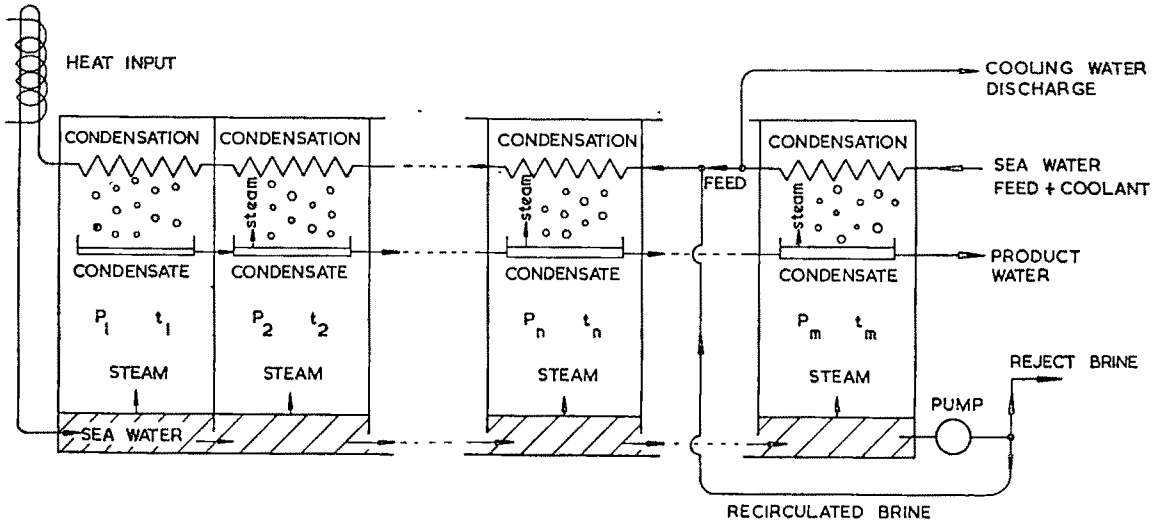


Fig. 1. Multistage flash distillation.

at a temperature t_3 above t_1, which can therefore be used to repeat the original evaporation process at t_1. Thus water can be evaporated with the expenditure of the work needed for the compression, which need be only about 3–4 per cent of the heat of evaporation. Work energy can be produced from heat energy with a loss factor of about three, so that the actual heat consumption in this case represents about 9–12 per cent of the heat of evaporation. This offers roughly the same heat economy as flash desalination with twenty stages, but it gives it in a single stage of operation as the recovered energy can be fed back into the same evaporator. The equipment is thus smaller but requires expensive compressors and comparatively large heat exchangers. It has an obvious advantage for the production of water on a small scale, but it is not clear whether it will compete economically with flash distillation on a large scale.

In both distillation processes the quality of the product water is very good; the solid content is usually guaranteed to be less than 25 p.p.m. and can be less than 1 p.p.m.

(*b*) *Freezing processes.* Because of their highly organized structure, ice crystals cannot accommodate other molecules or ions without severe local strain, and consequently salts are rejected by the advancing surface of a growing ice crystal. This offers the possibility of producing pure water from sea water in a single operation.

Thermodynamically, the process is very favourable, requiring (theoretically) only 6·3 kWh/1,000 gal. of product water. As a result of the irreversible effects in any practical system, however, the value which has so far been achieved is 40 kWh/1,000 U.S. gal. by Colt Industries, Inc., Beloit, Wisconsin, United States. One of their plants in Eilat, Israel, presumably as a result of the higher external temperature, shows a power consumption of 69 kWh/1,000 gal., which it is hoped will be improved to 49 kWh. These values have been achieved by evaporative cooling of sea water, forming a "mush" of small ice crystals, which are separated in a vertical column (by density difference) from the brine, and washed free from adhering brine by recycling some of the purified water. The water–ice slurry is then moved to a melting unit where the latent heat of the compressed water vapour is used to melt the ice.

Theoretically there is no limit to the purity of the water produced, but the considerable difficulties of washing the ice pure from adhering brine have so far led to a compromise at 500 p.p.m. of dissolved solids, which is adequate for drinking purposes. The cost of water has been estimated to lie between $0·5 and 0·7/1,000 U.S. gal. for a 5 MGD plant. Thus while energy consumption is considerably less than in the case of distillation process, the cost of the water is similar, and its quality is not nearly so good.

(*c*) *Electrodialysis.* Electrodialysis makes use of the transport of ions in solution in an electrical field. The most commonly applied method of electrodialysis uses multiple cell assemblies with alternating cation- and anion-semipermeable membranes through which an electric current is passed, so that alternate compartments will produce a reduction and an increase in concentration respectively.

Electrodialysis (and also ion exchange) has the great advantage over most other methods of desalination in

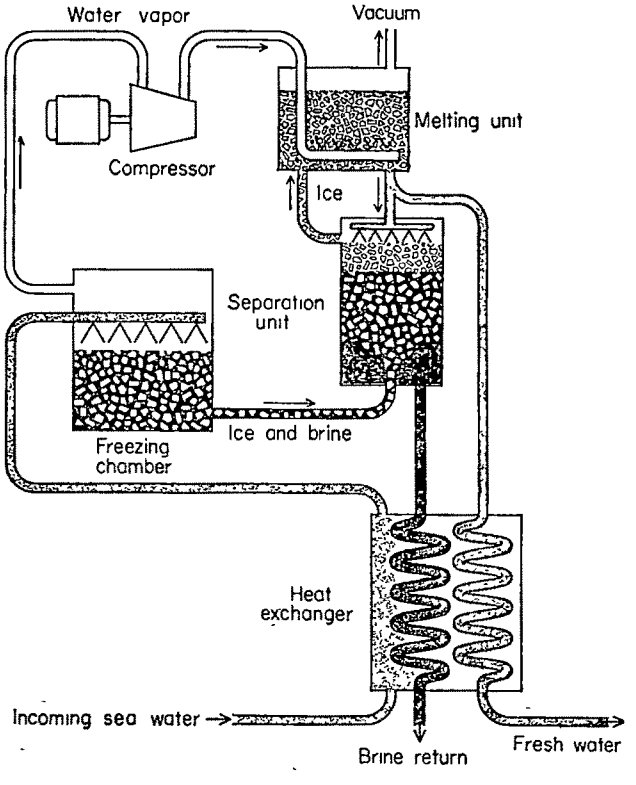


Fig. 2. Vacuum freeze-vapour compression method. [Reprinted from Spiegler, K. S., *Principles of Desalination*, by permission of Academic Press Inc. (London), Ltd.]

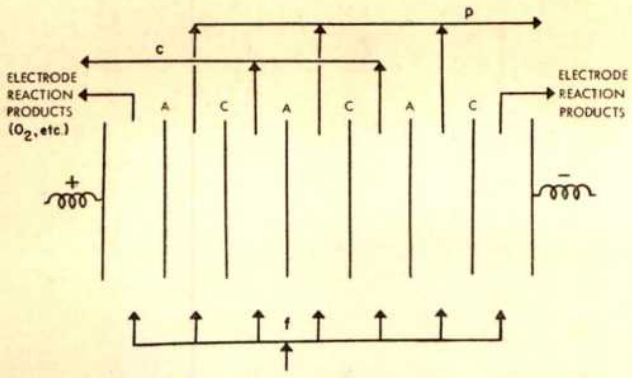


Fig. 3. Conventional electrodialysis. *A*, Anion-permeable membrane; *C*, cation-permeable membrane; *f*, feed; *p*, product; *c*, concentrate. [Reprinted from Spiegler, K. S., *Principles of Desalination*, by permission of Academic Press Inc. (London), Ltd.]

that it removes the minor component (the salt) from the major component (water). As a result of this, power consumption is more or less proportional to the salt content of the feed solution: roughly 2 kWh/1,000 gal. for a feed concentration of 2,000 p.p.m., 5 kWh for 4,000 p.p.m. and about 80 kWh/1,000 gal, for a feed concentration of 40,000 p.p.m., if a product of 300 p.p.m. is required in each case. Further reductions of the salt content are possible, but power consumption then increases as a result of increased electrical resistance of the dilute salt solutions. Though power consumption is low for brackish water, equipment cost remains high, so that the final water cost is not very much less than that of flash desalinated sea water.

On the basis of present-day experience the cost of desalinating water from 3,000 p.p.m. to 500 p.p.m. (on a 10 MGD plant scale) has been estimated as high as \$0.35/1,000 U.S. gal.[4]. It is conceivable that these costs can be substantially reduced by the manufacture of cheaper membranes, and by electrodialysis at higher temperatures. Any increase in temperature could be particularly important for the desalination of sea water, as the resultant reduction of electrical resistance and the higher permissible current densities permit a reduction of both power and capital expenditure. This problem is being seriously attacked at the Institute for Arid Zone Research at Bersheva, Israel, where solar pond heat could provide the energy for an operating temperature of 90° C, at which temperature sea water can be desalinated with an energy consumption of 22–29 kWh/1,000 gal.—a saving of 50 kWh/1,000 gal. If the technological development of membranes which can stand up to an operating temperature of 90° C is successful—and there are indications that this may be so—then this technique of desalting may well become the most economic method in hot climates, even for sea water.

(*d*) *Hyperfiltration* (*reverse osmosis*). Hyperfiltration is achieved when a salt solution is forced under pressure through a membrane which has a high permeability for water, and very little permeability for electrolytes. The pressure must be large enough to overcome the osmotic pressure and the friction loss in the membrane. As the salt rejected at the membrane surface must diffuse back into the solution, the salt concentration at the membrane surface can become considerably higher than that of the water to be desalted (concentration polarization). Agitation of the feed water is therefore important (high Reynolds numbers). Under ideal conditions the osmotic pressure of sea water is about 25 atm. at room temperature, but concentration polarization can increase this pressure considerably. Thus in order to obtain reasonable water throughput (10 gal/ft.²/day or more), pressures of 60–100 atm. have to be applied, as well as adequate agitation of the flowing saline solution.

The membrane material used for this purpose to-day consists of a partially hydrolysed cellulose acetate of 39·8 per cent acetyl content in which 5/6 of the available OH-groups are acetylated. Lower acetylation gives materials showing too little salt rejection; higher acetylation reduces the water throughput below the level of usefulness. The water flux through a membrane is given by the equation

$$f_w = \frac{P_w}{RT} \cdot \frac{\Delta(P - \pi).\bar{v}}{\Delta x}$$

where f_w is the water flux in cm³/cm²/sec; P_w, the permeability of the membrane for water in cm²/sec; $\Delta(P - \pi)$, the excess pressure above the osmotic pressure π, in atm.; $\bar{v}$, the molar volume of water (18 cm³); RT, the product of gas constant and temperature = 24,000 cm³ atm. at room temperature; Δx, the effective thickness of membrane in cm. It follows from this that for a flux of 10 gal./ft.²/day = $4{\cdot}7 \times 10^{-4}$ cm/sec and $\Delta(P - \pi) = 50$ atm., $P_w = 2 \times 10^{-7}$ cm² sec⁻¹, we require a membrane thickness Δx of $1{\cdot}6 \times 10^{-5}$ cm and smaller thicknesses for larger fluxes. Such a thin membrane is quite an obstacle for use in technical equipment, and so hyperfiltration only became feasible after Loeb and Sourirajan (at the University of California, Los Angeles) succeeded in producing a composite membrane where this thin "active" film was backed by a relatively thick porous layer of the same material (about 0·01 cm or more). Rejection of up to 99 per cent of the salt combined with water flow rates of 7 gal./ft.²/day have been obtained with these membranes. Larger water flow rates can be achieved, at the expense of a lower salt rejection.

Although hydrolysed cellulose acetate is so far the only known material which gives the right degree of water permeability and salt rejection, many other polymers and liquid films are known to show similar behaviour. It is therefore likely that increased research into the nature of these polymers will eventually lead to materials with superior properties. This is particularly desirable, as these cellulose acetate membranes, when exposed for longer periods to pressures of 100 atm., lose much of their water permeability, probably as the result of internal structural deformation.

Another feature of cellulose acetate is that it hydrolyses slowly when in contact with aqueous solutions of low and



Fig. 4. Mobile 10,000 gal./day desalination unit produced by Havens Industries Inc.

high *p*H. The speed of hydrolysis is lowest at a *p*H of about 5 so that for solutions of a *p*H between 4 and 6 a lifetime of at least 1 year can be expected.

The mechanism of the transport of salt and water through hyperfiltration membranes in general and cellulose acetate membranes in particular has not yet been fully established. The salts exist in the membrane as dissociated ions despite the low dielectric constant of cellulose acetate, which presumably means that they must be surrounded by a considerable shell of water molecules in order to avoid ion pair formation. The diffusion of ions is very slow ($D \sim 10^{-9}$ cm²/sec for sodium chloride) and their permeation coefficients are highly dependent on ionic charge. With water the permeation coefficients are surprisingly high for a polymer containing only about 15 per cent of water. Also, if calculated from osmotic flow they are higher by a factor of 2·5 than those obtained from the self-diffusion through the membrane of isotopically traced water[5]. This indicates a substantial amount of water–water friction in the latter case. This can be interpreted either as diffusion through bulk material—a view expressed by Merton of General Atomic—or as flow of water through pores of about 9 Å diameter, a view which I favour. Clearly much more work needs to be done.

It is apparent from this that the hyperfiltration technique has not been as fully developed as other methods of desalination. There is, therefore, still a considerable development potential. It is also not yet clear which type of physical arrangement of the membrane surface offers the most economical way to provide the vast areas required for large scale technical application.

Loeb, at the University of California, Los Angeles, favours a tubular system with the cellulose acetate membranes on the inside of perforated metal tubes. Havens Industries, San Diego, California, use 0·5 in. fibre glass tubes with the cellulose acetate cast directly on to the inside of the tubes. Seven of these are then assembled within a plastic tube acting as conduit for the purified water (see Fig. 3). Aerojet General Corp., Azusa, California, have developed a flat plate assembly within a pressure containing vessel. General Atomic at San Diego, California, use a spiral arrangement of alternating membrane—porous support—membrane—spacer layers with withdrawal of the purified water from a central perforated metal tube, the whole being pressurized inside a 3 in. metal tube.

There have been only few economical assessments for plants on a large scale. Energy consumption (assuming recovery of energy from the rejected brine) lies in the region of 20 kWh/1,000 gal. and Aerojet General have estimated a capital cost of $0.55/gal./day for a 10 MGD system, and a total water cost of $0·44/1,000 gal. water produced[6]. This presumably includes membrane replacement at intervals of 6 months. That this estimate is reasonably realistic can be seen from the fact that Havens Industries offer a plant 1,000 times smaller mounted on a trailer for a unit capital cost which is only four times larger. Further improvements on this cost are possible if further research produces a membrane which permits use at somewhat higher temperatures and pressures. The flow viscosity of water at higher temperatures permits larger water flow rates and so reduces the comparatively high capital cost component in terms of water produced. Even so, it is hard to see that the cost of water could fall much below $0·33/1,000 gal.

(*e*) *Ion exchange.* We shall not discuss here the various methods of using ion exchangers, because they seem economical only for the desalting of brackish waters of lower salt content.

Conclusions

It is useful to compare the optimized cost estimates for the different techniques of sea water desalination on the 10 MGD scale approximately (Table 1).

Table 1

Type	Unit capital cost of plant ($/gal./day)	Water cost ($/1,000 (U.S.) gal.)	Purity (p.p.m.) of product water
Flash distillation*	0·69	0·35	< 25
Freezing†	1·4	0·5–0·7	30–500
Electrodialysis‡	(0·5 to 1·7)	0·48	500
Hyperfiltration (reverse osmosis)§	0·55	0·44	500

These figures do not include the cost of distribution.

* To make the data of ref. 2 comparable with United States estimates, they have been converted to an interest rate of 3 per cent and a capital amortization over 30 years, which is the usual practice in the American estimates.

† Ref. 7, p. 338.

‡ Ref. 7, pp. 280–282.

§ Ref. 6.

Bearing in mind the uncertainties of extrapolation in scale for all cases except flash desalination, there is little significant difference in the water costs, except that flash distillation provides purer water and that the other methods, not being fully developed, still have a greater development potential than flash distillation, which has already produced plants capable of desalinating several million gallons of sea or brackish water each day. But the component of power cost of all these methods is such that, without reduction of power cost, the cost reduction potential is limited. Power cost reduction can arise apparently only from the use of super-reactors.

In any event, available information and cost studies do not permit us to predict how the different desalination processes will compete against each other. The outcome may depend not only on the "local conditions"—the size of a given plant and maintenance cost—but also to a considerable extent on the financial terms offered by the supplier.

There are a number of points which are often forgotten when discussing the economics of water desalination, namely, that the water which is desalinated may have to be distributed over some distance. If the intake of water is at the shore of the sea, the pure water may have to be pumped inland. If the supply is a brackish inland water, the concentrate may have to be disposed at some distance, otherwise the salinity of the source water will gradually increase. If water consumption fluctuates, considerable storage may be necessary to smooth out the fluctuations, otherwise the desalination equipment cannot work at its design level of production, which would greatly increase the capital charges.

Even when there is no desalination necessary, the cost of impounding, treatment and distribution is of the order of $0·2/1,000 gal. for a 10 million gal./day supply, and pumping over some distance would easily add another $0.1 to this figure.

As matters stand now, desalinated water at these prices is cheap for domestic purposes, is acceptable for industrial consumption, but is too expensive for indiscriminate agricultural irrigation. Only when used for high priced products (fruit or market gardening) or with sophisticated irrigation systems avoiding surface evaporation could it contribute to raising the food production in the arid zones of the world to a desirable level.

References

So far as possible, this article has avoided references, as these would have taken up too much space. For a detailed description of the processes discussed and extensive references, I recommend the recent book edited by K. S. Spiegler[7].

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POPULATION DYNAMICS OF THE BLACKBIRD

By Dr. D. W. SNOW
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Studies of population dynamics in birds have usually been concerned with comparatively small sections of a much larger population, and these small sections have been intensively and professionally studied with the view of elucidating the main factors regulating numbers. With such an approach it is rarely possible, however, to obtain all the information that one would wish on both recruitment and mortality. Often there is some phase of the life cycle where consistent observation is difficult, and in species with populations continuous over wide areas, movements in and out of the study area may upset calculations to an unknown degree.

The present study is of the opposite kind: it is based on numerous data collected on a country-wide scale by many observers, but in the main without any intention that they should be brought together for a study of population dynamics in the way attempted here. Thus it suffers from the opposite disadvantages from those affecting a more intensive local investigation, in that the records are patchily distributed in time and place, and the standard of accuracy cannot be expected to be as high as in a professional investigation. Against these defects can be set the sheer quantity of the data available; there is also an advantage in the fact that the records are derived from several different schemes and were supplied by many independent observers without previous knowledge of the uses to which they might be put.

The data are drawn primarily from the "Ringing Scheme" and the "Nest Records Scheme" of the British Trust for Ornithology. A third major enquiry by the Trust, the "Common Birds Census", has not been in operation for a sufficient time for its results to be more than briefly mentioned, though they will eventually be relevant to the questions under consideration. These schemes have been in operation for various lengths of time, and have expanded with the years. Results of the schemes for ringing and nest records have been the subject of many analyses, which have thrown light on mortality and reproductive rates, migrations and movements; however, it has usually been necessary to treat all years collectively in order to obtain a sample adequate for analysis. It is only in the past ten years or so that they have been operating at a sufficiently high level to permit analysis on an annual basis.

The blackbird (*Turdus merula*) is by far the most suitable species for a study of this sort. It is common almost everywhere, is ringed in larger numbers than any other species, and is recovered in good numbers. Its nests are easy to find and are recorded in greater numbers than those of any other species. Finally, it offers the advantage that not only are the sexes distinct but also birds in their first year can be distinguished from older birds with a high degree of certainty. It has the slight disadvantage of being a partial migrant—a proportion of the population, especially from northern Britain, migrates to Ireland in the winter, and a very small proportion, especially from southern England, migrates south to France. The tendency to migrate is, however, independent of age[1].

Annual Mortality

Coulson[2] described a method of calculating annual mortality for each year separately from recoveries of ringed birds. He presented annual mortality figures for the starling and later[3] for the blackbird from 1932–33 to 1953–54. The samples for the earlier years were, however, so small that the significance of the annual differences is doubtful. In Fig. 1 the same method has been used to calculate the annual mortality of the blackbird from 1948–49 up to 1963–64. Instead of the calendar year, a "mortality year" running from April 1 to March 31 has been adopted, so that the whole of the winter falls within one year.

The numbers of recoveries on which the percentages for recent years are based are large (more than 300 from 1955–56, more than 1,000 from 1960–61), so that if sampling biases could be discounted, the standard errors of the percentages are so small that the larger annual differences could be confidently interpreted as reflecting real differences in the mortality of the blackbird population as a whole. It is certain, however, that those blackbirds that are ringed and recovered are not drawn at random from the entire blackbird population. For example, birds are more likely to be ringed in some habitats (for example, gardens) than others, and in some parts of the country than others, and these biases may not be quite the same every year. It is impossible to guard against these and other sources of bias, and the only practicable course is to divide the data and compare the sub-samples. When the simplest division is made, into the north and

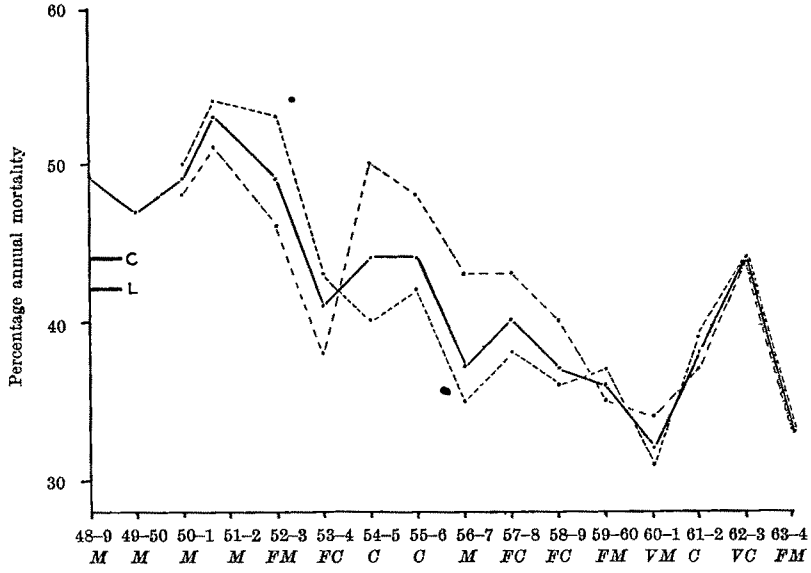


Fig. 1. Annual mortality of British blackbirds, 1948–64. ——, Data from all areas combined. – – – –, Data from southern half of Great Britain. —·—·—·—·—, Data from northern half of Great Britain. *C* and *L*, Average annual mortality calculated by Coulson and Lack respectively. The "mortality years" (abscissa) run from April 1 to March 31, winters being classified as very cold (*VC*), cold (*C*), fairly cold (*FC*), fairly mild (*FM*), mild (*M*) and very mild (*VM*).

south of Great Britain, the calculated mortality rates for recent years, for which the largest samples are available, are extremely similar for the two halves of the country (Fig. 1); thus it may be concluded that sampling bias is not important and the annual differences in calculated mortality are genuine and refer to the total population.

The most striking point shown by Fig. 1 is that from 1951–52 to 1960–61 there was a decrease in the annual mortality, from about 50 per cent down to 32 per cent. The decline was not quite steady; there were irregularities in it which are probably attributable to the differing severity of the winters. Thus the winters of 1954–55 and 1955–56 were cold and the annual mortality was higher than in 1953–54, while the winters of 1956–57 and 1960–61 were mild or very mild and the annual mortality was correspondingly low. The general decline over these years cannot, however, be attributed to weather. For example, the four winters preceding the decline were all mild and yet the annual mortality in those years was higher than in any of the subsequent eleven years. In 1961–62 the winter was cold and the annual mortality rose to 38 per cent, and in the following year the winter was exceptionally severe and mortality rose to 44 per cent. The following year it was down again to a very low level.

Fig. 1 also shows the average annual mortality of the blackbird as calculated by Lack[4] and Coulson[3]. From 1948–49 to 1952–53 the annual mortality exceeded these averages in each year, whereas in most subsequent years it was lower. It is noteworthy that even in 1962–63, when the winter was the most severe for many years, the mortality only just reached the average calculated by Coulson.

Relation between Mortality and Recruitment

The causes of the progressive decline in the annual mortality will be discussed later. Its effect on the numbers of the blackbird must have depended on what happened to the recruitment rate over the same years. If the recruitment rate remained constant, at the level originally needed to maintain the population, numbers would have increased by about 70 per cent over the years 1952–61. But it may be that the recruitment rate fell correspondingly, so that the population level remained much the same. Or the recruitment rate might have fallen a little, but not enough to balance the reduced mortality, resulting in a smaller increase in population.

There is no really satisfactory information on the level of the blackbird population over these years. A small population studied at Oxford for five years (1953–57) reached its highest level in 1957 (the year after the greatest decrease in calculated mortality)[5], and a farmland population of which a census was taken during 1954–57 was also highest in 1957[6]. Data on these and three other small populations over the years 1952–58 (summarized by Beven[7]), however, suggest no general rise in this period, the annual changes being largely attributable to the severity of the winters. Finally, a suburban population of which a census was taken by Simms[8] remained extraordinarily constant from 1951 to 1962, but this was probably a saturated population, because first-year blackbirds tend to settle in gardens in preference to more open habitats[5]; thus it might not have reflected changes in the general population level. While, however, the data are inconclusive, they strongly suggest that there was no marked increase in population over the years 1952–61.

The recruitment rate, that is the number of young birds, born in one year, which survive to form part of the breeding population of the following year, can be measured by the proportion of first-year birds in the population at the beginning of the breeding season. For this purpose, information on the age (whether first-year or older) of all male blackbirds ringed in March in each year has been extracted from the ringing schedules, and in Table 1 the results have been compared with the adult annual mortality for the years 1957–58 onwards (in earlier years ringers were not consistent enough in ageing blackbirds for the information to be reliable).

Table 1. ANNUAL MORTALITY AND RECRUITMENT RATE CALCULATED FROM RINGING DATA

Year (April–March)	Annual mortality	Percentage of first year birds (March)	Calculated population level (March)
			100
1957–58	38	29	87
1958–59	36	31	81
1959–60	37	34	78
1960–61	31	41	92
1961–62	39	37	89
1962–63	44	41	85
1963–64	33	46	105

Data are for the southern half of England only. The "percentage of first year birds" is based on males only (mortality of males and females does not differ significantly). Calculated population level is based on an index of 100 for the breeding population in 1957.

Table 1 indicates that in the three years 1957–58 to 1959–60 the recruitment rate was even lower than the mortality, so that there was probably a slight decline in the population. In 1960–61, the annual mortality was the lowest in the years under review but the recruitment rate increased. In 1961–62 and 1962–63, with respectively hard and very hard winters, mortality and recruitment were almost equal and in 1963–64, with a mild winter, recruitment was considerably higher than mortality, suggesting a population increase of about 20 per cent.

In a few more years, with the "Common Birds Census" in full operation, it will be possible to test the validity of such calculations by the observed population level on a country-wide basis. At present, a comparison can be made only with the census figures for the years 1962–64. Between 1962 and 1963 the census showed a decrease in the blackbird population of 18 per cent, and between 1963 and 1964 an increase of 57 per cent. Thus the changes are in the same sense as those calculated from ringing data but are much more marked. Because the census methods were being modified in these years, and the blackbird is a difficult species of which to take an accurate census, the agreement is probably as good as can be expected.

Nesting Success and Post-Fledging Survival

The recruitment rate depends both on the number of young successfully reared by each pair in the previous breeding season, and on the subsequent survival of these young birds through the autumn and winter. Nest record cards help to provide information on the first of these, while later survival can be calculated from ringing data. For an understanding of population dynamics the main questions to be asked are: whether recruitment rate is related to the number of young produced in the previous breeding season, and whether either or both of these are related to the population level. Until more census data are available for further years, only the first of these questions can be tackled.

Table 2 summarizes breeding statistics for the years 1951–65, derived from nest record cards. There are a

Table 2. SUMMARIZED BREEDING STATISTICS OF BLACKBIRD, 1951–1965

Year	Mean clutch	Family size	Partial loss	Hatching success	Fledging success	Percentage of desertions	Percentage of success
1951	3·9	3·2	0 7	89–94	82	13	38
1952	3·8	3·2	0 6	88–93	83	14	34
1953	3 9	3 3	0 6	92–94	87	14	37
1954	3·7	3·2	0·5	90–95	84	13	39
1955	3·9	3·3	0·6	91–96	83	12	33
1956	3 8	3·3	0·5	91–95	83	13	42
1957	3·7	3·1	0·6	89–94	82	13	28
1958	3·8	3·2	0 6	89–95	83	13	21
1959	3·7	3·0	0 7	88–94	82	14	29
1960	3·8	3·1	0·7	90–94	83	12	28
1961	3·7	2·9	0·8	87–93	81	16	30
1962	3 9	3·2	0·7	89–95	83	12	30
1963	3·9	3·3	0·6	90–94	86	16	40
1964	3·9	3·3	0·6	92–97	85	11	36
1965	3·9	3·3	0·6	91–95	85	14	33

few salient features. Clutch size varied little, with no obvious long-term trends, most of the variation being attributable to weather[9]. Hatching success (excluding predation and desertion) was nearly constant. The number of nestlings surviving the age of 9 days or more showed, however, an irregular fall from 1956 to 1961, owing to the fact that partial losses of young in the nest increased slightly in these years. The survival of young rose again in 1962 and remained high from 1963 to 1965.

The figures for percentage nest success (the percentage of all nests started which produced one or more fledged young) are much influenced by the chances of predation, both human and natural, in which sampling biases may have a very marked effect. It seems, however, that the generally low values for the years 1957–63 may be significant. The percentage of nests reported to have failed through desertion was nearly constant throughout the period.

Table 3. Percentages of Young Birds in the Population in September

Year	North	South
1961	64	61
1962	68	72
1963	86	84
1964	79	79
1965	74	82

Table 4. Survival of Adults, Juveniles and Nestlings, 1959–1961

Year of ringing	Age when ringed	Percentage recovered after following April 1	Percentage of survival until following breeding season
1959	Adult	6·1	63
	Juvenile	3·7	38
	Nestling	0·9	9
1960	Adult	5·0	69
	Juvenile	3·1	43
	Nestling	1·7	23
1961	Adult	3·7	61
	Juvenile	2·5	41
	Nestling	1·3	21

A much better idea of the ultimate success of the breeding season is given by the percentage of juvenile birds in the population in the autumn. Table 3 gives the figures for 1961–65, based on ringing schedules. The annual variations are much more marked than could be accounted for by variations in nesting success in the previous season, suggesting that variations in post-fledging survival were playing the chief part. A comparison of the survival of nestlings born in 1959–61 with the survival of adults in the same years shows that this was indeed the case (Table 4). The survival after leaving the nest of birds born in 1959 was less than half as good as that of birds born in 1960 and 1961. In 1959 there was a hot and dry summer, which lasted to the end of September. There seems little doubt that drought caused heavy mortality among young blackbirds, which at this season feed largely on the ground fauna; it probably affected them mainly in the immediate post-fledging period, because birds ringed as juveniles, which had thus survived the earliest post-fledging period, survived about as well as in the other two years. This calculation cannot unfortunately be made for birds born in 1962 or later, as insufficient recoveries have accumulated.

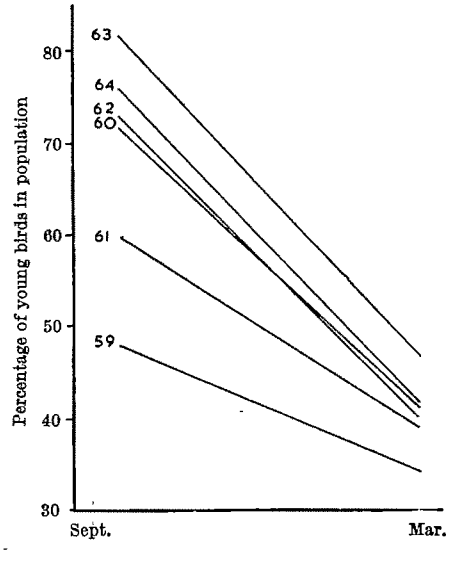


Fig. 2. Change in percentage of young birds in the population of blackbirds between September and the following March, for the six years beginning September 1959 (southern England only); based on male birds trapped for ringing.

Fig. 2 shows that despite a very different proportion of juveniles alive in the population in September each year, by the following March the percentage of first-year birds was rather similar in each year. This suggests that the autumn and winter mortality of young blackbirds is largely determined by the "carrying capacity" of their environment. There is, however, a tendency for a high or low proportion in September to be followed by a respectively high or low proportion in the following March, so that the level of the breeding population is probably affected to some extent by the success of the previous breeding season.

Discussion

Because no increase in the breeding population of blackbirds was recorded over the years 1951–61, despite a reduction of about 10 per cent in the annual mortality, the recruitment rate presumably decreased. The alternatives are that the surplus numbers were absorbed by an expansion of range, that numbers increased in marginal habitats and peripheral areas, or that there was an increase in non-breeding birds unable to acquire territories; good evidence does not exist for any of these alternatives, at least so far as the population of British blackbirds is concerned.

The analysis of many thousands of nest record cards gives no evidence of any change in nesting success sufficient to balance the decreased mortality. The post-fledging survival of young birds may, however, have changed, and this is suggested by the low percentages of first-year birds in March in the years 1958–60 (Table 1). It should be emphasized that a change in the mean post-fledging survival only would be expected, not in that for each year, because survival in any one year depends very much on weather, as already mentioned.

This analysis does not show what was the cause of the reduced annual mortality. It could either have been a change in the environmental conditions affecting the survival of adults (for example, improved winter food supply), in which event the reduction in the survival of young birds would be a consequence of increased competition with adults for the limited territories available. It might be a change in conditions affecting the survival of young birds in their first months of life (ringing shows that after the beginning of January young blackbirds survive as well as old ones), in which case the reduced adult mortality would be a consequence of reduced competition with young birds. If the permanent enquiries of the British Trust for Ornithology continue to operate at their present level, it will be possible to follow the fortunes of the blackbird population year by year and eventually to test such possibilities, and much should be learnt about the factors controlling bird populations.

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BOOK REVIEWS

MUTUAL IMPROVEMENT

Benjamin Franklin and Eighteenth-Century American Libraries

By Margaret Barton Korty. (Transactions of the *American Philosophical Society*, n.s. LV, pt. 9, 1965.) Pp. 83. (Philadelphia: American Philosophical Society, 1965.) $2.00.

NOBODY who knows the academic centres of the United States can be unaware of the tremendous enthusiasm with which great libraries are endowed, maintained and enriched by private benefactions. That it is not a new trait and that it showed itself at a very early stage in the colonial period is a reminder that this regard for the high educational value of libraries is indeed a part of the Anglo-Saxon tradition.

When, in 1728, the young Benjamin Franklin drew some of his friends together in a club "for Mutual Improvement" which he called the "Junto", his chief aim was to facilitate the study and discussion of new books on every subject, from history and art to medicine and the "mechanic arts". Only three years later, the books which the Junto had brought together became the nucleus of a private subscription library incorporated on July 1, 1731, as The Library Company of Philadelphia.

"So few were the Readers at that time in Philadelphia", wrote Franklin in his *Autobiography*, "and the Majority of us so poor, that I was not able with great Industry to find more than Fifty Persons, mostly young Tradesmen, willing to pay down for this purpose Forty shillings each, and Ten Shillings per annum. On this little Fund we began."

The importance of this act of faith can hardly be overestimated, for the Library which was thus established became not only a great educational influence in the lives of the colonists but also a political institution of such consequence that no less than ten of its members signed the Declaration of Independence and six the United States Constitution, and the Library served as the Library of Congress during the early years.

Franklin's own scientific bent, which was shared by a number of his associates, was reflected in the types of books which accumulated on the Library's shelves. The very first book order included such titles as *Boerhaave's Chemistry*, *Drake's Anatomy*, *Dechall's Euclid*, *Johnson's History of Animals*, *Keill's Astronomical Lectures*, *L'Hospital's Conic Sections*, *Parkinson's Herbal*, and several others. In 1738, the Library became a centre of scientific experiment with the gift by John Penn of an air-pump, later to be joined by a microscope and electrical apparatus which became the nucleus of a scientific museum and the occasion for several courses of public lectures on scientific subjects. In 1749 Franklin brought out his plans for an Academy and in 1750 he became the first President of the Board of Trustees of the institution which is now the University of Pennsylvania.

Benefactions allowed the purchase of books for the Academy's sole use, and its library was stocked "with suitable books in the different Branches of Science". About half the fund was also used to purchase "mathematical and philosophical apparatus", which emphasizes the strong bias towards scientific subjects apparent in the Academy from the start. In this project Franklin had secured the support of James Logan, a wealthy scholar who had a private library of over 2,000 volumes, including a 1516 edition of Euclid for which Franklin printed a facsimile title-page as a substitute for the missing original. Logan had carried out a series of experiments on the maize plant of which he published an account in Latin, later to be translated into English by the well-known English physician John Fothergill, who was Franklin's personal physician when he was in London. On his death, Logan left his library for the use of the public, which it continued to serve for forty years until, in 1792, it was absorbed into the Library Company, bringing with it many early scientific books which reflected Logan's own interests.

Franklin was also the moving spirit in expanding the scientific section of the Harvard College Library, especially after the disastrous fire of 1764, when 5,000 volumes were destroyed. He also encouraged scientific experiments, especially in electricity, at both Harvard and Yale, where he sent an electrical machine in 1749.

It was among the members of the Library Company that Franklin found his greatest support when he promoted the foundation of the American Philosophical Society in 1743 on the lines of the Royal Society. Franklin was its first Secretary and later its President for more than twenty years. The first volume of its *Transactions* appeared in 1771. The respect which it won from foreign savants is indicated by the names to be found in the lists of donors, for among them are those of Buffon, Lavoisier, Ingenhousz, Herschell, Fothergill, Priestley, Pringle, Banks, and Torbern Bergmann. Its success was the chief factor in encouraging the establishment of the Boston and other scientific societies, including medical societies such as the Medical Society of New Haven County.

The Pennsylvania Hospital, America's first permanent hospital and home of the first American medical library outside the Spanish American colonies, was born from the inspiration of Dr. Thomas Bond and the promotion of Benjamin Franklin, who became secretary, and later president, of the Board of Managers. Fothergill sent books, drawings and anatomical casts from London and after his death his protégé, Dr. J. C. Lettsom (founder of the Medical Society of London), became London agent for the library there.

This inspiring story of self-help and communal effort, which can only be hinted at in a review, is well told in Miss Korty's closely documented study. When asked by a young member of the Junto how to judge a good book, Franklin replied that "it ought to benefit the reader, by improving his virtue or his knowledge . . . it should be *smooth*, *clear*, and *short*". One feels that Franklin himself would have been satisfied that his requirements had been met in this account of his own great services to libraries and to science in the British colonies in America.

F. N. L. POYNTER

THE CHANGING ZULU

Zulu Tribe in Transition

The Makhanya of Southern Natal. By D. H. Reader. Pp. xii + 363 + 8 plates. (Manchester: Manchester University Press, 1966. Distributed in the U.S.A. by The Humanities Press, Inc., New York.) 45*s*. net.

To those for whom the name "Zulu" conjures up a picture of fierce and intractable warriors conquering all before them under their colourful ruler Shaka, Dr. Reader's

conscientious and carefully constructed if perhaps rather pedestrian monograph will make somewhat depressing reading. After a short but useful account of the history of the tribe and of its brief years of glory, the social system of one of the several small tribal fragments of the Zulu, the Makhanya, who live, about 15,000 strong, on a location about 25 miles south-west of Durban, is described in detail. The book has three main parts, dealing informatively with land use and economics; family, kinship, descent and marriage; and the politico-legal system. It concludes with two brief chapters on theoretical aspects of the study of social change which, like most theorizing by social anthropologists in this field, add little to our already reasonably clear picture of what is going on.

When Dr. Reader studied the Makhanya in the early fifties they had long ceased to be the proud warrior tribe familiar to Victorian schoolboys. With traditional values appropriate to unlimited land resources, a largely pastoral way of life, and shifting cultivation, the restricted area they now occupied was quite inadequate for their support. They had shown little response to the Administration's sporadic and somewhat feeble attempts to persuade them to adopt the changed methods of cultivation and animal husbandry which might have enabled them to sustain a wholly agrarian economy in modern conditions. Instead, 50 to 80 per cent of adult men engage in migrant labour (mainly in Durban) to provide themselves with the means for participation in the cash economy in which all Makhanya are now involved. The consequent increase in individualistic values, paralleled in almost every part of Africa, is expressed in numerous modifications of the traditional culture, ably documented by the author.

But, surprisingly, some traditional institutions, notably the basic essentials of the former military regimental system, survive with astonishing tenacity. Although the Makhanya are down they are by no means out. Traditional values, though changing, still survive and a sense of Makhanya identity is still strong. As the author rightly observes, the society he studied (like many another) is neither wholly "tribal" nor quite assimilated to Western norms, but is in a state of transition. And he wisely declines to prophesy what the final result of this process may be.

J. H. M. BEATTIE

CARNAP'S PHILOSOPHY

Philosophical Foundations of Physics

An Introduction to the Philosophy of Science. By Rudolf Carnap. Edited by Martin Gardner. Pp. x + 300. (New York and London: Basic Books, Inc., 1966.) 45*s*.

RUDOLF CARNAP is one of the most distinguished American philosophers of this century. Most of his writings have been on formal logic and on the philosophy of logic, but he began his career as a philosopher of science with the publication of his doctoral thesis on "Space" in 1922, and has always retained his interest in it. The present work originated from a lecture course given regularly by him. A transcript of the course was taken, and was edited and simplified by Martin Gardner. The result is a brilliantly clear introduction to the philosophy of science. There is considerable lack of good introductory textbooks on the philosophy of science. Such textbooks as there are either make very awkward reading or presuppose too much in the way of logic or mathematics. Carnap's book suffers from neither of these deficiencies. It is clear and simple, and will prove a great boon to teachers of the subject.

Topics covered include explanation and prediction, probability, measurement, the structure of space, causality and determinism, and the structure of scientific theories. Carnap deals more thoroughly with measurement and the structure of space than with the other topics. He analyses what rules we must have in order to make a measurement of extensive or intensive magnitude, and shows to what extent our reports of measurements made reflect our arbitrary conventions and to what extent they are informative about the physical world. He deals with other topics more briefly, merely setting forward alternative points of view and stating without argument his preference. He does this, for example, when considering whether a numerical measure can be given to the likelihood of a scientific theory being true, and, if so, whether it is a measure of probability in the sense in which we use that term to make judgments about the probability of the occurrence of events. This approach is inevitable in a book dealing briefly with many different topics within a controversial area, and Carnap, unlike many philosophical writers, has recognized the inevitability.

It is hard to fault this book, but one general criticism of Carnap's approach might be appropriate. In many passages Carnap seems to assume that the only evidence for the correctness or incorrectness of a scientific theory is that predictions made from it turn out to be true or false. But there is at least one further criterion used in judging the correctness of scientific theories—their relative simplicity. If one theory orders the data in some field in an evidently simpler way than another theory, that is grounds for saying that the first theory is correct and the second incorrect. I do not think that Carnap would wish to deny this point without qualification, but there are many passages in the book where he seems to ignore it. Thus, in discussing alternative time scales, he rightly claims that if we choose a man's pulse beat rather than the tick of a pendulum as our clock, the laws of physics would become extremely complicated. If we record our observations in terms of the former time scale and devise a theory which adequately accounts for them more simply than any other theory, that theory will still be an extremely complicated one. But, he argues (page 83), "there is no sense in which it is 'false' to measure time on such a basis". But surely the vast complexity of the resulting theory is ground for claiming that the alternative theory which accounts more simply than any other theory for the observations described in terms of the pendulum time scale is more likely to be true and so for saying the same of the time scale which it uses.

R. G. SWINBURNE

AVIATION PHYSIOLOGY

A Textbook of Aviation Physiology

Edited by J. A. Gillies. Pp. viii + 1226. (London and New York: Pergamon Press, Ltd., 1965.) 200*s*. net.

THIS book is the combined work of 22 authors, and presents, as Air Vice Marshal W. K. Stewart says in the preface, "a picture of the principles practised at the R.A.F. Institute of Aviation Medicine" at Farnborough. The Institute houses extremely well-equipped laboratories of human physiology, and, while the research at the Institute must embrace immediate practical problems, the Institute has been continuously concerned with their underlying fundamental basis. The high standards set and maintained at the Institute will be apparent to the readers of this volume.

The book is addressed to those attending courses in aviation physiology, to practitioners in aerospace and aviation medicine, and as a supplementary text to students reading for an honours degree in physiology or ergonomics. It will, however, appeal to many readers outside these fields. Aviation physiology is concerned with defining the limits of human tolerance to a great many different stresses, and the nature of the response to such stresses. There is no aspect of physiology which is unimportant to the aviation physiologist, although from the practical point of view some aspects are more important than others.

This book assembles and synthesizes in a lucid and convenient form information that is otherwise scattered.

It starts with an account of the physics of the atmosphere, and of the physiologically relevant aspects of aerodynamics. All aspects of the effects of reduced barometric pressure are then considered, including decompression sickness and the effects of the rapid decompression that follows failure of a pressure cabin at high altitude. The effects of hypoxia, thermal stress, acceleration in various axes, and of noise and vibration, and the means that may be adopted to counteract these stresses occupy a major portion of the book. Visual auditory and spatial perception are accorded an important place. The concluding sections on factors affecting aircrew performance, and on the causation, results and prevention of accidents contain lessons that could be, and should be, immediately applied more generally, and in particular in relation to the quantitatively much more important case of road accidents. It is remarkable that only very recently has there been any widespread interest in applying to road vehicle design and passenger protection principles that have been well understood for many years by aviation physiologists.

The editor and authors are to be warmly congratulated. The production is good, but the weight (3·2 kg) restricts in some measure the circumstances in which the book can comfortably be read. A. D. M. GREENFIELD

RUSSIAN TREMATODOLOGY

Essentials of Trematodology

By K. I. Skrjabin. (Trematodes of Animals and Man, Vol. 18.) Translated from the Russian. Pp. 532. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 135*s*.

THIS book is a translation of Volume 18 of a Russian series about which I have made some comments in a previous review[1].

It provides a good impression of the tremendous effort which Academician K. I. Skrjabin and his colleagues have made to record what is known about the structure of the digenetic trematodes and to use this information to formulate a system of classification. Descriptions of species are transcribed in great detail from original papers and books; accounts from various authorities sometimes given, always separately. Such a huge compilation may represent a sense of fairness as well as a craving for completeness down to the last detail, but the reader must often use his own judgment and sometimes his discretion because, sad to say, many examples of bad workmanship have been accepted at their face value. The text is overburdened with unsifted information and loaded with unusual terms such as "pedicellate suckers", "large scanty eggs", "cuticle unspinose", "ovate unlobed ovary" and "sexual anlage", as well as innumerable roundabout expressions. The edges of the body may be "decurving ventrally", "eyelets" are said to be "strewn over the 'cervical' region" and one organ may wind "convolutedly anteriorly directly behind" another. Measurements are given (in mm) to one, two or three places of decimals indiscriminately, the dimensions of eggs sometimes to four places, with a pretence of accuracy to one-tenth of a micron. Figures are often devoid of scales indicating sizes and captions are generally inadequate. There are 21 diagrams in Figs. 128 and 129 representing developing cercariae and their parts but the single scale which each figure carries clearly does not refer to all the diagrams, none of which is explicable in terms of a common caption which simply states "developmental cycle".

All these shortcomings transcend mere blemishes and the reader can hardly be expected to agree that the "essentials of trematodology" have been presented succinctly. I have already expressed my opinion (loc. cit.) about the use of keys, which entails constant revision as the bounds of knowledge expand, sometimes revealing previous misconceptions. It is certain that we shall need to know much more about the development and life cycles of the Digenea before we can contemplate "the creation of a new and superior system". Russian attempts to formulate such a scheme may be praiseworthy but they also seem to be premature. We may have to apply the principles of numerical taxonomy by the use of computors and with greater international co-operation than has been practised so far before attempting to formulate a practicable scheme. Some readers may welcome the compilation of such information as appears in these pages but others may feel disposed to check original descriptions and formulate more usable diagnoses for themselves. This appraisal is not intended to be harsh but only to indicate that the approach to "truth" must be gradual and not final and irrevocable. BEN DAWES

[1] Dawes, B, *Nature*, **203**, 1318 (1964).

MICROBIOCHEMISTRY

Chemical Microbiology

By Anthony H. Rose. Pp. viii+247. (London: Butterworth and Co. (Publishers), Ltd., 1965.) 37*s*. 6*d*.

ONE of the signs of the rapid growth of both biochemistry and microbiology in recent years has been the proliferation of textbooks for these subjects. Well over twenty-five texts, published *de novo* or revised since 1960, are available for undergraduate students of biochemistry. A similar situation exists in the case of microbiology. In spite of the increasing use of biochemical concepts and techniques by microbiologists, and the immensely useful contributions that have been made to general biochemical knowledge as the result of using micro-organisms, relatively few textbooks exist which cover the interdisciplinary area of chemical microbiology. This may be a consequence of the compartmentalization of the physical and biological sciences in institutions of advanced education, and it is certainly not a reflexion of the research situation, in which rapid progress continues to be made in the study of microbial life at the molecular level.

In the forties Marjorie Stephenson's *Bacterial Metabolism* inspired a whole generation of chemical microbiologists, and a limited number of monographs on the life and chemical activities of bacteria have since stimulated the enthusiasm of undergraduates for the subject. Such monographs rapidly become outdated, however, and if revised tend to increase in size and cost. The time was ripe for the appearance of a concise, well balanced, intellectually rigorous and yet readable undergraduate text of chemical microbiology. Dr. Rose's book suits the need admirably.

The book is intended for students with a basic knowledge of both microbiology (*pace* bacteriology) and biochemistry, but is introductory for those wishing to combine such knowledge in further studies. Familiarity with basic biochemistry is essential for an understanding of the various and numerous metabolic schemes, most of which indicate the nature of intermediates by name only. Although about half the text is devoted specifically to different aspects of microbial metabolism, additional chapters are concerned with "chemical anatomy", the environment, growth, reproduction and survival. In general, the author's intention to balance a consideration of microbial metabolisms against the relationship between structure and function and the more physiological aspects of microbial activity, has been realized. This balance, together with the carefully planned structure evident in each chapter, and the pleasing narrative style should make the book popular with its intended readers. References at the end of each chapter are mainly to review articles, although particularly significant reports in the primary literature are also included. It is noteworthy that more than 70 per cent of the references are to material

published since 1960, and that one-quarter of the references are to material appearing within the two years preceding publication of the book.

The format and lay-out of the book are pleasing, and the rather extensive list of *errata* and *addenda* appears to be complete. The book should be valuable to students taking a variety of courses in biochemistry, microbiology, or both, and at the price is extremely good value for money. J. M. TURNER

GRADUATE INORGANIC CHEMISTRY

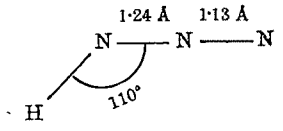
The Structure of Small Molecules

By W. J. Orville-Thomas. (Principles of Modern Chemistry: a Guide to Advanced Study, Monograph 1.) Pp. ix+189. (Amsterdam, London and New York: Elsevier Publishing Company, 1966.) 52*s*. Paperback 30*s*.

THIS book is the first volume in a series, edited by Profs. Forbes and Orville-Thomas, aimed at North American graduate students. The object is to provide material for courses intended to take the student beyond his own speciality.

This volume may be roughly classified as the structural inorganic chemistry of the first short period of the Periodic Table. About half the book is devoted to explaining the qualitative and quantitative principles for understanding structures, and the rest shows how the principles may be applied by discussing the structures of a number of individual molecules, classified by the bonding of one of their central atoms. The balance is right for the American market, where the students have a markedly professional approach and demand facts well mixed with modern theory.

At present the teacher of structural inorganic chemistry is faced with a difficult situation. To account for a bond length or an angle he may apply, qualitatively, molecular orbital theory, delocalized bonding, resonance structures, $p_\pi \rightarrow d_\pi$ bonding, varying hybridization, hyperconjugation and so on. These effects are combined until a "plausible" explanation is obtained; all the arguments are, necessarily, after the fact. This is illustrated by the following argument. Hydrazoic acid has the structure



From the bond angles, a hybridization can be assigned to each nitrogen atom (sp^2, sp, ground state) and then Hückel theory applied to the π electron system. The result is that one bond between the nitrogen atoms is shown to be stronger, and presumably shorter, than the other. What has been shown by this argument? If the angles could be specified the bond lengths were already known. So may the agreement of the theoretical explanation with the experimental facts be merely a consequence of the initial specifications? Has the language of theory said merely what was anyway known?

Prof. Orville-Thomas has tried to find a way out of these problems; he has not succeeded completely, but he has produced an interesting book with a worth-while quantitative approach. He has made much use of molecular orbital theory and compared his results with force constants and dipole moments, as well as bond lengths and bond energies. Empirical correlations are used to relate these quantities, but one thing that is clearly lacking is any reliable overall correlation between them. It is a pity that only the first short period of the Table is dealt with, since by this restriction discussion of the ubiquitous $p_\pi \rightarrow d_\pi$ bonding is precluded, together with that of many interesting molecules including, of course, the rare gas halides, which provided many teachers and students with their only experience of using principles to predict structures before the structures were actually known.

In short, it is an interesting book and should provide both teachers and students with a more genuine approach to the subject and with useful sidelights on some familiar problems as well. PETER BORRELL

DRY SOLVENTS

Inorganic Chemistry in Non-Aqueous Solvents

By A. K. Holliday and A. G. Massey. (The Commonwealth and International Library of Science, Technology, Engineering and Liberal Studies: Chemistry Division.) Pp. viii+143. (London and New York: Pergamon Press, Ltd., 1965.) 17*s*. 6*d*.

THIS is a concise, easily read monograph nicely balanced with respect to the solvents it covers. A quarter of the book deals with liquid ammonia and a good proportion of this space is devoted to the properties of metal solutions in this medium and theories relating to them as well as their reactions with elements, ions, and covalent compounds. The ordinary chemistry of reagents in liquid ammonia is dealt with concisely. Two pleasing features of the book are the tabulations of pertinent physical data for the solvents discussed and some degree of detail in describing experimental manipulations. The contrasting properties of amine solvents, notably ethylenediamine, receive brief mention before passing on to other protonic solvents of current interest. Sulphuric acid, exemplifying a hydrogen-bonded self-ionizing medium with its specialized self-ion conduction mechanism, is treated fairly fully and its interactions with salts, acids, bases and covalent compounds (free radical formation) described. Liquid hydrogen fluoride receives slightly more space; its probable ionization and fluoride ion conduction mechanisms are discussed and its mode of interaction with covalent fluorides and other substances to give the fluoronium ion is explained. The other hydrogen halides, as solvents, receive appropriately brief treatment. Protolysis, metatheses and ion association in glacial acetic acid, its differentiating action on the "strong" acids and its suitability as a medium for quantitative iodine and permanganate oxidations end the protonics section.

The non-protonic field represented by bromine trifluoride, sulphur dioxide, dinitrogen tetroxide and phosphorus oxychloride is briefly but interestingly reviewed. The analogues of protolysis in these media are considered and the once-postulated self-ionization of sulphur dioxide dismissed. As with all media, solvation, complex formation and metatheses receive mention. The effects of co-solvents, particularly with otherwise uninteresting solvents such as dinitrogen tetroxide, are treated, especially bromine trifluoride in the latter. The self-ionization of phosphorus oxychloride and the structure and properties of its solvate compounds are critically treated. Xenon hexafluoride, its properties and possible self-ionization briefly complete this section.

The final chapter of the booklet is devoted to the high-temperature solvent systems afforded by fused salts, their degrees of ionic structure and the physical chemistry of mixed melts. Protolysis in fused ammonium salts and analogous behaviour in non-protonic systems, notably mercuric halides and antimony trifluoride, is discussed. The Lux–Flood acid-base classification dependent on oxide ion donation provides a contrast with the more generalized Lewis theory. It is interesting to see silicate slag formation and the "borax bead" test interpreted on this basis, which is extended to the polymerization equilibria of sulphates, phosphates and chromates and also to the halide melts of aluminium, beryllium, titanium, cadmium and cobalt. Reductions in melts notably by

dissolved metals, the aqueous electrode potentials of which are broadly related, and also by anions; oxidations by anions and free halogens; metatheses involving the reciprocal Coulombic effect complete a neatly arranged section.

It is a matter for conjecture whether the page title heading "Non-aqueous Solvents in Inorganic Chemistry" might not have opened up a slightly wider field than the cover title but the authors are presumably the best judges of the area to which they wished to confine themselves.

S. R. C. HUGHES

Molecular Beams

Edited by John Ross. (Advances in Chemical Physics, Vol. 10.) Pp. viii+419. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 113*s*.

THIS volume of the *Advances in Chemical Physics* is in the form of a series of review articles by leaders in the field of elastic and inelastic molecular scattering: polarizabilities, repulsive intermolecular forces, quantum effects on elastic scattering, chemical reactive scattering, excited atom collisions, charge transfer, ion-molecule reactions and supersonic molecular beam sources are discussed.

Although the upsurge of interest in these and related topics has produced much understanding of importance, it is likely that the decisive working out of the subject will take place over the next five or ten years. This volume is therefore not definitive but it does represent a great advance on ten years ago when Taylor and Datz discovered the first molecular beam detector capable in itself of responding differently to different molecules; this can be said to have made possible the commencement of study of a limited number of chemical reactions by particle scattering technique, years before it could otherwise have been achieved.

The field is one which the British chemical fraternity largely ignores, to its ultimate discomfort. This volume should go some distance towards stimulating the necessary interest.

J. B. HASTED

Solar-Activity Forecasting

By Yu. I. Vitinskii. Translated from the Russian by Z. Lerman. Edited by R. Hardin. Pp. iv+129. (Jerusalem: Israel Program for Scientific Translations; London: Oldbourne Press, 1965.) 54*s*.

SOLAR radiation rules the lives of everyone on Earth, and its fluctuations, though barely noticed by the sunbathers on our beaches, are vitally important for satellites in low orbits, which end their lives prematurely if the Sun is unduly active and makes the air density increase. The fluctuations will be even more vital for interplanetary travellers, who may end their lives prematurely if the Sun is unduly active in shooting out streams of ions.

The prediction of solar activity is at present even more chancy than long-range weather forecasting, because there is no accepted physical theory of solar disturbances and no agreement on the best index of activity. The Wolf sunspot numbers are usually regarded as the best index, although, as the author of this book points out, the Wolf numbers are even to-day slightly subjective and used to be far less reliable. Nevertheless the Wolf numbers provide the only index which goes back more than 100 years, and have to be used by anyone attempting long-term prediction.

Sunspot maxima recur on average every 11·1 years, but the period can be as low as 7 years (as in the 1830s) or as high as 17 years (1788–1805). The maximum monthly smoothed sunspot number in a cycle can be as low as 50 (in 1805) or as high as 200 (in 1957–58); the minimum is usually less than 10. So far no satisfactory method of predicting the dates and intensities of future cycles has been developed, though various recurrence tendencies have been detected. Predicting the monthly smoothed numbers a year ahead offers a better chance of success, essentially because each cycle, once it has started, shows some resemblance to a previous cycle, or to the average of several previous cycles, and is open to statistical treatment. On a still shorter time-scale, prediction is bedevilled by the unexpected appearance of new sunspot groups.

Vitinskii's book, published in the Soviet Union in 1962, is the most thorough survey available and can be strongly recommended to anyone interested in the subject. The author reviews all the methods that have been suggested, with admirable impartiality, and does not hesitate to conclude that many of them are worthless. It still seems likely, however, that a causal relationship underlies the seemingly irregular variations; and if any mathematician, intoxicated by such a thought, wishes to enter this dangerous field, the book will provide him with both an excellent guide and a sobering view of the reputations lying slaughtered among the man-traps.

D. G. KING-HELE

Solids

Elementary Theory for Advanced Students. By Gabriel Weinreich. Pp. xii+163. (New York and London: John Wiley and Sons, 1965.) 53*s*.

THIS textbook is based on lectures to American graduate students and presupposes a thorough grounding in quantum mechanics, including operator theory, second quantization, and perturbation theory using Feynman diagrams. The term "elementary" in the subtitle is to be understood in the Gibbsian sense.

The ground covered consists mainly of selected portions of the quantum theory of solids and includes Brillouin zones, band theory, the use of Bloch and Wannier functions, vibrational properties of solids and singularities in the vibrational spectrum. The treatment is succinct but thorough, and can be studied with profit given the requisite background.

The opening chapter is devoted to crystallography and is particularly well set out; the illustrations both of lattices and of crystallographic point groups are presented with unusual clarity.

The experimental consequences of the theory have been consciously—one might also add conscientiously—ignored. In a book of limited size, this attitude is certainly justified.

The book is written in a pleasant as well as lucid style and the presentation leaves nothing to be desired. It is warmly recommended.

M. BLACKMAN

Dog Breeding

Reproduction and Genetics. By S. A. Asdell. Pp. viii+194. (London: J. and A. Churchill, Ltd., 1966.) 45*s*.

THE dog is much the oldest of domesticated animals. He is also, not excluding man, physically, emotionally and mentally the most diverse. The breeding of dogs is therefore, as Darwin has explained, of evolutionary interest beyond the range of dog-lovers. Prof. Asdell, however, has written his book from the veterinary or dog-lovers' point of view. To be sure he mentions Darwin by name and he also devotes a chapter to "The Science of Genetics". But the account he gives us is a personal one in which he adds to the established misconceptions a few new ones of his own. The dog breeder will do well to skip this chapter.

On the practical side Prof. Asdell describes the breeding experiments of Stockard—which were carried out in his own university 25 years ago—but he misses the point of this classical work. The four photographs he gives from Stockard will, however, show the reader something of what he has missed.

C. D. DARLINGTON

MEETINGS

BIOCHEMISTRY OF MITOCHONDRIA

A COLLOQUIUM on the biochemistry of mitochondria was included as part of the third meeting of the Federation of European Biochemical Societies, which was organized in Warsaw during April 4–7, 1966. The colloquium was arranged by Prof. E. C. Slater (Amsterdam) and organized by Dr. Z. Kaniuga and Dr. L. Wojtczak (Warsaw). Seven invited addresses were included and delivered by B. Chance (Philadelphia), J. B. Chappell (Bristol), L. Ernster (Stockholm), M. Klingenberg (Marburg), H. A. Krebs (Oxford), P. Mitchell (Glynn Research Laboratories, Bodmin) and E. C. Slater (Amsterdam), together with eighty-one short communications by research workers from seventeen countries. Although almost all current problems of mitochondrial biochemistry were discussed, the invited addresses and most of the short communications were concerned with the following topics: (1) the structure and function of the respiratory chain; (2) the mechanism of energy conservation; (3) transport mechanisms in mitochondria; (4) the regulation of substrate utilization; (5) activation and oxidation of fatty acids; (6) metabolic interrelationships between mitochondria and the cytoplasm.

Immediately after the Federation's meeting a "closed" symposium was held in Warsaw during April 8–9. This was organized by the departments of biochemistry of the Universities of Amsterdam and Warsaw and of the Nencki Institute of Experimental Biology in Warsaw. The symposium, which was attended by sixty-five invited scientists from fourteen countries, was devoted to discussions of data already presented during the Federation's colloquium. The following six topics were discussed: (1) the place of ubiquinone and cytochrome *b* in the respiratory chain; (2) the chemiosmotic theory of oxidative phosphorylation; (3) uptake of cations by mitochondria; (4) the chemical coupling theory of oxidative phosphorylation; (5) regulatory mechanisms of substrate utilization; (6) oxidation and activation of fatty acids.

The present report is concerned with both meetings.

Two alternative theories dealing with the role and the position of ubiquinone in mitochondrial respiration were proposed by M. Klingenberg (Marburg) and E. R. Redfearn (Leicester), respectively. M. Klingenberg, in his invited address, presented several lines of evidence in favour of the view that ubiquinone is located on the main pathway of electron transport, and that it acts as a common pool for reducing equivalents from various flavoproteins. This was supported by data on the redox state of ubiquinone, compared with nicotinamide-adenine dinucleotide and cytochromes, for both forward and reversed electron transfer, and by studies of the kinetic of either the reduction of ubiquinone after substrates have been added to the terminally inhibited respiratory chain, or its oxidation after oxygen has been added in the anaerobic state. E. R. Redfearn postulated the existence of a branching pool of ubiquinone in the respiratory chain. Related problems concerning the place of cytochrome *b* were not discussed in detail, presumably because of the lack of sufficient new experimental data.

As E. C. Slater pointed out in his introductory address, three theories have been proposed for the energy coupling mechanism in mitochondria: (i) the chemical coupling theory; (ii) the concept of phosphorylation driven by proton transport (proposed by P. Mitchell); (iii) the concept of conformation changes in specific proteins (proposed by P. Boyer). Lively discussions of both the first two theories took place at the colloquium and the "closed" symposium. P. Mitchell presented new experimental evidence in favour of his concept. He postulated that both the *p*H changes produced by submitochondrial particles catalysing forward and reverse electron transport, and the burst of proton ejection after a rapid transition from the anaerobic to the aerobic states support the concept that adenosine triphosphate is synthesized by a mechanism involving the ejection of protons and which is intimately connected with the respiratory chain. On the other hand, B. Chance, J. B. Chappell and J. M. Tager presented evidence that proton transport is a secondary effect of the conservation of energy. The basic differences between these two theories were depicted by E. C. Slater in the following scheme:

CHEMICAL COUPLING THEORY

Respiratory chain → high-energy intermediate ⇌ ATP

⇅

cation uptake = H^+ production

CHEMIOSMOTIC THEORY

Respiratory chain → H^+ production ⇌ ATP

↓

cation uptake

A new concept of the action of arsenate on the energy-coupling mechanism was proposed by L. Ernster. He postulated the formation of a relatively stable arsenylated derivative of a high energy intermediate.

Ion transport in mitochondria was extensively discussed by J. B. Chappell, B. Chance, E. Carafoli (Modena), G. F. Azzone (Padua) and A. Azzi (Padua). The main topic in the discussion of cation transport was the stoichiometry (or its lack) between the calcium and oxygen taken up by, and the protons ejected from, mitochondria.

Of considerable importance for a better understanding of the utilization of substrate in mitochondria are the problems of anion transport. J. B. Chappell classified anions (including respiratory substrates) into four groups: (1) those which easily penetrate mitochondrial membranes, for example phosphate and arsenate; (2) those which penetrate mitochondrial membranes only in the presence of phosphate (for example, D- and L-malate, succinate and *meso*-tartrate); (3) those which penetrate mitochondrial membranes in the presence of phosphate and L-malate (for example, citrate); (4) those which are unable to penetrate the mitochondrial membrane, such as chloride ions and fumarate. He also postulated the existence of specific carriers for these different groups of penetrants, as did E. J. de Haan (Amsterdam) for α-oxoglutarate.

Another factor which controls the citric acid cycle is oxaloacetate. Its effect on the oxidation of succinate and the factors controlling the level of oxaloacetate itself were discussed by A. B. Wojtczak (Warsaw), A. M. Roberton (Oxford) and S. Papa (Bari).

New concepts on the activation and oxidation of fatty acids were discussed both at the colloquium and at the "closed" meeting. The group at the University of Bristol working under P. B. Garland was represented at the meetings by D. Shepherd and D. W. Yates. The essential point of their theory is that an intramitochondrial pool of coenzyme *A* involved in the β-oxidation of fatty acids can be acylated only by acyl-carnitine formed either extramitochondrially or intramitochondrially. On the other hand, S. G. van den Bergh presented evidence in favour of his view that carnitine is not involved in the normal pathway of fatty acid oxidation.

Sir Hans Krebs dealt with the problem of the transport of reducing equivalents between mitochondria and the cytoplasm. He showed that the reduced nicotinamide-adenine dinucleotide which is required in the cytoplasm for glucogenesis is formed primarily within the mitochondria and is transferred to the cytoplasm by way of an oxaloacetate-malate system. The same system, working in the reverse direction, transports reducing equivalents, which are produced during glycolysis, from the cytoplasm to mitochondria.

L. WOJTCZAK
Z. KANIUGA

OBITUARIES

Evgeniya Nikolaevna Sinskaya

THE death occurred on March 4, 1965, in Leningrad of Professor Evgeniya Nikolaevna Sinskaya, the distinguished Soviet biologist, well known for her studies in taxonomy, plant breeding, ecology, genetics and physiology.

E. N. Sinskaya was born into the family of a teacher in the city of Pskov on November 25, 1889. After she had obtained her school-leaving certificate, she studied from 1911 until 1917 as an external student at the former Petrov Academy of Agricultural Sciences in Moscow. In the summers she used to carry out practical agricultural work and also participate in scientific expeditions. Graduating as an external student from the Academy in 1917, she obtained a diploma with the title of scientific agronomist. In 1918 she joined an expedition to Central Asia to study the plants of the solonchaks (saline soils), and in 1919 started her work as a specialist on meadow culture at the Saratov Province Land Department. In Saratov she happened to meet Professor N. I. Vavilov, who then held the chair of plant production at the Saratov University, and was invited by him to join his department. In 1921, together with other colleagues of N. I. Vavilov, Sinskaya moved from Saratov to Petrograd. Here, N. I. Vavilov became head of the institution then named the Bureau of Applied Botany which in 1925 became the All-Union Institute of Plant Industry (VIR). E. N. Sinskaya worked at this Institute up to the last days of her life. She took an active part in the organization and direction of various divisions of the Institute—the Technical Crops Division, the Forage Crops Division, and the Ecology and Taxonomy Division.

A woman of great erudition, tireless activity and energy, Sinskaya soon became a leading scientist of the Institute. Her scientific activity led to the publication of more than 140 works. Among the most important are: *Oleiferous and Root Crops of the family Cruciferae* (1928), *Breeding of Forage Crops* (1936), *Meadows of the Mountain Foothills in the North-western Caucasus* (1939), *Dynamics of Species* (1948), *Alfalfa* (1950), *Annual Forage Crops of the USSR* (1957), *On Categories and the Laws of Variability within Populations of Higher Plants* (1963). A large work of forty printed sheets on *Historical Geography of Cultivated Plants* was left in manuscript. In 1936 the degree of Doctor of Biological and Agricultural Sciences was conferred on her for her studies on oleiferous Cruciferae and on forage crops.

Each year, Sinskaya actively participated in expeditions collecting plant resources. She organized collections of local cultivated and wild plants in the Far East, in the Altai Territory, and her most detailed investigations were carried out in the Caucasus and Transcaucasus regions. She brought back from her Far East expeditions many samples both of cultivated and of useful wild plants from Japan.

Prof. Sinskaya was a person of ready sympathy. She was always greatly interested in the work of other research workers, and gladly shared her knowledge and experience with those who asked her help. She maintained wide relations with many scientists both in the Soviet Union and elsewhere.

E. N. Sinskaya was decorated in 1957 with the Order of Lenin.

V. BORKOVSKAYA

Dr. Erna Bennett adds:

Professor Sinskaya's death at the age of seventy-five is a grievous loss to biology. Her work extended over more than four decades which saw deep and fundamental changes in the concepts of evolutionary genetics. Her own contribution to these changes has been very considerable.

Although her biological interests were diverse to an unusual degree, and enriched not only ecology, geography and taxonomy but also plant breeding and population genetics with a wealth of new data and ideas, throughout her whole work there can be found a strikingly single-minded concern with the nature and causes of genetic variability. From her first published paper in 1922—"On the Study of the Laws of Variability in the Family Cruciferae"[1]—to one which appeared in the last year of her life—"On the General Regularities of Ecological–Geographical Variability within Populations of Wild and Cultivated Plants"[2]—Sinskaya's preoccupation with this problem is very clear. It is a problem to which she brought a fluent and erudite knowledge of plant taxa and the skilled application of the phyto-geographical techniques of Vavilov.

Working with oil and forage crops and their related wild species for most of her life, Sinskaya drew heavily on the evidence they evinced of speciation processes in order to build into the growing structure of genecology her own specific contribution of the ecoelement concept. Sinskaya's ecoelements—character complexes, maintained by linkage groups of adaptive origin—carried the ecotypic analysis of plant populations a stage further than did most concepts by other genecologists of her day. Ecoelements resemble the genetic inertias formulated by Darlington and Mather which rested on the polygenic theory of inheritance, and the cohesion theory of Anderson. They also supplied the link between ecology and genetics from the direction of ecology that the work of Dobzhansky and others provided from genetics. Recognizing the importance of Sinskaya's work, J. W. Gregor invited her to prepare one of her very rare contributions in English[3].

[1] *Trudy Priklad. Bot. Genet. Seleks.*, **13** (2), 15 (1922).
[2] *Trudy Priklad. Bot. Genet. Seleks.*, **36** (2), 3 (1964).
[3] *Report Scott. Pl. Breed. Sta.*, 31–40 (1958).

J. K. Dixon

DR. J. K. DIXON, a prominent research scientist and director of the Department of Scientific and Industrial Research Soil Bureau, Taita, died on July 30, 1966, at his home in Karori, Wellington, New Zealand.

Dr. Dixon was educated at Christchurch Boys' High School and Canterbury University from which he graduated in 1929. For the next two years he worked on causes of bush sickness in the Te Kuiti area; then in 1931 he received a National Research Scholarship to spend two years at the Imperial College of Science and Technology, London. In 1933 he gained a Ph.D., was awarded the Diploma of Membership of Imperial College of Science and Technology (London) and was elected a Fellow of the Royal Institute of Chemistry.

After his return to New Zealand Dr. Dixon worked for three years at the Cawthron Institute, Nelson, and established that "Morton Mains disease" in animals in Southland was a disorder resulting from cobalt deficiency. He joined the D.S.I.R. Soil Bureau in 1936 and became its chief soil chemist in 1940, assistant director in 1952, and director in 1962.

Dr. Dixon served as president of the Royal Society of New Zealand, the New Zealand Institute of Chemistry, the New Zealand Soil Science Society and the New Zealand Institute of Agricultural Science. He was the senior New Zealand Government representative at the eighth International Congress of Soil Science in Bucharest in 1964.

Dr. Dixon was well known for his research on problems associated with cobalt deficiency in animals and for his work on the use of organic waste matter in composting. I knew Dixon personally for the past thirty-six years and, together with all those with whom he was associated, shall sadly miss a former colleague who not only left his mark in the field of soil science but has at the same time been outstanding in his dedication to the service of his fellow men. He is survived by his wife and two sons.

L. W. TILLER

STRUCTURE OF THE RADIO SOURCES 3C 46, 66, 129 AND 465

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A SERIES of observations has recently been carried out with the Cambridge one-mile radio telescope[1,2] to determine the angular structure of a large number of relatively intense radio sources. Many of these sources comprise two components, as Maltby and Moffet[3] have already demonstrated, but a number have been found to be of considerable complexity and their structure has not previously been known. This article describes four of these complex sources which are believed to be extra-galactic; any theory of radio galaxies should account for these sources in addition to the more numerous double sources.

The observations have been made at frequencies of 408 Mc/s and 1,407 Mc/s, using a method which has already been described[2,4,5] in which 12 h observations are made with the movable aerial at each of a number of positions along the full length of the rail track. These observations, when combined in the computer, provide a pencil beam surrounded by a series of elliptical grating responses; the angular separation of the first grating response from the main beam can be increased by the use of a larger number of aerial positions, but for minimum observing time it is normally arranged to be at an angle rather greater than the greatest extent of the source being observed.

In the present observations four positions of the movable aerial were used for 3C 46, 66 and 129, and eight positions were used for 3C 465; because the signals from the movable aerial are combined with those from two fixed aerials, these observations provide eight and sixteen spacings respectively. The main response has widths, to half power, of 23 sec arc and 80 sec arc in right ascension and (23 cosec δ) and (80 cosec δ) sec arc in declination at the two observing frequencies of 1,407 Mc/s and 408 Mc/s. The first grating response is an ellipse with a semi-minor axis of 4·8 and 16·4 min arc at the two frequencies for the observations of 3C 46, 66 and 129, and a semi-minor axis of 9·5 and 32·8 min arc in the observations of 3C 465.

These angles are sufficient to provide maps, unaffected by grating responses, of all the sources, except for the 1,407 Mc/s observations of 3C 66 and 129; for these two sources a complete map has been obtained at 408 Mc/s, but the present 1,407 Mc/s observations have only been used to investigate the detailed structure of the two main components.

The maps are presented in Figs. 1–4. The declination scales have been reduced by sin δ in order to make the aerial response circular and thus simplify the distinction between resolved and unresolved features; the factors by which the scales are compressed are indicated in the figure legends. In each case the half-intensity beamwidth is indicated by the shaded area. The observations were made using linearly polarized radiation with the E-vector in position angle 0° at 1,407 Mc/s and 90° at 408 Mc/s except for 3C 465, where these angles were reversed.

3C 46. This source has been reported as double[3] but in fact it has four elongated components (Fig. 1), although the preceding pair is not fully separated; none is resolved perpendicular to the axis of the source. Their approximate flux densities at 1,407 Mc/s are 0·5, 0·12, 0·1 and 0·48 × 10^{-26} Wm^{-2} $(c/s)^{-1}$.

The separation of the two intense components is 144 sec arc in position angle 67°. They are connected by a strong ridge of emission which contributes about 15 per cent of the total integrated flux. The relative intensities of the components at 1,407 and 408 Mc/s are similar, and correspond to a spectral index $\alpha = 0{\cdot}97$. The source closely resembles 3C 452. which has already been described[5].

Three optical objects in the region of the source have been measured from the *Palomar Sky Survey* prints, and their positions marked with crosses. Object a is a faint red galaxy of $m_{pg} \simeq 19{\cdot}5$ suggested as an identification by Wyndham[6] and b is another slightly nebulous red object of $m_{pg} \simeq 18{\cdot}0$; both are probably members of a cluster of galaxies. Object c is stellar with $m_{pg} \simeq 17{\cdot}0$. There are no other optical objects within 30 sec arc of the centres of the sources.

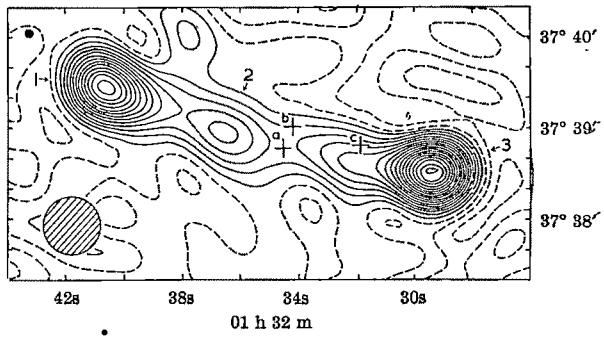


Fig. 1. Map of 3C 46 at 1,407 Mc/s. a and b are faint red galaxies and c is probably a star. Assuming that a is the identification with $M_{pg} = -20{\cdot}5$, the red-shift is about 0·12 and the separation of components 1 and 3 is 260 kparsec. The declination scale is compressed by a factor of 0·61.

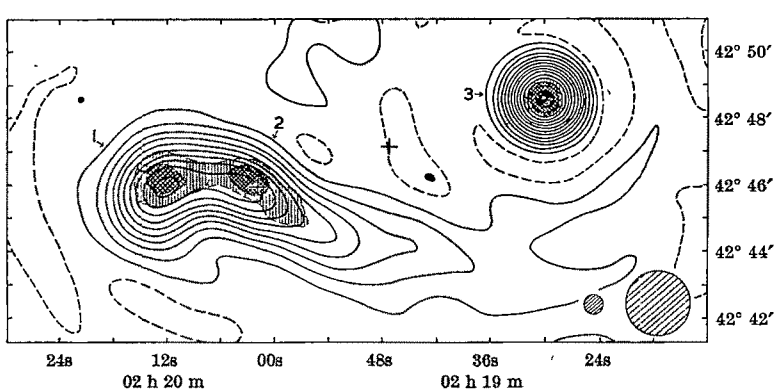


Fig. 2. Map of 3C 66 at 408 Mc/s; the structure revealed by the 1,407 Mc/s observations is shown by shading. The 408 and 1,407 Mc/s beams are shown by shaded circles. The object coincident with component 2 is a 14m 2 elliptical galaxy with red-shift 0 0215; another elliptical galaxy of 15m lies between components 2 and 3. The declination scale is compressed by a factor of 0 68.

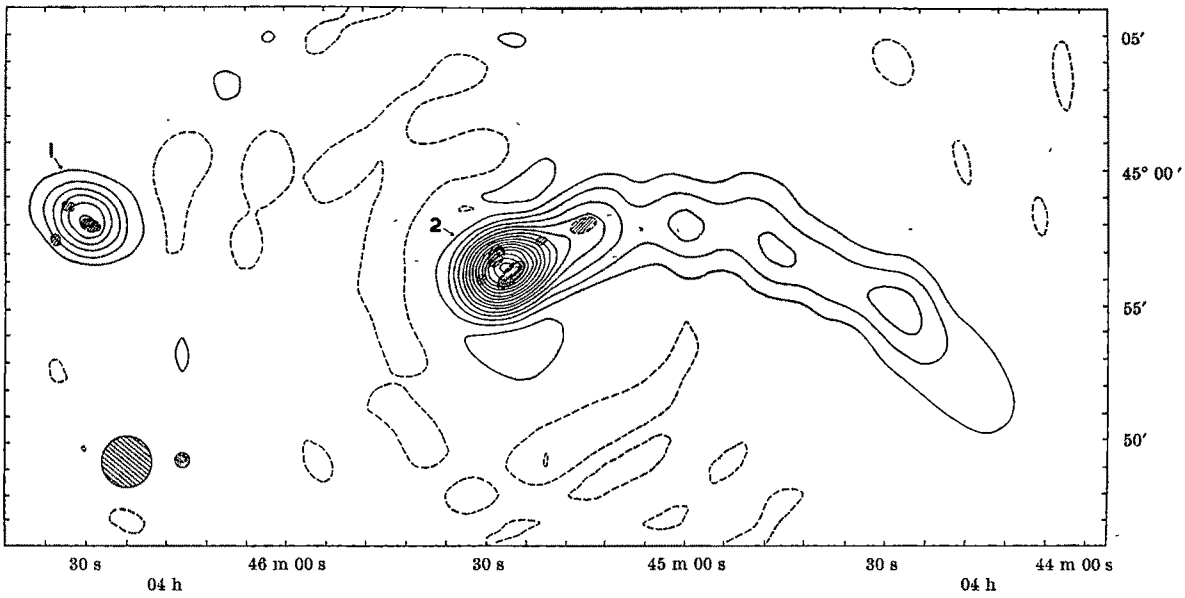


Fig. 3. Map of 3C 129 at 408 Mc/s; component 1 is 3C 129.1. At 1,407 Mc/s both sources are resolved into a number of components as indicated. The 408 and 1,407 Mc/s beams are shown by shaded circles. The declination scale is compressed by a factor of 0·71.

3C 66. Although previous observations have revealed the presence of double structure[3,7] these observations show that one of the components is very much extended and also shows fine structure. Fig. 2 shows the 408 Mc/s contours, with shadings which represent the areas within three of the contours obtained at 1,407 Mc/s; the extended component may be seen to consist of two main sources (1 and 2) linked by a bridge which extends south of, and beyond, the compact component 3. The latter is unresolved at both frequencies and therefore has an angular diameter < 10 sec arc.

An elliptical ($ED2$) galaxy of $m_{pg} = 14{\cdot}2$ coincides precisely with component 2. The position was measured by Griffin[8] and its red-shift has been found to be 0·0215 (ref. 9). There are no optical objects which can be associated with components 1 and 3, but a second elliptical galaxy of $m_{pg} = 15{\cdot}0$ lies almost exactly mid-way between components 2 and 3. Both galaxies appear to be members of Abell[10] cluster A-347.

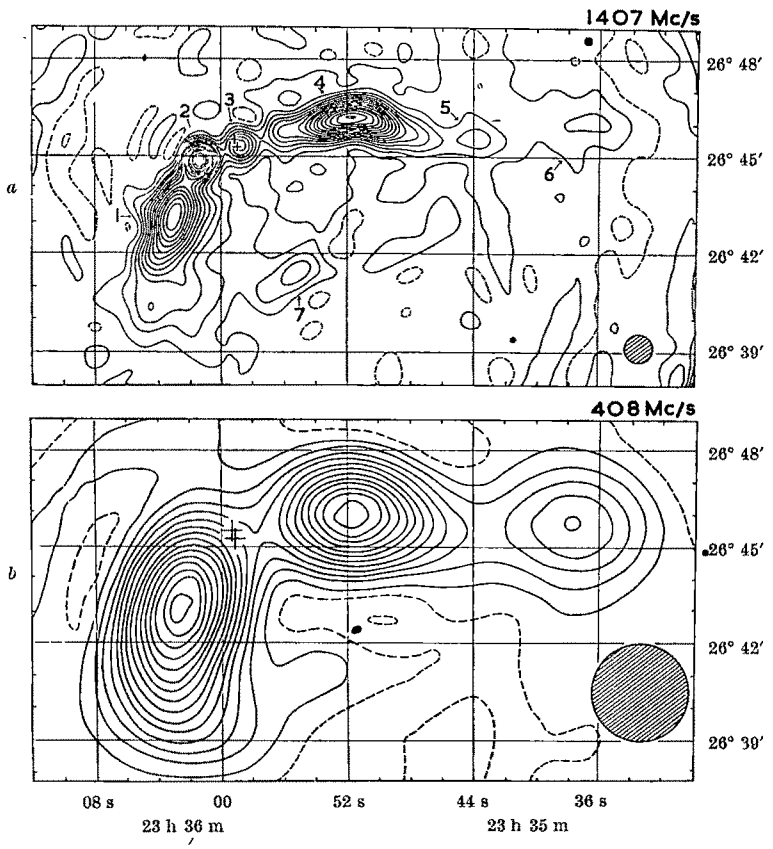


Fig. 4. *a*, Map of 3C 465 at 1,407 Mc/s. The position of the double galaxy *NGC* 7720 is marked with two crosses. There appear to be no other optical objects associated with the radio source. *b*, Map of 3C 465 at 408 Mc/s. It may be seen that component 6 is here relatively much more intense than at 1,407 Mc/s. The declination scale is compressed by a factor of 0·45.

3C 129. Previous observations[3] showed this source to have an angular extent > 6 min arc with structure < 1 min arc. The present observations at 408 Mc/s are presented in Fig. 3 which shows that the intense central source has an extension which develops into a curved ridge of emission, 13 min arc in length and about $\frac{1}{2}$ min arc wide with three weaker peaks at almost equal intervals of 3 min arc; the ridge contributes about 20 per cent of the total flux density. It seems likely that the source (1) lying 11 min arc east of the central source (3C 129·1) is associated with 3C 129.

The observations of 3C 129 at 1,407 Mc/s are confused by grating responses beyond 4·8 min arc from the central source, but they may be used to investigate the detailed structure of the two main components. It is found that the central source is resolved into five separate components, two of which are intense and elongated almost parallel to the extension of the 408 Mc/s map with three weak ones in line with the extension. These are indicated by shaded areas superposed on Fig. 3. 3C 129·1 is similarly resolved into three components, one with about twice the intensity of the other two.

The source lies close to the galactic plane in an area where there is appreciable obscuration. Although it is possible that 3C 129 might be a supernova remnant, the well-defined limits of the arc and the very large

Table 1

Component	Right Ascension h m s	Declination ° ′ ″	S_{1407} ($\times 10^{-26}$ Wm^{-2}(c/s)$^{-1}$)	S_{408}	α	d (kpc)	Volume ($\times 10^{66}$ cm^3)	Luminosity ($\times 10^{41}$ ergs sec^{-1})	E_{min} ($\times 10^{57}$ ergs)	H ($\times 10^{-5}$ gauss)
3C 46										
1	01 32 40·7	37 39 29	0·5	2·1	0·97	153	250	9·0	32	3·7
2	01 32 36·7	37 38 58	0·12							
3	01 32 29·5	37 38 32	0·58	1·9	0·97	113	250	8·5	32·0	3·7
3C 66										
1	02 20 12·4	42 46 09	3·3	18·0	0·6	35	670	2·7	20·0	2·0
2	02 20 02·5	42 46 06	5·0							
3	02 19 30·1	42 48 37	1·3	5·0	1·1	121	<2	0·7	<0·7	>6·3
3C 129·1 and 129										
1	04 46 29·9	44 58 14	(6·5)	2·1	(0·6)	–	–	–	–	–
2	04 45 26·8	44 56 33	(1·1)	17·0	(0·7)	–	–	–	–	–
3										
3C 465										
1	23 36 03·1	26 43 06	3·0	7·7	0·77	60	53	2·0	5·5	3·0
2	23 36 01·4	26 44 48	0·76	1·9	0·72	18	5	0·5	1·1	5·2
3	23 35 58·8	26 45 19	0·26	0·47	0·48	2	<3	0·2	0·56	>4·5
4	23 35 51·8	26 46 09	2·81	6·4	0·66	47	91	1·8	9·5	3·4
5	23 35 44·0	26 45 31	0·61	1·2	0·57	86	21	0·4	2·0	3·1
6	23 35 36·8	26 46 04	0·25	1·6	1·5	129	68	0·6	3·8	2·4
7	23 35 55·4	26 41 33	0·34	0·9	0·77	97	26	0·2	1·5	2·5

surface brightness of the main component make an extragalactic origin seem more likely. Wyndham[6] has suggested as a probable identification a 19^m red galaxy which lies close to component 2, but the area within which a related object might lie is too large for this to be regarded as definite.

3C 465. The association of *3C* 465 with *NGC* 7720 was originally suggested by Dewhirst[11]. Previous radio observations[3] showed it to extend over several minutes of arc with complex structure. Fig. 4*a* is the map of the radio emission observed at 1,407 Mc/s which shows seven components. The positions of the two components of the double galaxy *NGC* 7720 as given by Griffin[8] are marked with crosses. They are associated with the only unresolved feature of the 1407 Mc/s map (component 3), which has an angular size less than 10 sec arc. There are a number of bright galaxies, members of Abell[10] cluster *A*-2634, in the region of the source, but none appears to be related to any other radio component; a 16^m galaxy lies 19 sec arc west of the centre of component 4.

Although the resolution of the 408 Mc/s map (Fig. 4*b*) is inadequate to show all the details visible at 1,407 Mc/s, it is clear that most of the features are similar at the two frequencies. There is, however, a remarkable exception in component 6, which is strong at 408 Mc/s, but extremely weak in the 1,407 Mc/s map.

By convolution of the 1,407 Mc/s map with the appropriate function to reduce the resolving power to that of the 408 Mc/s beam, a direct comparison can be made of the total integrated flux of the seven components at the two frequencies. An estimate of these fluxes and the corresponding spectral indices α are given in Table 1.

Matthews, Morgan and Schmidt[12] have investigated *NGC* 7720 in detail. It is a $13^m{\cdot}5$ *D*-galaxy, with an elliptical nucleus surrounded by an extensive asymmetric envelope of higher ellipticity. There is a circular 15^m companion galaxy in the same envelope. The spectrum shows a weak emission line of OII and absorption lines which give a red-shift of 0·0301[13]. At the distance of *NGC* 7720 (88 Mparsec), the upper limit of 10 sec arc on the angular size of component 3 corresponds to a linear dimension < 5 kparsec. This component appears to be associated with (but is smaller than) the brighter member of *NGC* 7720, and it is probably significant that this is the galaxy showing an excited emission line in its spectrum.

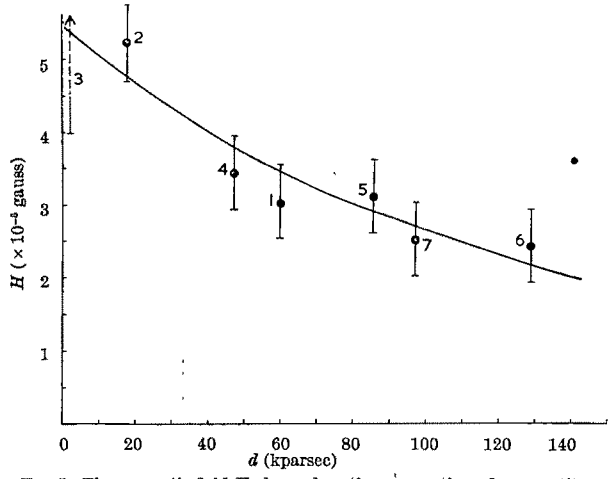


Fig. 5. The magnetic field H, derived on the assumption of equipartition between particle and field energies, is plotted against d, the projected distance of each component of 3C 465 from the brighter member of *NGC* 7720.

The details of the four sources are summarized in Table 1, which gives the co-ordinates, flux densities and spectral indices of each main component.

Red-shifts are available for *3C* 66 and 465, and the projected physical separation (d) of each component from the optical object has been derived using a value of the Hubble constant $H = 100$ km sec^{-1} Mparsec^{-1}. In the case of *3C* 46 an approximate red-shift of 0·12 has been obtained assuming that source *a* is the identification, with $M_{pg} = -20{\cdot}5$. The volume of each component has also been computed; in the case of elongated features, it has been assumed that they have a circular cross-section, with an extent in the line of sight comparable with the width. For elongated features making a small angle with the line of sight, this assumption will lead to an underestimate of the volume.

From the corresponding luminosities and volumes it is possible to determine the minimum energy requirements for each component assuming that there is equipartition between the particle energy and the magnetic field; it is assumed that the protons have 100 times the energy of the electrons[15]. These figures, and the corresponding values of magnetic field, are given in columns 10 and 11.

Since the volumes of the components must originally have been considerably less (and presumably less than that of the galaxy) the adiabatic losses require the initial energy to be greater by a factor of at least the ratio of the linear dimensions. In the case of *3C* 46 and 66 the minimum total energies are therefore of the order of 2 and 0·7 × 10^{59} ergs respectively.

The magnetic fields for all the components in *3C* 465 are plotted in Fig. 5 as a function of d, their projected distance from the brightest member of *NGC* 7720. It is probable that the value of d is related to the age of the component, and this relationship may be of importance in connexion with theories of the evolution of radio galaxies.

The evidence provided by the present observations of *3C* 46, 66, 129 and 465, the earlier observation of *3C* 452 (ref. 5) and the multiple source 5*C* 1·47 and the association of 5*C* 1·71, 76, 77 and 79 (ref. 16), confirm the occurrence of multiple explosions in galaxies. The marked curvature in *3C* 129 suggests that ejection may have occurred along a fixed axis in a rotating galaxy. The relative magnitudes of the energy released in successive explosions, and the intervals between them, are clearly of great importance in understanding the mechanisms involved in radio galaxies.

We thank Mr. B. Elsmore for assistance with the observations. One of us (G. H. M.) is indebted to the Science Research Council for a Maintenance Award.

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SONIC BANG SIMULATION BY A NEW EXPLOSIVES TECHNIQUE

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THE study of the effects of the so-called "sonic bangs"—that is the disturbance produced by the passage of a supersonic aircraft—requires an experimental approach. The response of most real systems, both animate and inanimate, to this type of stimulus is so complex that useful theoretical work is limited respectively to statistical studies or to simplified model treatments. On the grand scale, experiments can be conducted using supersonic aircraft, as at Oklahoma City[1], but these are expensive and for small-scale experiments a simulation technique has the advantages of cheapness, localization of effects and the potential ability to produce bangs characteristic of future aircraft types, for example *Concord*.

A typical sonic bang waveform at ground level (Fig. 1) is an *N*-wave (ref. 2) with front and rear shocks of equal amplitude (about 1 mbar), lasting up to 300 msec for supersonic transport aircraft. Waveforms vary somewhat according to atmospheric conditions, the shock rise-times being normally between 0·1 msec and 20 msec.

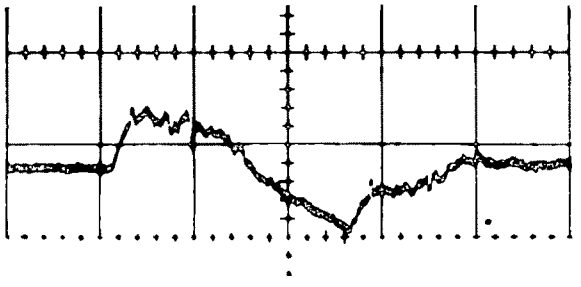


Fig. 1. Typical sonic bang waveform at ground level. Ordinate, 0·5 mbar per large division; Abscissa, 50 msec per large division.

To simulate waveforms of these durations using conventional "point source" explosive techniques is impracticable; charges of many tons weight would be required situated many miles distant and no control of waveshape would be possible.

We were led to investigate the new line charge technique reported here by the realization that an extended explosive charge must give rise to a pressure waveform the duration of which—whatever its shape—is approximately its line-of-sight extension divided by the average air shock velocity across that extension. For charges of small weight such as ours the air shock rapidly decays to sonic velocity (1·1 ft./msec), and because of this a distant observer experiences a pressure wave the duration of which in milliseconds is roughly equal to the extension, in feet—the shape is governed by the distribution of explosive along the extension.

Theoretically, we consider the extended charge as a spatial array of infinitesimal point charges detonated simultaneously, the combined effects of which we approximate by the assumption of linear superposition. Thus if

$$p = p(m,r,t-\theta) \qquad (1)$$

is the pressure waveform from a point source explosion, m,r,t and θ being respectively the explosive mass, observation distance, time and propagation delay (that is, the time for the wave front to reach the observer), then our assumption yields a formal description of the pressure waveform from a charge distribution of density σ by integration of p over an appropriate range of a certain variable x. This variable must be such that σ, r and θ may be expressed as unique functions of it and must also be such that the pressure increment $dp(t)$ can be expressed in terms of the first power of the increment dx in x. It can be shown that the variable r is itself a suitable x.

We have used an empirical form of equation (1) due to Friedlander[3], together with simplified explosive scaling laws to obtain this integral in the form

$$p(t) = \int \frac{a\sigma^n}{r} \left\{1 - \frac{t-\theta}{b\sigma^n}\right\} \exp\left\{-\frac{t-\theta}{b\sigma^n}\right\} dr \qquad (2)$$

A solution for the simple case of a uniform straight linear charge observed from a distant point on the axis produced is shown in Fig. 2 and is in qualitative agreement

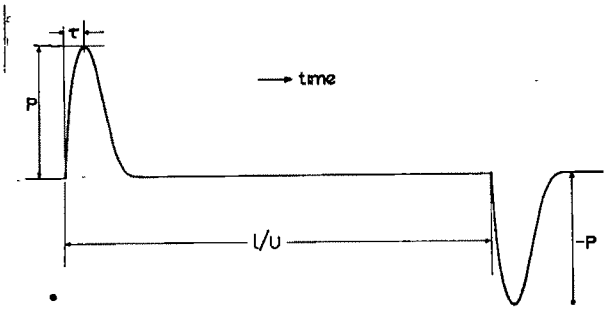


Fig. 2. Calculated pressure waveform from straight linear charge of uniform explosive linear density.

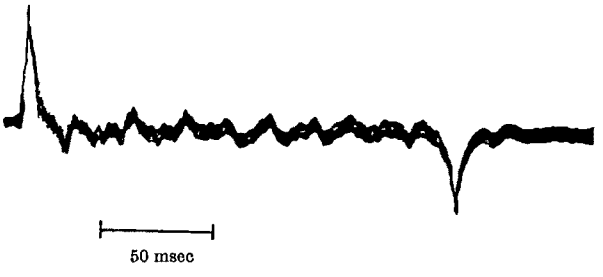


Fig. 3. Experimental pressure waveform from straight linear charge of uniform explosive linear density, length 60 m.

with the waveform observed experimentally (Fig. 3). This example is of practical interest because the only readily available distributed explosive is in this form (detonating fuse).

Many complex waveforms can be synthesized by detonating linear charges constructed from multiple strands of detonating fuse of different lengths. Fig. 4 shows schematically how sonic bang simulants (Mark I) have been developed in this way. The simulant of the sonic bang from a *Lightning* aircraft, with a duration of about 100 msec, has an explosive weight of only 1·5 kg. Fig. 5 is the experimentally determined waveform from another *N*-wave simulant, having a duration of 200 msec and a charge weight of 4·4 kg.

The waveform is dependent on the angle of observation, tending to the shape characteristic of a point source explosion at the 90° orientation, an acceptable *N*-wave being obtained within a cone of 8° semi-angle about the charge axis. Overpressures in the range 1·5 mbar–0·5 mbar are obtained at distances from the near end of the charge between 100 m and 200 m. This type of simulant sometimes shows detailed differences from actual sonic bang waveforms. A need therefore arises for some criterion with which to assess the importance of such differences in specific applications of the simulant. The criterion which we have used is the correlation between the energy spectra of the simulant and sonic bang waveforms over the frequency range in which the given "target system" responds—that is, absorbs energy. Building structures for example respond only to frequencies below about

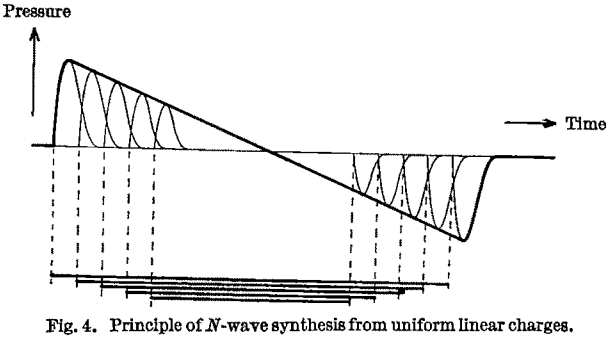


Fig. 4. Principle of *N*-wave synthesis from uniform linear charges.

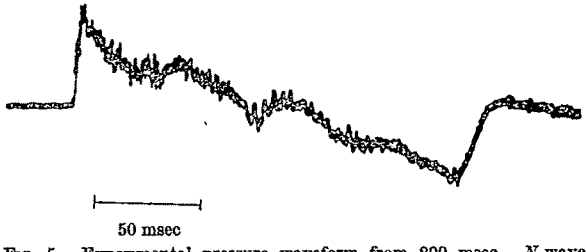


Fig. 5. Experimental pressure waveform from 200 msec. *N*-wave simulant (Mark I).

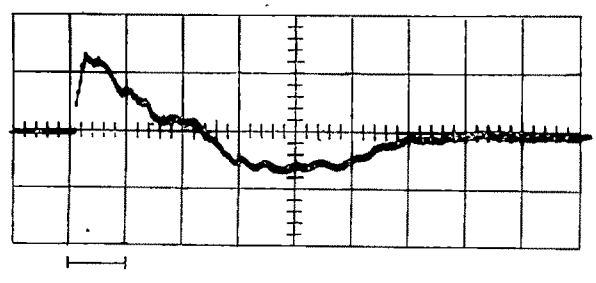


Fig. 6. Experimental pressure waveform from one half of Mark II sonic bang simulant.

50 c/s, whereas in audiometric studies the frequency range of interest lies almost wholly above this.

Energy spectra of the pressure waveforms of actual sonic bangs and explosive simulants have been obtained by digitizing the waveforms and performing the necessary Fourier analysis and summation into 1/3rd octave bands on an Elliott 503 computer. These show the Mark I simulants to be very suitable for structural studies, but in the higher frequency range divergencies appear between the spectra owing partly to the higher inherent noise level of the simulant and partly to the different pressure rise rates in its shock fronts. The extent to which these affect the suitability of Mark I for loudness studies, for example, is difficult to measure, but attempts are being made to do this using two different loudness calculating procedures.

The recent work of Zepler and Harel[4] on sonic bangs and other impulsive sounds has shown that the two most significant parameters in studies of loudness are the rate with which pressure rises and the peak overpressure. These observations together with the uncertainties already discussed concerning the Mark I simulants have led us to develop a second type of simulant which is complementary to the first type in that it simulates the energy of sonic bangs in the higher frequency range and is therefore very suitable for ear response studies. This type of simulant is composed of two identical linear charges arranged side by side and detonated consecutively with an appropriate time delay. It is designed to reproduce in amplitude and rise rate the bow and stern shocks of the sonic bang waveform—the acoustically insignificant slow linear fall in the middle of the wave is replaced in the simulant by an equally acoustically insignificant slow asymptotic return to ambient pressure; this is not possible with point source explosive charges. Fig. 6 shows the experimentally recorded waveform from one half of the Mark II simulant.

We thank Mr. M. J. Harper of this establishment for calculations of energy spectra and of loudness values.

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COMPOSITION OF CRYSTALLINE SHIELD ROCKS AND FRACTIONATING EFFECTS OF REGIONAL METAMORPHISM

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THERE is particular interest at present in the thermal history and chemical composition of the crust. The metamorphic facies of crustal rocks may be used to estimate temperatures that existed in parts of the crust during the early epochs and the composition of rocks of various metamorphic facies may be used to calculate the redistribution of crustal heat and heat sources that resulted from regional metamorphic events. During regional metamorphism, heat is transported by the movement of more volatile components and by the differential movement of heat-producing elements such as potassium, uranium and thorium, as well as by conduction. The data presented here represent analyses of bedrock samples from a semi-systematic array of col-

lection points in New Quebec, Canada. The analytical data for the major contributors of radiogenic heat are incomplete because thorium and uranium values are not yet available.

The rocks of New Quebec, Canada, consist in large part of weakly to strongly foliated Archaean granodioritic gneisses that are probably of metasedimentary origin. Early geological work in a part of this area[1] suggested that only rocks of amphibolite facies were present, but recent reconnaissance mapping of 200,000 square miles of the area[2–4] has revealed the widespread occurrence of rocks of the granulite facies. A large number of hand specimens was collected in the course of systematic traversing by helicopter, and chemical analyses of groups of these specimens have made possible a comparison of the composition of rocks of the granulite facies with that of adjacent rocks of the amphibolite facies. These analyses provide a unique opportunity to determine directly the composition of a large area of the surface rocks of the Canadian Shield which presumably was at one time deeply buried in the crust. Although this approach to the determination of the abundance of the constituent elements of the crust, that is, through the analysis of a large number of hand specimens, is admittedly unimaginative, it has certain real advantages over indirect techniques[5,6] that depend on a number of assumptions. This approach was made possible by the emphasis placed in recent years by the Geological Survey of Canada on the expansion of facilities for the rapid analysis of rocks.

The rocks of the granulites facies (shown in black in Fig. 1) are, at the one extreme, very coarse grained, rusty and white weathering and exhibit a faint irregular gneissosity. This rock type grades, as a result of an increase in finer grained, biotite-rich, metasedimentary material, into a strongly banded rusty and grey weathering paragneiss. Biotite is ubiquitous in all the rocks of this facies and is pleochroic from pale yellow to deep red. Plagioclase is the dominant feldspar, locally has an antiperthitic texture and its fresh surfaces are pale green or brownish green with a greasy lustre. Quartz is blue or dark grey. Hypersthene, hornblende and locally garnet and augite are common constituents.

These rocks are broadly similar in origin and composition to the common lower grade granitoid gneisses of the Canadian Shield. The banding of the gneiss and the inclusions of banded biotite-rich material suggest a derivation from predominantly sedimentary strata. The absence of notable quantities of crystalline limestone, lime silicate or highly quartzose layers suggests a greywacke-type of primary sediment. Mineralogical peculiarities, notably the presence of hypersthene, calcicplagioclase and garnet, and textural features such as large grain size, are thought to result from metamorphism under condition of high pressure and temperature. The presence of biotite and, less commonly, of dark hornblende in the association suggests the hornblende granulite subfacies. These gneisses of New Quebec may constitute one of the world's largest areas of high grade metamorphic rocks.

The rocks of the amphibolite facies (Fig. 1) are grossly similar in appearance to their neighbours of higher metamorphic grade and the structures of one facies are continuous into rocks of the other facies. The granitoid varieties of the amphibolite facies are chiefly pink or buff on a fresh surface and rocks of this facies abound in minerals such as epidote, microcline and muscovite that are considered characteristic of lower temperature-pressure conditions. Locally, the preservation of structures in rocks of the amphibolite facies allows the

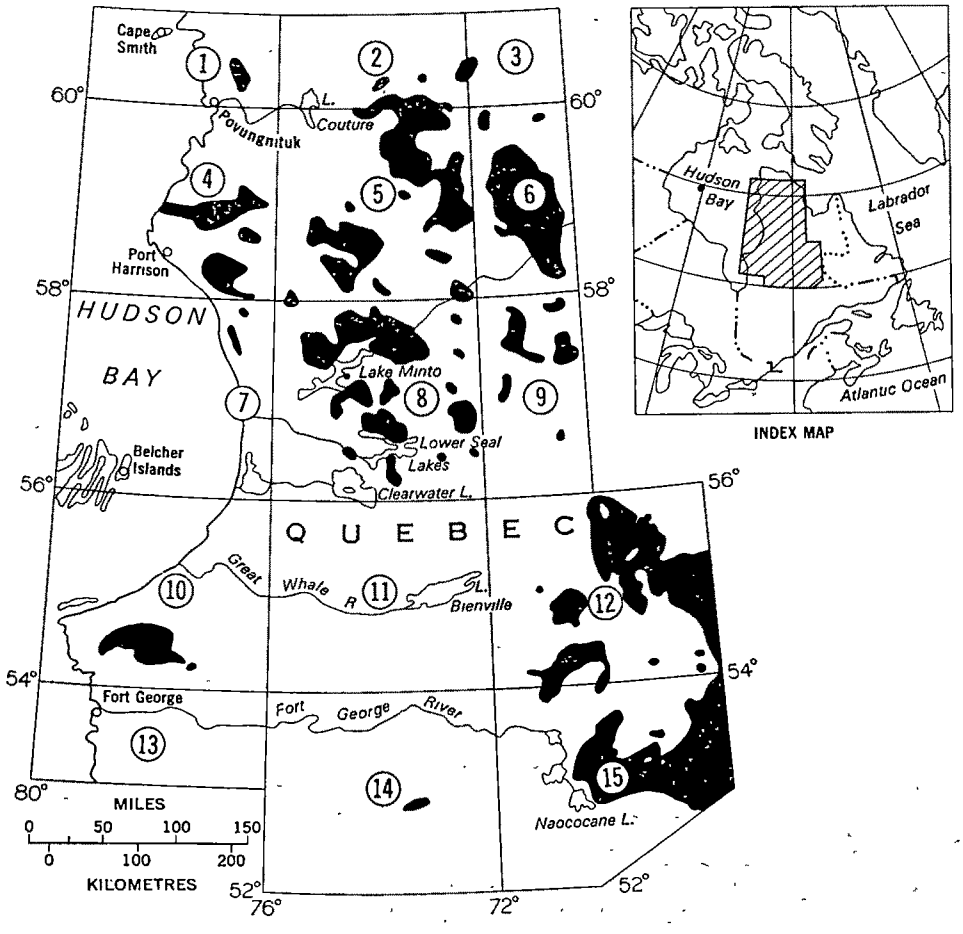


Fig 1.

identification of metasedimentary and metavolcanic rock types. For more detailed descriptions of rock varieties from both the hornblende granulite and amphibolite facies of New Quebec see Eade[2] and Stevenson[3,4].

Map-sheets constituting one quarter of primary quadrangles of the National Topographic System comprise the unit areas in this investigation. In Fig. 1 these map-sheets, or portions of them, are numbered 1 to 15 for ease of reference. The distribution of samples within one area is shown in Fig. 2 and is typical of the other areas. To prepare a sample for analysis the hand specimens of each rock unit, as determined by the geological mapping within each area, were grouped together. Approximately 20 g of each hand specimen were combined to form a composite sample for each rock unit in each area. After crushing, grinding and thorough mixing, a portion of each such area rock unit was analysed by a combination of X-ray fluorescence and chemical methods. The fifteen unit areas provided 78 rock unit samples.

From these 78 analyses the average composition of rocks of the amphibolite and granulite facies of each area studied was obtained by measuring the areas underlain by rock units of the two facies and using these measured areas to obtain a weighted average (Table 1). The average composition of all amphibolite facies rocks and of all granulite facies rocks, together with the average composition for the entire region, were obtained by weighting the data in proportion to the area occupied by each rock type (Table 2). Cratonic cover rocks were not included.

As a check on the validity of such calculated averages, a special composite sample representing areas 6, 9, 10, 11, 12, 13 and 14 was prepared by mixing portions of the area rock unit samples in proportion to the aerial extent of the rock units as measured on the geological maps. This special sample was analysed by classical chemical methods and its composition may be compared with that obtained by calculation from the rapid analyses of individual rock unit samples from the same area (Table 2).

The calculated comparative chemical analyses are shown in Table 1. There are no major differences between the chemical composition of the two facies probably because there were no primary differences in their protorocks. The values for Al_2O_3, total Fe (as FeO), P_2O_5 and, possibly, MgO, CaO and TiO_2, appear to be higher in the

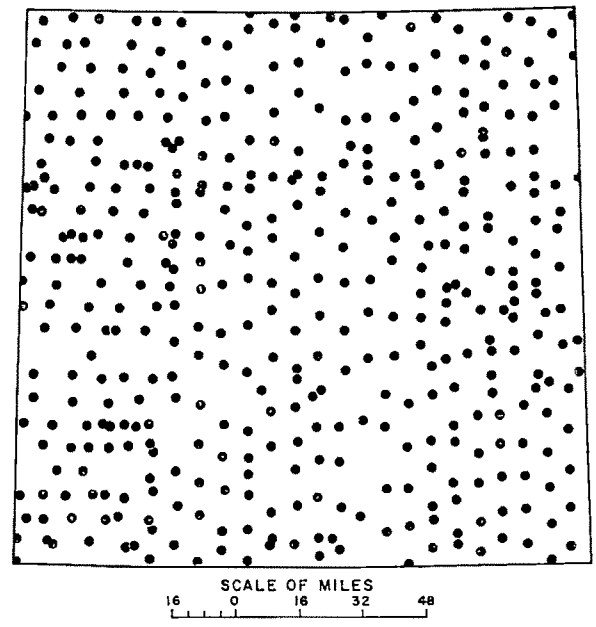


Fig. 2.

granulite facies, whereas K_2O, H_2O and, possibly, SiO_2 appear to be lower. The ferrous–ferric ratio also appears to be significantly higher in the granulite facies. These results seem to corroborate the views of Ramberg[7] and Heier and Adams[8] that high-grade gneisses tend to be more basic than low-grade gneisses. This basification of granulites results from the loss by upward movement of a mixture of more volatile constituents (K_2O, H_2O, SiO_2) in response to regional metamorphism. The loss of potassium from rocks of the granulite facies, and so presumably from deeper crustal levels during regional metamorphism, is particularly interesting as potassium is one of the chief sources of radiogenic heat. Table 1 thus gives some indication of the scale of this migration in one large area of Shield rocks.

The abundance of TiO_2 in the amphibolite facies and the corresponding granulite rocks of each of the sample areas is shown in Fig. 3 in which the values of TiO_2 in the

Table 1. SAMPLE AREAS SHOWN ON FIG. 1

		1	2	3*	4	5	6	7	8	9	10	11*	12	13*	14	15
SiO_2	*a*	64·1	64·2	63 6	62 1	65 3	65·5	63·3	66 4	67 2	63 8	63 2	65·1	64·3	65·0	66 3
	g	57·3	64·2		63 3	62 5	64·3	61·7	62·4	61·7	62·3		65·3		65 0	63·5
Al_2O_3	*a*	16·7	16·8	16 1	16·5	16·9	14 3	16 0	16·9	14·6	16·0	15·6	15·8	16 8	15·5	16·1
	g	17 9	17·9		17·4	17 7	14·4	17·1	17·4	15·5	13 9		17 4		16·2	16·5
Fe_2O_3	*a*	1·3	1 3	1·3	1·7	1·9	1·5	1·5	1·3	1·5	1·8	2 0	1·2	1·5	1·1	0·7
	g	1·4	0 6		1·8	2·4	1·4	1·0	1·2	2·5	0 2		1 4		0·7	1·1
FeO	*a*	3 0	3·2	3 0	3 7	2·9	3·0	2·7	2·7	2·4	3·1	2 8	2·9	2·7	2 8	2·7
	g	5·0	3·0		3·6	3·6	3·6	4·4	3·2	3·2	5 0		2·8		4·1	3·5
MgO	*a*	2·4	2 3	2·3	3·2	1·7	2·3	2·0	1·5	1·3	2·7	3 1	2 7	2·0	2·5	2·0
	g	3·5	1·0		2·5	2·5	2·0	3·7	2 3	1·7	5·5		1·3		4·0	2 2
CaO	*a*	3 4	3·9	4·3	4·7	3·4	3·7	4·3	2·9	2·6	3·0	3·3	3 0	3·7	3 0	2 8
	g	5 9	3·6		4·2	4·3	4·3	4·6	4·4	3·9	4·3		2 5		3·2	2·8
Na_2O	*a*	4·2	4·2	4·7	4·0	4 4	4·1	4·1	4 3	4·3	3·2	3 8	3 8	4·1	4 3	3 8
	g	4·2	4·4		3·8	4·5	4 2	4·7	4·3	4·4	3·2		3·8		4 2	3·8
K_2O	*a*	2·74	2·27	1·87	2 38	2·72	2·41	2 95	3·06	3·10	3·11	3·10	2 98	2·46	2·62	3·09
	g	2 33	2·14		2·34	1·99	1·83	1·34	2·48	2 23	2·32		3·48		1 46	3·01
H_2O (total)	*a*	0·9	0·7	0 8	0·8	0·7	0·7	0·8	0·8	0 8	1·0	0 9	0 8	0 9	1 0	1·1
	g	0·8	0·6		0·7	0·6	0 5	0·6	0·7	0 6	1·1		0·7		0·8	0·7
TiO_2	*a*	0 50	0 54	0·47	0·53	0·51	0·49	0 50	0·48	0·41	0·47	0·51	0·45	0 45	0·41	0·44
	g	0·90	0 51		0 52	0 68	0·57	0 48	0 51	0·69	0·46		0·38		0·44	0·54
P_2O_5	*a*	0·13	0·13	0 09	0 10	0·12	0·13	0·19	0·15	0·16	0·17	0·21	0 18	0·18	0·16	0·17
	g	0·29	0 21		0 08	0·19	0 20	0 13	0 17	0·30	0·15		0 21		0·27	0 18
MnO	*a*	0 06	0·08	0 08	0·11	0 08	0·07	0 10	0 07	0·06	0·07	0·08	0 08	0·08	0·08	0·08
	g	0·11	0 08		0 09	0 09	0·09	0·11	0 08	0·06	0·10		0 03		0·12	0 08
CO_2	*a*	0·2	0·04	0·3	0 4	0·1	0·03	0·9	0 1	0·1	0 1	0·2	0 06	0·12	0·31	0·12
	g	0·2	0 2		0 1	0·1	0·2	0·2	0·6	0·1	0·01		0 01		0·10	0 10
Total Fe	*a*	4·2	4·4	4·2	5·5	4 6	4·3	4·0	3·9	3·7	4·7	4·6	4 0	4·0	3·8	3·3
as FeO	*g*	6·3	3·5		5 2	5·8	4·9	5 3	4·3	5·4	5·2		4 1		4 7	4 5
FeO/	*a*	2·3	2·5	2·3	2·2	1 5	2 0	1·8	2 1	1·6	1·7	1·4	2·4	1·8	2·5	3·9
Fe_2O_3	*g*	3·6	5·0		2·0	1 5	2·6	4·4	2·7	1·3	25·0		2 0		5·9	3 2

a, Rocks of amphibolite facies. *g*, Rocks of granulite facies. * No rocks of granulite facies were mapped in those areas.

These calculated values are based on 78 rapid rock analyses provided by the Analytical Chemistry Section of the Geological Survey of Canada. Silicon, aluminium, total iron, calcium, magnesium, potassium, titanium and manganese were determined by X-ray fluorescence using an Applied Research Laboratories X-ray spectrograph (Model *VXQ*–25,000) and a fusion method based on that of Rose *et al* (ref. 12). Sodium was determined by flame photometry, iron-21 titrimetrically, phosphorus colorimetrically and total water and carbon dioxide gravimetrically. The accuracy of the 78 analyses from which the above averages were calculated may be stated as follows, in terms of the possible maximum percentage variation in the reported value: SiO_2 (±1 2); Al_2O_3 (±0·7); Total Fe, as Fe_2O_3 (±0·5); CaO (±0 3); MgO (±1·0); K_2O (±0·1), TiO_2 (±0·05); MnO (±0 02); FeO (±0 2); Na_2O (±0 15); CO_2 (±0·1); Total H_2O (±0·1).

amphibolite facies are plotted in a sequence such that the TiO_2 increases to the right. Titanium is generally higher in the granulite rocks and has a much greater range than in the amphibolite facies. As previously noted, biotite is ubiquitous in New Quebec rocks of the granulite facies and the stability of high-temperature biotite may be the result of a concentration of titanium[9]. Titanium is so much higher in granulite rocks of some of the areas, for example, 1, 5, 9, relative to adjacent rocks of the amphibolite facies that addition of titanium to the granulites is indicated. The source of the titanium may be a deeper, higher-grade granulite in which biotite was unstable and from which titanium was expelled.

Inspection of the analyses indicates little or no regional compositional variation and hence no great variation in the primary rock types throughout the area.

The accompanying data contribute in some measure towards the solving of the problem of the bulk chemical composition of the continental crust. Specifically, of course, the data relate to the surface crystalline rocks of the continental shield. Analysis 1 of Table 2 gives the average composition determined from the sampling of some 200,000 square miles of northern Quebec, Canada. We believe that, as a result of the sampling procedure used, this average closely represents the surface composition of this part of the crystalline shield. The composition of this large cratonic area may be of particular interest because the general absence of "high level" types of igneous intrusions and the widespread occurrence of gneisses of the granulite facies suggest that it represents an area of deep continental crust.

There are small but significant differences between our average composition and the estimate of Poldervaart[6] (No. 8, Table 2) for the average composition of continental shield crystalline surface rocks. The surface crystalline rocks of New Quebec are slightly higher in aluminium and sodium and slightly lower in silicon, potassium, titanium, calcium and phosphorus than the average composition given by Poldervaart[6]. The differences are attributed in part to the migration of silicon and potassium from the present exposed level of New Quebec during high-grade regional metamorphism. Poldervaart's average may represent a composition in which high level granitic intrusions play a more dominant part.

The average composition given by Reilly[10] (No. 7, Table 2) using samples from a Geological Survey of Canada project[11] for an area of 40,000 square miles in north-western Ontario is directly comparable with our data as it is based on a rather similar sampling scheme. The value for aluminium is significantly lower, and that for silicon significantly higher, in Reilly's areas than in New Quebec. The Reilly sample area represents a higher crustal level because both "high level" granitic intrusions and greenstone belts of low metamorphic grade are present in significant amounts.

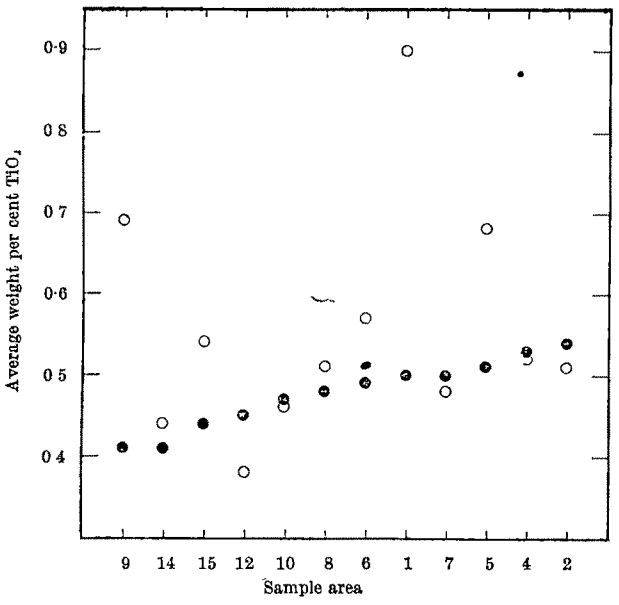


Fig 3. Average abundance of TiO_2 in rocks of amphibolite (●) and granulite (○) facies.

Table 2

	1	2	3	4	5	6	7	8
SiO_2	64·5	64·7	63·4	65·26	64 6	60 3	66 3	66·4
Al_2O_3	16 1	16 0	16·7	15 67	15 6	15 6	15 4	15·5
Fe_2O_3	1·5	1·5	1·5	1·44	1·5	—	1·3	1·8
FeO	2·9	2 9	3 4	2 82	2·9	7·2*	2·9	2 8
MgO	2 3	2·3	2 2	2·55	2·5	3·9	2·1	2 0
CaO	3 3	3·3	3·6	3·51	3·2	5·8	4·0	3·8
Na_2O	4 0	4·0	4·0	3 83	3 9	3·2	3·9	3 5
K_2O	2·78	2·81	2 58	2·93	2·85	2 5	2·3	3 3
H_2O (total)	0·8	0·9	0 7	0·82	0 9	—	0·87	—
TiO_2	0 48	0·47	0 53	0 46	0 45	1·0	0 47	0·6
P_2O_5	0·16	0·16	0·19	0 02	0·2	—	0·13	0·2
MnO	0 08	0 08	0 07	0 07	0 07	—	0 07	0·1
CO_2	0 2	0·2	0 2	0·16	0·2	—	0·15	—
Total	99·10	99·32	99·07	99 54	98 87	99 5	99 89	100·0

1. Average composition of the entire New Quebec sample area (Fig. 1) calculated from values given in Table 1.

2. Average composition of all amphibolite facies rocks of the sample area (Fig. 1), calculated from values in Table 1.

3 Average composition of all granulite facies rocks of the sample area (Fig. 1), calculated from values in Table 1.

4. Average composition of all rocks in areas 6, 9, 10, 11, 12, 13, and 14 (sample preparation described in the text) determined by classical chemical analysis, J. A. Maxwell, analyst, Analytical Chemistry Section, Geological Survey of Canada.

5. Average composition of all rocks in areas 6, 9, 10, 11, 12, 13 and 14 calculated from values in Table 1.

6. Average composition of the continental crust, one part basic and one part felsic rock (ref. 5).

7. Average composition of an area of 40,000 square miles of Canadian Shield of Northwestern Ontario (ref. 10).

8. Average composition of shield crystalline surface rocks (ref. 6).

* Total Fe as FeO.

Taylor[5] has used an interesting method for calculating the composition of the continental crust. The distribution of rare earth elements in sedimentary rocks was studied and related to a mixture of the two most common igneous rock types, namely, granite and basalt. His data closely fit a 1 : 1 mixture of basalt and granite (No. 6, Table 2). A possible disadvantage to the calculation is that the results are based on sediments and hence on rare earth element abundances in that part of the crust that has been exposed to weathering. If the continental crust is significantly zoned in its upper part (say, 10 km) the 1 : 1 mixture may not represent continental crustal abundances. Possibly crustal averages from crystalline shield regions of high grade regional metamorphism where "high level" intrusions are lacking are more characteristic of a greater thickness of continental crust.

The data presented here have been considered on two levels, that of the relationship between chemical composition and metamorphic grade, and the average regional composition. It is concluded that there are minor but significant differences in composition between rocks of the amphibolite and granulite metamorphic facies as a result of the removal of some constituents from the granulite zone. The potassium content of rocks of the hornblende granulite facies is lower than in adjacent rocks of the amphibolite facies, suggesting that significant fractionation of this heat-producing element occurs during deep-seated regional metamorphism. There are differences between the average composition of this area of continental shield crystalline surface rocks and previously published average compositions for surface shield rocks. Because the New Quebec area probably represents a deeper crustal level, these differences reflect a chemical zoning of the crust resulting from the transport of low temperature constituents to higher crustal levels. The average composition of New Quebec rocks may be close to the average composition of the

continental crust, or at least that part that lies above the Conrad seismic discontinuity.

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THE SOLOMON ISLANDS AS AN ISLAND ARC

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OF the various categories of island arcs which have been proposed[1], the "fractured arcs" are the least known and have received the least attention. So far as routine geological literature is concerned this category of the little known includes the Solomon Islands, a straight-line chain, but with arcuate features, which makes up some 800 miles of the border of the south-west Pacific Ocean.

The Solomon Islands are comparatively new to geology for it was not until 1950 that systematic investigations began. The islands have now been covered by general or semi-detailed reconnaissance surveys. A gravity survey of the land areas has been completed by the Geophysical and Polar Research Centre, University of Wisconsin, and sea gravity and aeromagnetic surveys are planned for the near future*. This article is based primarily on stratigraphical investigations, the results of which, however, are supported by the geophysical information so far available. It aims to give a summary of the island arc features which distinguish this island chain. It would be premature, however, to use these as a basis for interesting speculations on various aspects of the grand tectonics of the region; for this, more detailed information is required, especially on bathymetry and on the gravity picture.

Disposition and Bathymetry

The Solomon Islands lie east of New Guinea (Fig. 1). As a geological entity the Solomons probably include the Lihir group of small volcanic islands, but not the Santa Cruz group to the east which geologically is part of the New Hebrides archipelago.

The axis of the Solomon bathymetric high appears distinctly sigmoidal. Over the middle part the islands form a double chain around a rhomb-shaped basin up to 2,500 m deep. The bathymetry is confused about Malaita and San Cristoval, where there are rhomboidal, linear and small "pocket" depressions. On the Pacific side the islands Bougainville-Choiseul-Santa Ysabel-Malaita are progressively offset so that they lie *en echelon* to each other. This arrangement persists in the bathymetric lines, but is not followed by the islands on the opposite flank.

On the Australian side the Solomon high is bordered by the Solomon Trough which includes part of the Planet Deep (over 9,000 m), unnamed deeps of over 5,000 m, and the San Cristoval Trench (about 7,000 m). Somewhat sinuous in plan, it is roughly arcuate and convex towards Australia. The Solomon high is separated from the large platform of the Ontong Java Rise by minor linear deeps.

* August, 1966: the aerogeophysical survey is now nearly completed

Stratigraphy and Structure

The account which follows is based on the work of British Solomon Islands Geological Survey personnel (in particular that of J. C. Grover, Chief Geologist; R. B. Thompson, P. Pudsey-Dawson and B. D. Hackman); R. L. Stanton, University of New England; F. K. Rickwood, British Petroleum Company, and that of the writer. Unpublished information on Bougainville was supplied by J. G. Speight, Commonwealth Scientific and Industrial Research Organisation, and D. H. Blake, Bureau of Mineral Resources, Canberra. Detailed references up to 1963 are given in ref. 2.

The Solomon Islands constitute an autochthonous stratigraphical system; that is, all the rocks present have originated within the region. On the bases of rock type and structure the group can be divided into three provinces (Fig. 2): the Volcanic, Central and Pacific Provinces.

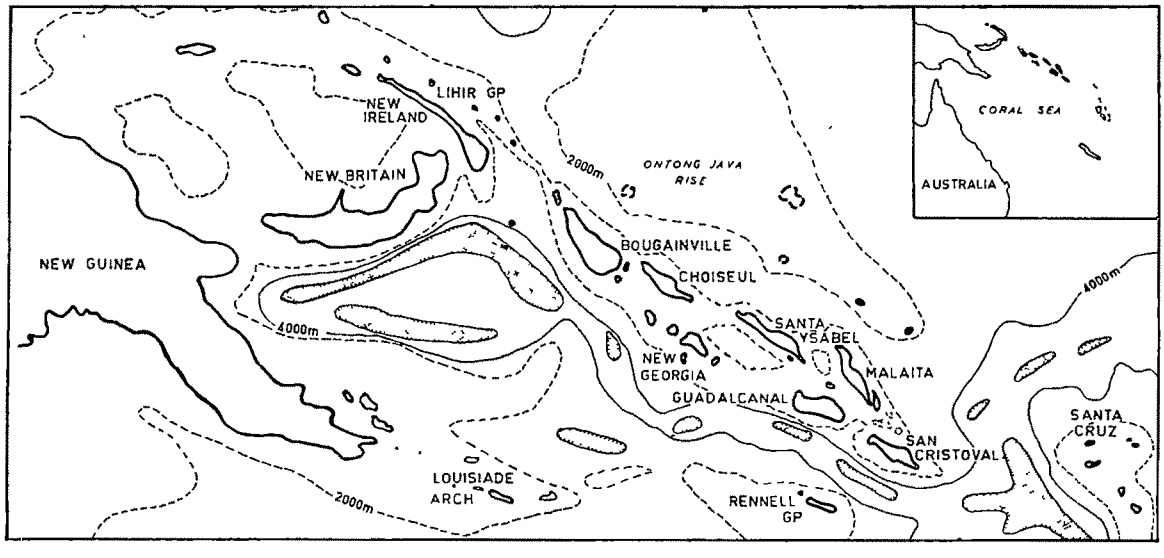


Fig. 1. Generalized locality and bathymetry map of the Solomon Islands region. As a geological entity the Solomons block probably includes the Lihir Group, but not the Santa Cruz Group

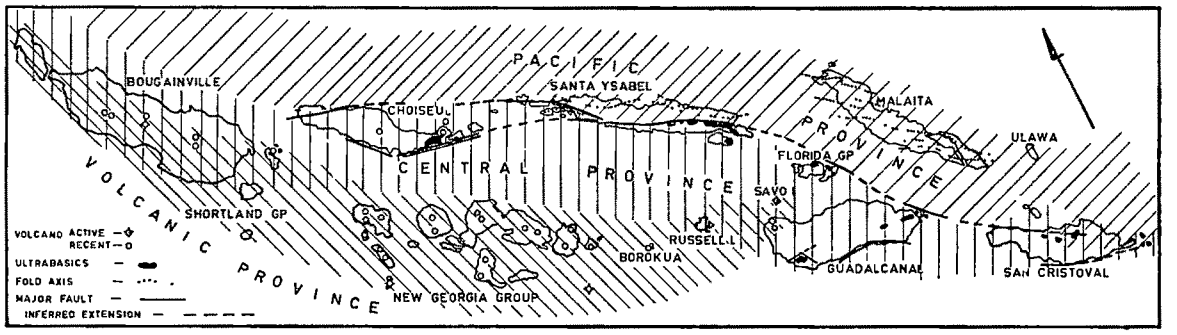


Fig. 2. Geological provinces in the Solomon Islands and the distribution of active and recent volcanoes, ultrabasic (serpentine) rocks and major structural features.

The arcuate Volcanic Province includes more than thirty well preserved recent volcanoes, but only a few are at present active. It is best exemplified by the New Georgia Group which is an assemblage of cones linked by their own extrusives and by fringing reefs. The lavas and pyroclastics are mainly andesitic and probably no older than Pliocene. Faulting is intense and many large faults are active. The three recent volcanoes on Choiseul are in an area of great fractures associated with the boundary between the Central and Pacific Provinces and may not be truly a part of the Volcanic Province. In terms of the continuing development of the Solomons, the Volcanic Province may be regarded as a youthful extension of the Central Province.

The Central Province has a Mesozoic (probably Cretaceous) igneous and metamorphic basal complex which includes chloritic and amphibolitic schists, a few patches of lightly metamorphosed pelagic sediments, and some gabbroic and granitoid rocks. Potassium–argon radiometric age determinations of the Choiseul schists indicate a Lower Eocene age of metamorphism. The results are described in detail in the accompanying paper[3].

The basal complex is intruded and covered by Upper Eocene–Oligocene lavas and occasional agglomerates, predominantly andesitic, although basaltic pillow lavas are also common. The lavas may be up to 3,000 ft. thick. They are overlain by a pile of reef-sediments, re-worked tuffs and material derived from the older lavas, which range in age from early Miocene (approximately equivalent to Aquitanian of the Lower Miocene) through Recent. The sediments accumulated in subsiding fault-bounded troughs. In one such trough on north-east Guadalcanal, there are more than 15,000 ft. of sediment, a large part of it derived from a source area, now many hundreds of feet below sea level, off the south coast. Regional dips are usually of the order of 5–15 degrees. Some Quaternary fringing reef masses have been uplifted more than 1,200 ft.

The Central Province is shaped and shattered by high-angle faults, which in plan form an irregular mesh-work, the result of the intersection of two major sets, one trending 80–100 degrees, the other 145–160 degrees. A less obvious set trends 30–50 degrees. Many faults have strong strike-slip components, but neither the lateral nor the vertical movements have acted to produce a systematic result, except on Guadalcanal which has a spine of step-faulted block mountains, asymmetric in cross-section and falling away steeply to the fault-bounded southern coastline. The south coasts of San Cristoval and east Choiseul also have tensional features.

The boundary between the Central and Pacific Provinces is marked by an apparently sigmoidal "lineament", almost certainly a major crustal fracture system, expressed emphatically by great faults in Choiseul and particularly by those running through the length of Santa Ysabel, and delineated also by a serpentine belt (Fig. 2). The first erosion products from these serpentinites appear in Lower Miocene sediments (approximately Burdigalian).

The Pacific Province contrasts markedly with the Central Province. It is demonstrated best on Malaita where basal basaltic lavas, which show evidence of submarine emplacement, are overlain in large part by about 4,000 ft. of calcareous organogenic sediments which range in age from Upper Cretaceous through Quaternary. Some of the Upper Eocene sediments show contemporaneous intrusion by younger lavas. These include types akin to alnöite and ankaratrite; it is worth noting that similar alkaline lavas occur in the Lihir Group[4]. The older sediments consist essentially of planktonic foraminifera and their clayey detritus, although radiolarian cherts are common and at least one benthonic fauna (of Upper Eocene age) is present. The Upper Miocene and younger sediments show an increasing amount of terrigenous (volcanic) material.

The whole sequence is folded with moderate intensity to give a series of rather irregular folds disposed *en echelon* to the general trend of the island. The older sediments are crumpled in cascade fashion on the limbs of the folds and show other evidence of deformation before consolidation. These folds may well be of the *Bruchfalten* type, brought about as much by movements in the faulted lava basement as by lateral compression.

The marginal areas on Santa Ysabel and San Cristoval have thinner sequences with a much higher terrigenous content than those on Malaita. So far, no basal complex of the Central Province type has been found in the Pacific Province.

Island Arc Features

Although it lacks much significant detail, the outline just given provides a minimal background for the following generalizations.

The Solomon Islands chain is an autochthonous geological system which had its origin in the Mesozoic, probably in Cretaceous time.

The Solomon block is not sharply demarcated from New Ireland nor the Ontong Java Platform, but is clearly separated from the easterly extension of New Guinea (Louisiades–Rennell Rise) by the Solomon Trough, a deep which is subarcuate and convex towards Australia.

The crustal thickness of the Solomon block has been estimated to be about 15 km, a result[5] based on the rather slender seismic evidence then available. The recent gravity work suggests a lower figure. It also suggests that the region is a gravity high with large positive and negative anomalies superimposed, having gradients as steep as any so far recorded (personal communication from T. S. Laudon, University of Wisconsin). Significantly, the positive anomalies on Guadalcanal and San Cristoval increase in magnitude (to over 200 milligals) from north to south, towards the Solomon Trough. Data from gravity, structural and stratigraphic investigations indicate that the Solomon block is in a state of isostatic imbalance, with great vertical displacement of crustal and subcrustal material, and that it is made up of elongated

rhomboidal blocks defined by fractures with preferred orientations of 80–100 degrees and 145–160 degrees (usually the long dimension). The common *en echelon* arrangement of these blocks, due to differential movement between them (as reflected by Bougainville–Choiseul–Santa Ysabel), gives rise to asymmetric flattened sawtooth lineaments, thus ⩘⩘. If the angles of such lineaments be rounded off (subjectively, by the observer) then sigmoidal lines result and this is the probable explanation of the many sigmoidal trends observed in the region[6], both large and small scale; for example, the axis of the Solomon block, the border between the Pacific and Central Provinces and the north and south coasts of Guadalcanal.

A belt of high seismicity lies between the Solomon Trough and the axis of the Solomon block, with shallow shocks greatly predominating, most of them probably tectonic. Intermediate and deep focus earthquakes are comparatively rare and tend to be concentrated at the Bougainville and San Cristoval ends. In profile, the seismic zones appear to be either vertical or steeply inclined to the Pacific, but they are neither clearly defined nor continuous, with a near-gap between 170 and 300 km depths, and finishing at 500 km.

The oldest unaltered lavas (Lower Cretaceous to Eocene) are basic, probably submarine in large part and are found on the Pacific side. The youngest (Quaternary) are on the Australian side, are predominantly andesitic and have been produced by a volcanic belt which is subarcuate and convex to Australia. The lavas of the central region are mainly of Upper Eocene–Oligocene age, and are generally andesitic.

Folded terrains are found only on the Pacific side. Folding probably began in Upper Eocene time and reached its greatest intensity in the late Miocene.

The folded Pacific Province alone has pelagic sediments in quantity, but pre-Pleistocene reefal sediments are not found.

The central region is shattered by a trellis pattern of high-angle faults which have dominated the structure and sedimentation since at least Upper Oligocene time. The faulted Central Province alone has large areas of regional metamorphic rocks and great thicknesses of terrigenous sediment. The last and possibly the only major episode of metamorphism occurred in the Lower Eocene.

The present-day proximity of the Central and Pacific Provinces, with their contrasting stratigraphies, poses a problem centred on the presence of widespread Eocene metamorphics in the Central Province and unmetamorphosed contemporaneous lavas and pelagic sediments in the Pacific Province. This problem can be resolved by postulating a past shift of the Pacific Province relative to the Central Province; that is, strike-slip movement along a fault or system of faults during the Upper Eocene–Oligocene, a time fixed by stratigraphic evidence. An obvious target here is the fracture system/serpentine belt separating the two provinces and manifestly a belt of some tectonic significance. Alternatively, the total movement involved could have resulted from the cumulative effect of small incremental shifts by a number of blocks making up the region. Neither idea is supported unambiguously by investigation of the lesser faults, many of which have strike-slip components. Regionally, the progressive off-setting of the islands on the northern flank, the trends of the largest faults and lineaments and tensional features such as the central rhomboidal bathymetric depression suggest past movement of the Solomon blocks to the north-west in the relative sense. It is conceivable that the Pacific Province either did not share this movement, or, more likely, moved at a slower rate and was, as it were, overtaken by the Central Province. The overall structure of the Solomons is consistent with Carey's Tethyan Torsion Zone[7] and the offsetting which resulted in the Melanesian Re-entrant (Solomons–Fiji–Tonga–Kermadec).

The build-up of the Volcanic Province has been accompanied by tension in, and collapse of, parts of the Central Province.

No true granite plutons have been found.

The Solomon chain can be compared most closely with the New Hebrides group (a conclusion based largely on unpublished information). Its central Province has some features in common with the Fiji group, but no relationship is suggested: Fiji remains a tectonic enigma. The Solomon chain is quite distinct from the other larger Pacific arcs, or parts thereof, including the herniated arcs of the Caribbean and Nova Scotia.

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POTASSIUM–ARGON MEASUREMENTS OF THE AGE OF BASAL SCHISTS IN THE BRITISH SOLOMON ISLANDS

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This article reports the results of the first radiometric age determinations on basal schists from Choiseul Island in the Solomon Islands group, located east of New Guinea in the south-west Pacific Ocean. In the preceding article, Coleman summarizes the present understanding of the geology of this group. Conventional biostratigraphic methods have unravelled the basic history of the Pacific Province in these islands as far back as the Upper Cretaceous, but only to the early Miocene in the adjacent Central Province. Here, the fossiliferous Miocene sediments overlie a pile of andesitic lavas and older metamorphosed volcanics of the basal complex[1]. The time of this metamorphism, an important parameter of the tectonic history of the region, seemed to offer the best target for an exploratory venture in potassium–argon dating.

Amphibolites are widely distributed on Choiseul Island and have similar petrological characteristics. They were probably derived from rather basic lavas

(basic andesites, basalts) and their equivalent pyroclastics; a few samples show what appears to be relic bedding. From the twenty samples available from the collection of the Geology Department, University of Western Australia, three were selected. The criteria for selection were: freedom from weathering effects (this factor alone, in such a tropical terrain, barred most samples); a location removed from faults (again, an almost prohibitive condition); the sample should be "typical"; the samples should not all be from the same locality, to lessen the possibility of unsuspected contact-metamorphic effects brought about by a hidden igneous intrusion.

Sample *GA* 698 (53878) met these requirements rather better than the others, which were both about 50 ft. from faults, judged to be small ones.

The first number refers to the Australian National University geochronology collection and applies to a split of the main sample (number in parentheses) in the collection of the Department of Geology, University of Western Australia. *GA* 698 (53878): from the west coast of the northern end of a small un-named island a few hundred yards to the east of the mainland, north of Siutu Island, Eastern Choiseul. Co-ordinates on the 1,000 metre southern New Guinea Zone, British Metric Grid, Bessel Spheroid, are 3834697/1063559, eastings first.

The sample was taken at the northern end of a beach outcrop, from an unweathered ledge which was broken up to give the freshest possible sample.

GA 699 (53879): same locality as 53878, but taken from a spot about 80 ft. south.

GA 700 (53880): collected from outcrop filling small stream bed, 50 ft. from east bank of the Kolumbangara River, on upstream limb of large open hairpin bend about 6 miles from the river mouth, western Choiseul. Co-ordinates are 3750369/1103545.

The samples are similar in hand specimen and in thin section. They are dark green, fine grained, finely foliated amphibolite schists. The foliation is due to the partial segregation of the hornblende and feldspar, which are in roughly equal proportions, and together make up 95 per cent of the whole. The hornblende is a bright green pleochroic variety common to such rocks, and occurs in streams of aggregated shreds and elongated crystals; these may contain grains of apatite, clinozoisite, epidote and sphene, and wholly or partially envelop feldspar crystals. The feldspar, a plagioclase close to andesine in composition, shows crystals which are more tabular and less elongated than those of the hornblende. Epidote occurs as veinlets of tiny anhedral grains and as granular aggregates. The grains of iron ore (commonly magnetite) are dragged out into strings. The quartz is probably secondary, occurring as veins which are often distorted and faulted.

A block was cut from the centre of each sample, and crushed. Because of the small size of the grains and their original interlocking relationship, composite grains could only be avoided by separations performed on material retained on the 250 Tyler screen but passing through the 115 screen. Ten grams of hornblende and 5–10 grams of plagioclase were obtained for each sample.

Potassium was determined by flame photometry by intercomparison with standard solutions, using a lithium internal standard and a sodium buffer[2]. Argon was determined by isotope dilution, following extraction procedures very similar to those described by Evernden and Curtis[3], with the exception that acid-washing was not used.

The results are summarized in Table 1. The potassium contents of these minerals are low, but the analyses should be good to better than 2 per cent at the 95 per cent confidence level. The low potassium content, comparatively young age, and fine particle size with the consequently high surface area available for adsorption, all lead to a high proportion of air argon in the analysed gas. Thus

Table 1. CHOISEUL RESULTS

Sample No.	Mineral	per cent K	Ar^{40}/K^{40} *	Air Corr. (per cent)	Age (m.y.)
GA 698	Hornblende	0·168	$3·047 \times 10^{-3}$	91·9	51·5
(53878)	Plagioclase	0·114	$2·931 \times 10^{-3}$	90·4	49·5
GA 699	Hornblende	0·219	$2·575 \times 10^{-3}$	76·7	43·6
(53879)	Plagioclase	0·138	$2·397 \times 10^{-3}$	88·1	40·6
GA 700	Hornblende	0·233	$2·621 \times 10^{-3}$	78·2	44·3
(53880)	Plagioclase	0·1068	$1·910 \times 10^{-3}$	93·2	32·4

differences in the ages of less than 10 per cent may well not be significant[4].

The observed spread of ages is very high. On the not unreasonable assumption that metamorphism of all three samples took place at the same time (the first two came from closely related localities, the second and third yield closely similar hornblende ages), we deduced a standard deviation of $\pm 6·8$ m.y. for any one of the six ages. This gives 95 per cent confidence limits (for five degrees of freedom, $t = 2·57$) of $\pm$ 17·5 m.y., amounting to $\pm$ 40 per cent, based on the mean value of 44 m.y.

We therefore look for any special, probably geological, circumstances which may enable us to give greater credence to some results, or to reject others as of lesser significance. Previous examination of thin sections had given no cause to reject any of the three samples. Other sets of results[4] suggest that air corrections of the magnitude reported here are insufficient to account for this high scatter of ages. Next we observe a general tendency for the plagioclase to appear younger than the corresponding hornblende. This is small for the first two samples, larger for the third. As plagioclase in general responds more readily than hornblende to factors causing argon loss, we have some grounds for placing less weight on the result for *GA* 700 plagioclase. Third, and possibly most significantly, we recall that *GA* 698 was the only sample for which close proximity (less than 50 ft.) to a fault was not recorded.

Thus we conclude that the time of metamorphism of the original rocks to the amphibolite schists lay somewhere within the range 44 $\pm$ 18 m.y. (at the 95 per cent confidence level), with a preference for the figure 50 m.y. (average of *GA* 698 results). On the currently accepted time-scales[5] this implies a geological age of Lower to Middle Eocene, with bias towards Lower Eocene (Lutetian, possibly Ypresian—Evernden *et al.*[6]).

The metamorphism cannot have occurred more recently than Upper Eocene, but this younger limit, based as it is on the *GA* 700 plagioclase result, cannot be regarded seriously. These conclusions represent a good geological fit for Choiseul and, indeed, if extrapolated to the Guadalcanal schists, for the whole of the Central Province.

The fixing of a Lower–Middle Eocene time of metamorphism provides a causal connexion between the Lower Tertiary lava pile in the Central Province and the stresses which were responsible for the metamorphism of the underlying schists, and disposes of a time-gap which, on previous evidence, could have existed between the schists as such and the lavas.

Taken with the other evidence[1,7], our results remove the need to suppose that the Solomons Group began its history any earlier than the late Mesozoic (? Cretaceous) and pose the problem examined by Coleman[1], of reconciling the near-juxtaposition of two contrasting geological terrains: Eocene metamorphics in the Central Province, and unmetamorphosed Upper Cretaceous–Eocene lavas aad sediments in the Pacific Province.

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IRON-55 IN MARINE ENVIRONMENT AND IN PEOPLE WHO EAT OCEAN FISH

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THE concentrations of iron-55 in human beings and their food both in Alaska and in the State of Washington were recently reported[1]. During the course of this investigation, it was found that ocean fish had higher concentrations of this radionuclide than did the Alaskan caribou. This finding suggested that individuals whose diet contained large quantities of ocean fish could have body burdens several times higher than those of the caribou-eating Eskimos, and perhaps several orders of magnitude higher than those measured in residents of Richland, Washington. This article details the measurements that have been made on samples from the marine environment, and includes estimates of the body burdens of iron-55 of individuals who derive a major portion of their diet from ocean fish.

The concentrations of iron-55 in ocean fish have been reported previously[2,3], but the measurements were made before the last series of atmospheric nuclear tests. The concentrations in salmon and tuna fish are now 100–200 times those reported for these earlier measurements. We have analysed a number of marine samples in an attempt to (*a*) indicate the iron-55 concentrations in the various trophic levels of the marine environment and (*b*) point out the relative concentrations of iron-55 in those marine organisms which serve as foodstuffs for human beings.

Table 1. IRON-55 IN OCEAN FISH DURING THE LAST SIX MONTHS OF 1965

Type of fish (flesh)	No. of samples in average		Average stable iron content (mg/kg)	Average iron-55 content (nc./kg)
Salmon	1	Ocean at Kotzebue, Alaska	12·0	93·0
Salmon	1	Ocean at Kotzebue, Alaska	4·6	48·0
Salmon	3	Yukon River in Alaska	13·2	37·0
Salmon	1	Cook Inlet, Alaska	14·2	34·0
Salmon	1	Washington coast	5·8	14·0
Albacore tuna	1	Northern Pacific Ocean	19·1	42·0
Albacore tuna	1	Southern Pacific Ocean	16·3	1·6
Albacore tuna	1	Northern Atlantic	11·1	8·4
Albacore tuna	1	Southern Atlantic	4·5	0·78
Ocean perch	1	Pacific	1·9	0·85
Cod	1	Pacific	—	0·20
Whitefish	3	Northern Alaskan coast	11·0	0·10
Shee fish	2	Northwestern Alaskan coast	—	0·48
Sea bass	1	Washington coast	63·7	0·27
Swordfish	1	Pacific	—	0·33
Herring	2	Packed in Norway	—	20·0
Herring	3	Canadian West coast	11·2	0·79
Sardines	2	Packed in Norway	—	15·0
Sardines	2	Packed in Maine, U.S.A.	—	2·6
Smelt	2	Washington coast	18·2	0·41
Saury	(Several whole fish)	Oregon coast	52·3	106·0

Table 1 contains the results of the analysis of a variety of ocean fish using the methods previously described[1]. Several interesting points are evident from these data. First, it is seen that salmon, tuna and saury fish have the highest content of iron-55. Saury fish is processed in Japan and sold for human consumption, but only in a very small amount compared with the consumption of salmon and tuna. The saury fish that we analysed were taken in the plume of the Columbia River outlet, and some of the iron-55 in these fish probably comes from the cooling water at the Hanford nuclear reactors which empties into the river. The concentrations of iron-55 in herring and sardines canned in Norway are relatively high at about 15 nc./kg, whereas iron-55 concentrations in the same type of fish from the United States and Canada are lower by a factor of ten. We have not measured enough samples to determine if this difference is consistent or why the difference exists. Secondly, tuna caught in the northern oceans have more iron-55 than those caught in the southern oceans, and those in the Pacific Ocean have more iron-55 than those in the Atlantic Ocean. These differences in iron-55 concentrations of ocean fish from different geographical locations are consistent with the differences in concentration of caesium-137 (ref. 4), cobalt-60 and silver-110 (ref. 5); however, the interesting observation is that the iron-55 concentrations are about 1,000 times higher than the reported concentrations of the other three isotopes.

The relatively high concentrations of iron-55 in the marine species measured largely results from the low concentration of stable iron in sea-water (~ 10 μg/l.). Most of these marine species also have a lower iron content than do land animals. Furthermore, radioactive iron falling on land surfaces will be diluted by the large amount of stable iron existing there, and as a result the uptake of iron-55 by plants and animals will be greatly reduced because of this dilution. Salmon and tuna fish, on the other hand, are fast growing fish and living in a low iron environment probably take up and retain most of the iron available to them. The slower growing fish such as sea bass, perch, and whitefish contain much less iron-55. The lower ratio of iron-55 to stable iron which was measured in these fish confirms the possible lower uptake and slower growth.

Table 2 contains data on the relative concentrations of iron-55 in the liver, roe, and muscle of two chum salmon caught at Kotzebue, Alaska. The livers of these salmon contain almost 2 μc. of iron-55/kg of liver (wet weight) and represent the highest concentrations we have measured in any organism or tissue. It is also interesting to note the rather uniform labelling of the salmon as evidenced by the ratio of radioactive to stable iron in the various tissues.

Since salmon are known to feed on a variety of marine organisms (plankton, euphausiids, herring, squid, etc.), it is difficult to attribute their high iron-55 concentrations to the consumption of a specific species. Indeed, these high concentrations probably result from intake both of small fish of relatively high activity, such as herring or saury, and of organisms of lower activity such as plankton, euphausiid and squid. Table 3 lists some representative values of the iron-55 concentrations in these trophic levels. The barnacles are surface-living organisms which were taken from glass floats off the California coast, and their high iron-55 content indicates a large uptake from fresh fallout on the ocean surface. The higher availability of iron-55 at the surface may be the reason for the high concentrations in saury fish because they also live close to the surface. The sea cucumbers are mud scavengers and these samples were taken from the Columbia river plume which probably accounts for their high content of iron-55. Other marine organisms such as oysters, scallops, clams, shrimp and crab have very

Table 2. IRON-55 AND STABLE IRON IN SALMON CAUGHT AT KOTZEBUE, ALASKA

	Muscle			Liver			Eggs		
Sample	Fe^{55} (nc./kg)	Iron (mg/kg)	nc. Fe^{55} / mg Fe	Fe^{55} (nc./kg)	Iron (mg/kg)	nc. Fe^{55} / mg Fe	Fe^{55} (nc./kg)	Iron (mg/kg)	nc. Fe^{55} / mg Fe
No. 1	93	12	7·8	1,620	175	9·3	275	32	8·6
No. 8	48	4·6	9·6	1,860	205	9·1	222	23	9·6

Table 3. CONCENTRATION OF IRON-55 IN SEA-WATER AND SOME MARINE ORGANISMS

Species	Location	nc./kg
Sea-water	California coast	<0·00004
Phytoplankton (dinoflagellata)	California coast	0·5
Euphausiids	Oregon coast	1·5
	Gulf of Alaska	0·16
Mussel (*Mytilus*)	California coast	0·36
Kelp (*Nacrystis*)	California coast	0·03
Barnacles (*Lepas*)	California coast	140·0
Squid muscle (*Lolego*)	California coast	0·76
Squid liver	California coast	8·6
Purple sea cucumbers (whole)	Oregon coast	76·0
Sea urchins (whole)	Oregon coast	0·48

Table 4. COMPARISON OF IRON-55 BODY BURDENS IN ALASKAN NATIVES WITH MEAT AND FISH CONSUMPTION

Alaskan village	Ratio of fish consumption*/ animal meat consumption	Average adult male body burden (nc.)
Anaktuvuk Pass	0·19 (ref. 8)	81
Allakaket (Tanana Area)	0·7 (ref. 9)	252
Southwestern Alaska (Bethel Area)	4·4 (ref. 9)	900

* The type of fish consumed is mostly salmon except at Anaktuvuk Pass where salmon are not available.

low concentrations of iron-55 (ref. 1) and probably do not contribute much to the iron-55 body burdens of either other marine species or humans.

As might be expected, these high concentrations of iron-55 in the salmon produce higher than average concentrations of iron-55 in populations who eat substantial quantities of these fish. The caribou-eating Eskimo of Anaktuvuk, Alaska, had an average iron-55 body burden of 61 nc. in January 1965 (ref. 1). In November 1965, this same group of people had an average iron-55 body burden of 81 nc., an increase of some 13 per cent. By way of comparison, adult male Eskimos from Tanana, Alaska, who eat large quantities of salmon in addition to caribou and moose meat, showed an average iron-55 body burden of 252 nc. Furthermore, recent blood samples received from natives in south-western Alaska near Bethel, Alaska, who eat more salmon than the Tanana natives show that the average body burden of iron-55 in the adult male is about 900 nc. in some villages, with one individual having a body burden of 2,300 nc. Data on fish consumption from studies of Alaskan diets are compared with these body burdens in Table 4, and these show a definite correlation between salmon consumption and body burden of iron-55.

In the analysis of the blood specimens obtained from the Bethel natives it was found that the average body burden of iron-55 in the females was twice that of the males. The opposite is true in the case of caesium-137 in the Alaskan natives where the content in women is consistently two-thirds that in the males[8] due to their lower dietary intake. Because the food intake of women is generally lower than that of men, the higher iron-55 body burdens observed in this case probably reflect the more rapid turnover of iron by females. This probably results from the loss of iron through the menstrual blood with the result that their body burdens are closer to an equilibrium with the iron-55 in their diet than are those of the males.

Despite the fact that some of the Alaskan natives have body burdens approaching 2·3 μc., which is about 100 times the average level of residents of the State of Washington, it must be emphasized that these levels are far below the maximum permissible body burden of 100 μc. for members of the general population who are not exposed to iron-55 in the course of their occupations[7]. The radiation dose to the erythrocyte fraction of the blood of a person with a constant iron-55 body burden of 1,000 nc. would be approximately 30·0 m rem per year. This is only about 30 per cent of the yearly dose to the body from natural sources such as cosmic rays and internally deposited potassium-40 and radium.

As a result of these investigations it can be predicted that populations in northern countries such as Japan and the Scandinavian countries who eat large quantities of ocean fish probably have relatively high iron-55 body burdens as do the Alaskan natives. In our present investigations we are measuring the iron-55 content in various populations of the world.

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EFFECTS OF IRRADIATED SUCROSE ON THE CHROMOSOMES OF HUMAN LYMPHOCYTES *IN VITRO*

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EVIDENCE is available that suggests that certain food substances, or their derivatives, may be toxic and/or mutagenic if exposed to irradiation before being utilized by micro-organisms, plants and animals. Stone *et al.*[1] reported that broth irradiated with ultra-violet light is capable of inducing mutations in *Staphylococcus aureus*. Several investigators[2–4] have demonstrated an increase in mutations when *Drosophila melanogaster* are reared on irradiated food. Others have reported cytological aberrations in higher plants produced by irradiated carbohydrates[5–8]. These include chromosome stickiness, lagging anaphase fragments, nuclear disintegration, formation of micronuclei, increased chromosome breakage at the centromere region, and arrest of prophase. Toxicity to mammalian cells *in vitro*, as shown by growth inhibition produced by irradiated glucose and fructose in the culture media, was demonstrated by Berry *et al.*[9].

To our knowledge, no studies have been reported on the effects of irradiated media (or irradiated components of the media) on the chromosomes of mammalian cells. This article describes experiments concerned with the effects of irradiated sucrose on the chromosomes of human lymphocytes *in vitro*.

The test solutions used in these experiments were autoclaved sucrose solutions which had not been exposed to radiation (control) or which had received 2 Mrads (2 million rads) from a cobalt-60 source (treated). Irradiation and autoclaving were carried out several months

before the experiments, and the solutions were stored at room temperature or at 5° C. The sucrose solutions were irradiated at a concentration of 2 per cent and were evaporated for shipment to 20 per cent. Just before use, various dilutions of the test solutions were made with 0·9 per cent sterile sodium chloride solution.

Heparinized syringes were used to obtain venous blood from four healthy adult males and females. Each culture tube contained 5 ml. of culture medium with phytohaemagglutinin (Grant Island Biological Company, Medium 1*A*), 0·25 ml. whole blood, and 0·25 ml. of appropriate dilutions of the sucrose test solutions. In some experiments the sucrose was added at the beginning of the culture period, while in others it was added 24 h before collecting the cultures.

All cultures were incubated for 72 h at 37° C. Three hours before gathering them, colchicine was added to give a final concentration of 0·6 μg/ml. culture medium. The cultures were collected and slides prepared using minor modifications of the method of Moorhead *et al.*[10]. Many replicate slides of each culture were made and assigned code numbers by individuals who did not participate in the microscopic studies. Five well spread mitoses per slide were chosen under low power for the purpose of scoring the number of chromosome abnormalities : scoring was done directly with oil immersion phase microscopy.

Aberrations were classified as "breaks" only if a distinct discontinuity of the chromosome matrix was visible. If two breaks were visible in homologous regions of sister chromatids they were scored as a single isochromatid break. If a region of the chromosome appeared pale, stretched, uncoiled, or attached by a tiny thread it was scored as a "gap" but not included in the calculations of breakage rates. Single acentric fragments were counted as chromatid breaks while double fragments were included among isochromatid breaks. Exchange figures were scored separately.

The following results were obtained:

(*A*) *Seventy-two hour treatment.* Preliminary experiments suggested that when the irradiated sucrose solutions were added at the beginning of the culture period at concentrations of 0·55 per cent and more, they were extremely toxic to the lymphocytes. Mitoses were inhibited and many of the non-dividing cells appeared pycnotic and had not undergone normal "blast" transformation. Degenerated mitoses were observed and the chromosomes were grossly damaged. The chromatin material was clumped or the chromosomes appeared shattered or pulverized. In the few dividing cells where definite counts could be made the chromosomes appeared "sticky" with attenuated regions and telomere strands (Fig. 1*a*). In contrast, treatment with unirradiated sucrose for 72 h at the same concentration had no apparent effect on the mitotic rate and the chromosomes were not visibly damaged. Table 1 gives the mitotic rates for both treated and control cultures.

(*B*) *Twenty-four hour treatment.* In subsequent experiments the sucrose solutions were added 24 h before the cultures were collected. Cultures containing irradiated or unirradiated sucrose at eight different concentrations were prepared. In these cultures an adequate number of

Table 1. MITOTIC RATES AND FREQUENCIES OF CHROMOSOME BREAKS IN HUMAN LYMPHOCYTES TREATED WITH IRRADIATED AND UNIRRADIATED SUCROSE

Final concentration in culture (per cent)	Time treated (h)	Mitoses per 1,000 cells	Breaks per 100 mitoses	Percentage of mitoses with breaks
		Irradiated sucrose		
0·91	72	0	*	*
0·73	72	2	*	*
0·55	72	11	*	*
0·91	24	23	68 + †	34 + †
0·73	24	26	89	41
0·55	24	37	54	37
0·36	24	62	43	29
0·18	24	71	24	21
0·09	24	73	11	10
0·045	24	82	10	9
0·009	24	82	16	13
		Unirradiated sucrose		
0·91	72	89	0	0
0·73	72	74	2	2
0·55	72	80	3	3
0·91	24	69	6	6
0·73	24	80	3	3
0·55	24	72	7	7
0·36	24	78	3	3
0·18	24	81	4	4
0·09	24	78	4	4
0·045	24	91	6	4
0·009	24	71	6	4

* Frequencies of breaks could not be obtained because of the poor condition of the cells and the rarity of mitotic figures. The chromosomes appeared to be "shattered".

† Many mitoses could not be scored because of the severe erosion of the chromosomes. The number of breaks given is therefore a minimum figure.

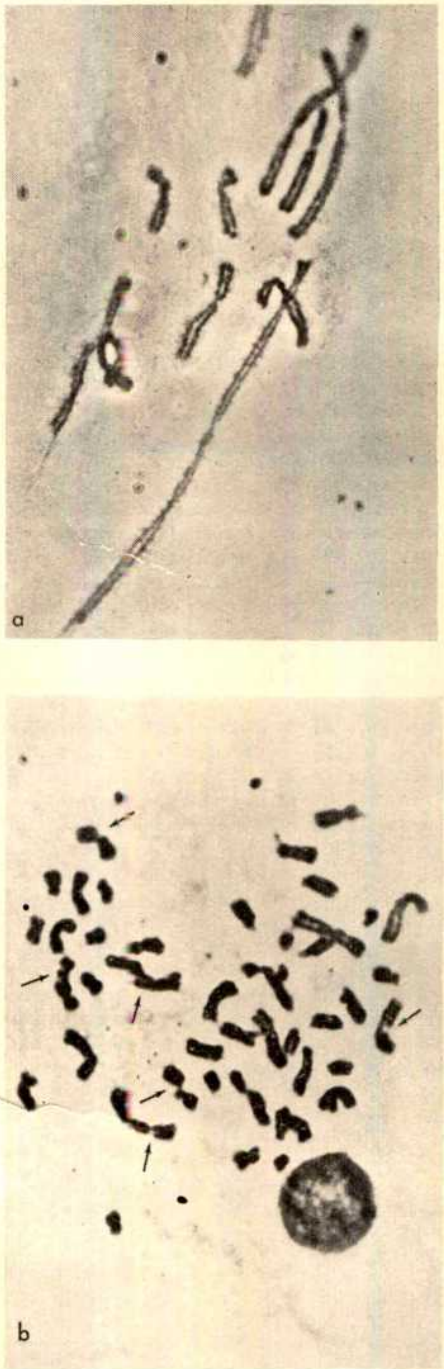


Fig. 1. Effects of irradiated sucrose solutions on the chromosomes of cultured human lymphocytes. *a*, 72-h treatment, 0·55 per cent sucrose. Note extreme attenuation of the chromatin material, the "sticky" appearance of the matrix, and the telomere strands. *b*, 24 h treatment, 0·91 per cent sucrose. Arrows point to chromatid and isochromatid breaks and gaps.

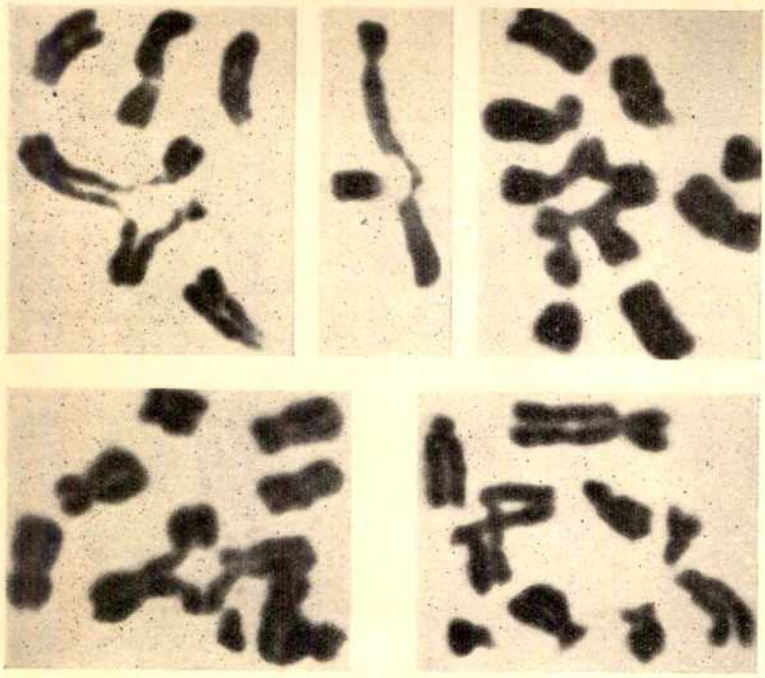


Fig. 2. Five exchange figures produced by treatment with irradiated sucrose solutions for 24 h. A complex exchange involving three chromosomes is shown in the upper right.

mitoses were present and could be scored for chromosome abnormalities. These results are also given in Table 1. At concentrations of 0·36 per cent or greater, the mitotic rate was lower in the treated cultures. At all concentrations of irradiated sucrose tested there was an increase both in the total number of breaks and in the percentage of mitoses with one or more breaks. Fig. 1*b* illustrates a metaphase from a cell which had been exposed to irradiated sucrose for 24 h. The proportion of breaks which were of the isochromatid type was much higher in the treated cells (52/234) than in the control cells (2/37). Among the treated cells there were eight double fragments and two single fragments, compared with one single fragment in the controls. Fig. 2 illustrates five of the nine exchange figures which were scored among the treated cells. Undoubtedly they were induced by the irradiated sucrose solutions because no exchanges were observed in the controls. Endoreduplication was encountered four times among treated cells when mitotic rates were being scored; this phenomenon was not seen on the control slides. Dicentric chromosomes and rings were not observed.

(*C*) *Specific effects of irradiated sucrose.* The distribution of breaks among specific chromosomes or groups of chromosomes is given in Table 2. The observed distribution agrees closely with that expected, based on random breakage per unit of chromatin length calculated from the Denver measurements[11]. Although breaks may be randomly distributed among chromosomes it does not follow that they occur within a given chromosome randomly along its length. Several regions of several chromosomes were quite frequently broken and may represent "hot spots". For example, thirty-one of forty-one breaks found in the *D*13–15 group occurred in the middle third of the long arm; seven of eleven breaks in the *F*19–20 group occurred in the proximal third of one arm; and twenty-two of thirty-nine breaks in *A*1 occurred in the middle third of one arm rather than at the paracentric secondary constriction.

Table 2. DISTRIBUTION OF CHROMATID AND ISOCHROMATID BREAKS ACCORDING TO SPECIFIC CHROMOSOMES OR GROUPS OF CHROMOSOMES

Chromosome or group	Chromatid breaks	Irradiated sucrose Isochromatid breaks	Total breaks	Expected No. of breaks*	Unirradiated sucrose Total breaks
*A*1	36	3	39	28·6	4
*A*2	24	6	30	26·7	4
*A*3	14	3	17	22·4	2
*B*4–5	37	4	41	40·2	3
*C*6–12–*X*	105	21	126	127·0	13
*D*13–15	34	7	41	32·4	4
*E*16	4	0	4	10·0	0
*E*17–18	15	4	19	18·0	3
*F*19–20	9	2	11	15·0	3
*G*21–22	4	2	6	10·6	1
Y	0	0	0	2·8	0
Total	282	52	234	233·7	37

* Expected values derived from measurements given in the Denver report (ref. 11). Breaks are assumed to be random according to unit chromatin length.

Berry *et al.*[9] using a human aneuploid line (HeLa) and a mouse aneuploid line (strain *L* fibroblasts) measured the cytotoxicity of glucose and fructose solutions which had been exposed to 2·5 Mrads of cobalt-60 gamma irradiation. It is interesting to note that this radiation dose and the concentrations of irradiated carbohydrates they used are directly comparable with our experiments and their results are in agreement with our findings. Apparently there is a rather sharp threshold concentration for demonstrating toxicity of irradiated carbohydrates to mammalian cells *in vitro*.

The chromosome breaks reported in our experiments were observed in the first (or rarely, in the second) mitotic division *in vitro*. Counts were made and breaks were scored under direct observation but only a few of the 1,900 metaphases were analysed and karyotyped. Therefore, structural rearrangements would, for the most part, have gone undetected. Exchange figures, however, are immediately evident. It thus seems reasonable to conclude that permanent chromosomal rearrangements can be induced *in vitro* and passed on to daughter cells.

Our experiments have no bearing on the question of whether point mutations are produced by irradiated sucrose. Neither do they shed light on the important question of whether foods which have been preserved by irradiation and ingested by mammals produce chromosome damage *in vivo*. These questions are particularly pertinent because evidence of increased mutations in *Drosophila*[2–4] suggests that the toxic products of irradiated media can indeed pass the gastro-intestinal barrier, and mass human consumption of irradiated foods is being seriously considered. Feeding experiments with deer mice are now in progress in order to assess somatic damage to the chromosomes in bone marrow and germinal damage to chromosomes of testicular cells undergoing spermatogenesis.

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EFFECT OF FORMIC ACID ON THE STRUCTURE OF α-KERATIN

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It has long been believed that the crystalline reflexions in the X-ray diffraction pattern of keratin disappear when the material is exposed to concentrated formic acid[1–5]. Some of the work referring to this[1,2] has been based on an early report by Speakman[6], in which he quotes the finding of Meyer and Mark[7] that the fibre pattern of silk disappears when the fibre is placed in formic acid; at that time Speakman considered it probable that the same effect would also be observed with wool. This view appeared to be confirmed later, when Sikorski[8] tested wool fibres immersed in 98–100 per cent formic acid and failed to detect any crystalline reflexions, although the fibre pattern was found to be present at lower acid concentrations (74 per cent). It has also been reported[3,9] that a dilute solution of monochloroacetic acid in water destroys the X-ray diffraction pattern of crystalline keratin. In view of the importance of the effect of organic acids on the structure of keratin these problems have now been re-investigated, and it has not been found possible to substantiate these claims. This article presents infra-red and X-ray diffraction evidence which establishes that at least part of the crystalline α-helix structure of keratin, though modified, is not destroyed by formic or mono-chloroacetic acids.

Earlier, on the basis of mechanical properties and the dimensions of wool in formic acid/water mixtures, it was suggested by Mason[10] that a proportion of aligned α-helices are still present in formic acid. Feughelman[11] concluded from the birefringence of wool fibres in formic acid that about half the original α-helical molecules were present in the helical form. Furthermore, values of the dielectric constant of wool in water and in formic acid indicated[12] that at least some α-helices must be stable to the acid. It is also relevant to mention here that the helix-containing soluble fraction of wool keratin (*S*-carboxymethylkerateine *A*), which is 50 per cent helical in water in dilute solution, has a helix content in formic acid which is still of the order of 15 per cent[13], although here cystine cross-links are absent and stabilization by aggregation is not likely. The helical structure is preserved even when films of this material are prepared by casting from cold or boiling formic acid or cold trifluoroacetic acid, for the infra-red spectrum[14] indicates a helix content as high as in films cast from water.

Apart from the earlier work by Sikorski[8], only one X-ray test of the effect of formic acid on keratin seems to have been reported. Skertchly[4] showed that, compared with dry keratin, the intensity of the "9·8 Å" equatorial reflexion is reduced relative to the intensity of the so-called halo near 4 Å. He concluded that the major part of this reflexion had been removed, and implied that most, if not all, of the crystallinity had disappeared. It is difficult to accept this interpretation, because the acid makes a very substantial contribution to the intensity of the halo: as the "9·8 Å" reflexion is as strong as the halo in his diagram, Skertchly's results indicate that considerable crystallinity is retained in formic acid.

The infra-red spectrum in the 6μ region of the soluble wool protein *S*-carboxymethylkerateine *A* (kindly supplied by Dr. J. M. Gillespie, C.S.I.R.O. Division of Protein Chemistry, Melbourne) is shown in Fig. 1. The spectrum of the dry film (curve *a*) exhibits the diagnostic α-helix peak at about 1,545 cm^{-1}, and that of the wet film (in the presence of saturated water vapour) is very similar (curve *b*), except for the underlying water band and a general shift to higher wave numbers. The spectrum of the film exposed to an atmosphere of 98–100 per cent formic acid vapour (curve *c*) also has a very similar shape, although somewhat modified by the presence of a strong underlying band due to the acid. Curve (*c*) is broader on the low-frequency side than curves (*a*) or (*b*), and this probably reflects the presence of additional non-crystalline material, which in dry keratin has been shown[15] to absorb near 1,520–1,525 cm^{-1}, and 5–10 cm^{-1} higher in keratin exposed to saturated water vapour.

The presence of helices is confirmed by the X-ray diffraction pattern of a horsehair immersed in formic acid (Fig. 2), which is typical of α-helical material. For this test, a short piece of horsehair of circular cross-section was placed in a tight-fitting, thin-walled glass capillary containing formic acid. This procedure was adopted in order to reduce interference from the acid; it is possible that previous tests (for example, ref. 9) with organic acids failed to detect the presence of crystalline reflexions because wool bundles were used, so that the strong halo due to acid between fibres completely swamped the X-ray diffraction pattern. Before the glass capillary was sealed and the specimen pushed into position, the specimen was placed in a wider part of the tube; in an atmosphere of dry nitrogen, acid was allowed to flow past it for 15 min. The effluent was tested and found to contain 99·5 per cent formic acid, which leaves no doubt that the X-ray pattern shown in Fig. 2 was obtained with almost pure formic acid. After sealing, the fibre was held in the acid for 15–30 min before the X-ray diffraction pattern was recorded in order to ensure proper penetration of the acid; the X-ray diffraction procedure itself took about 2 h. These times can be compared with that taken for a horse-

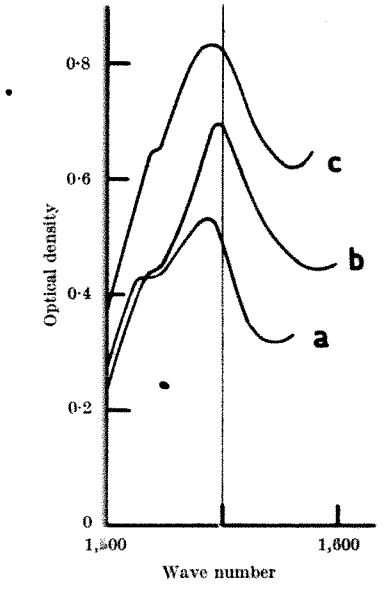


Fig. 1. Infra-red spectrum of a film of the helix-containing soluble wool fraction *S*-carboxymethylkerateine *A*. (*a*) Specimen dry; (*b*) specimen exposed to saturated water vapour; (*c*) specimen exposed to the vapour of 98–100 per cent formic acid.

hair to swell to equilibrium in formic acid, which is of the order of a few minutes.

Exposure of keratin to formic acid for extended periods weakens the X-ray fibre diffraction pattern. In one test, a horsehair specimen was kept immersed for 10 months, and both the 5·15 Å and "9·8" Å reflexions were very considerably reduced in intensity. This partial destruction of the ordered helical structure may have been one of the reasons for Sikorski's[8] failure to observe the crystalline reflexions, as his specimen had been kept immersed in formic acid for some 6 weeks before testing[16].

A single Lincoln wool fibre (60μ in diameter (dry)) was also tested in a thin-walled glass capillary containing formic acid. Once again the X-ray diffraction pattern exhibited the meridional and equatorial reflexions diagnostic of α-keratin.

Similar tests on horsehair using aqueous solutions of monochloroacetic acid (2·7 N) have shown that, contrary to previous claims[9], the crystalline X-ray diffraction pattern is again not destroyed by the reagent; the pattern is, in fact, somewhat better than that of a specimen immersed in formic acid. The halo which results from the liquid is less pronounced than for formic acid, and this reflects the degree of lateral swelling in these reagents (about 55 per cent for concentrated formic acid and about 25 per cent with 2·7 N monochloroacetic acid). A test with 22 N monochloroacetic acid (lateral swelling about 45 per cent) gave an X-ray diffraction pattern similar to that for formic acid.

Additional conclusions can be drawn from the X-ray diffraction patterns, but before considering these it is desirable to outline some aspects of keratin structure. α-Keratin is thought to consist of microfibrils, which contain the crystalline component, embedded in a non-crystalline matrix[17]. The microfibrils are many thousands of Ångströms long, about 75 Å in diameter, and have a centre-to-centre distance of 85–95 Å depending on the type of keratin. They are formed by an assembly of protofibrils, each consisting of two- or three-strand ropes of α-helices. A possible model of the distribution of α-helices in the microfibril is shown in Fig. 3. The main meridional reflexion at 5·15 Å results from the axial repeat of coiled α-helices, while the prominent equatorial reflexion near 10 Å is approximately equal to the lateral distance between α-helices within the protofibril. This equatorial reflexion exhibits a multiple structure under high resolution, owing mainly to the interference produced by the arrangement of protofibrils within the microfibril. The reflexion is usually quoted as 9·8 Å for dry keratin, but precise measurements[19] using markers indicate that it is significantly lower at 9·4–9·5 Å. It is worth noting that the shape and position of the

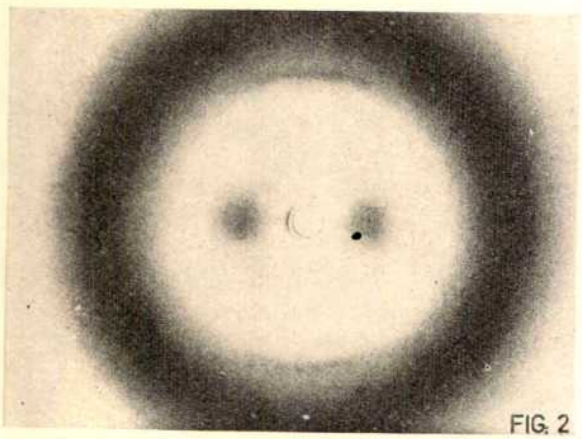


Fig. 2. X-ray diffraction pattern of horsehair (dry diameter 180μ), immersed in 99·5 per cent formic acid. Nickel filtered copper *kα* radiation from a Philips fine focus X-ray tube, lead glass collimator 300μ diameter, specimen-film distance 24·8 mm.

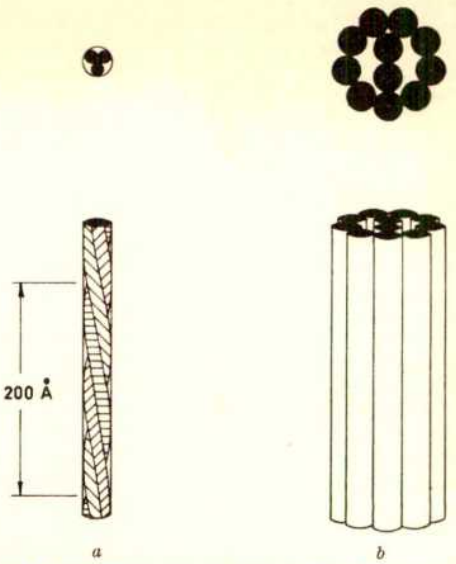


Fig. 3. Model of microfibril structure (*b*) showing the "9 + 2" arrangement of protofibrils (*a*) based on a diagram reported by Fraser *et al.* (ref. 18).

envelope of the 10 Å group of reflexions reported by Fraser *et al.*[20] for dry porcupine quill suggests that the peak under low resolution should be in the region of 9·5 Å. The microfibrils themselves give rise to three prominent reflexions in the low-angle X-ray diffraction pattern of keratin; in dry porcupine quill these occur at 83 Å, 45 Å and 28 Å.

Returning now to the X-ray pattern shown in Fig. 2 (formic acid), the spacing of the main meridional reflexion appears to be unchanged at 5·15 Å, although the precision with which this measurement can be made is limited by the broad halo due to the acid. This indicates that the configuration of those α-helices which are not destroyed by the reagent is very similar to that of the α-helix in dry keratin; however, it is possible that in formic acid the longitudinal order of the helices within the protofibrils is disturbed, as the usual[21] off-meridional reflexions near 5·15 Å do not appear to be present in Fig. 2.

For horsehair, the main lateral spacing is changed from 9·5 Å in the dry state to 11·1 Å on immersion in formic acid. This is an increase of 17 per cent, but it does not necessarily indicate a corresponding dimensional change in the protofibril or microfibril. In the parallel case of keratin fibres in water, the spacing increases by about 5 per cent[21], but it can be inferred from recent work by Fraser *et al.*[22], that only part of this increase is the result of dimensional change.

It is not clear, at this stage, whether the microfibrils retain their identity in formic acid, as the present equipment does not permit the innermost equatorial reflexions (at 83 Å and 45 Å in dry keratin) to be observed. If microfibrils are present, their diameter is likely to increase by about the same extent as the distance between helices, that is, of the order of, but less than, 17 per cent. As there is only a very small length change when a wool fibre is placed in formic acid, the volume swelling of the microfibril is less than 30 per cent. In contrast, the volume swelling of the whole fibre is about 150 per cent, and as the microfibrils in dry keratin occupy about half the space, the volume swelling of the matrix will be of the order of 250–300 per cent. Even if the microfibrils are disrupted, and if the interferences in the X-ray diffraction pattern shown in Fig. 2 result from protofibrils dispersed throughout the structure, the volume swelling of non-helical material would still be well in excess of 150 per cent. There is little doubt that the

protofibrillar arrangement of α-helices is retained in formic acid, because single dispersed helices could not account for the 5·15 Å meridional and the 11·1 Å equatorial reflexions.

This high degree of matrix swelling cannot easily be reconciled with its disulphide content, which averages about one half-cystine group per five residues. It is clear that these cystine cross-links cannot be distributed uniformly in the matrix, and it is necessary to postulate concentrations of disulphide groups (perhaps as high as 1 in 2) arranged in such a way as to permit the rest of the matrix protein to swell several times over. It is therefore tempting to regard these concentrations as clusters or sheets surrounding the microfibril. Such local concentrations, cross-linked to the microfibril in possibly only a few places, could act as "keepers" in certain sections along the microfibril and provide the viscous element opposing extension in the stiff post-yield region of wool (above 30 per cent extension), while the sections along the microfibril between the "keepers" would correspond to the easily extended yield region.

These considerations should be taken account of in discussions of keratin structure and its relationship to mechanical properties.

In summary, it has been shown that part of the helix structure in α-keratin is not destroyed by formic or monochloroacetic acids. The spacings of the X-ray diffraction patterns indicate that the helices are present in the form of protofibrils, but it is not certain whether the protofibrils remain in the microfibril arrangement or are dispersed throughout the structure. In either event, the high degree of swelling in the non-helical part of the structure can be reconciled with the high density of disulphide cross-links only by postulating local concentrations of very high cystine content. It is suggested that these concentrations may be arranged along the helices to act as "keepers", in order to provide the viscous element opposing extension in the stiff post-yield region of keratin.

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SULPHYDRYL—DISULPHIDE INTERCHANGE AND THE STABILITY OF KERATIN STRUCTURE

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WHEN a horsehair fibre was stretched in water at 20° C to 140 per cent of its normal length for 10 min, dried in the extended state, and its X-ray diffraction pattern recorded, the characteristic 5·1 Å meridional reflexion of α-keratin and the 4·7 Å equatorial reflexion of β-keratin were both present. This is in accord with previous work[1,2]. When the fibre was placed, still held extended 40 per cent, into distilled water at 100° C for 2 h, removed from the water and air dried still extended, the X-ray diffraction pattern showed a complete absence of α-keratin, and a sharper β-keratin reflexion at 4·7 Å on the equator. In another test, horsehair fibres were stretched in boiling distilled water in small steps over a period of 1·5 h to 140 per cent of their normal length and held extended a further 30 min in the boiling water. After drying them still in the extended state, they gave X-ray reflexions showing both the presence of α- and β-keratin similar to the first case. When an aqueous solution of hydrochloric acid at *p*H 2 and 100° C was substituted for the boiling distilled water, the X-ray diffraction pattern obtained showed the presence of β-keratin only. When a fibre was extended rapidly in boiling water (in less than 0·5 min) to 40 per cent extension, held extended for 2 h in the boiling water, removed from the water and air dried still stretched, the X-ray diffraction pattern obtained also showed the presence of only β-keratin. These results are summarized in Table 1.

The stability of the α-keratin structure in a keratin fibre in water will depend on the temperature and the stress on the α-keratin[5,8]. Under stress-free conditions[3,4] the α-keratin "melts", that is becomes unstable, in water at about 130° C. In the presence of stress on the structure this temperature of melt is reduced. The X-ray diffraction results obtained under the varying conditions of extension of the horsehair fibres to 40 per cent can be explained in terms of this variation of stability of the α-keratin with stress and temperature. A diagrammatic representation of this relationship is shown in Fig. 1.

In the situation corresponding to (*a*) in Table 1 the α-keratin present in the extended structure is stable. The stress level on the α-keratin is high at room temperature, but not sufficient to cause "melting" because of the low temperature. When the fibre is plunged into boiling water

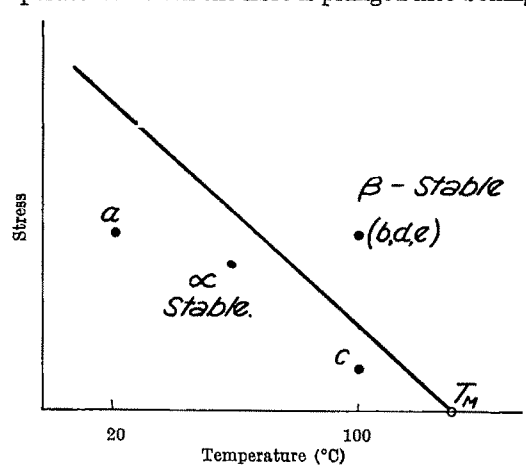


Fig. 1. Diagrammatic representation of the relationship between stress, temperature and the stability of α- and β-keratin in water. T_M is the stress-free melting temperature of the α-keratin phase. The points (*a*), (*b*), (*c*), (*d*) and (*e*) correspond to the same fibres as in Table 1.

Table 1

Conditions of extension in water to 40 per cent	Crystalline state
(*a*) Extended at 20° C	α and β
(*b*) Case (*a*) plunged still extended into water at 100° C for 2 h	β only
(*c*) Extended slowly to 40 per cent over 1·5 h at 100° C and then held 30 min at 100° C	α and β
(*d*) Extended as in (*c*) except aqueous hydrochloric acid at *p*H 2 is substituted for water	β only
(*e*) Extended rapidly in less than 0 5 min in water at 100° C and held at 100° C for 2 h	β only

the initial high stress level combined with the high temperature results in the instability of the α-keratin material present and thus "melting" occurs with a disappearance of the 5·1 Å meridional repeat for fibre (*b*). If a fibre is extended very slowly at an elevated temperature as in the conditions corresponding to fibre (*c*), the level of stress in the fibre remains very low and is insufficient to upset the stability of the α-keratin present in the extended fibre. The low stress level during slow extension in boiling water mainly results from sulphydryl-disulphide interchange eliminating bonds stressed by the extension of the fibre[6]. However, if the fibre is either extended slowly in an acid solution (*p*H 2) at 100° C or extended rapidly in water at 100° C, a high level of stress will result. In the former it will result from reduction[3] of the rate of sulphydryl-disulphide interchange at *p*H 2; in the latter it will result from the fact that insufficient time has been allowed for the interchange. Because of this high level of stress at 100° C the remaining α-keratin becomes unstable, and in both cases only the presence of β-keratin is observed in the X-ray diffraction pattern when the fibre is removed from the water and air dried while still stretched.

When a horsehair fibre which has been extended by 40 per cent in water at 20° C is released in water at 20° C the β-keratin disappears and the native α-keratin structure is returned[1]. For the fibre corresponding to (*b*), the α-keratin does not return on release in water either at 20° C or 100° C. The β-keratin appears to be quite stable. If the fibre is only held extended in the boiling water for a short time (about 0·5 min) and then released, however, the α-keratin structure returns and the β-keratin disappears[7]. From these results it seems that the major portion of the retraction force in a fibre must be present at 100° C, if the "melted" α-keratin structure is to return on release of the fibre. It has been shown[6,7] that this force of retraction rapidly relaxes in distilled water at 100° C after a few minutes because of scission of strained disulphide bonds by way of sulphydryl-disulphide interchange. From these observations it appears that once the α-keratin structure has melted it will only return so long as a sufficient force of retraction is still present, that is, a sufficient number of uninterchanged disulphide bonds are present. This non-reversal of α-keratin structure, when the disulphide interchange process has gone too far, could arise in a number of ways:

(i) The force of retraction as supplied by the presence of the disulphide bonds provides part of the "memory" directing the structure back to the native α-keratin.

(ii) Disulphide interchange may interpose new cross-links, which interfere sterically with the reformation of the helices of the native α-keratin configuration.

(iii) Reformation of disulphide bonds in equilibrium with the extended state may stabilize the β-keratin crystallites formed in the extended state, and thus prevent their return to their native configuration.

(iv) The α-keratin structure is directly dependent on disulphide bridges to provide stability to the structure, and their removal by interchange makes the α-keratin structure less stable.

In summary, the stability of the native α-keratin structure is dependent on temperature and stress. This stress arises from the straining of disulphide bonds in the structure, and is transferred to the α-keratin components of fibre by these bonds. Sulphydryl-disulphide interchange in an extended fibre interferes in a number of ways with the ability of the native α-keratin to recover its configuration, when the fibre is later released.

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ANTI-VIRAL ACTIVITY OF AZATHYMIDINE AND URACIL METHYL SULPHONE

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AZATHYMIDINE, the deoxyribonucleoside of azathymine (6-methyl-*as*-triazine-3,5(2H, 4H)-dione), has been prepared by enzyme[1] as well as by chemical synthesis[2] and the biological properties of this analogue have been reviewed[3,4]. Azathymine and its deoxyriboside are competitive inhibitors of the utilization of thymine or thymidine for the growth of *Streptococcus faecalis*. Although this analogue is incorporated into the DNA of *S. faecalis*, the amount of incorporation is not related to its lethal effect. Marsh and King[5] observed that azathymidine was a competitive inhibitor of the enzyme *trans*-N-glycosidase derived from *Thermobacter acidophilus*. In mammalian cells the incorporation of formate into DNA is effectively inhibited by azathymidine, but thymidine incorporation is much less sensitive[6]. Because azathymidine appeared to be relatively non-toxic to mammalian cells, we decided to investigate its potential anti-viral activity.

6-Uracil methyl sulphone (UMS) was designed as a potential metabolic antagonist of orotic acid[7]. This analogue inhibits the reproduction of several bacteria[8] and murine lymphomata[9,10]. Metabolic investigations indicate inhibition of (*a*) the formation of orotidylate from orotate and 5-phosphoribosyl-1-pyrophosphate by a partially purified enzyme preparation from yeast[11]; (*b*) the utilization of orotate or formate but not of thymidine for the biosynthesis of DNA-thymine in Ehrlich ascites cells *in vitro*[12]; (*c*) the utilization of orotate for the formation of RNA-pyrimidines in regenerating liver slices[13]; and (*d*) the conversion of ribonucleotides to deoxyribonucleotides by mammalian cells[14]. Because UMS reacts rapidly and non-enzymatically with several sulphydryl compounds such as glutathione it was postulated that this analogue may function as an alkylating agent for a sensitive protein containing sulphydryl that is critically involved in cellular reproduction[15]. The present report includes a study of the anti-viral activity of UMS and of the inability to prevent its inhibition by various compounds that were chosen on the basis of the postulated[11–14] biochemical sites of inhibition of UMS.

A monolayer of 2-day old chick embryo fibroblasts in 3 oz. prescription bottles was prepared by the procedure described by Hsiung[16] and inoculated with 0·1 ml. of herpes simplex virus, *HF* strain (obtained from Dr. J. McCrea), having a titre of 10^6 plaque-forming units (P.F.U.)/ml. The infected cells were incubated for 1 h at 37° C, washed once with Hanks's buffered salt solution and layered with 10 ml. of Earle's maintenance medium[17]. These were examined for cytopathological changes (CPE) 24 h later.

The supernatant fractions were stored at −70° C before determination of their virus titre. The cellular debris was removed by centrifugation and serial ten-fold dilutions were made in Eagle's growth medium supplemented with 10 per cent calf serum. Chick embryo fibroblasts in 3 oz. prescription bottles were prepared as already described and inoculated in triplicate or quadruplicate. After incubation at 37° C for 1 h, the inoculated cells were washed with Hanks's buffered salt solution. Agar overlay medium (7 ml.) was added and the formed plaques were counted 5 days later.

The results shown in Table 1 show that azathymidine markedly inhibited the formation of herpes simplex virus; however, 5-iodo-2′-deoxyuridine (IUdR) was found to be seventy times more potent on a molar basis.

Because with mammalian cells a thousand-fold molar excess of azathymidine was required to inhibit the utilization of thymidine labelled with carbon-14 for the biosynthesis of DNA-thymine[2,6], the effect of thymidine on the inhibition of viral reproduction exerted by azathymidine was investigated. Thymidine added within 2 min of the addition of azathymidine completely prevented the inhibitory effect of azathymidine when the former was present in a 0·015 molar equivalent (Table 2). More than 90 per cent inhibition was achieved when the ratio of azathymidine to thymidine was 330.

An attempt was made to determine which phase of the growth cycle of herpes simplex virus was most susceptible to the inhibitory effect of azathymidine and of IUdR (Table 3). The cultures were prepared and inoculated with virus as described above and either azathymidine (2 mmoles/l.) or IUdR (0·06 mmoles/l.) was added at various intervals of time. The inoculated cultures were incubated for a total of 24 h and the virus titre determined by the plaque technique as already described.

Although both these compounds are analogues of thymidine and exerted a similar pattern of inhibition under these conditions it must not be assumed that a similar molecular mechanism of inhibition is involved.

Table 1. EFFECT OF AZATHYMIDINE ON THE REPRODUCTION OF HERPES SIMPLEX VIRUS*

Concentration of azathymidine (mmoles/l.)	Virus titre P.F.U./ml.
None	2×10^8
0·23	2×10^6
0·45	8×10^4
0·90	6×10^4
1·35	3×10^3

* Azathymidine was added 2 h after inoculation of chick embryo fibroblasts with herpes simplex virus.

Table 2. EFFECT OF THYMIDINE ON THE INHIBITION EXERTED BY AZATHYMIDINE ON THE REPRODUCTION OF HERPES SIMPLEX VIRUS

Concentration of thymidine (mmoles/l.)	Virus titre* P.F.U./ml.
None	$5\,5 \times 10^4$
0·0002	7×10^4
0·001	$1{\cdot}5 \times 10^5$
0·005	$8\,0 \times 10^5$
0·025	2×10^7

* Titre of control: $1{\cdot}7 \times 10^7$.
Concentration of azathymidine: 1·65 mmoles/l.

Table 3. EFFECT OF TIME OF ADDITION OF AZATHYMIDINE OR IUdR ON THE REPRODUCTION OF HERPES SIMPLEX VIRUS

Time of addition of analogue (h)	Virus titre* (P.F.U./ml.) Azathymidine (2 mmoles/l.)	IUdR (0·06 mmoles/l.)
4	$2{\cdot}7 \times 10^4$	1×10^2
8	$1\,2 \times 10^5$	1×10^2
12	$2\,6 \times 10^5$	4×10^4
16	$1{\cdot}0 \times 10^6$	$2\,5 \times 10^6$
20	$1{\cdot}3 \times 10^7$	$2{\cdot}3 \times 10^7$

* Control titre: $2{\cdot}4 \times 10^7$.

Table 4. EFFECT OF 5-URACIL METHYL SULPHONE ON THE REPRODUCTION OF HERPES SIMPLEX VIRUS*

Concentration of UMS (mmoles/l.)	Virus titre (P.F.U./ml.)
None	$2{\cdot}4 \times 10^7$
0·026	4×10^6
0·105	$1\,9 \times 10^6$
0·53	1×10^5
1·59	$5\,3 \times 10^4$
5·25	4×10^2†

* UMS was added 2 h after inoculation of a monolayer of chick embryo fibroblasts with the virus.

† On the basis of additional experiments this figure is interpreted as residual input virus.

The results shown in Table 4 indicate that UMS inhibits the formation of infective herpes simplex virus. This analogue is considerably less potent than 5-iodo-2′-deoxyuridine but approximately equal to azathymidine. UMS has no direct inactivating effect on herpes simplex virus particles *in vitro*. The virus (10^6 P.F.U.) was incubated for 1 h at 37° C in the presence or absence of UMS (2·6 mmoles/l.) and treated as already described.

Attempts to reverse or prevent the inhibitory effect of UMS (1·3–2·6 mmoles/l.) with thymidine (0·41–2·05 mmoles/l.), uridine (0·4–2·05 mmoles/l.) or orotic acid (0·64–3·2 mmoles/l.) failed. A similar failure was obtained with combinations of thymidine, uridine, cytidine and deoxycytidine (0·41–2·05 mmoles/l.) or thymidine, deoxyadenosine, deoxycytidine and deoxyguanosine (0·04–0·4 mmoles/l.).

Thus inhibition by UMS of the reproduction of herpes simplex virus is neither the result of failure to form pyrimidine nucleotides by the *de novo* pathway, nor the result of failure to inhibit the conversion of ribonucleotides to deoxyribonucleotides. UMS may be exerting a non-specific alkylation[15] of an induced or cellular sulphydryl enzyme critically involved in the formation of the infectious virus particle.

An anti-viral action of 6-uracil methyl sulphone has been demonstrated, but the failure of many "logical" metabolites either to reverse or to prevent the inhibition makes it difficult to speculate on its mechanism or site of action.

It has been shown that azathymine deoxyribonucleoside, an analogue of thymidine, is an inhibitor of the reproduction of herpes simplex virus and that this inhibition can be completely prevented by thymidine. This analogue appears to affect the formation of the infective virus particle in the same phase of growth as IUdR, but is not so potent an inhibitor. It would be of interest to determine the anti-viral activity of azathymidine under conditions *in vivo*, because it presumably has a higher therapeutic index than IUdR. The very limited availability of azathymidine, however, at present limits investigation.

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SPECTROSCOPIC EVIDENCE OF ALLOSTERIC EFFECTS IN PHOSPHORYLASE B

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It is generally accepted that allosteric effects in enzymes[1] are mediated by conformational changes in the tertiary structure of the protein. Few observations have yet been made, however, of the physico-chemical changes which occur in the enzyme molecule during allosteric activation or inhibition. We decided to work on the problem spectroscopically and chose phosphorylase B (ref. 2) as a suitable object molecule because its active centre is marked by a phosphopyridoxal group with a characteristic absorption band near 330 mμ (ref. 3). Measurements of absorption spectra and ultra-violet dichroism in the region 300–400 mμ showed that this absorption band has a measurable dichroic ratio (0·002) but both absorbance and dichroism are insensitive to the presence of the activator—adenosine monophosphate (AMP), and its inhibitor—adenosine triphosphate (ATP). We therefore concluded that the active form of the enzyme must be a small equilibrium fraction of the protein, even if the substrate is present in excess.

To substantiate this assumption we have made measurements with a differential spectrophotometer (*SF*–4, Milano) with a xenon lamp and a filter with a transmission band between 300 and 400 mμ. Four identical quartz cells (2 cm path) were used, two in each beam. One of the two in the first beam contained phosphorylase B (20 mg/ml.) and cysteine (0·03 M) in glycerophosphate buffer (0·03 M, *p*H 6·8). The second cell contained the allosteric activator, AMP (0·001 M), in the same buffer and 0·01 M glucose-1-phosphate. Two cells were placed in the second beam; in one of them all the components (enzyme, substrate and activator) were mixed together in equal volumes, while the second contained buffer.

We observed a new absorption band near 360 mμ and a decrease of extinction near 330 mμ (Fig. 1). Thus it would seem that some of the protein of the common form (absorption band at 330 mμ) is transformed into a new species with an absorption band at 360 mμ. We found that we could estimate the concentration of this new form

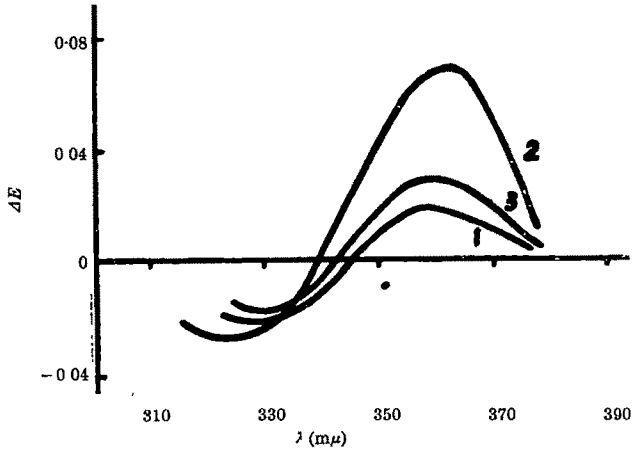


Fig. 1. Differential spectra of phosphorylase B in the presence of substrate glucose-1-phosphate (0 01 M), activator (0 001 M) and inhibitor (0 01 M). (1) Enzyme + adenosine monophosphate, (2) enzyme + adenosine monophosphate + glucose-1-phosphate, (3) enzyme + adenosine monophosphate + glucose-1-phosphate + adenosine triphosphate. The protein was dissolved in 0·03 M glycero-phosphate buffer *p*H 6 8, containing 0 03 M *L*-cysteine. The enzyme concentration was 20 mg/ml.

to within 2 per cent of the total amount of enzyme. Two control experiments were carried out. First, the activator, AMP, was omitted. The protein solution was treated with charcoal. Both beams were symmetrical and the spectrophotometer traced a zero-line. Second, in the absence of AMP, the substrate glucose-1-phosphate was then placed separately from the enzyme in one beam and mixed with the enzyme in the second beam. The extinction was near to the background. Next we omitted the substrate and retained the activator separate from the enzyme in one beam and mixed the two in the second beam. We observed a weak absorption at 360 mμ very near to the background. When we added both substrate and activator and varied the concentration of the protein we found that the intensity of the new absorption band increased linearly. The intensity of the band at 360 mμ does not depend on what order both reagents (substrate and activator) are added to the protein solution.

The action of ATP was found to bring about the most significant spectroscopic changes. When we added equal quantities of 0·01 M ATP to the cell containing enzyme, substrate and AMP in the first beam and to the cell without enzyme (with the AMP solution) in the second beam; we found that the intensity of the spectral band at 360 mμ was reduced 2·5 times. This is exactly what we can expect from kinetic studies[4] for the interaction between the enzyme, the activator and the inhibitor.

The experiment which resulted in the appearance of the 360 mμ band during activation and its disappearance during inhibition was repeated many times with different samples of purified crystalline phosphorylase B. Irreversible inactivation or denaturation of the enzyme causes the effect to vanish. We also found that the magnitude of the effect depends on the presence of a reducing agent (cysteine) in the enzyme solution. This point is a very characteristic feature of phosphorylase B, which loses its activity in the absence of cysteine. From these results we concluded that the band near 360 mμ must be due to a Michaelis complex of the enzyme with the first substrate. This complex cannot lead to the reaction of glucose transfer because the second substrate—glycogen—was not present. If instead of glucose-1-phosphate we took glycogen as the only substrate, we did not find any absorbance near 360 mμ.

To investigate what part of the substrate molecule is participating in the complex, we tried the substrate of the back reaction, for example orthophosphate. When we took 0·04 M phosphate as substrate in the presence of AMP, we observed a differential spectrum (Fig. 2, curve *a*) with the same characteristic band. Thus it seems that the phosphate group is involved in the bond with the pyridoxal moiety. It is well known that glucose is a competitive inhibitor of glycogen phosphorylase. We tried to add glucose (2 mg/ml.) to the mixture of enzyme, AMP and phosphate (Fig. 2, curve *b*). We observed that the 360 mμ band was strongly reduced.

The appearance of the 360 mμ band during the interaction with phosphate is sensitive to the presence of both allosteric activator and inhibitor. Without AMP we obtain a zero line in the spectrophotometer. If we add the inhibitor, ATP (0·01 M), to the same solution (containing phosphorylase, AMP and phosphate), we get the curve *c* in Fig. 2, that is, the background. If we then

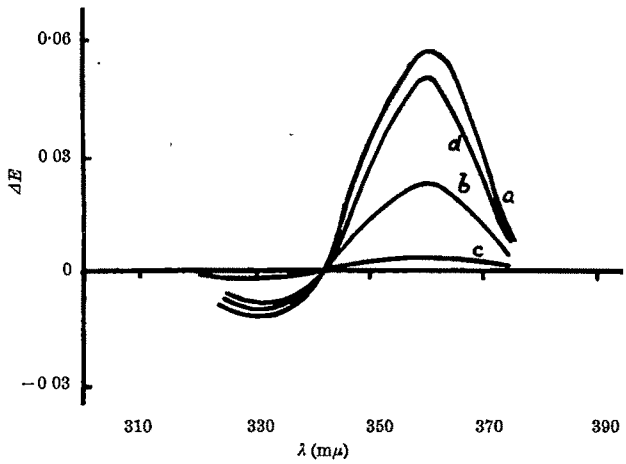
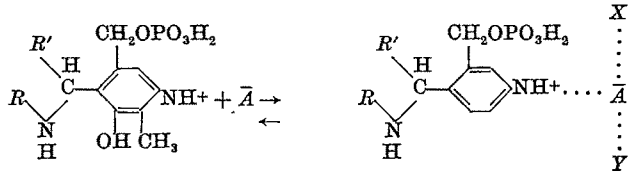


Fig. 2. Differential spectra of phosphorylase B in the presence of inorganic phosphate. (*a*) Enzyme + adenosine monophosphate + phosphate; (*b*) enzyme + adenosine monophosphate + phosphate + glucose; (*c*) enzyme + adenosine monophosphate + phosphate + adenosine triphosphate; (*d*) the same as (*c*) with additional phosphate (0·1 M).

increase the concentration of phosphate to 0·1 M the absorption band appears anew (curve *d*). This is a very characteristic property of allosteric enzymes; they can be inhibited to a certain degree but the effect is overcome by an excess of substrate.

It is well known from kinetic studies that ammonium sulphate is an inhibitor of phosphorylase B. We tried instead of substrate a solution of sulphate (0·1 M). We registered the band near 360 mμ sensitive to the presence of the activator. We suggest that the general explanation of the spectral band near 360 mμ is that a reversible equilibrium exists between the two forms of the prosthetic group (dissociated and undissociated).



Here R, R', X, Y are groups on the protein.
Here A is an anion (either sulphate, phosphate or glucose-phosphate). The undissociated form is the source of the spectral band. The attachment of the anion is specific. For instance the chloride ions do not interact with the enzyme in the presence of AMP. The undissociated compound formed by the reaction of the phosphate or sulphate ion with pyridoxal must, therefore, be stabilized by interactions with adjacent groups on the protein. The band near 350 mμ cannot be explained in terms of simple protonation of the pyridoxal ring. When the *p*H in the cell was kept at 5·5 and in the reference cell at a value of 7, we did not observe the band in question in either the presence or absence of AMP and phosphate. The band appeared at *p*H 6·1. The action of the activator and inhibitor is easily explained by supposing that deformation has occurred on the protein macromolecule, as a result of which some groups on its surface are more or less accessible to interaction with the anion.

In the case of the specific substrate (glucose-1-phosphate) we use a low concentration of the anion (5.10^{-3} – 10^{-2} M) to obtain the same amount of enzyme-anion complex, because of the existence of a special group on the protein for the attachment of the glucose moiety. If we add glucose we get inhibition because the corresponding group is occupied. Even if the anion A is simply phosphate, glucose interferes with its adsorption because of steric hindrance. From our point of view it is easy to explain the data of Hendrick and Fischer[5], who showed that pyridoxal phosphate without the protein does not catalyse the transfer of glucose to glycogen. This accords with the fact that the apoenzyme participates in the binding of glucose-1-phosphate.

In summary we can state that by means of differential spectrophotometry we successfully observed an enzyme-substrate complex with a characteristic band near 360 mμ. This complex exists only in the presence of the allosteric activator AMP and disappears when the inhibitor ATP is added. It is evident that the phosphate ion of the substrate participated in the binding with pyridoxal and we can speculate that it is a compound stabilized by additional bonding of the anion to the adjacent groups of the aboenzyme.

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MIRROR-IMAGES AND THE FOREBRAIN COMMISSURES OF THE MONKEY

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Midline section of the optic chiasma in the monkey and cat restricts the primary visual input of each eye to the hemisphere of the same side. Such a preparation, when trained monocularly to discriminate between pairs of simultaneously presented patterns or objects, shows good interocular transfer of the discrimination[1–3]. In these experiments the stimuli used were pairs of dissimilar objects or patterns, such as + and ○, or ||| and ≡. Note that each of these stimuli is bilaterally symmetrical.

This article describes the results obtained using pairs of stimuli, one of which is a mirror-image of the other, for example ⌈ and ⌉. Three monkeys (*Macaca mulatta*) were used, *M*1, *M*2 and *M*3, with their optic chiasms sectioned in the midline. Each was trained monocularly to discriminate between pairs of solid black objects, except for *M*1 which received training on black planometric stimuli. A food reinforcement was hidden underneath the "correct" object. Usually, thirty trials, twice daily, were presented for 5 days a week. The position of the objects was varied randomly from side to side by the Gellerman sequence[4]. When 90 per cent correct choices on two successive sessions of thirty trials had been reached, the other eye was tested for transfer. For these interocular tests, response to either stimulus was reinforced, except on two occasions when either the negative or the positive stimulus only was reinforced.

The results, in terms of percentage errors over successive sessions, are shown in Fig. 1, and are of transfer trials only. *M*1 was given only one problem. These stimuli were similar on one side but the other sides were mirror-images of each other (Fig. 1, *A*). *M*2 learned to solve problems of mirror-image pairs (Fig. 1, *B* and *D*) and one non-mirror-image pair (Fig. 1, *C*). *M*3 learned to discriminate three pairs of left–right mirror-image problems (Fig. 1, *E*, *G* and *H*) and one pair differing only in an up/down orientation (Fig. 1, *F*).

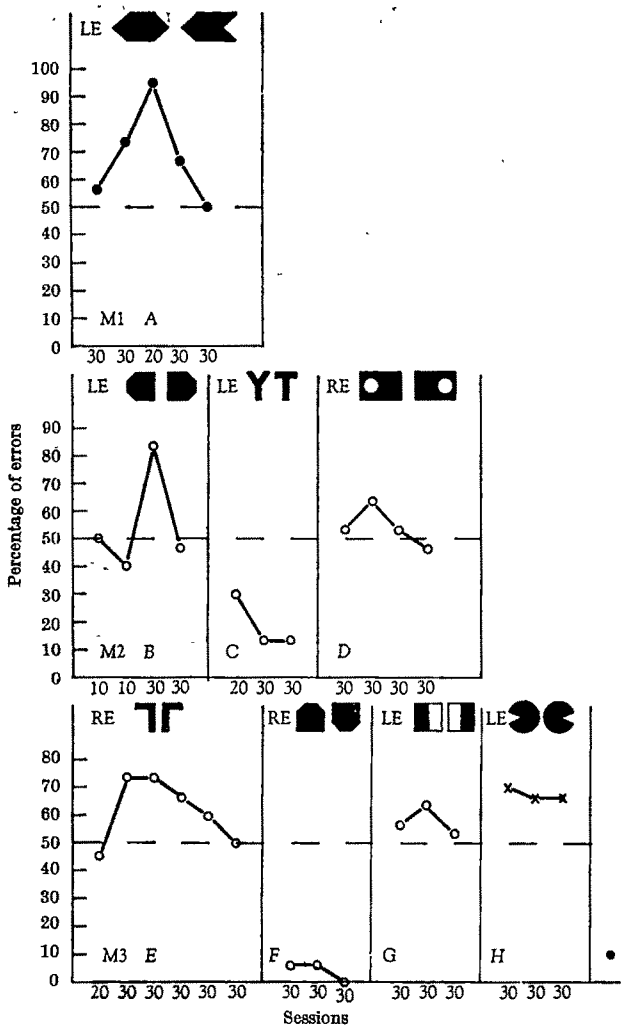


Fig. 1. Interocular transfer tests, percentage responses to the negative, incorrect stimulus, over successive sessions (the numbers refer to the number of trials on that particular session). LE and RE, in upper part of graphs, refer to left or right eye. The stimuli discriminated are shown next to the eye used. *M*1, *M*2 and *M*3 are three monkeys with optic chiasma sectioned. The horizontal dashed line indicates responses at chance level. ●, Responses when positive stimulus only rewarded; ○, responses when both stimuli rewarded; ×, responses when negative stimulus only rewarded.

As had been expected, the non-mirror problem was solved correctly by the untrained eye, although a few errors were made initially (Fig. 1, *C*). Similarly, where the problem differed only in an up/down orientation, there was positive interocular transfer (Fig. 1, *F*). There was, however, no positive transfer with left–right mirror-image pairs. After responding at chance level for twenty or thirty trials, the negative stimulus, not previously rewarded, was selected most frequently. Then after a session or two the responses dropped back to chance level.

Free use of either hand was allowed throughout, except on one occasion; on the first session of the last problem (Fig. 1, *D*) *M*2 was forced to use its non-preferred hand. The previously non-preferred hand was used spontaneously by *M*1 towards the end of the second session and throughout the third session (Fig. 1, *A*). No obvious change in the distribution of the animals' responses resulted from these changes of hand preference.

Apart from the initial chance level responding, the mirror-image of the previously reinforced stimulus was responded to most frequently. In other words, the negative stimulus was treated by the "untrained" eye as if it was the positive one. The performance of *M*1 is particularly convincing in that, on the transfer test, responses to the negative stimulus were never reinforced (Fig. 1, *A*). Yet, in one session of twenty trials only one correct choice was made. The first discrimination by *M*2 and *M*3 shows similar transfer curves but in these cases responses to both the negative and positive stimuli were reinforced (Fig. 1, *B* and *E*). The third discrimination presented to these two animals shows a similar trend, but the magnitude of the effect is less (Fig. 1, *D* and *G*). This probably occurs because the monkeys learn, because of the experience acquired since their first problem, more quickly to discriminate between the situation in which only responses to one stimulus are rewarding and the situation in which responses to both stimuli are rewarding. The latter is an extinction procedure. Compare this with the problem for *M*3, where only the negative stimulus was rewarded (Fig. 1, *H*), in an attempt to enhance the effect. Again, more responses were made to the negative stimulus and, moreover, this procedure eliminated the initial chance level responding.

The drop to chance level after the brief response to the negative stimulus in the first two mirror-image problems of *M*2 and *M*3 can be attributed to reinforcing both stimulus objects (Fig. 1, *B*+*D* and *E*+*G*). That this did not occur with non-mirror stimuli can be explained on the basis that few wrong choices were made, and so there was little opportunity for the animals to learn that both stimulus objects would be rewarding. This extinction procedure was continued for a further number of trials (not shown in Fig. 1) on these animals and responses continued to be at chance level.

It is of interest that mirror-image reversal in interocular transfer has been reported in the unoperated pigeon[5,6]. In this animal, the optic fibres from each eye cross completely in the optic chiasma and terminate in the tectum of the opposite side of the brain. In the monkey with its optic chiasma sectioned, the surviving optic nerve fibres from each eye pass only to its ipsilateral hemisphere. Apart from the hemisphere of termination, the two preparations are similar in having each eye projecting only to one hemisphere.

Midline section of the forebrain commissures in addition to section of the optic chiasma prevents interocular transfer. These commissures must therefore play a part in mirror-image reversal. Fig. 2 illustrates, diagrammatically, a possible explanation. Each primary visual area (striate

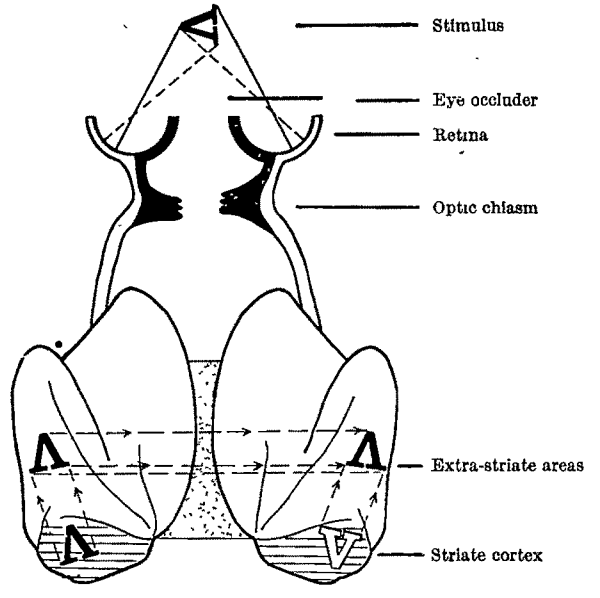


Fig. 2. Diagram to explain mirror-image reversal. With right eye occluded the input to left striate cortex is propagated to extrastriate areas (Λ) and is laterally reversed in crossing the midline via the forebrain commissures (stippled). When the left eye is occluded the input to the right striate area (V) does not match the stimulus (Λ) transposed from the left hemisphere; it does, however, match its mirror-image.

cortex) receives input from half the visual field, the right striate area from the left visual field and the left striate area from the right visual field. The projections of these half-fields are topologically organized so that the striate areas are symmetrical about the midline[7-9]. The midline of the visual field projects to the anterior border of the striate cortex. The representation of the stimulus of Fig. 2 in the striate cortex is such that in one hemisphere its open end is directed towards the anterior border whereas in the other hemisphere it is directed away from the anterior border. Apart from the striate cortex the prestriate and infero-temporal regions (extra-striate areas) are known to be important in the retention or learning of visual pattern discriminations[10,11]. There is no evidence that there are direct connexions between the two striate areas. The extra-striate areas are, however, connected in large part to homologous areas of the other hemisphere by way of the corpus callosum and the anterior commissure. The results of anatomical and physiological investigations emphasize that these connexions are between specifically symmetrical areas, that is, they are homotopic connexions[12-14]. There is some evidence that the topology inherent in the striate cortex is maintained into the extra-striate cortex, at least as far as the prestriate area[7,15]. If this is correct, the representation of a stimulus in these areas will be laterally reversed, because of the homotopic arrangement, in crossing the midline (Fig. 2).

In the monocularly trained monkey, with its optic chiasm sectioned, the stimulus is received by only one striate area and propagated to the extra-striate areas. It is not known whether or not the information passes directly to the other hemisphere or remains lateralized to the side of reception but is available at a later time for reference by the other hemisphere. In either case, if mirror-image pairs of stimuli are used, the information that crosses the midline (Fig. 2, $\wedge$) does not match that which would result from stimulation of the previously unstimulated striate area (Fig. 2, $\vee$) when this hemisphere is presented with the problem. The representation of the positive stimulus in the extra-striate areas of the untrained side matches the presentation of the negative stimulus as made through the untrained eye. Thus the animal shows a preference for the negative stimulus when made to use this representation. Bilaterally symmetrical pairs of stimuli, even though laterally reversed, maintain the same spatial arrangement, consequently the originally positive stimulus is still preferred.

This explanation does not account for the initial interocular transfer responses being at chance level, or for the fact that reinforcing the negative stimulus (Fig. 1, *H*) produces less than 100 per cent responses to the negative stimulus. This is not a regular feature in the interocular transfer of response to non-mirror image stimuli where the response is usually positive and immediate. Since normal monkeys can learn binocularly to discriminate mirror-image problems, it follows that if information crosses the midline during training it must produce a weaker increment to learning than does a direct sensory input, because such problems would never otherwise be learned. Let us assume then, that, in the initial trials of transfer testing, both hemispheres contribute to the response. In the non-mirror image problem, that is, with dissimilar bilaterally symmetrical stimuli, both hemispheres will receive the same information because lateral reversal will not alter their configuration. With mirror-image problems, because of the lateral reversal in crossing the midline, a positive sign is attached to one stimulus in one hemisphere and the opposite sign attached to it in the second hemisphere, although the latter may have a smaller value. As both stimuli will now have both positive and negative reinforcement, no discrimination is possible and chance-level responses will be made.

It has been shown that, in monkeys with unilateral occipital lobectomies, or with one optic tract sectioned, the addition of a unilateral inferotemporal lesion produces a greater deficit in the binocular learning of a visual discrimination if the inferotemporal lesion is on the side opposite the occipital or tract lesion. The more direct route from the striate area to the inferotemporal region, the one on the same side, is the most efficient one[16,17]. This might explain why the negative stimulus is preferred after the initial random responding in the present experiment. The untrained side, which is now receiving input from its own striate area, being the more efficient, becomes dominant, with the consequent preference for the mirror-image stimulus. The fact that even when responses to the negative stimulus are reinforced (Fig. 1, *H*) there are still a number of responses made to the other stimulus, suggests that the hemisphere trained initially exerts some influence for a considerable time. The same operations will apply to non-mirror-image stimuli but in this case they support the original learning.

It follows that monkeys with sectioned optic chiasmas should show positive interocular transfer when trained monocularly if dissimilar, bilaterally symmetrical stimuli are used (○ and +). Pairs of dissimilar, asymmetrical stimuli (F and Ⅎ, F and ⅁, F and ⅃) might show some initial difficulty as both stimuli will be reversed in crossing the midline. It is possible that sufficiently distinctive features will generate stimulus equivalence, for example, the curve in D may be a sufficient cue to evoke or inhibit a response. Stimuli differing only in up/down orientation (▲ and ▼) should transfer normally (as demonstrated) as they are bilaterally symmetrical, or in the case of F and Ⅎ have a distinctive feature—the horizontal line is at the top or bottom. Confusion should be evident only where exact mirror-image pairs of stimuli are used. These various categories of stimulus pairs are now being tested.

The mirror-image phenomenon, on this hypothesis, is only elicited if there is no primary visual input to the striate cortex to override the weaker trans-callosal input during training. This will happen only in animals with sectioned chiasmas. Normal monkeys should show normal, positive, interocular transfer since the primary visual input is relayed to both hemispheres from each eye. The same should apply to animals with only the forebrain commissures sectioned, leaving the optic chiasma intact, since again there is a bilateral input from either eye. A monkey with its optic tract sectioned on one side should also show positive transfer, as only one striate area is being used, irrespective of the eye open. Completely "split-brain" monkeys, with forebrain commissures and the optic chiasma sectioned, should show no interocular transfer of response to mirror-image pairs, or to any other pairs, as no visual information can cross the midline.

Experiments using two intact monkeys, two monkeys with corpus callosum section, one with additional section of the anterior commissure and one monkey with optic tract sectioned unilaterally have confirmed these predictions.

Finally, if in the normal, binocularly trained animal visual information crosses the midline during training, then both the positive and the negative representations of a mirror-image pair will receive both positive and negative reinforcement. This will not be true for non-mirror-image pairs of stimuli. Whether or not this is an explanation for the difficulty of mirror-image problems in normal animals is worth investigating. The often-reported difficulty of discriminating oblique lines (\ and /) as opposed to the ease of discriminating between a horizontal and a vertical line (— and |), where the only difference between the problems is in terms of the orientation of the lines, is a special instance of mirror-image and bilaterally symmetrical pairs[18,19]. It is predicted, from the above theory, that, in binocularly trained monkeys with forebrain commissures sectioned but optic chiasma intact, the difference in the number of trials to learn \ versus / and — versus | will be smaller than in unoperated animals.

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SULPHUR METABOLISM: THE USEFULNESS OF *N*-ETHYLMALEIMIDE

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A SERIOUS problem encountered in the study of the metabolism of sulphur compounds is the extreme reactivity and instability of many of the intermediate compounds[1]. The reaction[2] between sulphydryl groups and *N*-ethylmaleimide (NEM) has been widely used to stabilize thiol compounds in both free[3,4] and protein-bound forms[5]. NEM is not specific for thiols but reacts also with imidazole derivatives and peptides[6]; this lack of specificity can be disregarded if sulphur-35 is used to label the NEM-thiol adducts[7,8]. NEM reacts not only with organic thiols but also with sulphite, thiosulphate, and sulphide[9–11], and the adducts can be separated by paper chromatography[9]. The potential usefulness of NEM for studying sulphur metabolism is thus greater than is generally realized. This article reports on the use of NEM to trap both the organic and inorganic intermediates in sulphate reduction by intact cells of *Escherichia coli*[12].

The scheme at present accepted for the pathway of sulphate reduction in *E. coli* can be summarized as follows[13]:

$$\text{sulphate} \rightarrow \text{sulphite} \rightarrow \text{sulphide} \rightarrow \text{cysteine} \begin{cases} \nearrow \text{glutathione} \\ \searrow \text{methionine} \end{cases}$$

This scheme relies heavily on data derived from mutant and enzyme studies. Although such investigations are valuable, they do not always yield unequivocal conclusions, and confirmatory evidence obtained from feeding experiments with intact cells is desirable[14,15]. The reactions between NEM and the postulated intermediates were therefore investigated.

The kinetics of these reactions were measured by observing the extinction of NEM at 300 mμ, since this extinction disappears when NEM is added to thiols, including sulphide[10]. Fig. 1 compares the effects of sulphite, thiosulphate, sulphide, cysteine and reduced glutathione at *p*H 7·4. The complete loss of extinction produced by equimolar cysteine and glutathione is rapid compared with the loss produced by equimolar sulphite and sulphide, but with the latter compounds the reaction is approaching completion by 5 min. Thiosulphate does not cause any loss of extinction in this time at a *p*H of either 7·4 or 4·5, or if the concentration is increased by a factor of five. The reaction of NEM with sulphite seems to be equimolar because the extinction is reduced by a factor of a half when half the molar quantity of sulphite is added; this stoichiometry suggests that the loss of extinction could be used to assay sulphite in the same way that it has been used to assay sulphydryl groups[16]. The reaction of NEM with sulphide is not equimolar, as previously noted[11]. Similar results were obtained in phosphate buffer dissolved in 70 per cent ethanol, which matches more closely the extraction conditions used in the feeding experiments.

Feeding experiments were performed with cells of *E. coli* A.T.C.C. 9723 grown on a minimal medium as previously described[17], with sulphate as the sulphur source. Cells were washed once with a simplified medium (10 mM glucose, 10 mM ammonium chloride, and 10 mM phosphate buffer, *p*H 7·4) and resuspended in the same simplified medium at a cell density of 3–5 mg dry weight/ml. A simplified medium was used rather than the original minimal medium because there were sufficient traces of sulphate in the components of the latter to reduce the incorporation of sulphur-35 to low levels. The concentrated cell suspension (9 ml.) was placed in a sparging tube and aerated at 20° C for 5 min. Carrier-free ^{35}S-sulphate (1 mc. in 1 ml.) was then added; 0·5 ml. samples of the suspension were removed into 1·5 ml. of either pure ethanol or ethanol containing 20 μmoles NEM. The cells were centrifuged down and 0·5 ml. of the supernatant placed on Whatman 3 *MM* paper. The paper was run in two dimensions as originally described[9]. Fig. 2 shows the autoradiographs obtained from extracts of cells exposed to ^{35}S-sulphate for 1 min. Chromatograms of extracts made in ethanol (Fig. 2*A*) show methionine and its sulphoxide, but those of extracts made in ethanol

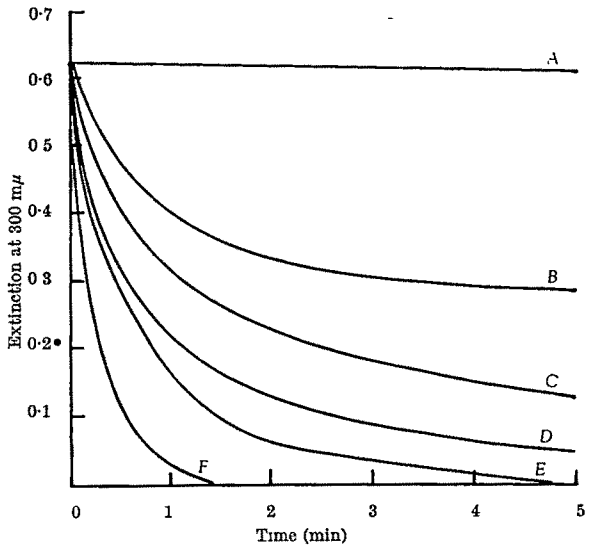


Fig. 1. Reaction of sulphur compounds with NEM. NEM (3 μmoles) was dissolved in phosphate buffer (0·1 M, *p*H 7·4) and placed in a 1 cm silica cell at 20° C. The sodium salts of the sulphur compounds were dissolved in the same buffer immediately before use and added to the cell at time zero (final volume 3 ml.). *A*, Blank cell and also cell containing 3–15 μmoles thiosulphate; *B*, 1·5 μmoles sulphite, *C*, 1 5 μmoles sulphide, *D*, 3 μmoles sulphite; *E*, 3 μmoles sulphide; *F*, 3 μmoles of either cysteine or reduced glutathione. The molar extinction coefficient of NEM at 300 mμ is 620 (ref. 10).

containing NEM show several other labelled compounds in addition (Fig. 2*B*).

The adducts of NEM with sulphite, sulphide and cysteine were identified by co-chromatography with the authentic labelled compounds, while methionine, methionine sulphoxide, and NEM-glutathione were identified by "fingerprinting" the labelled areas with the ninhydrin-positive spots produced by authentic markers. The NEM–cysteine adduct gives a negative reaction with ninhydrin because exposure to the first alkaline solvent causes cyclization to a thiazane derivative[6,18]. The identity of the NEM–sulphite spot was confirmed by two other pieces of evidence: (*a*) the radioactivity coincided with the faint red spot produced when marker NEM–sulphite was run with the extract and developed with excess NEM[9]; (*b*) chromatograms of extracts of *E. coli* mutant 8/6*G* fed with ^{35}S-sulphate revealed a heavily labelled spot in the NEM-sulphite area but no label at all in the NEM–cysteine, NEM–glutathione, or methionine areas. Mutant 8/6*G* fails to grow on sulphate because it lacks sulphite reductase[17], but it will grow on glutathione which derepresses the enzymes of sulphate reduction[13].

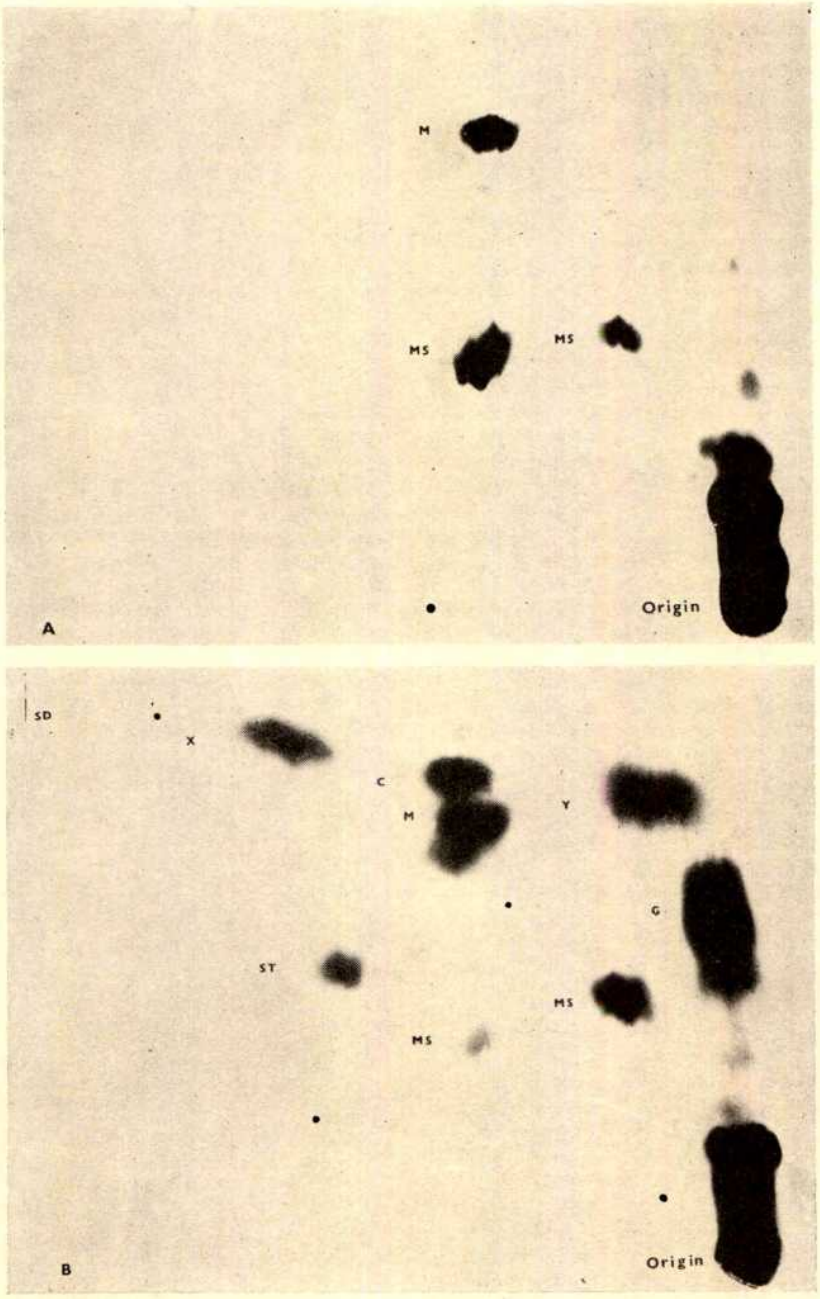


Fig. 2. Chromatography of extracts of *Escherichia coli*. A thick cell suspension of *E. coli* (4·3 mg dry weight/ml.) was fed with ^{35}S-sulphate for 1 min, as described in the text, and extracts chromatographed in ethanol–*tert*. butanol–aqueous ammonia (specific gravity 0·88) –water (300 : 100 : 4 : 75, by volume) in the first dimension (right to left), and in *tert*. butanol–formic acid–water (14 : 3 : 3, by volume) in the second dimension (bottom to top). The dried chromatograms were exposed to 'Kodirex' X-ray film for 5–10 days. *A*, Extract made in ethanol; *B*, extract made in ethanol + NEM. *M*, Methionine; *MS*, methionine sulphoxide; *C*, NEM-cysteine; *G*, NEM-glutathione; *SD*, NEM-sulphide (very faint); *ST*, NEM-sulphite; *X* and *Y*, NEM-unknowns. There are two methionine sulphoxide spots because a variable oxidation of methionine takes place during chromatography in the second solvent (ref. 9). Sulphate stays near the origin and streaks in the second solvent.

Additional evidence to support these identifications was obtained from experiments where cells were exposed to cysteine for 1 min before ^{35}S-sulphate was added. The addition of cysteine causes a rapid inhibition (more than 90 per cent) of sulphate uptake, this probably being the site of control by end-product inhibition[12]. Nevertheless some sulphate still enters the cells under these conditions and its fate can be followed by the NEM technique. Fig. 3 illustrates the chromatographic pattern observed in such experiments; radioactivity is present in the NEM-sulphite and NEM-cysteine areas but none in the methionine or NEM-glutathione areas. This is the result predicted if the above identifications are correct because the small amount of ^{35}S-sulphate taken up can be reduced to cysteine, but the subsequent metabolism of this labelled cysteine to methionine and glutathione will be masked by the large amount of unlabelled cysteine entering the cells. By the same arguments, the occurrence of label in compound NEM-*X* in Fig. 3 would suggest that it is an unknown intermediate between sulphate and cysteine; its identification is being pursued.

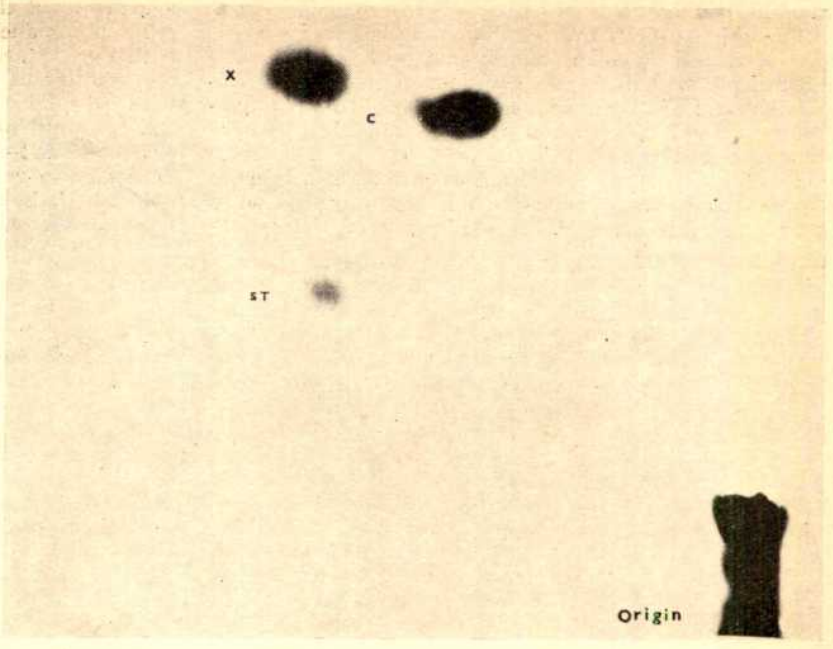


Fig. 3. Effect of cysteine on sulphate reduction. A suspension of *E. coli* was exposed to 0·5 mM L-cysteine for 1 min before a labelling experiment with ^{35}S-sulphate was carried out as described in Fig. 2. Extracts were made in ethanol + NEM 1 min after the addition of ^{35}S-sulphate. Symbols as in Fig. 2.

No radioactivity was detected in any experiment at the position on the chromatogram where NEM-thiosulphate was reported to run[9]. A similar lack of radioactivity was found when mixtures of thiosulphate and ^{14}C–NEM were chromatographed after incubation at *p*H 7·4; an absence of reaction between NEM and thiosulphate is suggested also by Fig. 1. The production of a red colour when mixtures of NEM and thiosulphate are made alkaline[11] suggests that a reaction between them does occur under these conditions, but it is clear that the present method cannot determine whether thiosulphate is an intermediate in sulphate reduction (as has been suggested for *Aspergillus nidulans*[19] but ruled out for *Salmonella typhimurium*[15]).

Table 1. TIME COURSE OF SULPHATE REDUCTION IN *Escherichia coli*

Time	Sulphite	Sulphide	Cysteine	Methionine*	Reduced glutatione	*X*
5 sec	0·24	< 0·02	0·13	0·04	0·03	0·06
10 sec	0·49	0·06	0·68	0·24	0·28	0·28
15 sec	0·58	0·09	1·11	0·51	0·63	0·58
30 sec	0·79	0·14	2·00	2·38	2·49	1·40
1 min	1·15	0·25	4·60	8·25	9·50	1·31
2 min	1·75	0·53	5·0	11·23	19·0	4·04
3 min	2·32	0·80	4·65	11·71	28·0	4·31
5 min	2·67	1·57	3·23	10·14	42·0	5·36

* Includes methionine sulphoxide.

A thick cell suspension (3·8 mg dry weight/ml.) of *E. coli* was fed with ^{35}S-sulphate as described in the text and samples placed in ethanol containing NEM at the times indicated. The figures represent the radioactivity in the various compounds as determined directly on the chromatograms with a large end-window counter, and are expressed as c.p.m. $\times 10^{-3}$.

The time course of sulphate reduction is shown in Table 1. At 5 sec sulphite is the most heavily labelled compound, followed by cysteine; methionine and glutathione are only very slightly labelled at this time. As the time of exposure to ^{35}S-sulphate increases, the radioactivity of all compounds increases. With sulphite, cysteine, methionine, and compound *X*, an approximate steady state is reached at about 2 min, whereas the amount of label in glutathione and sulphide continues to rise. After 5 min glutathione becomes far more heavily labelled than all the other products of sulphate reduction, confirming earlier findings that glutathione is the major soluble sulphur compound in this organism[20]. The data for sulphide can be reconciled with the postulated pathway if it is assumed that as well as being an intermediate between sulphite and cysteine, sulphide is also produced by the desulphurylation of cysteine. The sulphide pool is initially small in comparison with the pool sizes of the other intermediates; this is in strong contrast with the high concentration of sulphide that is required to demonstrate the conversion of sulphide to cysteine by the *in vitro* action of cysteine synthase[13].

These results demonstrate that the NEM technique can detect very small amounts of some unstable sulphur compounds; this technique should prove useful in other studies of sulphur metabolism.

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EFFECT OF DISSEMINATED LUPUS ERYTHEMATOSUS SERA ON THE RESPIRATORY ACTIVITY OF GUINEA-PIG LEUCOCYTES

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Since Hargraves's original description of the lupus erythematosus (LE) cell[1] the morphological changes associated with LE cell formation have been extensively investigated. In spite of refinements in methodology and technique which have increased its usefulness as a diagnostic test[2,3] little is known about the basic cellular alterations which initiate the formation of the LE cell. Godman and Deitch[4] have described the nuclear changes which accompany the formation of LE cells, beginning with the entry of foreign protein into the nucleus of the white blood cell and the loss of ability to stain for nuclear histone. They felt that this foreign protein displaced histone from its association with nuclear DNA. This protein has been shown to be a gamma globulin, presumably LE cell factor itself[5-7]. The altered nucleus is then extruded from the cell as the LE cell body, and afterwards engulfed by viable leucocytes to form the typical LE cell[8].

It seems unlikely that such profound morphological changes could occur without alterations in the basic metabolism of the cell. Conceivably, the formation of LE cells might be initiated by metabolic changes within the cell, mediated by serum containing the LE cell factor. If a serum effect on white blood cell metabolism could be demonstrated *in vitro*, then the possibility exists that a similar mechanism might be operative *in vivo*, perhaps relating to the disease process itself.

For these reasons we have been interested in the possible changes in cellular metabolism which occur when viable white blood cells are incubated with disseminated lupus erythematosus (DLE) serum. Using leucocytes obtained from guinea-pig peritoneal exudates, we have investigated the changes in respiratory activity which occur during incubation with DLE serum, and compared these changes with those induced by normal human serum. The respiratory effects of sera from patients with other disorders of connective tissue and a variety of other illnesses have been tested for comparison, and some of the properties influencing the effect of serum on leucocyte respiration have been described.

Casein-induced peritoneal exudates were obtained by the method of Stahelin *et al.*[9] from 300–400 g guinea-pigs. These exudates contained 80–90 per cent polymorphonuclear leucocytes. The leucocyte concentration was standardized by adjusting the optical density of the leucocyte suspension (wavelength 550 mμ in a Bausch and Lomb 'Spectronic 20'). Rabbit leucocytes were obtained from 4–6 lb. rabbits by the same procedure.

Serum was obtained from patients at the Robert Breck Brigham Hospital, Children's Medical Center, and the New England Baptist Hospital. Diagnoses were established after evaluation[7] of the clinical history, laboratory results, and subsequent course of the disease. Normal human serum was obtained from healthy laboratory personnel. Guinea-pig and rabbit sera were obtained by cardiac puncture. Horse serum was obtained from the Massachusetts State Laboratories, Department of Public Health. Serum not used immediately was stored by freezing or by refrigeration for periods not exceeding 7 days.

Respiratory measurements were carried out using conventional Warburg manometry[10]. One ml. of leucocyte suspension containing 50–90 million cells was added to each Warburg flask. Unless otherwise noted, 0·6 ml. of serum was placed in the sidearm and the total volume in the flask brought to 3·0 ml. with Krebs–Ringer phosphate buffer at *p*H 7·4. 0·2 ml. of 20 per cent potassium hydroxide was placed in the centre well to absorb liberated carbon dioxide. After equilibration, serum and cells were combined and incubation continued at 37° C for 2 h. Oxygen consumption was recorded every 15 min. Two control flasks containing leucocytes in media alone (without serum) were included in each experiment to determine baseline cell respiration.

Experimental tests were carried out on LE cells according to the method of Zimmer and Hargraves[2]. Fluorescent antibody tests were performed by the indirect technique[5] on sections of unfixed human skin. Fluorescent antibody tests on leucocytes were performed after fixation of cells in absolute ethanol.

Normal serum consistently produced a marked reduction in oxygen uptake compared with leucocytes incubated in media alone. For example, after incubation for 60 min, normal serum depressed leucocytic respiration by approximately 60 per cent when compared with leucocytes respiring in the presence of media alone (Fig. 1). No such inhibition could be noted when DLE serum was substituted for normal serum and the respiratory activity of DLE exposed leucocytes was identical to the baseline respiration. This difference in the respiratory effect of DLE and normal serum remained constant over a wide range of leucocyte concentrations (16–114 million cells per flask). It should be noted that no macroscopic or microscopic differences between cells from any of the flasks could be noted after incubation for 60 min. Extended periods of incubation often resulted in macroscopic differences between the cells in the different flasks (probably because of accumulation of acids and insufficient buffering capacity). No concomitant changes in respiratory activity were noted, however. When serum volumes less than 0·6 ml. were used, normal serum showed decreased inhibition, and at 0·2 ml. or below did not depress leucocyte respiration. With serum volumes greater than 0·6 ml. both sera showed increasing ability to depress respiration, but at a given serum concentration DLE serum always caused less respiratory depression than did normal serum.

The respiratory effect of a single serum specimen was reproducible when used in the same concentration in

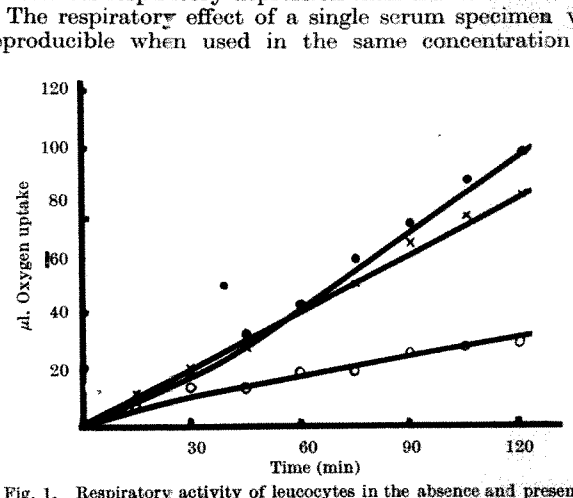


Fig. 1. Respiratory activity of leucocytes in the absence and presence of different sera. ×, Cells in absence of sera; ○, cells in presence of normal human serum; ●, cells in presence of DLE serum; serum concentration, 20 per cent; $9{\cdot}1 \times 10^6$ cells per flask. See text for additional details.

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successive experiments. For example, 0·6 ml. of normal serum used in eight different experiments caused an oxygen uptake varying from 34 to 46 per cent of baseline cell respiration, with an average of 40 per cent and a standard error of 1·5.

Clinical Correlations

132 normal and pathological human sera were tested for their effect on guinea-pig leucocyte respiration. Eleven normal sera had average respirations at 40 per cent of baseline cell respiration, while sera from eighteen patients with active DLE averaged 103 per cent. Sera from patients with rheumatoid arthritis (R.A.) with a positive LE cell preparation, polyarteritis, scleroderma, ulcerative colitis and rubella arthritis have an effect on leucocyte respiration similar to that of active DLE serum.

Sera from patients with typical DLE in remission (inactive), or in patients in whom the clinical picture was atypical but who probably had either DLE or rheumatoid arthritis, or from rheumatoid patients with a negative LE cell preparation, or from patients with other types of joint diseases and, finally, from patients with liver disease, caused significantly more respiratory depression. Thirty-four patients with a variety of non-rheumatic diseases showed respiratory inhibition similar to that of normal serum. These data are shown in Tables 1 and 2.

The 132 sera tested were arbitrarily divided into two groups: those which caused depression of baseline leucocyte respiration (ninety sera, 21–89 per cent), and those which did not (forty-two sera, 90–145 per cent). These two groups were compared with respect to the patients' age and sex and to pertinent laboratory tests, and can be seen in Table 3. The forty-two sera which did not depress baseline leucocyte respiration showed a higher percentage of positive LE cell preparations and fluorescent antibody tests, but in other respects the two groups appeared comparable.

Two separate serum samples were obtained from twelve patients with active DLE, at intervals varying from 1 week to 2 years. These patients were divided into three groups, depending on the activity of the clinical disease during the interval between the first and second serum samples: four patients had no change in their clinical disease activity, four patients showed some improvement, and four patients had become essentially asymptomatic during the interval. The percentile oxygen uptakes of the first and second sera were averaged for each of these three clinical groups. No difference in respiratory activity could be demonstrated between the first and second sera when the disease activity remained unchanged or showed only moderate improvement. When the clinical disease became essentially asymptomatic during the interval, depression of leucocyte respiration appeared in the follow-up sera, a change which can be considered significant ($P < 0.05$).

The eighteen patients with active DLE were receiving many types of therapy at the time their sera were tested, including aspirin, antimalarials, large and small doses of steroids, white blood cell injections, injections of crude nucleoprotein extracts, and no medication. There was no correlation between the type of therapy received and the serum effect on leucocyte respiration.

Table 1. RESPIRATORY ACTIVITY OF LEUCOCYTES IN THE PRESENCE OF SERA FROM DIFFERENT PATIENTS WITH RHEUMATIC DISEASES

Disease	No. tested	Oxygen uptake* (μl.)	P† Normal serum	P Active LE serum
Normal human serum	11	40 ± 4·4	—	< 0·001
Lupus erythematosus				
Active	18	103 ± 4·5	< 0·001	—
Inactive‡	3	81 ± 4·2	< 0·001	< 0·01
Questionable lupus erythematosus				
Questionable rheumatoid arthritis§	5	72 ± 6·1	< 0·001	< 0·001
Rheumatoid arthritis‖, with				
Positive LE cell preparation	10	89 ± 6·2	< 0·001	> 0·1
Negative LE cell preparation	16	70 ± 6·1	< 0·001	< 0·001
Polyarteritis	6	86 ± 7·0	< 0·001	> 0·1
Scleroderma	5	104 ± 7·0	< 0·001	> 0·3
Dermatomyositis				
Active	1	105		
Inactive	1	54		
Apresoline lupus	1	100		
Other joint diseases¶	9	61 ± 4·5	< 0·01	< 0·001

* Percentage of baseline leucocyte respiration at 90 min (baseline respiration set at 100 per cent). The mean and standard error of the mean are given for each group.

† P has the usual significance of a probability value referring to differences between the effect of normal serum and each group of disease sera, calculated using the t test for small samples.

‡ Patients with documented lupus erythematosus in the past (including a positive LE cell preparation) who had reverted to a negative LE cell preparation at the time of testing and had no evidence of disease activity (two of the three also had normal sedimentation rates).

§ Patients whose disease was compatible with either lupus erythematosus or rheumatoid arthritis, but in whom a definite diagnosis could not be made. All had positive LE cell preparations.

‖ Classical or definite disease, according to revised A.R.A. criteria.

¶ These consisted of one case of spondylitis, three of gout, two of degenerative joint disease, one of adult Still's disease, one of Reiter's disease and one of polymyositis.

Table 2. RESPIRATORY ACTIVITY OF LEUCOCYTES IN THE PRESENCE OF DIFFERENT SERA FROM PATIENTS WITH NON-RHEUMATIC DISEASES

Disease	Number tested	Oxygen uptake* (μl.)	P† Normal serum	P Active LE serum
Ulcerative colitis‡	2	101 ± 11·1	< 0·001	> 0·2
Rubella, with arthritis	3	104 ± 3·2	< 0·001	> 0·9
Liver disease§	7	78 ± 5·9	< 0·001	< 0·01
Other medical diseases‖	34	50 ± 2·5	> 0·1	< 0·001

* Percentage of baseline leucocyte respiration at 90 min (baseline respiration set at 100 per cent). The mean and standard error of the mean are given for each group.

† P has the usual connotation as a probability value referring to differences between the effect of normal serum and each group of disease sera, calculated using the t test for small samples.

‡ Without joint disease.

§ Laennec's cirrhosis, 4; post-necrotic cirrhosis, 1; homologous serum jaundice, 1; adenocarcinoma (site unknown) with extensive hepatic metastases, 1.

‖ Acute bacterial pneumonitis, 6; fever of unknown origin, 1; acute viral bronchitis, 1; acute idiopathic pericarditis, 1; acute upper gastrointestinal bleeding (gastric or marginal ulcer), 3; carcinoma, 5; iron deficiency anaemia, 1; polycythaemia (primary and secondary), 3; chronic lymphatic leukaemia, 1; diabetes mellitus, 4; hyperthyroidism, 2; hypothyroidism, 1; acromegaly (active), 1; acute myocardial infarction, 2; hypertension, 4; rheumatic heart disease (inactive), 1; phlebitis, 1; acute renal tubular necrosis, 1; nephritis following renal transplant, 1; pyelonephritis (acute and chronic), 1; chronic glomerulonephritis with nephrotic syndrome, 3; cerebral thrombosis, 1; Parkinson's disease due to arteriosclerosis, 1; asthma (intrinsic and extrinsic), 1. Since many patients had more than one diagnosis, the number of diseases represented (48) exceeds the number of patients tested (34).

Table 3. RELATIONSHIP OF PERTINENT CLINICAL AND LABORATORY DATA TO THE SERUM EFFECT ON LEUCOCYTE RESPIRATION

	Depression of oxygen uptake (21–89%)	No depression of oxygen uptake (90–145%)
Number of patients	90	42
Average of:		
Patients' age	49	44
Sex (per cent female)	65	69
Blood sedimentation index* (mm/min)	0·91	1·17
White blood count	7,900	5,300
Total serum protein (g per cent)	6·8	6·5
Albumin	3·39	3·06
Globulin†	3·36	3·47
Alpha 1	0·36	0·40
Alpha 2	0·76	0·77
Beta	0·84	0·89
Gamma	1·65	1·53
Positive serological tests (per cent):		
LE cell preparation	36	64
Fluorescent antibody	44	73
Latex agglutination (positive serum, euglobulin and inhibition)	65	60

* According to the method of Rourke-Ernstene. Normal range 0·05–0·40 mm/min.

† Determined by paper strip electrophoresis.

Effects of Temperature

The specific respiratory effects of DLE and normal human serum were not altered by refrigeration at 10° C for periods up to 15 days, or by repeated freezing and thawing. When normal serum was heated to 56° C for 30 min, however, the ability to depress baseline leucocyte respiration was completely destroyed. This can be seen in Table 4. Heat did not alter the effects of DLE or guinea-pig serum on leucocyte respiration, but heating rheumatoid arthritis serum (negative LE preparation) caused complete loss of its inhibitory effect. The four heated sera showed no significant statistical differences in their effect on leucocyte respiration. When DLE and normal human serum were heated to 65° C for 30 min, results were identical to those shown for 56° C. The addition of guinea-pig complement to both heated and

Table 4. EFFECT OF HEATED SERA ON THE RESPIRATORY ACTIVITY OF GUINEA-PIG LEUCOCYTES

Addition*	Respiratory activity (%) 100†
—	
Guinea-pig serum	98
Heated guinea-pig serum‡	96
Normal human serum	40
Heated normal human serum	101
RA serum	92
Heated RA serum	110
DLE serum	102
Heated DLE serum	101

* 20 per cent serum added in all cases.
† Oxygen uptake in μl., at 90 min set at 100 per cent.
‡ Heated at 56° C for 30 min.

unheated DLE and normal serum before incubation did not change the results shown.

Heated normal serum did not depress baseline leucocyte respiration; it behaved, in effect, like DLE serum. This similarity in behaviour suggested that heated normal serum might also be capable of forming LE cells. When tested with autologous human leucocytes, however, no LE cells, rosettes, or extracellular material could be demonstrated.

Homologous and Heterologous Sera

The effect of species difference on leucocyte respiration was investigated using normal human, guinea-pig, rabbit and horse sera with both guinea-pig and rabbit leucocytes. Normal human serum caused depression of baseline leucocyte respiration with both guinea-pig and rabbit cells, but none of the other sera showed this effect.

Normal human serum caused a depression or inhibition of the oxygen consumption of guinea-pig leucocytes, while serum from patients with DLE and the other connective tissue diseases did not. This difference in respiratory effect may be explained by postulating (1) the presence of an inhibitory factor in normal serum that is absent or different in DLE serum, (2) a stimulatory factor in DLE serum that is not present in normal serum, or (3) a combination of 1 and 2. The possibility that an inhibitory factor(s) is present in normal serum which is absent, decreased or masked in DLE serum is indicated by the data presented. Certain characteristics of this inhibitory factor(s) emerge from the experimental data. It is labile to heat (destroyed by heating at 56° C for 30 min). It is stable at low temperatures, and resists repeated freezing and thawing. It is inhibitory to both guinea-pig and rabbit leucocytes, but is absent in the other animal sera tested (guinea-pig, rabbit, horse).

The possibility that the inhibitory effect of normal human serum is due simply to an agglutination of the leucocytes caused by an antibody-like substance present in normal human serum and missing in DLE serum is attractive and must be considered. The fact that no early microscopic or macroscopic differences were noted between the leucocytes incubated in the absence and presence of different sera, however, would tend to weaken this line of reasoning; also the fact that no change was found in the respiratory rate of the leucocytes in the presence of normal serum would indicate that death of the cell due to agglutination had not occurred. In addition the factor is destroyed by heating (56° C, 30 min). This would eliminate most of the conventional type of antibodies. This possibility is still attractive, however, and is at present being explored.

Merchant[11] has described the deleterious effects of normal human serum on guinea-pig peritoneal exudate cells. By observing the cells under dark field microscopy, he noted a marked reduction in the various parameters of leucocyte activity during incubation with normal human serum, compared with the effects of guinea-pig serum. Although it is attractive to attempt to correlate these observations with the inhibitory effect of normal serum on leucocyte respiration, it should be noted that Merchant also found that the reduction in leucocyte activity was not limited to human serum but was also present with other heterologous sera, and when leucocytes were incubated in inorganic media alone (Locke's solution). Washed peritoneal exudate cells were used in these experiments which may account for the difference in results with heterologous sera, since our data showed that only human serum was able to inhibit leucocyte respiration.

The respiratory effect of normal serum was simulated by sera from patients with a variety of illnesses including bacterial and viral infections, cancer, blood loss, blood dyscrasias, endocrinopathies, and cardiovascular and renal diseases. The other sera tested all showed significantly less respiratory depression, although inactive DLE, questionable DLE, questionable RA, RA with a negative LE cell preparation, other joint disease and liver disease sera caused more respiratory inhibition than did active DLE serum. RA with a positive LE cell preparation, polyarteritis, scleroderma, active dermatomyositis, apresoline lupus, ulcerative colitis and rubella arthritis all behaved similarly to active DLE serum in that they did not appreciably inhibit leucocyte respiration, and indeed sometimes showed stimulation above baseline respiration. The connective tissue diseases—disseminated lupus erythematosus, rheumatoid arthritis, polyarteritis, scleroderma and dermatomyositis—have been noted to have many common clinical, laboratory and even pathological findings, and stigmata of several of these diseases are sometimes observed in the same patient[12]. It is not surprising that a failure to inhibit leucocyte respiration is shared by serum from patients with these disorders. Apresoline lupus ulcerative colitis and rubella arthritis, although indirectly related, have been noted to show as yet poorly understood similarities to certain of the connective tissue disorders, similarities which the present investigation extends to include the effects of serum on leucocyte respiration.

Another possible mechanism for the difference in the respiratory effects demonstrated by DLE and normal serum can be suggested. The phagocytosis of inert particles is known to require an increase in glycolytic energy and is accompanied by a marked increase in oxygen consumption[13–15]. Thus, the inhibitory factor would be present in both sera, but in DLE serum a stimulatory factor, perhaps an antinuclear factor, could be present and subsequently "phagocytized" and an increased oxygen uptake could thus occur. Some evidence for this line of reasoning is seen where the serum concentration is reduced to 10 per cent. At this level, inhibition with normal serum is absent and an increase in respiration is noted with DLE serum[16]. From these data a hypothesis has been developed which relates lysosomes, aetiology, LE cell and appearance of antinuclear factor in patients with DLE[16,17]. The data presented in this paper are not inconsistent with the proposed hypothesis. In both instances, a stimulation of respiratory activity of leucocytes results when the cells are allowed to incubate in the presence of DLE sera and are compared with those respiring in the presence of normal serum. This stimulation could be "triggered" by the entry of an aetiological agent (viral, bacterial, fungal, etc.) into the cell. This would result in the activation of the lysosomal enzymes (DNase, cathepsins, etc.), which would now be free to attack nuclei and connective tissue components. The former would result in the appearance of LE cells and antinuclear antibodies and the latter would be responsible for some of the clinical manifestations often seen in DLE[16,18]. Further, the hypothesis that collagen diseases may have a basis in hypersensitivity against nucleoproteins being derived primarily from viral, bacterial or animal sources[19] also favours the proposed hypothesis and the concept that a stimulatory factor is present in DLE sera.

The failure to detect any evidence of LE cell formation in our reaction mixtures was not unexpected, as guinea-pig leucocytes from the peripheral blood are known to possess ten times the avidity for LE cell formation that is shown

by human cells[20]. Cells from guinea-pig peritoneal exudate also failed to form LE cells when tested with known positive sera by routine LE cell test procedures. Guinea-pig peritoneal exudate cells have been shown to possess a surface inhibitor substance which can prevent the phagocytosis of inert particles in the absence of serum, an inhibitor which can be removed by washing the cells[21]. Since washed cells were not used in the present experiments, this offers a possible explanation for the failure to demonstrate LE cells in the incubation mixtures.

The significance of these observations for the aetiology and pathogenesis of DLE and the other connective tissue diseases remains speculative. Nevertheless, they offer an avenue of approach to the investigation of possible metabolic abnormalities associated with these diseases.

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RELATIONSHIP OF GLYCOLYTIC AND OXIDATIVE METABOLISM TO PARTICLE ENTRY AND DESTRUCTION IN PHAGOCYTOSING CELLS

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The metabolic activities accompanying phagocytosis by polymorphonuclear neutrophils (PMN) are well known. For example, it has been shown that the uptake of particles is accompanied by a stimulation of oxygen consumption, as well as by increases in aerobic and anaerobic glycolysis, glycogenolysis, flow of glucose through the hexose monophosphate pathway (HMP), lipid turnover[1-4], and formate oxidation[5]. These metabolic alterations occur with a wide variety of particulate materials (inert matter and bacteria) and it has been thought that some of these changes represent an increased demand for energy that is required for the entry of particles. The finding that glycolytic inhibitors (that is, sodium fluoride, and iodoacetate, IAA) inhibit phagocytosis, whereas the respiratory inhibitors (that is, potassium cyanide and antimycin *A*) do not, indicates that the energy requirement for particle uptake is derived from glycolysis[2]. It is clear that phagocytosis is an active process requiring a net expenditure of energy by the phagocytosing cell.

The fate of the ingested particles, especially in relation to the alterations in the metabolism of the phagocyte after particle uptake, has also been considered by some investigators. For example, the increased release of hydrolytic enzymes and/or bactericidal agents from cytoplasmic granules of PMN after phagocytosis has been implicated in the destruction of ingested bacteria[6,7]. On the other hand, Iyer, Islam and Quastel[5] have postulated that the increased production of hydrogen peroxide by the direct oxidation of glucose during phagocytosis by PMN is responsible for the functional bactericidal action. Obviously much has been learned. It should, however, be pointed out that many of these metabolic stimulations are oxidative in nature. Since it is well known that phagocytosis occurs anaerobically[2] the question regarding the function of these oxidative changes becomes important. As yet, the significance of many of these findings and their subsequent correlation with the eventual fate of the ingested material remains unresolved.

The object of this article is to show that the increased metabolic activities associated with phagocytosis may be separated into those events which are involved with entry of the particle and those which are apparently triggered by the particle once it has entered the cell. It will further be shown that these latter events (that is, stimulated metabolic oxidative activities, such as increased oxygen uptake and increased HMP activity) are apparently not involved in particle uptake. This will explain why phagocytosis can occur equally well in aerobic and anaerobic conditions. Finally, a correlation between bactericidal activity and increased oxidative activities of the phagocytosing cells will be shown.

Polymorphonuclear neutrophils were obtained from guinea-pig peritoneal exudate by previously published procedures[2]. Respiration was measured by standard Warburg manometric techniques. Anaerobic conditions were obtained by flushing the reaction flasks for 15 min. with nitrogen. Traces of oxygen present in the commercial nitrogen used were removed by passing the gas over hot copper powder. Lactate estimations were carried out using the method of Barker and Summerson[8]. The production of carbon-14 dioxide was measured as labelled barium carbonate as described by Calvin *et al.*[9]. The bactericidal activities of the cells were measured as described in a previous publication[10]. Cultures of *Staphylococcus albus* and *Escherichia coli* were maintained and used as previously described[10].

Intact cells were incubated in Krebs–Ringer phosphate medium (KRPM). PMN homogenates were prepared by suspending the washed PMN pellet in 0·25 M sucrose and homogenizing the resulting suspension in a motor-driven Potter–Elvehjem homogenizer with a 'Teflon' pestle for 2 min. The homogenate preparations contained a negligible number of whole cells, swollen and disrupted nuclei and intact granules, as observed by phase microscopy. The reaction mixture for the incubation of homogenates consisted of 133 μmoles of sucrose, 30 μmoles of potassium phosphate buffer at *p*H 7·4, 33·3 μmoles of potassium chloride, 6·7 μmoles of magnesium sulphate,

1 μmole of adenosine-5-phosphate, 10 mμmoles of cytochrome-*c* and 40 μmoles of nicotinamide in a total volume of 1 ml. Substrates and/or inhibitors were added when desired.

For the measurement of hydrolytic enzymes, PMN suspended in Krebs–Ringer phosphate medium were incubated in small homogenizer tubes in a Dubnoff metabolic shaker. After incubation, the tubes were plunged in an ice bath and 9 ml. of ice cold medium was added. The cells were separated by light centrifugation (400*g* for 4 min) and homogenized for 1 min in 2·5 ml. of 0·25 M sucrose. An additional portion of 2·5 ml. of 0·25 M sucrose was now added and the suspension was mixed vigorously in a 'Vortex' mixer for 1 min. Portions of this homogenate were taken for the estimation of total enzyme activities and the remaining homogenate suspension was centrifuged in a 'Serval *RC*-III' centrifuge at 18,000*g* for 15 min. Portions of this supernatant were used to assay the enzyme activities in the soluble (cytoplasmic) fraction of the homogenate. The difference between the total and soluble enzyme activities has been shown to reside in the lysosomal granules[6].

Acid and alkaline phosphatase activities were measured by the rate of hydrolysis of *p*-nitrophenylphosphate[11] at two different enzyme concentrations. The incubation mixture for acid phosphatase estimation consisted of 5 μmoles of *p*-nitrophenylphosphate, 22·5 μmoles of citric acid, 2·5 μmoles of hydrochloric acid and 45 μmoles of sodium hydroxide and enzyme preparations in a total volume of 0·5 ml. The final *p*H of the reaction mixture was 4·8. For alkaline phosphatase measurement the reaction mixture consisted of 5 μmoles of *p*-nitrophenylphosphate, 25 μmoles of glycine, 0·25 μmoles of magnesium chloride, 21·3 μmoles of sodium hydroxide and enzyme preparation in a total volume of 0·5 ml. The final *p*H was 10·5. After incubation for 30 min the reaction was stopped by the addition of 2·5 ml. of 0·02 N sodium hydroxide and the yellow colour of *p*-nitrophenol was estimated in a Beckman *DB* spectrophotometer at 410 mμ. β-glucuronidase was estimated by the rate of hydrolysis of phenolphthalein-β-glucuronidate[12]. The incubation mixture consisted of 0·5 μmole of substrate, 28 μmoles of sodium acetate–acetic acid at *p*H 4·5 and enzyme preparation in a total volume of 1 ml. After incubation for 60 min, the reaction was stopped by the addition of 2·5 ml. of 0·5 M glycine–sodium chloride–sodium hydroxide buffer at *p*H 10·4. The liberated phenolphthalein was estimated spectrophotometrically in a Beckman *DB* spectrophotometer at 540 mμ.

Metabolic stimulation is shown only by intact and viable phagocytosing PMN. This is clearly shown by the results presented in Table 1. The addition of particles to PMN homogenates does not significantly change any of the metabolic activities of the homogenates. Intact cells, on the other hand, show striking stimulation of oxidative metabolism. The PMN homogenates used in these experiments possess in a functional state the necessary enzyme systems responsible for the stimulated metabolic activities. This is shown by the fact that when TPN is added to homogenates the stimulation in the oxidation of glucose-1-^{14}C is readily apparent. TPN has been shown to be the rate limiting factor for the operation of the HMP in human leucocyte homogenates[13]. In intact cells TPN does not stimulate the HMP, presumably because of a transport barrier. Finally, it has been shown that sodium fluoride will cause a marked stimulation of respiratory and HMP activities in leucocytes[2]. PMN homogenates do not show these stimulations with sodium fluoride.

It is clear that an intact cell structure is necessary to produce these metabolic changes accompanying phagocytosis. Yet it is not clear whether these changes occur at the same time as particle uptake or whether they occur as a result of an alteration in the properties of the phagocyte. However, Zatti, Rossi and Meneghelli[14] have shown that the stimulation of HMP activity occurs as early as 30 sec. after phagocytosis. In order to investigate this problem, pre-incubation experiments were undertaken using heat killed *S. albus* as a test particle. PMN were incubated with bacteria in 50 ml. glass centrifuge tubes in a Dubnoff metabolic shaker. After incubation for 15 min. the PMN were washed free of extracellular bacteria with 40 ml. of ice cold KRPM and centrifuged at 400*g* for 4 min. This procedure was repeated twice. These washed PMN (95 per cent containing more than twenty-five particles per cell, measured under a light microscope) and also homogenates prepared from them were now incubated with labelled substrates in a Warburg monometric apparatus. Table 2 shows that these washed cells, in spite of the absence of extracellular particles, showed stimulated oxidative metabolism similar to that observed with phagocytosing PMN. It is also of interest to note that homogenates prepared from washed PMN containing particles also have an increased metabolic activity.

Thus, the sustained, stimulated oxidative metabolism of phagocytosing PMN does not appear to depend on the simultaneous act of engulfment. Apparently it occurs as a consequence of particle uptake. On the other hand, it has been shown conclusively that glycolytic activity is involved in particle uptake[2]. Also, it has been suggested that the increased glycolytic activity of phagocytosing PMN causes the increased oxidation of glucose-1-^{14}C by a TPN-linked lactic dehydrogenase[15]. Further, it has

Table 1. EFFECTS OF SODIUM FLUORIDE AND TPN ON THE METABOLISM OF GUINEA-PIG EXUDATE POLYMORPHONUCLEAR NEUTROPHILS AND HOMOGENATES

Additions	Glucose-1-^{14}C				Formate-^{14}C			
	Intact cells		Homogenates		Intact cells		Homogenates	
Particles	−	+	−	+	−	+	−	+
None:								
Q_{O_2} (μl.)	10·9 ± 0·7	29·9 ± 1·1	7·5 ± 0·6	7·1 ± 1·3	11·2 ± 0·6	38·6 ± 0·9	8·1 ± 0·1	8·3 ± 0·1
$^{14}CO_2$ (mμmoles)	38·6 ± 1·6	372·0 ± 7·1	17·1 ± 1·4	18·6 ± 1·0	8·6 ± 0·5	46·0 ± 2·3	10·2 ± 0·5	16·0 ± 2·3
Lactate (μmoles)	3·6 ± 0·4	3·8 ± 0·3	2·2 ± 0·1	2·3 ± 0·1	1·4 ± 0·1	1·6 ± 0·1	1·1 ± 0·1	1·1 ± 0·1
NaF (20 mM)								
Q_{O_2}	41·5 ± 1·8	40·3 ± 2·1	7·3 ± 0·2	7·1*	42·7 ± 0·4	39·6 ± 0·7	8·7 ± 0·4	7·9*
$^{14}CO_2$	31·5 ± 8·6	298·0 ± 13·1	17·2 ± 1·3	18·9*	56·9 ± 3·1	43·9 ± 2·7	13·9 ± 0·7	14·9*
Lactate	0·8 ± 0·1	0·9 ± 0·2	0·6 ± 0·1	0·6*	0·8 ± 0·1	0·8 ± 0·1	0·4 ± 0·1	0·5*
TPN (2 mM)								
Q_{O_2}	10·6 ± 0·6	31·1 ± 2·3	8·1 ± 0·2	8·9*	12·7 ± 1·0	41·6 ± 1·3	8·9 ± 0·2	9·1*
$^{14}CO_2$	41·2 ± 2·3	391·0 ± 12·6	152·0 ± 7·0	167·0*	9·1 ± 0·7	49·7 ± 3·1	21·1 ± 1·1	19·0*
Lactate	3·5 ± 0·3	3·9 ± 0·3	2·1 ± 0·1	2·3*	1·6 ± 0·2	1·8 ± 0·2	1·0 ± 0·1	1·3*

Results are given as the mean and standard error of the mean for four experiments, the mean for two experiments (*), for a 30 min reaction period at 37° C, for 100 γ cellular phosphorus (5·5 × 10^7 cells). Particles used were heat killed *Staphylococcus albus* at a particle : phagocyte ratio of 250 : 1. Initial concentrations of glucose 10 mM and formate 2 mM.

Table 2. EFFECTS OF PRE-INCUBATION WITH PARTICLES ON THE METABOLISM OF INTACT PMN AND PMN HOMOGENATES

PMN pre-incubated for 15 min. with additions	Glucose-1-^{14}C			Formate-^{14}C		
	Q_{O_2} (μl.)	$^{14}CO_2$ (mμmoles)	Lactate (μmoles)	Q_{O_2} (μl.)	$^{14}CO_2$ (mμmoles)	Lactate (μmoles)
None:						
Intact cells	10·8 ± 0·4	41·0 ± 1·5	3·5 ± 0·3	13·8 ± 0·8	9·8 ± 0·6	1·5 ± 0·1
Homogenates	6·9 ± 0·2	18·6 ± 1·0	2·5 ± 0·2	7·7 ± 0·1	15·9 ± 0·4	1·2 ± 0·1
Particles:						
Intact cells	30·0 ± 1·0	351·0 ± 6·8	4·2 ± 0·2	43·2 ± 1·0	49·7 ± 1·2	1·4 ± 0·1
Homogenates	14·7 ± 0·4	92·3 ± 4·5	2·3 ± 0·2	15·6 ± 0·2	47·2 ± 3·6	1·2 ± 0·1
Sodium fluoride (20 mM)						
Intact cells	44·4 ± 1·8	297·0 ± 14·5	0·8 ± 0·1	51·2 ± 5·1	58·1 ± 1·0	0·9 ± 0·1
Homogenates	16·1 ± 0·5	107·6 ± 5·7	0·2 ± 0·1	16·7 ± 0·5	71·8 ± 0·1	0·2 ± 0·1

Results are for 30 min reaction period following pre-incubation: see Table 1 and text for further details.

been shown that sodium fluoride will inhibit glycolysis and phagocytosis and simultaneously increase respiration and carbon-14 dioxide production from glucose-1-^{14}C and formate-^{14}C. This effect of sodium fluoride has not as yet been thoroughly investigated and explained. Experiments have now been carried out to elucidate the interrelationship of glycolysis and oxidative metabolism of PMN during phagocytosis and also to elucidate the apparently peculiar effects of sodium fluoride in PMN metabolism.

Sodium fluoride in increasing concentrations causes an increase in the respiration and glucose-1-^{14}C oxidation of PMN until a plateau is reached at about 30 mM concentration (Fig. 1). These data are similar to the results obtained when increased concentrations of particles are offered for phagocytosis[2]. Degranulation of PMN is also observed with added fluoride (Figs. 3 and 4) as has previously been observed with particles[6,7]. "Popping" of granules with fluoride has previously been alluded to in a statement by Karnovsky during a general discussion[16]. The similarities between the metabolic stimulations of fluoride and particles are further shown by the facts that fluoride does not stimulate the metabolism of PMN homogenates (Table 1) while it does stimulate the metabolic activities of intact cells and homogenates prepared from PMN pre-incubated with fluoride (Tables 1 and 2). These results are similar to those obtained with particles under identical conditions, and it is clear that fluoride stimulates the oxidative metabolism of PMN in the same way as do added particles. From our data, it would appear that this effect can be explained by the magnesium fluoro-phosphate complex formed on the addition

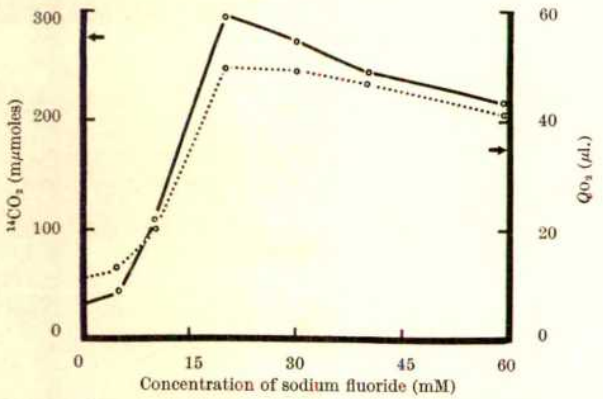


Fig. 1. Effects of sodium fluoride on respiration and glucose-1-^{14}C oxidation by PMN. Results are averages from two experiments for a 30 min. reaction period and 100 γ cellular phosphorus ($5{\cdot}5 \times 10^7$ cells). At concentrations of sodium fluoride higher than 20 mM, an equivalent amount of sodium chloride was removed from the medium to maintain isotonicity. ———, $^{14}CO_2$ produced from glucose-1-^{14}C; — — — —, oxygen consumption. Arrows indicate stimulation observed with polystyrene particles in absence of sodium fluoride.

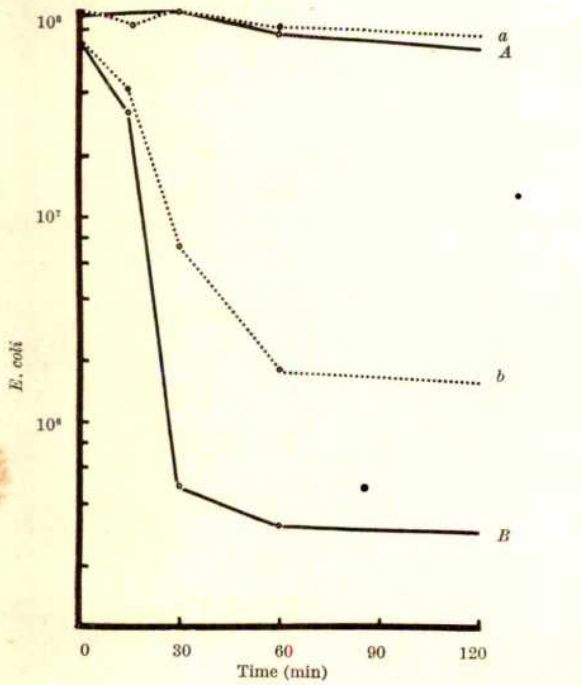


Fig. 2. Bactericidal activity of PMN under anaerobic and aerobic conditions. See text for details. *a* and *A*, Bacterial control under anaerobic and aerobic conditions respectively; *b* and *B*, phagocytic system under anaerobic and aerobic conditions respectively. (*E. coli*: PMN ratio 4:1.)

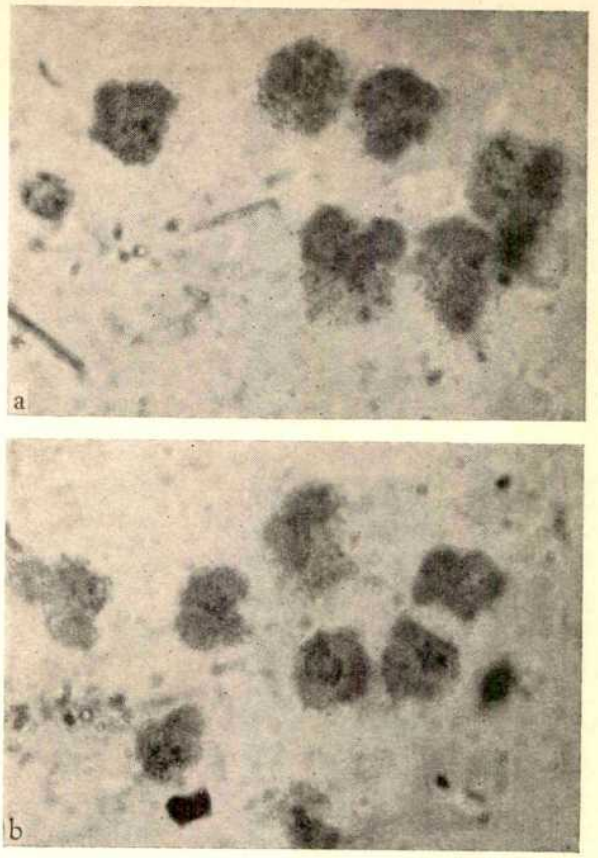


Fig. 3. Degranulation in PMN induced by sodium fluoride. *a*, Control, no fluoride; 15 min incubation period; *b*, 20 mM fluoride; 15 min incubation period.

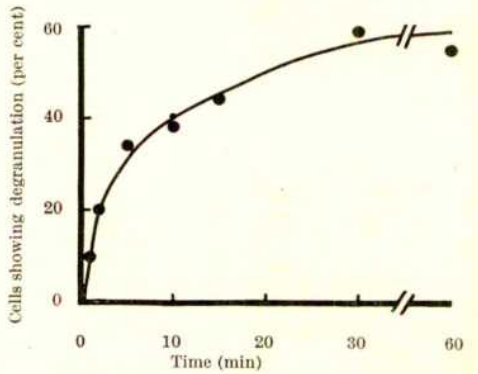


Fig. 4. Time-course investigations on degranulation induced by sodium fluoride. PMN suspensions in KRPM with and without sodium fluoride (20 mM) were incubated in test tubes held in a Dubnoff metabolic shaker at 37°C. Slides were made at the time intervals indicated and stained for 1 min with May-Grunwald (diluted 1 : 10 with 95 per cent ethanol). They were then washed with tap water and counter-stained for 6 min. with 1 : 200 Giemsa. The slides were then washed with tap water, dried in air and examined by light microscopy. Cells showing degranulation as shown in Fig. 3*b* were scored and are represented on the ordinate.

of fluoride to KRPM. The formation of this complex when fluoride is added to phosphate buffer in the presence of magnesium is well established[17]. This precipitate might therefore act as a stimulatory particle. Fluoride is also a glycolytic inhibitor, however, and depresses lactate production considerably and it effectively prevents phagocytosis. It is postulated that fluoride diffuses freely into the cell and forms magnesium fluoro-phosphate inside the cell and the resulting precipitate initiates the observed stimulated metabolic activities. If, however, sodium fluoride is added to magnesium sulphate and phosphate buffer in a test tube and the resulting precipitate is offered to PMN as a test particle, no stimulation of any of the metabolic activities usually seen with phagocytosing cells is observed. This is not unexpected as many insoluble inorganic precipitates (such as barium sulphate and manganese dioxide) have also been shown not to be phagocytosed by PMN[5].

To explore further the hypothesis that the observed increases in oxidative metabolic activities are the result of a stimulation inside the cell, pre-incubation experiments were conducted using IAA as a glycolytic inhibitor. A number of preliminary experiments were carried out by varying the time of pre-incubation and the concentration of IAA to obtain satisfactory conditions to prevent particle entry without causing an undue inhibitory effect on the metabolism of the resting cell. For these experiments, PMN were incubated with IAA in the main chambers of Warburg flasks, and glucose-1-^{14}C along with sodium fluoride or heat-killed *S. albus* were tipped from the side arm of the Warburg flask after the pre-incubation period. The results shown in Table 3 show that pre-incubation for 5 min with 0·5 mM IAA effectively prevents particle entry (as observed by light microscopy) and also inhibits significantly the metabolic stimulations observed with phagocytosis. Under identical conditions, however, sodium fluoride still stimulates respiration and oxidation of glucose-1-^{14}C. It is evident that fluoride diffuses into the cell and forms the magnesium fluoro-phosphate complex inside the cell, which then acts as a phagocytosed particle. Lactate production was inhibited considerably by fluoride, indicating that fluoride ions entered the cell, although the oxidative metabolism was stimulated.

These results not only throw light on the peculiar metabolism of PMN in the presence of fluoride, but they also allow one to draw some conclusions regarding the sequence and interrelationships of oxidative and glycolytic metabolism in phagocytosing cells. Evidence for the involvement of glycolytic energy for the engulfment of particles appears conclusive, since inhibition of the production of lactate by fluoride or IAA effectively inhibits particle entry. Convincing evidence that an increased glycolysis results in a stimulation of the flow of glucose molecules through the HMP, as has previously been suggested[15], is still lacking, however, and is inconsistent with the finding that fluoride stimulates the oxidation of glucose-1-^{14}C while lactate formation is considerably inhibited. These changes in the oxidative metabolism of phagocytosing PMN appear to be the result of changes that have already occurred in the phagocyte owing to the presence of the particle inside the cell. Although viable cells are required for the initiation of these events, neither an intact cellular structure nor simultaneous particle entry is necessary to produce these changes (Tables 1 and 2). The ingested particle might conceivably come into direct contact with the surface of the granules inside the cell, thus stimulating an increased interaction between the reduced pyridine nucleotides and their oxidases. The presence of "swollen" granules in phagocytosing cells has recently been reported by Zatti, Rossi and Meneghelli[14]. Further, these authors have also shown that this morphological change occurs during the period of stimulated metabolic activity.

Table 3. EFFECTS OF PRE-INCUBATION WITH IAA ON THE METABOLISM OF PMN

Additions	QO_2 (μl.)	Lactate (μmoles)	Glucose-1-^{14}C→$^{14}CO_2$ (mμmoles)
Control			
None	11·2	3·4	45·3
Particles	26·9	3·9	335·0
Sodium fluoride (20 mM)	42·0	0·9	296·0
IAA (0·5 mM) pre-incubated (5 min)			
None	8·9	0·5	31·3
Particles	13·0	0·5	32·1
Sodium fluoride (20 mM)	39·6	0·4	243·0

Results are averages of two experiments, for a 30 min reaction period following pre-incubation. For further details see Table 1 and text.

Since the glycolytic activity of PMN is involved in the uptake of particles and since it appears that the changes in oxidative metabolism occur as a result of this uptake, it would be of interest to examine the possible role of the stimulated oxidative changes in the digestion and destruction of phagocytosed particles. It is well known that phagocytosis occurs under anaerobic as well as aerobic conditions, although oxidative metabolism does not occur in the absence of oxygen[2,5,18]. These facts provide a basis for examining the possible relationship between the oxidative metabolic activity and the fate of the ingested material. It is evident from the results presented in Fig. 2 that the bactericidal activity of PMN against *E. coli* is significantly reduced under anaerobic conditions. Uptake of particles under anaerobic conditions was confirmed under the light microscope. Also, Table 3 shows clearly that the increased liberation of lysosomal enzymes associated with the entry of particles (increased activity in soluble fraction from phagocytosing cells) was also found to be comparable to that observed under aerobic conditions. These data would appear to indicate that the increased oxidative metabolism is associated with the higher bactericidal activity observed under aerobic conditions. Further, as lysosomal enzyme activities are increased equally under aerobic and anaerobic conditions, their role as bactericidal agents *in vivo* requires further investigation. The work of Zatti, Rossi and Meneghelli[14] is pertinent to this latter point. These authors have shown that lysosomal degradation is not related to the increased flow of glucose through the hexose monophosphate shunt nor to the increased respiratory activity associated with particle uptake. On the other hand, the increased TPNH oxidase activity associated with phagocytosis has been shown to result in increased formation of hydrogen peroxide which can be effectively prevented by anaerobiosis[5,19]. The increased bactericidal activity observed under aerobic conditions suggests that the role of hydrogen peroxide as a bactericidal agent in PMN requires further investigation. Such experiments are now in progress.

It thus appears that the metabolic activities of phagocytosing cells may be separated into those involved with actual engulfment (glycolytic) and those which are apparently triggered by the particle once it has entered the cell (oxidative). The latter activities would appear to be associated with inactivation and the eventual destruction of the phagocytosed particle.

We thank Miss C. Pilapil for technical assistance, and Mr. George Daynes for taking the photographs.

Table 4. RELEASE OF LYSOSOMAL ENZYMES BY PHAGOCYTOSING PMN UNDER AEROBIC AND ANAEROBIC CONDITIONS

	Aerobic				Anaerobic			
	Resting		Phagocytosing		Resting		Phagocytosing	
	Total	Soluble	Total	Soluble	Total	Soluble	Total	Soluble
Alkaline phosphatase	2·90 ± 0·18	0·16 ± 0·02	3·02 ± 0·05	0·40 ± 0·07	2·71 ± 0·04	0·17 ± 0·01	2·96 ± 0·02	0·39 ± 0·01
Acid phosphatase	2·74 ± 0·05	0·07 ± 0·01	2·77 ± 0·05	0·20 ± 0·04	2·71 ± 0·02	0·08 ± 0·01	2·77 ± 0·03	0·26 ± 0·02
β-Glucuronidase	0·06 ± 0·01	0·02 ± 0·01	0·07 ± 0·01	0·03 ± 0·01	0·07 ± 0·01	0·02 ± 0·01	0·07 ± 0·01	0·03 ± 0·01

Results are averages of four experiments. Enzyme activities are given as μmoles of substrate hydrolysed in 30 min by enzymes extracted from 100 cellular phosphorus (5·5 × 10^7 cells). Soluble fraction contains the enzymes released by lysed granules. For further details see text.

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MECHANISM OF RECURRENT INHIBITION IN THE LATERAL GENICULATE NUCLEUS OF THE RAT

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In a recent communication[1] a neuronal model was proposed to account for the deep and prolonged depression of transmission through the lateral geniculate nucleus (LGN) of the rat after a stimulus to the optic nerve[2]. This model was based on the finding of two types of cells in the LGN: *P* cells, believed to be principal cells projecting to the visual cortex, which constituted 88 per cent of the 255 cells examined; *I* cells, believed to be interneurones, constituting 12 per cent of all cells. Following the stimulus, both types of cell discharge in rhythmical bursts at intervals of about 150 msec. It was suggested that the *I* cells receive an innervation from recurrent collaterals of the *P* cells, responding to a single afferent volley with a burst of about ten action spikes, and synapse back on to the *P* cells, producing there a hyperpolarization (a summation of inhibitory postsynaptic potentials (IPSPs)). The *P* cells would be silenced during the hyperpolarization but would discharge again after about 150 msec as the hyperpolarization waned, perhaps because of an overshoot of potential beyond the resting level. The renewed *P* cell discharge would re-excite the *I* cells and the whole process would be repeated. Connexions between *P* and *I* cells appear to be diffuse. Possibly because of increasing asynchrony between the elements, the recurrent discharges do not continue indefinitely but cease after a few seconds.

The model is essentially similar to that of the spinal motor neurone–Renshaw cell loop[3] and similar models have also been proposed for several other nuclei in the central nervous system[4–8]. This article gives additional evidence in support of the model and suggestions are made concerning some still unexplained features. Recordings were made from single cells in the LGN of the rat, anaesthetized with paraldehyde or pentobarbital, using glass microcapillary electrodes filled with either 3 M potassium chloride or 2 M potassium citrate. Electrical stimuli were applied to the contralateral optic nerve or to the ipsilateral visual cortex.

Extracellular records from *P* cells in response to electrical stimulation of optic nerve show that the spike potential is usually followed by a slow negative wave. Although this is opposite in polarity to the field slow waves recorded in the ventro-basal nucleus of the thalamus by Andersen, Brooks, Eccles and Sears[9], there is little doubt that the two sorts of slow wave are homologous and accordingly, following the terminology of these authors, we refer to them as *P*-waves. The difference in polarity will be discussed later. The *P*-wave has been studied in extracellular records from forty-one *P* cells and the related intracellular potential changes in twenty-four *P* cells.

Fig. 1*A* shows a series of extracellular records from a *P* cell on successively slower time bases. The diphasic positive–negative spike potential is immediately followed by the negative *P*-wave, which commences during the negative phase of the spike. The time course of the *P*-wave is slightly longer than 100 msec and is then followed by a series of *P*-waves at intervals averaging about 150 msec and continuing for more than 1·5 sec. The later *P*-waves are always smaller than the initial wave and vary in amplitude, duration and interval. There is a rough rhythmicity in their occurrence, however, and their time course is not greatly different. An injury discharge in this cell (*A* potentials only) was suppressed during the *P*-wave.

Impalement of a cell always caused considerable damage; resting potentials were never more than 40 mV. The spike potential was usually lost within a few seconds and all responses were often lost within a few minutes; nevertheless, considerable information about the in-

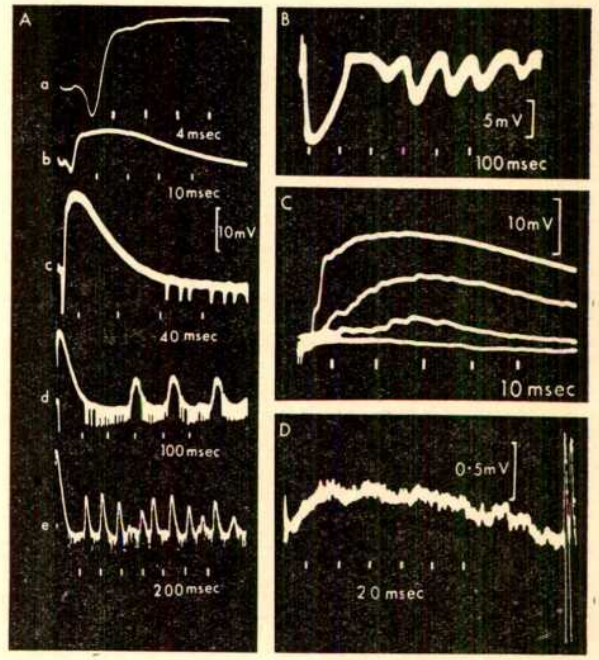


Fig. 1. Records from *P* cells in response to stimulus to optic nerve. Stimulus at commencement of each sweep. *A*, Extracellular. Negative upwards. Same strength of stimulus but successively slower sweeps. Spontaneous discharge of *A* spikes. *B*, Intracellular. Resting potential 30 mV. Positive upwards. Potassium citrate electrode. *C*, Intracellular. Resting potential at these records not recorded. Positive upwards. Superimposed successive depolarizing IPSP waves in response to a single optic nerve stimulus. Sweep for large response commences with stimulus; other sweeps are triggered by onset of IPSP wave. *D*, Extracellular. Negative upwards. Note that the negative *P*-wave is not preceded by a spike but is followed by spikes with good negative phase.

hibitory process was obtained during this time. The spike or excitatory postsynaptic potential (EPSP) is at first followed by a slow wave of hyperpolarization. This wave has a peak at about 20 msec, a duration of about 150 msec, and is followed by a series of waves of like form (Fig. 1*B*). There is no doubt that these are the intracellular counterparts of the *P*-waves and hence are referred to as IPSP waves[7]. If a potassium chloride electrode was used, the IPSP wave gradually became smaller, then reversed to give a wave of depolarization. If a citrate electrode was used there was no reversal (Fig. 1*B*). Evidently the ionic mechanisms underlying this potential change are similar to those at other postsynaptic inhibitory synapses[10]. Neither the *P*-waves nor the IPSP waves depend on the occurrence of a spike potential in the cell (Fig. 1*B–D*). Similar IPSP waves have been observed in cat LGN[11].

A similar series of slow waves may be obtained by cortical stimulation and all the other observations reported here are likewise reproducible. *I* cells can be reactivated by a second stimulus to the cortex shortly after the termination of the first discharge, that is at about 30 msec. Correspondingly, a second cortical stimulus prolongs a *P*-wave or IPSP wave. No slow waves were recorded from *I* cells. When *P* cells were tested with two cortical stimuli, in most cases they would not fire again until 130 msec or more had elapsed. The hyperpolarization is apparently sufficiently intense to prevent invasion of even the initial segment of the cell.

Fig. 2 shows the discharge of an *I* cell in response to two optic nerve stimuli (*a*, *b*). On each occasion the cell responded with nine bursts. These were recorded on moving film, each burst triggering the sweep, so that the vertical distance gives the interval between successive bursts. There is a variation in duration of burst, in pattern of discharge within the burst, and in interval between bursts. The first burst always has the highest frequency of discharge, however, and the intervals between bursts are comparable to those observed between *P*-waves or IPSP waves in a *P* cell. A high frequency of discharge and good synchronization between the first bursts of many *I* cells could account for the abrupt onset of the first *P*-wave or IPSP wave, as well as for its larger size (Fig. 1). Fig. 2 also shows that an *I* cell may discharge again after a longer interval than usual following a short discharge. Since its own short discharge would probably produce no rebound excitation in a *P* cell, the pause in the *I* cell discharge suggests a reciprocal innervation between a *P* cell and a given *I* cell. But the diffuseness of the system evidently enables one cell to pick up the rhythm from other cells and thus the discharges resume.

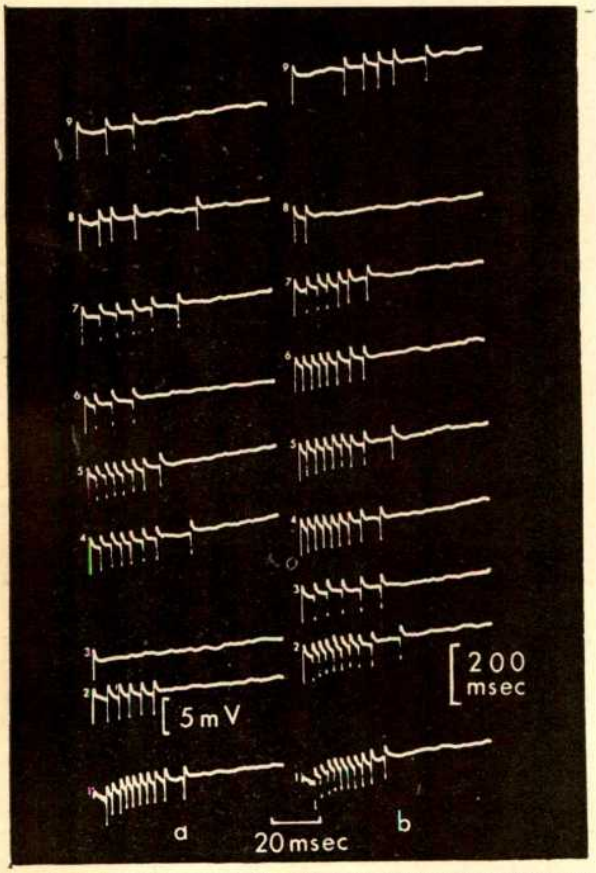


Fig. 2. Extracellular records from an *I* cell in response to two optic nerve stimuli (*a*, *b*). Each response consisted of nine bursts, photographed on slowly moving film (vertical calibration). Negative upwards. Responses commence at bottom of figure.

Rippling was observed in a few cells on the IPSP wave; the best example is shown in Fig. 1*C*. A potassium chloride electrode was used in this cell and at the time these records were obtained the IPSP wave had become depolarizing. The superimposed records are successive IPSP waves resulting from a single stimulus. The first wave (which is also the largest) commences with an EPSP of about 7 mV and is followed by an IPSP wave with a large initial step. The EPSP is absent in the later waves and the onset of the IPSP is much less abrupt.

Why is rippling not seen more frequently and why is it never seen in the LGN of the cat[11] nor in the ventro-basal nucleus of the thalamus[7] where analogous circuits apparently exist? Rippling might not be detectable if a large number of *I* cells converged on to each *P* cell and if their discharges were not precisely synchronous. In the rat LGN the amount of convergence is possibly less than in cat thalamic nuclei. In many records (such as Fig. 1*A*) there is a suggestion that the amplitudes of successive *P* waves are graded in a quantal fashion. That this is not exactly so is not surprising considering the variation in the successive discharges of an *I* cell (Fig. 2). It is not improbable, however, that the third largest wave in Fig. 1*C* results from a burst discharge of a single *I* cell; the pattern of rippling closely resembles the pattern of firing in the fifth burst in Fig. 2*a* (from another animal). The smallest wave in Fig. 1*C* seems to consist of only one IPSP such as would result from a single spike in an *I* cell (for example, the third burst in Fig. 2*a*). In the larger waves the rippling is faster and less obvious, as would be expected if more *I* cells were involved. The rippling would also be less obvious the closer the potential came towards the inhibitory equilibrium potential. Although we have insufficient data to make accurate estimates, a rough calculation will indicate the order of magnitude of the cross-innervation. It would not be unreasonable to suppose that about six *I* cells projected to one *P* cell. Since there are about eight *P* cells for every *I* cell, then each *I* cell would innervate on the average about fifty *P* cells.

An important point of difference between the rat LGN and thalamic nuclei in the cat (including the LGN) is that field slow waves are virtually absent in the rat. The *P*-waves described here are related to a single cell and disappear when the cell is lost or destroyed. In all except one of the forty-one *P* cells the extracellular slow wave was negative. This might have been because of injury by the electrode, but the same polarity was observed at a time when the diphasic spike potential also had a good negative phase (Fig. 1*D*), indicating that the electrode had not damaged the cell. Since most of the recordings are likely to be from the soma this result means that all or most of the inhibitory synapses are not on the soma, but on dendrites or possibly on the axon hillock. The single recording in which the *P*-wave was positive might have been from one of these sites. The field *P*-waves in the thalamus of the cat result from the flow of current from somas to axons and are therefore positive. These waves may very well obscure the single-cell *P*-wave. The reason for the insignificance of the field slow waves in the rat LGN may be the smallness of the structure.

This work was supported by a grant from the National Health and Medical Research Council of Australia.

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OCULOMOTOR FUNCTION AND THE ALPHA ACTIVATION CYCLE

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FOR many years the electroencephalogram (EEG) has been regarded as a useful, if somewhat crude, indicator of cerebral processes occurring in humans. In the detection of tumours, for example, certain electroencephalographic atypicalities can assist the skilled diagnostician in evaluating both their site and severity. Less reliably, modification of the occipital "alpha rhythm" has been used extensively as an index of such variables as the presence or absence of "attention" on the part of the subject. Recently one of us (T. M.) has been studying the ability of individual subjects to modify their own electroencephalograms at will. This has been achieved by setting up a "closed-loop" between the EEG and the activating stimulus—the subject "intervening", as it were, within the loop. A detailed description of the EEG-stimulus loop is given elsewhere[1], but the principle may be simply summarized here. The loop is effected by supplying the subject with information as to the presence or absence of recorded alpha rhythms from his own brain—this may be done by arranging for a predetermined persistence of the alpha rhythm to switch on a light in front of the subject or to sound a tone which continues as long as "alpha", according to the agreed criterion, is present. In such circumstances many subjects can give rather dramatic demonstrations of their ability to modify their own alpha-rhythm by "switching on" the light, tone, etc., on request[2,3]. In experiments recently performed in the Autonomics Division of the National Physical Laboratory, Teddington, a number of subjects were tested in this type of situation, one of whom (R. R.) became remarkably skilful at controlling the loop. In Fig. 1 we give examples from an experiment where this subject alternated between "alert" and "relaxed" states. The task was complicated by requiring the subject to keep his eyes open throughout the experiment and by coupling a light into the loop; the light was turned on when alpha rhythms occurred on the EEG and turned off when it ceased. Despite these somewhat stringent conditions it was clear that the subject was able to alternate reliably between the two states, and was also able to remain in any one state for a number of minutes.

In other experiments, the loop was set up so that a meter pointer moved to the right when alpha rhythms occurred and to the left when no alpha rhythms were present. The subject R. R., however, learned to regulate the needle so that it moved within a narrow range around the centre position—achieved by a rapid alternation of "alpha"–"no-alpha" states—with his eyes open for the whole time. In Fig. 2 we give a sample record taken at a time when the subject, with eyes open, was attempting to keep the needle at the midpoint.

The quite unexpected efficiency of this subject and others, in modifying their own alpha rhythm "at will", raises interesting questions about the nature of the variable they were controlling. For a number of reasons it now seems likely that the voluntary regulation of the EEG in a closed visual feedback loop was accomplished by voluntary regulations of oculomotor functions. For example, the performance of subject R. R. is reminiscent of that reported by Durrup and Fessard[4]. Their subject was able alternately to increase or decrease the amount of alpha in his EEG by "shifting his attention" alternately from visual to auditory stimuli. They reported that he also controlled the occurrence of EEG alpha by regulating visual accommodation, convergence and point of fixation. Likewise, our own subject R. R. reported that when attempting to move the meter pointer to one side, he would "look into the distance" with an indefinite fixation

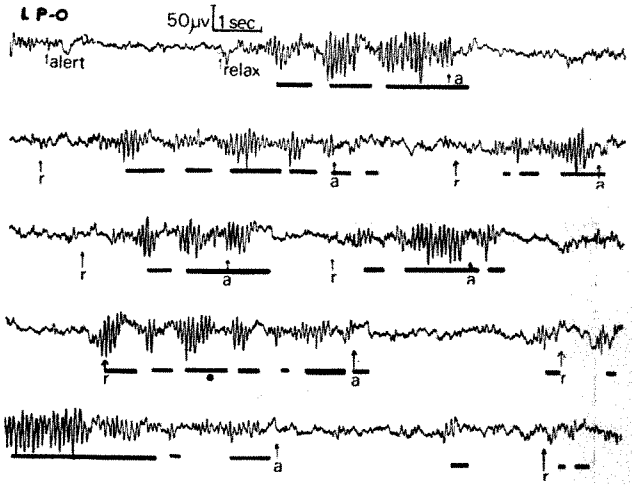


Fig. 1. Record of EEG taken when subject alternated on command between "alert" and "relaxed" states. Eyes were open throughout, and heavy bars indicate periods when the presence of alpha caused a bright light to be switched on.

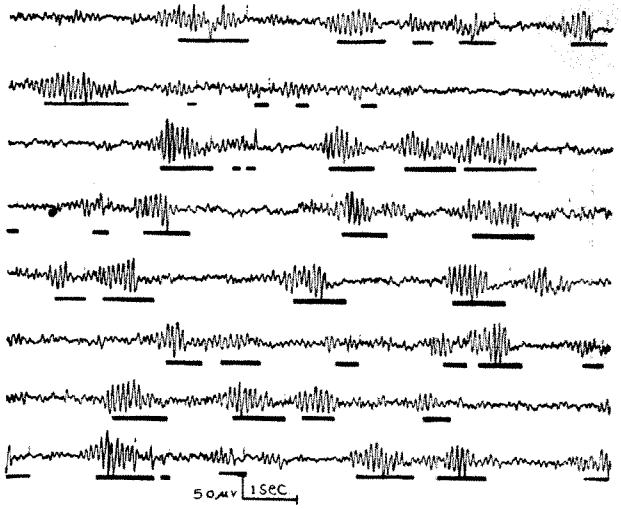


Fig. 2. Rapid alternation of "alpha–no alpha" as subject attempted to keep a meter needle at midpoint. Needle moved to right of dial when alpha occurred, and to left when alpha was not present. Heavy bars indicate periods of alpha as determined by feedback-loop analyser.

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point producing a blurred image of the pointer. Under these conditions, alpha rhythms were produced. To suppress alpha rhythms, he would bring the pointer into clear vision. It is evident that the concept of "attention" does not help much in understanding these results, for the subject is "attending" to his task (keeping the meter needle central) continuously.

Is the important variable the presence or absence of a clear, sharp image on the retina? In a recent paper describing tests on two subjects[5] we showed that alpha can be produced by the simple process of elevating the eyes, whether open or closed. Quite unusual bursts of alpha can be observed when these subjects roll their eyes back, and this may be sustained even when a clear pattern in the form of an after-image is present. In Fig. 3, EEG records from these two subjects are presented. Eyes were closed throughout both records. When the eyes were elevated and alpha occurred (subject T. M., top part of Fig. 3) the visual field was flooded with a homogeneous, very bright light. Despite this, alpha was abundant. In the lower part of the Fig. 3 the subject (R. R.) was observing a visual after-image with his eyes elevated (*U*) and with his eyes straight ahead (*A*). More alpha occurred during the "eyes up" condition, despite the fact that patterned stimulation was the same in each condition.

Repetition of these experiments on other subjects confirmed these earlier findings in all essentials. (There may, however, be occasional variations—for example, subjects may consistently show more alpha when eyes are elevated, and then may suddenly show a reversal of this effect.) In

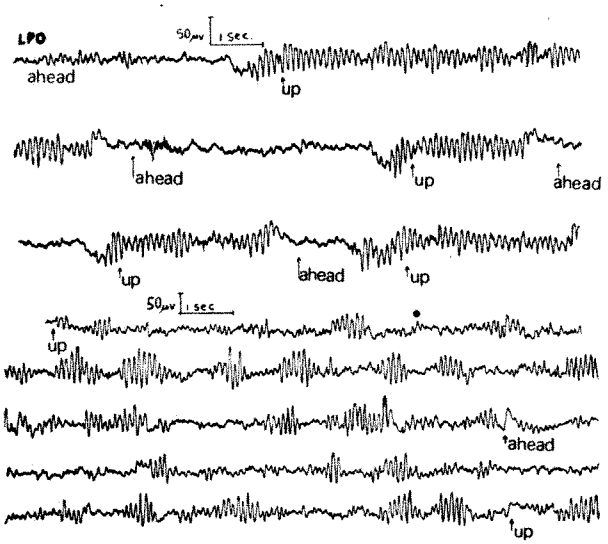


Fig. 3. Increase of occurrence of alpha with two subjects when eyes were rolled up. In top record presence of alpha switched on bright light. On lower record eyes were closed, but subject was viewing a bright patterned after-image with eyes closed.

one experiment, in which eight naïve subjects were tested, the amount of alpha rhythm was quantified by filtering the alpha component and integrating the filter output. With eyes closed, five subjects showed more alpha with eyes elevated. With eyes open, one subject only initially showed more alpha with eyes elevated, but in successive trials, the number who showed the effect with open eyes rose to five. It should be mentioned that in an early experiment, a power spectrum of the EEG was obtained from subject T. M. during two conditions; eyes closed and not elevated, and eyes open and elevated. Abundant alpha occurred during both conditions and it was found that the power spectrum obtained with eyes elevated did not differ from that obtained when eyes were not elevated.

These results show that oculomotor function may be dynamically integrated with those cortical or subcortical processes involved in the EEG alpha-activation cycle. Changing the optimum oculomotor configuration may change the response of the EEG to stimulation.

As we have stated here, the concept of "attention" in relation to the alpha-activation cycle is not particularly useful in the interpretation of these results. A search of most of the literature concerning human "attention" and the EEG does not reveal any experiment in which oculomotor functions have been excluded as a cause of the EEG phenomenon supposed to be associated with "attention". Attention, is, in fact, simply assumed to be the relevant variable. However, the results we have reported can be explained in terms of the hypothesis that the alpha-activation cycle reflects the movements, accommodation and position of the eyes. In sum, such a hypothesis would imply that the electroencephalographic and oculomotor components of the orienting reponse are not merely coincidental and independent[6], but are so dynamically linked that oculomotor processes can modify the EEG response to stimulation. From this point of view (which will need to be considered in all future experiments linking "psychological" variables with the EEG) if there is any affect of "attention", it is on oculomotor functions which in turn cause a modification of the alpha-activation cycle.

Since this article was prepared our attention has been drawn to the unusual EEG records taken from astronaut Frank Borman during the flight of the *Gemini* satellite *GT*-7. These show increases in alpha rhythm with weightlessness. This finding might be explained more simply on the basis of an oculomotor imbalance than by changes in "attention" or "arousal". We thank Dr. W. Ross Adey for allowing us to see a draft of his analysis of these results before publication.

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SPERMATOPHORES AND SPERMATOPHORIC REACTION IN THE GIANT OCTOPUS OF THE NORTH PACIFIC, *Octopus dofleini martini*

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In octopus as in other cephalopod molluscs, the spermatozoa are enclosed in spermatophores before they are transferred from the male to the female. At the time of mating the males take up the sperm-packed spermatophores with their hectocotylized arm and place them in the female. In the small species, as for example *Octopus vulgaris*, the spermatophores seldom exceed in length a few centimetres, but in some of the larger species, such as *Octopus hongkongensis* or *Octopus dofleini*, they are reputed to measure a metre or more. The present study concerns the spermatophores of *Octopus dofleini*, a species which, according to Pickford[1], exists in the form of at least three subspecies, namely *O.d. dofleini* (Wülker, emend. Sasaki) in Japan, *O.d. apollyon* (Berry) in the far North Pacific, and *O.d. martini*, which inhabits the more temperate waters of the North Pacific, along the coast of Washington State. It is the latter subspecies which we have studied and on which we wish to report briefly. The males which

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we examined ranged in weight from 10 to 47 kg, and were all caught in the Puget Sound—San Juan Archipelago area. Soon after an animal was taken in a trap, it was brought by seaplane to the laboratory at Seattle; there it was transferred to a large glass-panelled observation tank, filled with constantly aerated sea-water maintained at 8°–10° C. In these tanks the animals survived well for periods of many weeks or months.

To obtain a spermatophore, the animal was removed from the observation tank, and placed in a tub filled with cold sea-water where it was anaesthetized by slowly adding ethanol until a final concentration of 2 per cent was attained. When the animal became less mobile, and the muscles of the mantle more relaxed, it was possible to reach into the mantle cavity and to extract manually from the orifice of the penis a whole intact spermatophore. The animal was then either returned to the observation tank and allowed to recover, or killed if further spermatophores were required. Usually fewer than 10 spermatophores were found. In one male, however, suffering from occlusion of the terminal portion of the tract thirty-two spermatophores were present.

Male Reproductive Tract

In *Octopus dofleini* the male reproductive tract (Fig. 1) lies enclosed in a membranous capsule, the "genital bag", which occupies a large portion of the posterior region of the mantle cavity. The total weight of the reproductive tract is about 1 kg, of which one-fifth or so is taken up by the testis, a single, oval, porcelain-white gland, located in the posterior compartment of the genital bag. The remainder of the tract consists of the various accessory organs, which lie in the anterior compartment of the genital bag. In their general appearance these organs fit into the pattern described by Winkler and Ashley[2] in the animal presumed to be *Octopus apollyon* (Berry). Though much bigger and differing in shape from those of *Octopus vulgaris*, the accessory organs of *Octopus dofleini martini* tend to conform to the general distribution pattern characteristic of the common octopus[3–12].

When leaving the testis, the spermatozoa, not yet encased, pass first through the membranous, funnel-shaped ampulla into the long convoluted vas deferens proximale which in its gross appearance resembles the epididymis. Emerging from the vas the spermatozoa enter first one, and then another, system of tubular glands, each, when uncoiled, about one metre long; these two organs correspond roughly to the vesicula seminalis and prostata of the common octopus. In these two organs, perhaps best designated as the spermatophoric gland systems I and II, the spermatozoa are enfolded by the glandular mucilaginous secretions into tube-shaped membranes, and ultimately encased into the spermatophores. As it leaves these glands, each spermatophore traverses the vas deferens distale and enters the capacious spindle-shaped storage organ, known as the spermatophoric sac, or Needham's sac, so named after John Tuberville Needham[13], who in 1745 first described in detail the "milt-sac" of the "calamary" (squid). Later, the spermatophores (once called "Needhamia expulsatoria") enter singly the terminal spermatophoric duct, which penetrates the membranes of the genital bag and forms two tubes of unequal length: the longer and loop-shaped branch, named diverticulum, occupies a position closely adjacent to the genital bag, while the other shorter and straight branch, named terminal organ or "penis", hangs free outside the genital bag. During copulation a spermatophore is taken from the orifice of the "penis" and transferred to the female by the copulatory organ proper, that is the hectocotylized arm, which is the modified third right arm of the male.

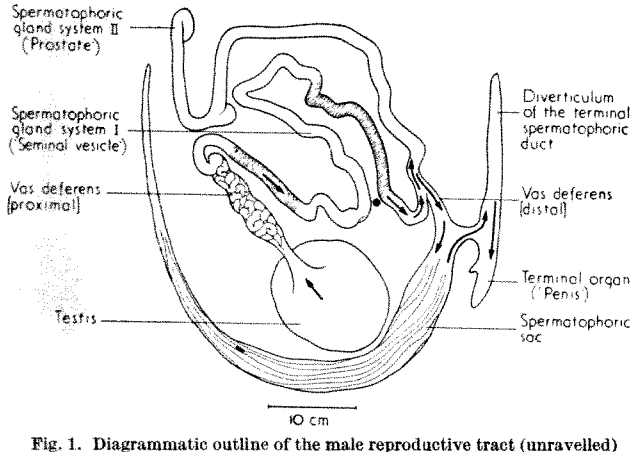


Fig. 1. Diagrammatic outline of the male reproductive tract (unravelled) of *Octopus dofleini martini*; arrows indicate the direction in which the spermatophores traverse the tract.

The Spermatophore

A complete spermatophore, freshly pulled out of the orifice of the "penis", is shown in Fig. 2. It is composed of two parts, the body and the cap thread (best seen in Fig. 3). The body, usually about a metre long, has the appearance of a slender but turgid cylinder, the turgidity being due to the tough, transparent covering tunic which is under considerable tension, so that when it is punctured the sperm contents of the spermatophore are explosively discharged. The body is much wider at one end, and consists essentially of two parts, approximately equal in length. The thicker portion, which at its round end reaches a diameter of 0·5–1 cm, emerges first from the orifice, and it might best therefore be referred to as the "proximal" portion. In terminology applied to the spermatophores of *Octopus vulgaris* and *Loligo pealii*, it would be designated as the "aboral" or "posterior" portion of the spermatophore[6,14–16]. In this part of the spermatophore is located the "sperm rope", that is the cylindrical, spirally-wound long thread consisting of a large white mass of tightly packed spermatozoa. When unwound, a thread of this kind measures about 4 m, and resembles somewhat the mammalian epididymal tubule in its general appearance. On the two occasions when spermatozoa were counted in the contents removed from such sperm ropes, we have found $1{\cdot}7 \times 10^{10}$ and $3{\cdot}7 \times 10^{10}$ spermatozoa, figures roughly equivalent to the entire sperm content of ten rams, or one hundred human semen ejaculates. On release from the sperm-rope and dilution with sea-water, the spermatozoa are immotile or only feebly motile, but their motility can be enhanced by the addition of a buffered solution of cystein or certain other amino-acids.

On the outside, the intact sperm rope is covered by 5–10 ml. of a colourless, transparent and highly viscous fluid which we propose to call the spermatophoric plasma (in analogy to the seminal plasma). The thinner half of the spermatophore, that is the "distal" portion (corresponding to "oral" or "anterior"), encloses the ejaculatory apparatus: a slender, membrane-coated rod-shaped hyaline plug, filled with yellow-coloured, markedly elastic material of rubbery consistency. The distal part of the ejaculatory apparatus is often slightly curved or even coiled, and at its tip, which is only 1–2 mm wide, the outer tunic thickens to form a firm round covering, the cap, and a fine filamentous appendage, known as the cap thread. The latter extends in two directions: proximally it runs firmly attached to the outer covering of the spermatophore, but distally it continues in a free form, as a filament about 0·5 m long (Fig. 3). The region which links the distal end of the sperm rope to the proximal end of the ejaculatory apparatus is filled with a small amount of a sperm-free, yellowish syrupy material, which in colour and consistency is quite unlike the spermatophoric plasma and therefore deserves a specific name, such perhaps as "cement liquid", without the implication,

however, that it possesses any cementing properties, nor indeed that it is analogous to the "cement body", "flacon" or "faux boyaux" in the spermatophores of other cephalopods, mentioned by Duvernoy[17], Racovitza[18,19] and Drew[9,15].

Several chemical analyses were performed on the mechanically separated components of the fresh spermatophores, namely sperm rope, spermatophoric plasma, cement liquid, outer tunic, and hyaline core of the ejaculatory apparatus.

For most determinations the sperm rope and spermatophoric plasma were collected together, by placing a ligature around the spermatophore in the region between the sperm rope and cement liquid, and emptying the entire contents of the proximal portion of the spermatophore through a cut in the outer tunic. In a few instances, the spermatophoric plasma was also obtained without the sperm, that is directly from the spermatophore, by aspiration with a syringe. The sperm and plasma were separated by centrifugation and washing. An interesting result of the chemical analyses was the identification of glycogen as a major constituent of octopus sperm. The content of glycogen in the different samples varied from 5 to 16 per cent of the dry weight. Its identity was confirmed by isolation, based on Pflüger's method of alkaline hydrolysis and ethanol precipitation, followed by acid hydrolysis and determination of glucose by means of glucose oxidase, ferricyanide reduction, and the reactions with orcinol and anthrone. The high glycogen content of the octopus sperm contrasts strikingly with the low values encountered in the sperm of mammals and echinoderms[20–22] but it recalls the elevated values reported in the sperm of oysters and certain ascidians[23,24].

In addition to glycogen some mucopolysaccharide was found in the sperm rope. Of that material, however, there was more in the spermatophoric plasma than in the spermatozoa. Most of it could be precipitated by ethanol and was composed of glucose, galactose, mannose, methylpentose and aminosugar. In addition, however, the spermatophoric plasma also contained some carbohydrate which was soluble in ethanol, including an unusually high concentration of aminosugar, up to 1 g/100 ml., and a variable quantity of free glucose (5–120 mg/100 ml.).

The cement liquid and ejaculatory apparatus, as well as the spermatophoric plasma, all showed exceptionally high dry weights (15–30 per cent), due in a large measure to the mucopolysaccharides. The outer tunic, on the other hand, was of a different composition. By stripping the tunic off the spermatophore, and then cutting it up and washing extensively with water, pieces of clean, transparent and remarkably elastic membranous material were obtained, which consisted mostly of protein, with about 10 per cent orcinol-reactive carbohydrate, and 10 per cent aminosugar. The relatively low aminosugar content shows that contrary to the views expressed by Williams[14] and other earlier investigators of molluscan spermatophores, the outer wall of the spermatophore cannot be regarded as being made up of "chitin-like material".

The Spermatophoric Reaction

In order to study the mechanics of ejaculation, that is the so-called spermatophoric reaction, *in vitro* as well as *in vivo* observations were made.

To examine the ejaculatory process *in vitro*, the spermatophores were placed in special troughs constructed of 'Perspex', 2 m long and 5 cm wide, which allowed a slow but steady flow of cold sea-water from one end to another. When a spermatophore had been placed in a trough, with the cap-thread facing in the direction of the flow, it usually took 15–30 min for the spermatophoric reaction to begin, and 1–3 h to reach completion. By tugging gently at the cap-thread it was possible to accelerate the initial, but not the later, stages of the reaction.

The first indication that the reaction is about to start comes from a change evident at the proximal end of the spermatophore. That portion, already very turgid to touch, swells owing to a rise in the volume of the spermatophoric plasma, and at the same time the sperm rope starts to uncoil and slowly moves forward, pushing before it the ejaculatory apparatus. Then, suddenly, a break occurs at the distal end of the body, that is at the point where the cap is joined on to the outer tunic, and, as the cap ruptures because of the pressure from the inside of the spermatophore, the ejaculatory apparatus immediately begins to evaginate, leaving the remnants of the cap and the cap-thread behind. As the evagination continues, the body of the spermatophore quickly increases in length by altogether about 50 per cent, so that ultimately it measures approximately 1·5 m. The next visible change associated with the spermatophoric reaction occurs in the ejaculatory apparatus. As the pressure from the spermatophoric plasma increases, and the sperm rope steadily advances, the space around

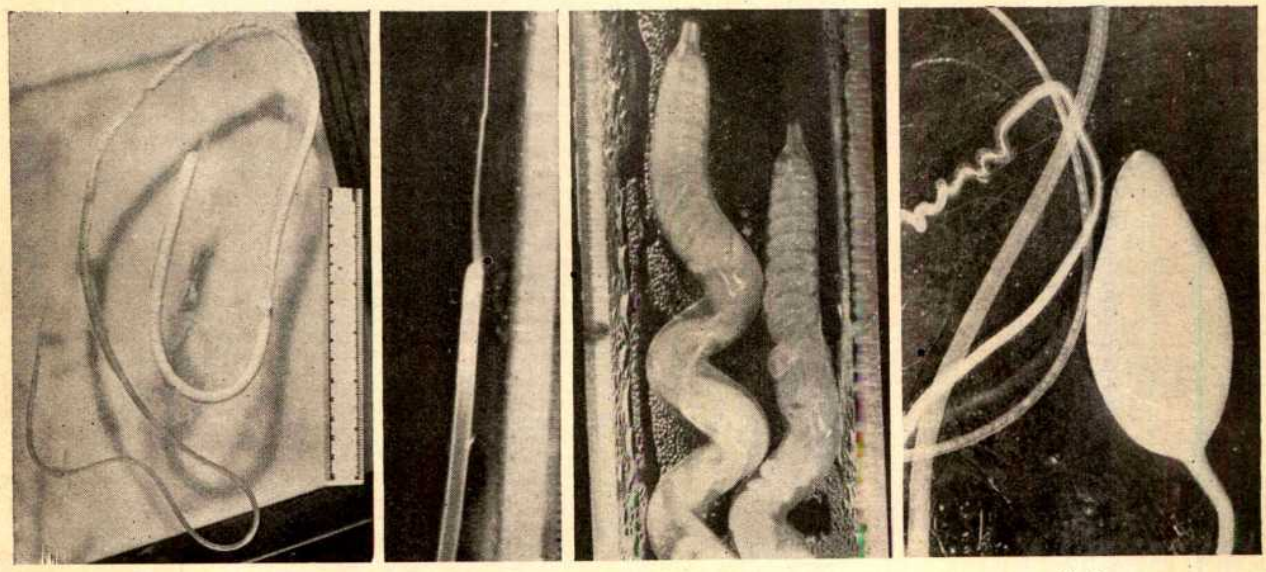


Fig. 2. Fig. 3. Fig. 4. Fig. 5.

Figs. 2–5. Spermatophores of *Octopus dofleini martini*. Fig. 2, Whole intact spermatophore. Fig. 3, Distal end of the body of the spermatophore showing the cap and cap thread. Fig. 4, Coiling of ejaculatory apparatus in two spermatophores undergoing the spermatophoric reaction. Fig. 5, Final stage of the spermatophoric reaction : formation of sperm bladder.

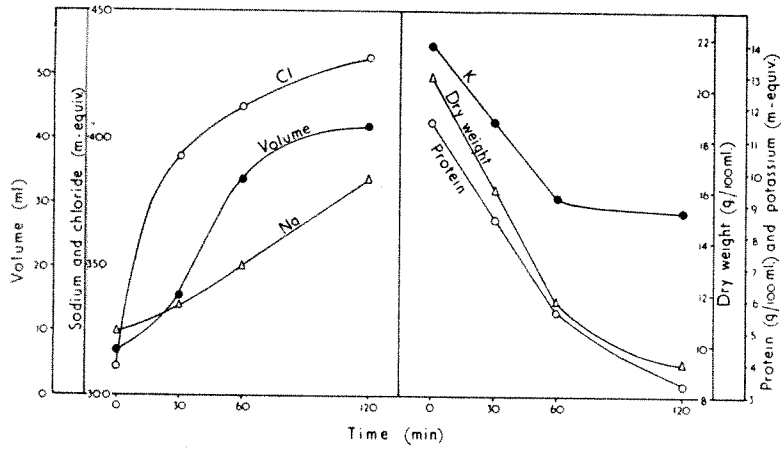


Fig. 6. Changes in the volume and content of dry weight, protein, sodium, potassium and chloride in the spermatophoric plasma of octopus during the spermatophoric reaction.

the core of the ejaculatory apparatus swells and becomes filled with fluid, while the apparatus itself undergoes a characteristic coiling (Fig. 4), followed by shortening and thickening, thereby making it possible for the sperm rope to advance still further in the direction of the distal end of the spermatophore. At this stage a definite pause occurs in the spermatophoric reaction until, again quite suddenly, the sperm rope pushes further forward and the whole of the distal portion of the spermatophore's body is blown out into a large egg-shaped bulb resembling a bladder. When completely filled with sperm, that "sperm bladder" measures roughly 8 × 4 cm. Behind it drags the remainder of the spermatophore, a long tube, still filled, though much less turgidly, with spermatophoric plasma, but more or less completely devoid of spermatozoa (Fig. 5).

Ostensibly the main purpose of the spermatophoric reaction, as observed in the giant North Pacific octopus, is to haul the whole mass of tightly encapsulated spermatozoa over a distance of about 1·5 m, from the proximal to the distal end of the spermatophore, and to provide in the form of the "bladder" a large sperm reservoir. This resembles the "reservoir spermatique de second ordre", described by Racovitza[19] in *Rossia macrosoma*, and the "sekundäre Spermabehälter" described by Marchand[8] in *Eledone moschata* and *Octopus macropus*. The two most important factors which provide the main driving force for the spermatophoric reaction as a whole, and the processes of evagination and bladder formation in particular, are evidently the elasticity of the outer tunic, and the pressure which arises from the influx of sea-water transmitted by the spermatophoric plasma to the sperm rope and the ejaculatory apparatus. This is borne out by experiments in which chemical analyses were performed on the spermatophoric plasma at different stages of the spermatophoric reaction. One such experiment is illustrated in Fig. 6. It records changes which have occurred in the volume, dry weight, and the concentration of sodium, potassium, chloride and protein of spermatophoric plasma in the course of the spermatophoric reaction, as observed in spermatophores which had been removed from the Needham's sac of an octopus, and placed in sea-water troughs. Four distinct stages were recorded: (i) at the moment of placing the spermatophore in sea-water; (ii) at 30 min, when the swelling of body and movement of sperm rope became apparent; (iii) at 60 min, coincident with the formation of the sperm bladder; (iv) at 120 min, when the sperm bladder had fully expanded. As can be seen from Fig. 6, the spermatophoric reaction was accompanied by a rapid increase in volume and sodium chloride concentration of spermatophoric plasma, owing to the influx of sea-water. Concomitantly with the uptake of sea-water the spermatophoric plasma became diluted, and consequently its dry weight, protein and potassium concentration rapidly declined. The final concentrations (at 120 min) of sodium and chlorine, respectively, were, in m.equiv./l., 386 and 433 in the spermatophoric plasma recovered from the sperm bladder; 440 and 451 in the sea-water taken from the observation trough; and 384 and 408 in a blood sample obtained from the same animal.

There is good reason to believe that events analogous to those which we have seen in octopus spermatophores undergoing a reaction *in vitro* also take place under conditions *in vivo*, during the copulation process. On three separate occasions we were able to follow the copulation of a male and female octopus placed together in an observation tank. On two of these occasions copulation was allowed to proceed to completion undisturbed: 2 and 3 h, respectively, elapsed from the moment when the male inserted the tip of its hectocotylized arm into the mantle cavity of the female, to the time when it withdrew, leaving the female not with one, but with two empty bodies of spermatophores, hanging freely from her mantle over a distance of about 0·5 m. On the next day one of these females was observed to lay eggs. On dissection, her oviduct was found to be filled with large masses of motile spermatozoa, particularly in the lumen of the oviducal gland, but there was no sign of the sperm bladder. Presumably by then it had ruptured, emptying its contents in this manner. On the third occasion, coitus was interrupted 30 min after insertion of the hectocotylus, by pulling the male away from the female. The spermatophore recovered from the hectocotylized arm of that male had the ejaculatory apparatus pointing towards the female, while the proximal portion was already exhibiting the swelling changes which are so characteristic of the early phase in the spermatophoric reaction.

The finer mechanism of the spermatophoric reaction remains to be investigated. The evidence so far obtained from both morphological and chemical observations, however, strongly favours the supposition that the main force which drives spermatozoa from one end of the long spermatophore to the other results from the tension which develops inside the spermatophore during the process of ejaculation as a result of the dilution of spermatophoric plasma with sea-water.

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LETTERS TO THE EDITOR

ASTRONOMY

Cosmological Significance of the Relation between Red-shift and Flux Density for Quasars

THE purpose of this report is to point out that recent measurements[1] of the red-shifts of quasars provide the most decisive evidence so far obtained against the steady state model of the universe. The previous evidence[2,3] consisted mainly of the anomalously[4] steep slope of the log N–log S relation for quasars (N is the number of sources with flux density greater than S). This evidence is ambiguous, however, because one could argue[5] that many of the quasars may have zero red-shifts and be situated inside our Galaxy. Their log N–log S relation would then have a purely local significance. If we can restrict the analysis to quasars of known red-shift we avoid this ambiguity, provided that we interpret the red-shift as being cosmological in origin, which in this communication we shall do.

Red-shifts are now known[1] for thirty-five quasars in the revised 3*C Catalogue*[6], which is complete down to 9 flux units at 178 Mc/s (1 flux unit is 10^{-26} W m^{-2}(c/s)$^{-1}$). To analyse the data we study not log N–log S, but the more detailed relation between red-shift z and flux density S for these sources (Fig. 1). A similar diagram was constructed by Hoyle and Burbidge[7], who claimed that the absence of a clear correlation between small S and large z shows that red-shift has nothing to do with distance. We shall see that this claim is not necessarily correct.

To test the steady state theory we draw in the diagram lines of constant intrinsic radio luminosity P evaluated at 178 Mc/s. If the sources are uniformly distributed then the steady state theory requires[8] that the number N of sources in a fixed range of P the red-shift of which is less than z should satisfy the relation

$$N \propto \ln(1+z) - \frac{z}{1+z} - \frac{z^2}{2(1+z)^2} \qquad (1)$$

P is given by

$$P = c^2\tau^2 z^2 (1+z)^{1+\alpha} S \qquad (2)$$

where τ is Hubble's constant and α is the spectral index of the source ($S \propto \nu^{-\alpha}$). The choice of α is not critical, and so we take the same value of 0·7 (K. I. Kellermann, unpublished results) for all the sources. To illustrate the analysis we draw two lines of constant P, the choice of which is also not critical.

To test the N–z relation (1) we divide the diagram into three regions of z in which we would expect to find equal numbers N of sources in any fixed range of P. Regions 1, 2, and 3 are defined by $z < 1{\cdot}02$, $1{\cdot}02 < z < 1{\cdot}55$, and $1{\cdot}55 < z < 2{\cdot}03$, respectively. We now find that there are five sources with $P > P_1$ in region 3, and because of the cut-off at 9 flux units the actual number may be greater. There is only one such source in region 2, however, and none at all in region 1. We cannot argue that we are dealing with an incomplete sample, and that the observers have a bias in favour of seeking the large red-shifts of region 3, because granted that the five sources exist at all, the corresponding sources in region 1 would have a flux density $S \gtrsim 50$ flux units, and could not have been missed. We find similarly that there are five sources with $P > P_2$ in region 3 (which is now very severely affected by the cut-off), five sources in region 2 (which is somewhat affected by the cut-off), and only two sources in region 1.

These discrepancies appear to be statistically significant and in so far as we may use relation (2) to calculate P they show that there is an intrinsic evolution of either P or N or both, with z, contrary to the predictions of the steady state theory. It is not possible to conclude from Fig. 1 that z has nothing to do with distance, because any reasonable distribution of the points could be accounted for by an appropriate rate of evolution.

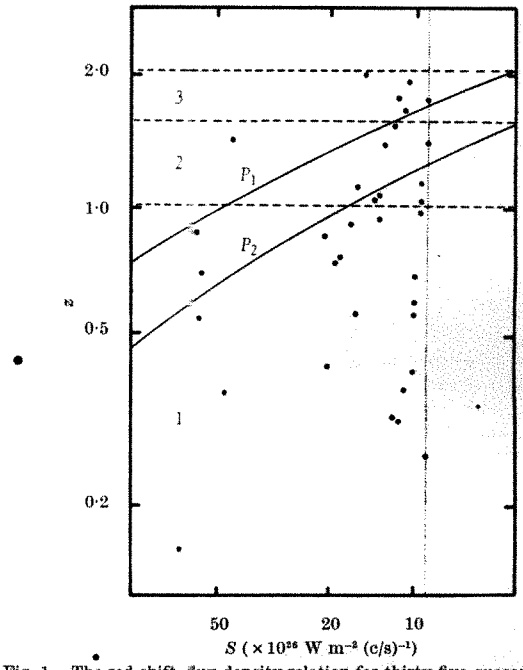


Fig. 1. The red-shift–flux density relation for thirty-five quasars in the revised 3*C Catalogue* (which cuts off at 9 flux units). The two curves are lines of constant source power according to steady state cosmology. This cosmology predicts equal numbers of sources of given power in the regions 1, 2, and 3 defined by $0 < z < 1{\cdot}02$, $1{\cdot}02 < z < 1{\cdot}55$, and $1{\cdot}55 < z < 2{\cdot}03$.

We conclude that if the red-shifts of quasars are cosmological in origin, then the present red-shift–flux density relation for quasars rules out the steady state model of the universe.

We acknowledge a helpful discussion with Mr. M. Rowan-Robinson.

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A Possible Galactic Origin for Quasi-stellar Sources

THE interpretation of the observed red-shifts of "quasars" as cosmological gives rise to severe difficulties concerning both the energy output and the variation of light because these objects have then to be placed at enormous distances.

We wish to suggest the possibility that the observed red-shifts result from an inflow of matter at relativistic velocities in the gravitational field of a highly condensed white dwarf with a very large escape velocity. To establish a starting point, consider a collapsed body with mass 10^{33} g and a radius of several kilometres; these stars must exist[1] and will strongly attract interstellar matter.

The high escape velocity—a large fraction of the velocity of light—implies that nothing will escape apart from photons and neutrinos, thus there can be no net flux of matter and thus no stellar wind. If the star is a member of a binary system there will be an outward flux of matter owing to the stellar wind of the companion, but if the condensed star is isolated it must accrete interstellar matter.

The accretion process should be quite efficient. If the interstellar medium has an ambient magnetic field accreted material will bring its own field lines with it which become trapped in the star. Also the star will possess in its wake a concentrated magnetic field directed so as to guide material into the star. If the interstellar gas has a uniform motion over distances of the order of GM/v^2, where v is the relative velocity of gas and star, then under the influence of gravitation all matter will concentrate in the wake which becomes a region of high density, temperature and magnetic field. The star accretes material at the rate (capture cross-section times velocity) $G^2M^2/v^3\ \rho$, where ρ is the ambient density of the interstellar material. Taking $M = 10^{33}$ g, $v = 5 \times 10^5$ cm/sec, and $\rho = 10^{-24}$ gm/c.c., the accretion rate is 10^{11} g/sec. With an escape velocity of $\frac{1}{2}C$ the accreted material supplies energy to the object at a rate 10^{31} ergs/sec. If this energy is radiated as black-body radiation the effective temperature of the star would be a few hundred thousand degrees. Thus this star would have a luminosity one hundredth of that of the Sun. Moreover, at a distance of 100 parsecs it would have a magnitude of about 15^m, which is within the observed range of quasar apparent magnitudes; the parallax of such a star $0{\cdot}01''$ would be very difficult to observe directly.

Material moving into the wake experiences a much greater magnetic field and incoming electrons will gain energy by the betatron effect and would be expected to produce large amounts of synchrotron radiation. (Such radiation would be expected to have a maximum intensity where the material first approaches the wake because here the energy associated with transverse velocity components would be dissipated. Thus we might expect a strong source of radio emission at a distance GM/v^2. With the values already given this would be at 10^2 astronomical units from the star.) Furthermore, the optical spectrum would be produced very near the stellar surface where densities and pressures are high so that the radiation would be strongly red-shifted by almost the amount of the escape velocity; the considerable gravitational red-shift of the star itself would be added to this.

In summary, a single highly condensed star interacting with the interstellar medium should give rise to the following effects: (1) A double radio source, the two members of which would be separated by about GM/v^2. (2) Associated with one radio source is a point source with a continuum characteristic of a very hot body with superposed very broad strongly red-shifted emission lines. The red-shifts observed depend on the orientation of the star with respect to the Sun. (3) Optical radiation of the synchrotron type is associated with the other radio source. (4) Both sources should be time-variable because the density, velocity and magnetic field of the interstellar field is dependent on time. (5) The optical point source should produce soft X-rays.

The sensitive parameter is v, the relative velocity of star and interstellar medium, because the accretion rate is proportional to v^{-3} and the source separation to v^{-2}.

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PLANETARY SCIENCE

Comparisons of Frequency by Long Path Very Low Frequency Transmissions

MOST of the reliable measurements of the frequency stability of very low frequency (VLF) propagation paths have been made over the North Atlantic path, and over paths of the same order of length. Pierce *et al.*[1] used atomichrons on each side of the Atlantic and found a standard deviation of 2 in 10^{11} for frequency comparisons consisting of 24 h averages for the *GBR* (Rugby, 16·0 kc/s) transmission over a path length of 5,200 km. It has been suggested by Watt and Plush[2] that the standard deviation of phase—and thus of frequency over a constant interval of time—would be proportional to the square root of path length. However, Chilton *et al.*[3] give figures suggesting that it may be proportional to the square of the path length squared.

Although the results presented here are not sufficient to resolve this question, it has been found that over very long paths there is little increase in the standard deviation above Pierce's value. Over the *GBR*–New Zealand path of 18,700 km the 24 h standard deviation of frequency is 5 in 10^{11}, and for the *WWVL* (Colorado, 20·0 kc/s)–New Zealand path of 12,200 km the corresponding figure is 3 in 10^{11}. These figures were obtained by intercomparisons between the received signals from both stations and a rubidium gas cell frequency standard. It has not been possible to determine to what extent they were affected by receiver phase shifts and they therefore represent an upper limit to the uncertainty due to purely propagation effects. The observations were made in December 1965; at this time of the year the anomalous phase effect observed at times over the *GBR*–New Zealand path does not occur[4]. National Physical Laboratory corrections were, of course, applied to the *GBR* figures, but the National Bureau of Standards corrections to *WWVL* are negligible for the present purpose.

It should be noted that the *GBR* path crosses the auroral zone and is, therefore, likely to be somewhat more disturbed than the *WWVL* path, even in the geomagnetically quiet conditions which prevailed during December 1965. The figures, therefore, give tentative support to the suggestion of Watt and Plush that a square root proportionality is in operation. Their main importance, however, lies in showing that, at least during geomagnetically quiet periods, VLF frequency comparisons over a 24 h period should, under normal conditions, be well within ± 1 in 10^{10} over the longest path lengths.

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A Submarine Outcrop of Lower Carboniferous Rocks off South-East Scotland

EXAMINATION by divers of a submarine section off the coast of south-east Scotland in which are seen beds of the Scremerston Coal Group has filled in the only major gap in the exposed Lower Carboniferous succession in this area.

The Southern Uplands of Scotland separate the Carboniferous basins of northern England from those of the Midland Valley and it is well established that in the centre and west the Uplands formed a relatively positive area during Carboniferous times[1]. Towards the north-eastern end of the Uplands considerable thicknesses of Upper Old Red Sandstone and Lower Carboniferous sediments encroach increasingly on the older rocks, which they largely overlap in the east; it is a reasonable inference that a short distance offshore a continuous Carboniferous outcrop joins those of northern England and the Midland Valley. The sediments deposited over the buried continuation of the Southern Uplands occur in faulted and folded basins, the largest lying in the Tweed Valley area and a smaller one on the coast south of Dunbar (Fig. 1).

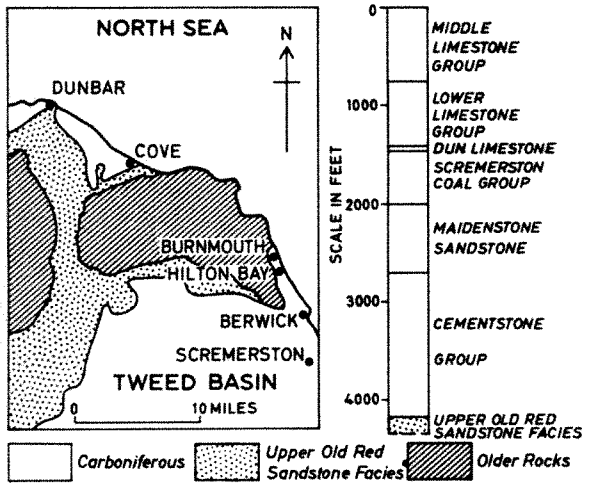


Fig. 1. Geological sketch-map of Burnmouth area, and Lower Carboniferous section between Burnmouth and Berwick.

There are excellent exposures of Carboniferous rocks on the coast of south-east Scotland, but it has not been possible to determine the total thickness of strata involved with any certainty, partly because of the presence of faults of unknown magnitude. Strike faults in Hilton Bay, a mile south of Burnmouth, and at Cove[2] farther north each cut out considerable parts of the succession.

On the foreshore at Burnmouth the base of the preserved Upper Palaeozoic section, which is faulted against older rocks, is formed by a 150 ft. bed of red sandstone with cornstones which has hitherto been classified with the Carboniferous[3]. This sandstone is indistinguishable from rocks of Upper Old Red Sandstone facies exposed near Dunbar and in the Tweed Basin so there seems no valid reason for regarding the bed as other than the topmost representative of rocks of this facies. Because the facies is, however, known to be diachronous it cannot be used for purposes of correlation over a wide area. The red sandstone is overlain by 1,500 ft. of strata of the Cementstone Group of northern England terminology, which are in turn followed by a sandstone, here called the Maidenstone Sandstone, lying approximately at the horizon of the Fell Sandstone of the area south of Berwick. At Burnmouth the Maidenstone Sandstone is seen to be at least 600 ft. thick but its top is not exposed on the foreshore. It strikes south along the coast to Hilton Bay, where the Dun Limestone, at the base of the Lower Limestone Group of northern England, is faulted against beds near the top of the sandstone. The fault in Hilton Bay thus cuts out the Scremerston Coal Group, but in the cliffs and foreshore between here and the Tweed a nearly continuous section is to be seen from about 250 ft. below the Dun Limestone to the top of the Lower Carboniferous.

Near low water mark at Burnmouth the Maidenstone Sandstone dips seaward at 75 degrees. Offshore the succession has now been shown to continue upwards through a further 75 ft. of this sandstone into 590 ft. of Scremerston Coal Group strata overlain by the Dun Limestone, and then into the Lower Limestone Group of northern England. The key horizon in the offshore section is the Dun Limestone, which consists of 4 ft. of exceedingly hard, dark grey, crinoidal limestone with productoid fragments. It is identified by comparison of the lithological sequence of which it forms part with that on the shore three-quarters of a mile to the south, beyond the fault in Hilton Bay. In each place the limestone is succeeded by a thick bed of dark mudstone passing up into banded siltstone which in turn is overlain by coarse-grained, current-bedded, red sandstone, seen to be at least 150 ft. thick in both the coastal and the submarine sections.

The only persistent marine limestones recorded at comparable stratigraphical horizons in the northern part of the Tweed Basin are in upward order—the Duddo, the Dun and the Woodend limestones. All three are believed to be represented on the coast between Hilton Bay and the River Tweed, but the probable equivalents of both the Duddo and the Woodend deteriorate northwards and neither is associated with overlying lithological sequences comparable with that above the Dun.

The measured thickness of 590 ft. for the Scremerston Coal Group compares with about 1,000 ft. for beds of the same facies 9 miles to the south at Scremerston[4], suggesting a marked change towards the northern margin of the Tweed Basin where the group encroaches on to the concealed continuation of the Southern Uplands.

It is to be noted that these measures are reported to thin out in a comparable fashion at the south[5] and west[6] margins of the Tweed Basin and that the base of the Scremerston Coal Group as at present mapped may be diachronous because it is not defined palaeontologically. The total thickness of accessible and measured Lower Carboniferous rocks between Burnmouth and the Tweed is now known to be 4,020 ft., compared with a calculated 10,000 ft. near Edinburgh[7] and at least 7,000 ft. without reaching the base just north of Brampton in the Northumbrian Trough.

The underwater section at Burnmouth was measured by examining a primary line along 900 ft. of permanent marked and buoyed rope laid downdip at right angles to the coast. Rock exposure on the line chosen is about 75 per cent complete and the consistently steep dip means that the sea-floor outcrop approximates to a vertical section of the strata. The rope was in independent 100 ft. sections in order to minimize the effects of snagging by fishing boats. With the resources available, work was most effectively undertaken by a team of three free-swimming 'Aqualung' divers, comprising a geologist who recorded the profile and indicated points for sampling, a collector and an attendant who carried samples and equipment. Depth of water varied from about 35 ft. to 80 ft. Underwater visibility was about 25 ft., but after periods of onshore gales or heavy swell it was so reduced as to be inadequate for the work; this happened frequently during winter months. Tidal currents did not normally provide a serious problem because near the irregular rocky sea-floor these are greatly reduced.

The Dun Limestone proved difficult to sample underwater because of its hardness and because it is deeply bored by modern molluscs, but specimens of most other rocks were easily collected with hammer and chisel. Below the *Laminaria* Zone, which here extends to about 25 ft. below low water, the rocks are not greatly obscured

by marine growth and even comparatively soft rocks, such as seatearths and coals, in places form ridges on the sea-floor. All samples of argillaceous rocks collected are bored by molluscs but otherwise little decomposed, and the fossils which they contain have not been affected by weathering.

This investigation arose from our duties with the Geological Survey. The offshore work was carried out by the Edinburgh Branch of the British Sub-Aqua Club, which is continuing detailed examination of the sequence.

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Depositional Environment of the Cleveland Ironstone Series

DR. HALLAM has recently restated some of the problems involved in an understanding of the oolitic ironstones of the British Lias, and has emphasized the importance of a close examination of their facies relationships[1].

In spite of Strakhov's observations[2] that ancient ironstone and deltaic deposits are antipathetic, the weight of recent evidence seems to imply that many oolitic ironstones are spacially related to deltas. Pattinson has described the occurrence of three sideritic chamosite oolites in a cyclothemic succession of silty shales, siltstones and fluviatile sandstones, in the Upper Limestone Group (Namurian E_1) of Weardale, County Durham[3]. This occurrence is reminiscent of the kaolinized oolites found in the roofs of three coal seams in the Coal Measures near Bradford and Leeds[4]. A similar association is known from the Liassic succession in Scania (Sweden), where sandstones, shales, coals and oolitic iron ores are cyclicly related[5]. Another important discovery has been that of fluviatile and deltaic lacustrine oolitic ores in Kazakhstan (Russia)[6,7]. The shoestring nature of the latter together with the occurrence of freshwater molluscs is incontrovertible evidence of their origin. Here the ooliths are largely limonitic, but chlorite appears as a constituent of the matrix and is also occasionally present in the ooliths. According to Giresse, goethite ooliths are forming at the present day in shallow water on the continental platform west of Cap Lopez (Gaboon, West Africa) at the mouth of the Ogooué delta[8].

These examples serve to illustrate a connexion between the already well known continental iron oxide accumulations of the high latitudes and the more problematic iron ores. All share evidence of association with a fluvio-deltaic environment, of a warm to tropical climate, and of heavy vegetation cover on land.

The Cleveland Ironstone Series of north-east Yorkshire was probably laid down in warm waters (oxygen-16/oxygen-18 ratios determined from belemnites from the Grey Shales, Upper Lias, Yorkshire, indicate a temperature of about 20° C)[9] and a heavy vegetation is suggested by the abundance of plant debris which is present throughout. The possibility of a nearby river delta was postulated by Dunham[10] and seems quite plausible. No direct evidence of an impinging delta, however, exists in the present outcrops. Hallam's supposition that the reworked shales and siltstones of the Middle Lias are distal pro-delta deposits can only be tentative at present.

The question of the derivation of these sediments is a difficult one. A Pennine landmass may be speculative but the extension of a delta complex from Scandinavia is even more so. The evidence at present available from the Middle Jurassic Deltaic Series suggests "a river or system of rivers entering the area approximately from the N.N.W.", which is compatible with a North Pennine landmass during this period[11].

A close examination of all the available sections of the Cleveland Ironstone Series reveals the following details. First, that a lateral passage from shales into silty shales and siltstones must not be over-emphasized. It rests in part[10,12] on the observations of Fox-Strangways *et al.*[13] of the incoming of siltstones along the western escarpments of the Cleveland Hills, which resulted from the correlation of the Ironstone Series with the underlying Sandy Series. Moreover, the presence of silty shales at the horizon of the Main Seam at Hawsker Bottoms[1] cannot be used to support the idea of a higher terriginous sedimentation rate to the south-east. In the North Sea basin it is well known that much of the sediment is derived from the basin itself. In the Wadden Sea, for example, it has been shown that mixed sand, silt and clay fractions are not derived from the land but from the North Sea[14].

The Main Seam and *Pecten* Seam were economically the most important ore horizons in the Ironstone Series. They appear to have been laid down on a south-easterly sloping sea bed eroded at the beginning of *spinatum* times. Fig. 1 shows the details of a section drawn in a north-west–south-east direction approximately along the line of the present coast. The *spinatum* beds thicken south-eastwards owing to a slight angular disconformity between F_1 and F_2, and also to the wedging of the *hawskerense* beds (E), which appear to be absent in the north-west part of the section as a result of a non-sequence. The chamosite oolite shoals of the Main Seam (A and $B_{1,2}$) apparently

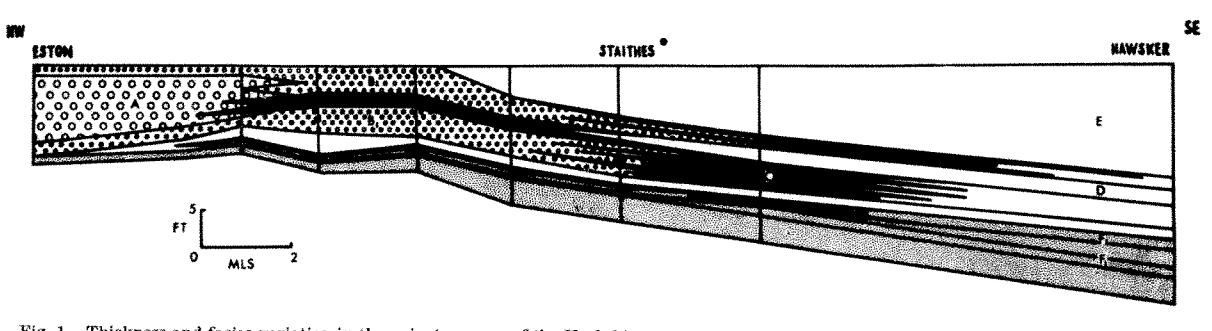


Fig. 1. Thickness and facies variation in the *spinatum* zone of the Yorkshire coast. *A–D*, Main Seam. *A*, Intraclastic chamosite oolite facies; *B*, chamosite oolite facies 2; Top Block; 1, Bottom Block and "Black Hard"; *C*, siderite mudstone and related facies; *D*, shale and siderite mudstone nodule facies. *E*, *Hawskerense* Beds (pars.), shales and siltstones with siderite mudstone nodules. $F_{1,2}$, *Pecten* Seam, siderite mudstones, and shales with siderite mudstone nodules. The top of the section is drawn at the "Sulphur Band" and its offshoal equivalent.

encroached on the basin from the north-west, thus derived fragments from the intraclastic oolite *A* are found in $B_{1,2}$, but there is no evidence of erosion in $B_{1,2}$ to provide the intraclasts for *A*. Offshoal the chamosite oolites pass into siderite mudstones (*C*) and eventually into shales (*D*). The oolitic facies of the *Pecten* Seam ($F_{1,2}$) are not preserved but a lateral passage from siderite mudstone into shale can be observed.

A diagnostic feature of the siderite mudstone facies (*C*) is the occurrence of cored siderite grains. It is tentatively suggested that the siderite mudstone facies may be diagenetically derived by the replacement of a limonitic silt and clay facies, the cloudy siderite cores representing replaced limonitic clay aggregates. Much of the iron may have been carried into the basin of deposition as a ferric coating on the surface of clay micelles as postulated by Carroll[15]. The dominantly kaolinitic nature of the shales of the Ironstone Series, as determined by X-ray diffraction, would support such a hypothesis.

Although evidence of a nearby shoreline is disputable with the Cleveland Ironstone Series, there are many instances in the literature of the association of oolitic ironstones with littoral facies. The repeated appearance of oolitic ironstones around the London–Ardennes landmass and the Morvan in the Jurassic is but one example[16].

On the basis of the evidence at present available it seems most plausible that iron reached the basin of deposition in north-east Yorkshire from a low lying, well vegetated landmass lying to the north-west. It may well have been carried by a river draining a north Pennine landmass.

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THE SOLID STATE

Thermal Decomposition of Irradiated Orthorhombic Ammonium Perchlorate

Isothermal decomposition kinetics of certain solids are markedly affected by previous exposure to ionizing radiation. The observed effects are usually interpreted as a change in the nuclei formation rate and, in the following stage, enhanced propagation of the reaction through the solid. For example, the effect of pre-irradiation by 1·2 and 1·3 MeV cobalt-60 gamma-rays on the subsequent thermal decomposition of the alkaline earth azides[1], or the permanganates of sodium, potassium, rubidium, caesium and silver[2], is to shorten the induction period and to accelerate the reaction. The solids studied so far all contain a metallic cation, thus it would be interesting to investigate the effect of pre-irradiation on compounds such as ammonium perchlorate which contain a non-metallic cation.

Investigations of Bircumshaw and Newman[3] on the isothermal decomposition of whole crystals of orthorhombic ammonium perchlorate show the following effects. The decomposition comprises an induction period, followed by an acceleratory period where the pressure versus time curves were fitted equally well by the Prout–Tompkins relationship

$$\log_{10}[p/(p_f-p)] = k_1t + c_1 \quad (1)$$

or the relation

$$p = k_2t^n = k_2t^6 \quad (2)$$

where p is the pressure at time t, p_f the final pressure and all c_1 and k_1 are constants. This stage was followed by a decay stage where the decomposition followed the unimolecular law

$$\log_{10}(p_f-p) = k_3t + c_3 \quad (3)$$

or, equally well, the expression

$$\tau\exp(-E/RT) = k_4 \quad (4)$$

where τ is the time to a fixed amount of decomposition. They obtained an activation energy of 27·8 kcal. mole^{-1} for all three stages.

Contradictory results were obtained by Galwey and Jacobs[5], who concluded that for whole crystals the acceleratory and decay periods were best described by the Avrami–Erofeyev equation[6]

$$-\log_{10}(1-\alpha) = k_5t^n \quad (5)$$

where $\alpha = p/p_f$ and n is equal to 4 for the acceleratory and 3 for the decay period. In both these studies only the non-condensable gases were measured (using McLeod gauges) and the effects of pre-irradiation were not investigated.

For this study, transparent crystals of ammonium perchlorate were prepared by the method of Galwey and Jacobs[5]. Only uniform crystals, approximately 1 × 1 × 1 mm obtained by careful selection, were used. The decomposition measurements were made in a grease-free, 'Pyrex' system using Dekker[7] valves for isolation. Gas pressures were measured with a fused quartz spiral gauge (Texas Instruments Corp., Houston). To effect a measurement of only those gases which would not be condensed at approximately −95° C, a trap at melting toluene temperature was inserted between the decomposition chamber and the spiral gauge. Irradiations with cobalt-60 gamma-rays were carried out at room temperature in air. The gamma-ray dose rate was $2{\cdot}05 \times 10^5$ rad h^{-1}.

Typical curves of α versus time for both unirradiated crystals and crystals subjected to a $9{\cdot}8 \times 10^6$ rad gamma-ray irradiation before decomposition are shown in Fig. 1. The irradiated salt is opaque and milky-white in colour. For equal masses of ammonium perchlorate, both unirra-

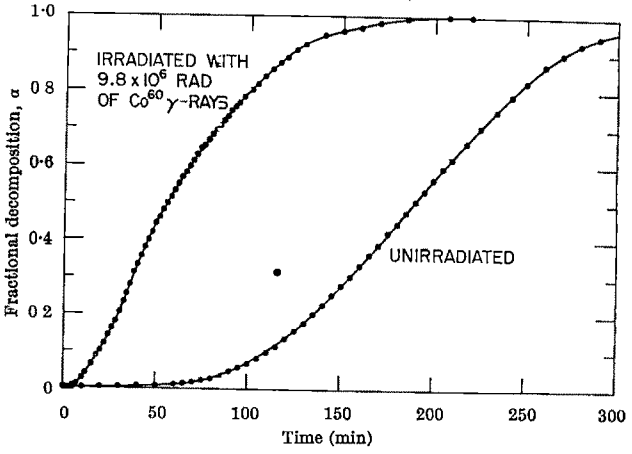


Fig. 1. Fractional decomposition, $\alpha = p(t)/p_f$, versus time for unirradiated and irradiated whole crystals of orthorhombic ammonium perchlorate thermally decomposed at 227° C. The samples weighed 20·0 mg and the same final pressure, p_f = 0·501 mm mercury, was obtained for both. The irradiated sample was exposed to $9{\cdot}8 \times 10^6$ rad of cobalt-60 γ-rays in air at room temperature.

diated and subjected to the $9{\cdot}8 \times 10^6$ rad dose, the final gas pressure is the same to within experimental error. This shows that, at this dose, any gases that may have been produced during irradiation remain trapped in the crystals. Neither the unirradiated crystals nor the irradiated ones appear to break up during decomposition.

The data from unirradiated crystals cannot be fitted by the Prout–Tompkins equation (1), or its modified form[8], nor does the unimolecular decay law (3) adequately describe the decay period. Curves from unirradiated material were successfully fitted to the Avrami–Erofeyev equation (5), with $n=4$ for the acceleratory period and $n=3$ for the decay period (Fig. 2). Preceding the acceleratory period is a linear induction period describable by the equation

$$\alpha = k_7 t + c_7 \qquad (6)$$

The curves of pressure versus time obtained from the irradiated material can also be fitted to the Avrami–Erofeyev equation (5), as is shown in Fig. 2. For a typical acceleratory stage curve (at 227° C), when $n=4$ in equation (5), a good fit is obtained only over the limited range $0{\cdot}07 < \alpha < 0{\cdot}25$. In the decay stage, when $n=3$ in equation (5), the fit is excellent over the unusually large range of $0{\cdot}40 < \alpha < 0{\cdot}98$.

From measurements made on unirradiated, whole crystals between 238° C and 217° C the rate constants, k_5 (acceleratory), k_6 (decay), and k_7 (induction period), were computed. Fig. 3 is a plot of $\log_{10} k_{5,6,7}$ versus $1/T$(°K). From this plot activation energies of 29·8, 26·9, and 24·6 kcal. mole^{-1} ($\pm 2{\cdot}5$ kcal. mole^{-1}) were found for the induction, acceleratory and decay periods respectively.

The nature of the decomposition in unirradiated, orthorhombic crystals has been attributed by Galwey and Jacobs[5] to a reaction occurring principally on mosaic boundaries after initiation by defects. The reaction proceeds three-dimensionally until the boundary material is all consumed. Assuming this mechanism to be correct, the effects of the $9{\cdot}8 \times 10^6$ rad gamma-ray irradiation appear to be a shortening of the induction period and an increase in the rate of three-dimensional growth during the acceleratory period, until a coherent interface is built up on the material between the mosaic blocks. At 227° C, the rate constant k_5 increases from $0{\cdot}39 \times 10^{-2}$ min^{-1} for unirradiated crystals to $1{\cdot}05 \times 10^{-2}$ min^{-1} for the irradiated crystals. The induction period is correspondingly shortened from 62 min to about 5 min. The increase in the rate constant during the acceleratory period might be attributed to an increase in the number of decomposition nuclei present.

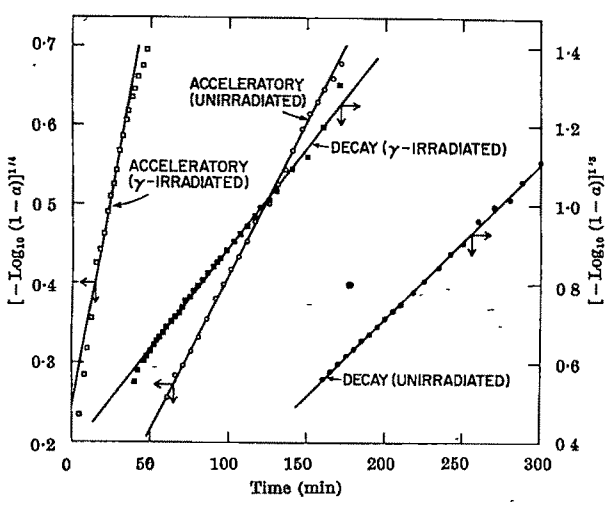


Fig. 2. Avrami–Erofeyev analysis of the data shown in Fig. 1. This demonstrates the applicability of this equation to both experimental curves. γ-ray irradiation decreases the range of fit of the acceleratory equation and greatly extends the range of the decay equation.

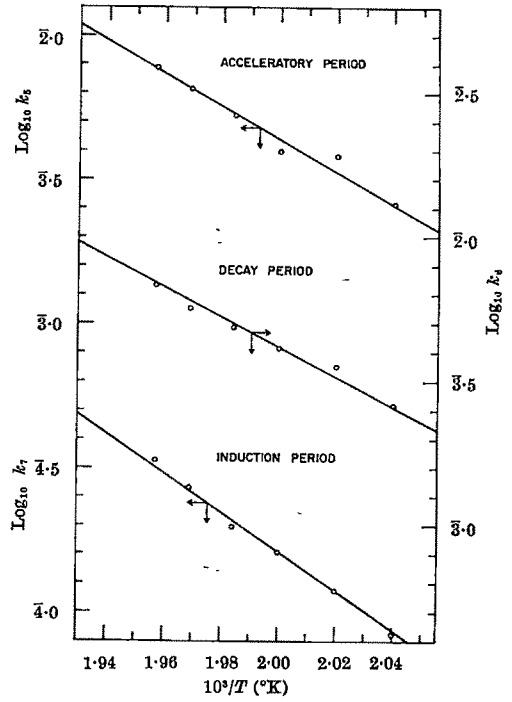


Fig. 3. Plots of $\log_{10}$ (rate constant) versus $1/T$ (°K) for the induction, acceleratory and decay periods of the thermal decomposition of whole crystals of unirradiated orthorhombic ammonium perchlorate.

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A Method of growing Anhydrous Single Crystals of Barium Azide

THE salts of hydrazoic acid are of military interest owing to the fact that many of them are explosive. They range in sensitivity from sodium azide, which is stable, to the heavy metal azides, such as lead and gold azides, which are highly explosive. Intermediate in its explosive character is barium azide, which is sufficiently safe to be handled conveniently, yet can be made to explode. Solid state studies of this material have been hindered by the lack of suitable single crystal specimens. In particular, in order to study the photolysis of barium azide by electron spin resonance, crystals with a minimum size of about 5 × 2 × 1 mm are required. Although crystals of barium azide monohydrate are relatively easy to grow[1], it was felt that anhydrous crystals would offer a

more simple system for study by electron spin resonance. This work was undertaken to develop a method of growing such crystals.

The primary difficulty was that of finding a suitable solvent. The only two liquids in which barium azide was found to have appreciable solubility were water and dimethyl sulphoxide. Water solutions, of course, yielded only unwanted hydrate crystals, while crystals grown from dimethyl sulphoxide were found by mass spectrometric analysis to contain excessive quantities of sulphur. Results of previous work[1] suggested that the proper mixture of water and some other solvent such as acetone might be made to yield anhydrous crystals. Three solvent pairs were tested: water–acetone, water–ethanol and water–methanol.

Growing solutions were prepared for test by mixing a known volume of saturated aqueous solution with a known volume of acetone (or ethanol or methanol). The precipitated azide was filtered off, and the saturated filtrate used as the growing solution. The actual amounts of water, acetone and azide in unit volume of the resultant growing solution are not yet known, but are at present being determined by radioisotope techniques. A knowledge of this is not necessary for growing the crystals, however, and in the following discussion growing solutions will be identified by stating the volume ratio of aqueous solution to acetone (or ethanol or methanol) used in their preparation.

The following method was used to test the growing solutions. Taking the water–acetone solvent pair as an example, a small test-tube filled with the saturated growing solution to be tested was placed inside a larger screw-cap test-tube containing pure acetone. When the large tube was capped, acetone vapour diffused into the growing solution in the small tube, thereby lowering the solubility of the azide. Crystals as long as 2 mm are obtained within a week. These were found to be anhydrous if the growing solution was prepared from 20 per cent (or less) aqueous solution and 80 per cent (or more) acetone. The corresponding percentages for the water–ethanol system were found to be 30 per cent aqueous solution to 70 per cent ethanol; and for the water–methanol system, 40 per cent aqueous solution to 60 per cent methanol. It is seen that the water–methanol system can contain a higher proportion of water while yielding anhydrous crystals than either the water–acetone or water–ethanol systems. This higher water content results in higher solubility of the azide. It was therefore decided to use the water–methanol system in an attempt to grow larger crystals by slow cooling,

A water bath was constructed, the temperature of which was regulated by a mercury thermoregulator through an electronic relay. A 1/12 r.p.h. synchronous motor was arranged to decrease slowly the setting of the thermoregulator. Varying the percentage of time during which the motor was switched on provided a means of adjusting the cooling rate.

Growing solutions were prepared as before, except that the aqueous solution and the methanol were mixed at 40° C. This temperature was chosen as the starting point for the cooling experiment because it was feared that a higher temperature would cause decomposition of the azide. Three mixtures were tested in the slow cooling experiment: these were prepared by mixing 30, 35 and 40 per cent aqueous solution with 70, 65 and 60 per cent methanol, respectively. The solutions were then transferred to 50 ml. test tubes. These were of a screw-cap design, with caps lined with 'Teflon', to prevent the methanol (which boils at 65° C) from escaping and thus changing the composition of the growing solution. The neck of each tube was plugged with a foam polyethylene plug, into which was inserted a 90 mm long melting point capillary. To the end of this was glued a seed cyrstal grown in a previous experiment, with a glue prepared by dissolving polystyrene in toluene. The tubes were capped and placed in the water bath, and cooling was begun at the rate of 1·2° C/day. When the temperature had reached 20° C, the experiment was terminated, and the crystals were removed and washed with methanol.

The largest crystals (up to 2 cm in length) were found to have been produced in the growing solution prepared from 40 per cent aqueous solution and 60 per cent methanol. The clearest ones, however, were found in the solution prepared from 35 per cent aqueous solution and 65 per cent methanol. Almost no growth was observed in the solution prepared from 30 per cent aqueous solution and 70 per cent methanol. In some cases crystallization occurred on the test-tube walls, as well as on the seeds, which indicates that a slower cooling rate is desirable.

No attempt has been made to grow larger crystals, since the crystals obtained in this experiment were of sufficient size for electron spin resonance studies. Work is at present being carried out to determine the amount of methanol included in the crystals, and to determine their lattice parameters.

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CHEMISTRY

Far Infra-red Studies of Hydrogen Bonding in Gaseous Mixtures

RECENTLY, there have been a number of reports on infra-red spectroscopic studies of hydrogen bonding in gaseous mixtures. These studies, chiefly by D. J. Millen and his colleagues, have included amine-alcohol[1], ether–nitric acid[2] and ether–hydrogen chloride[3] complexes. These workers have measured the infra-red spectra of these gaseous, hydrogen bonded complexes in the region of the O—H stretching frequency. After complexing, they found for all the systems a strong central band with a subsidiary band on both the high and low frequency sides. The central band has been assigned to the hydrogen bonded O—H stretching vibration and the side bands have been interpreted as sum and difference combinations of the O—H stretching vibration with the hydrogen bond stretching vibration. This interpretation has been offered as a general explanation for the broadening of the O—H stretching band on hydrogen bonding and has allowed a prediction of the hydrogen bond stretching frequency in these complexes.

It is claimed that these conclusions have been substantiated by the far infra-red study of the gaseous trimethylamine–methanol complex by Ginn and Wood[4]. In their work, a band which could be attributed to the complex was observed at 142 cm^{-1}; this is within experimental error of the value predicted by Millen and Zabicky[1] for the hydrogen bond stretching frequency.

We have studied three of these systems in the far infra-red in an attempt to observe additional hydrogen bond frequencies directly. For the nitric acid–dimethyl ether complex, a band at 175 cm^{-1} is attributed to the O—H - - - O stretching mode and not 340 cm^{-1} as predicted by Millen and Samsonov. In the case of the hydrogen chloride–dimethyl ether mixture, no evidence for complex formation could be found. For the methanol–trimethylamine mixture, the 142 cm^{-1} band reported by Ginn and Wood was found to be a methanol band shifted to higher frequencies by pressure and not the hydrogen bond frequency.

Far infra-red spectra (33–400 cm^{-1}) were obtained on a Beckman *IR*-11 spectrophotometer. Conventional 10 cm gas cells equipped with high density polyethylene windows were used. Differential spectra were obtained by running a 10 cm cell containing the complex against two tandem 5 cm cells containing equivalent amounts of the unmixed components placed in the reference beam. All complexes were prepared by mixing the components in the gas phase.

Far infra-red spectra for the nitric acid–dimethyl ether system are shown in Fig. 1. Only the relatively weak 175 cm^{-1} band appeared in the spectrum on complexing even though the entire 33–400 cm^{-1} region was carefully studied. Millen and Samsonov, using the sum and difference band interpretation to explain their results in the 3,200 cm^{-1} region, have predicted the O—H - - - O stretching frequency at 340 cm^{-1} although they questioned this value when it appeared to undergo a large shift with DNO_3. We have found no indication of a band at 340 cm^{-1} and conclude that the hydrogen bond stretching frequency for the gaseous ether–nitric acid complex is at 175 cm^{-1}.

Infra-red spectra in the 120–150 cm^{-1} region pertaining to the gaseous methanol–trimethylamine system together with conditions under which the spectra were obtained are given in Fig. 2. Millen and Zabicky have studied this system in the O—H stretching region[1]. Their work has shown: (*a*) methanol and trimethylamine form a 1 : 1 complex; (*b*) only about 3 per cent of the amine is present in the complexed form; (*c*) weak satellites at ± 145 cm^{-1} from the main hydrogen bonded OH peak may be sum and difference bands involving the hydrogen bond stretching frequency at 145 cm^{-1}. The work of Ginn and Wood[4] which reports the direct observation of a band at 142 cm^{-1} for this complex has added considerable support to the conclusions reached by Millen and Zabicky.

We feel that our work on the amine–methanol system does not support the observation of a hydrogen bond frequency at 142 cm^{-1}. The spectrum of methanol vapour shown in Fig. 1 has a weak doublet at 139 cm^{-1}. On addition of trimethylamine to the methanol this band becomes more intense and shifts to 141 cm^{-1}. This is in agreement with the observation of Ginn and Wood. Exactly the same result is obtained, however, when nitrogen or argon is added to the methanol, and in addition the intensity of the 141 cm^{-1} band increases with increasing

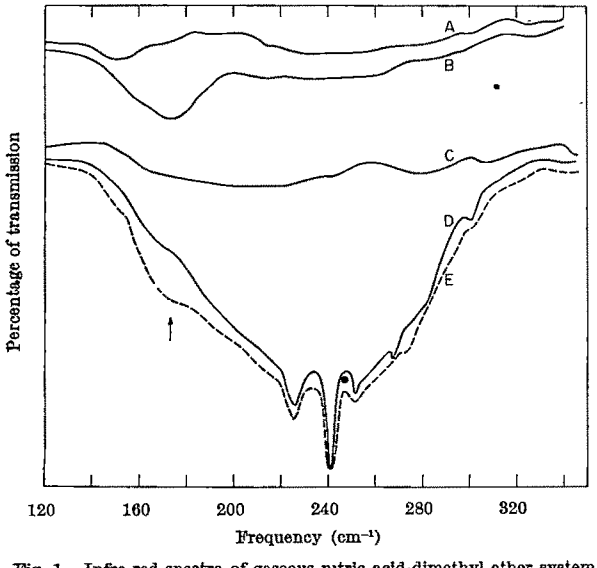


Fig. 1. Infra-red spectra of gaseous nitric acid-dimethyl ether system. *A* is spectrum of 150 mm ether in sample beam against 150 mm ether in reference beam, and *B* is differential spectrum after addition of 40 mm nitric acid to sample cell. *C* is spectrum of 45 mm nitric acid, *D* is spectrum of 400 mm ether, and *E* is mixture of 400 mm ether and 45 mm nitric acid. All spectra refer to 10 cm path length.

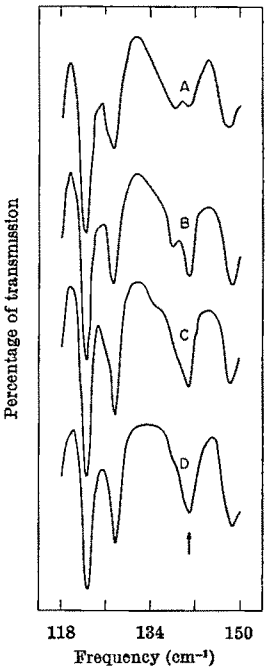


Fig. 2. Spectrum *A* is that of methanol vapour at 90 mm pressure, *B* and *C* are 90 mm methanol to which has been added 150 mm and 300 mm nitrogen respectively, and *D* is 90 mm methanol to which has been added 300 mm trimethylamine. Path length was 10 cm in all cases and spectral slit width was about 2 cm^{-1}.

partial pressure of nitrogen just as Ginn and Wood reported for trimethylamine. We therefore believe that Ginn and Wood's 142 cm^{-1} hydrogen bond frequency is the same band we have observed at 141 cm^{-1} and have shown is really a pressure enhanced methanol band. In fact, no evidence of complex formation could be found in the 33–400 cm^{-1} region, which is perhaps not surprising considering that only 3 per cent of the trimethylamine molecules are involved in complex formation.

Far infra-red spectra were also obtained on mixtures of 20 torr dimethyl ether and 30 torr hydrogen chloride. Addition of the ether produced no observable change in the position or intensity of the hydrogen chloride pure rotational lines. The differential spectrum of the gaseous mixture against equivalent amounts of the components in the divided reference cell showed no bands which could be attributed to a complex. Although Arnold, Bertie and Millen[3] have predicted from infra-red studies in the 2,500 cm^{-1} H—Cl stretching region an ←O - - - H—Cl→ frequency at 110 cm^{-1}, we could not confirm this. Apparently so little of the hydrogen chloride is involved in hydrogen bonding, as evidenced by the observation that the addition of ether caused no perturbation of the hydrogen chloride rotational lines, that any bands due to the complex are not detectable in the far infra-red.

It is concluded that with the ether–hydrogen chloride and trimethylamine–methanol systems, far infra-red studies offer no evidence for or against Millen's predictions based on measurements in the O—H stretching region. In both these systems the amount of hydrogen bonded complex formed is probably too small to be detected in the far infra-red region. We have shown, however, that a previously reported band at 142 cm^{-1} for the amine–methanol complex is not a hydrogen bond frequency. With the nitric acid–dimethyl ether complex, the observed hydrogen bond stretch at 175 cm^{-1} differs considerably from the 340 cm^{-1} value predicted by Millen and Samsonov, although there may be some significance in the fact that the observed frequency is almost exactly at half the predicted value. This could mean that the shoulders observed by Millen at ± 340 cm^{-1} from the O—H stretching band result from the ternary combinations, $\nu_{O-H} \pm$

$2\nu_{O-O}$, and that the corresponding binary combinations are not resolved from the strong central maximum. This work was supported by the National Institutes of Health.

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Band-broadening in Infra-red Spectra of Gaseous Hydrogen-bonded Complexes

AN infra-red investigation of hydrogen bonding in various gaseous mixtures showed the band arising from the hydrogen stretching vibration to be unusually broad even for gaseous complexes[1–3]. In favourable cases the structure of the broad absorption contour was clear, consisting of a central band with a subsidiary band on both the low and high frequency sides. An assignment was suggested of the central band to the hydrogen stretching frequency and of the side bands to sum and difference bands of this frequency and the stretching frequency of the hydrogen bond. The pattern of results for the complexes of hydrogen chloride and fluoride with ethers, and of methanol with amines, was found to accord with this view. On the other hand, the band structure found for mixtures of nitric acid and ethers had some unexpected features. The frequency of the hydrogen bond stretching frequency inferred from the sum and difference band interpretation was unexpectedly high by comparison with the other complexes, and the change in band structure found on transferring to deutero-nitric acid was also anomalous. Accordingly, we have made a further investigation of systems of nitric acid and ether. We are prompted to report briefly on this by the comments of Carlson, Witkowski and Fateley[4] on the far infra-red spectrum of this and other related gaseous systems.

Our new investigation leads to the conclusion that the side bands are displaced by about 185 cm^{-1} from the central peak. This value has been reached as a result of a systematic investigation, in which great care has been taken to avoid contamination by impurities. It will be sufficient here to quote one particular piece of evidence from which the conclusion can be reached directly. Fig. 1 shows the band structure observed for a mixture of hexadeutero dimethyl ether and nitric acid. The spectrum of the deuteroether, which is also included in Fig. 1, shows only a weak absorption in this region so that the material has distinct advantages over the light ether for this work. Mixtures were contained in a specially constructed infra-red cell. The inter or surface of the cell was gold-plated and silver chloride windows were fitted with 'Teflon' *O*-rings.

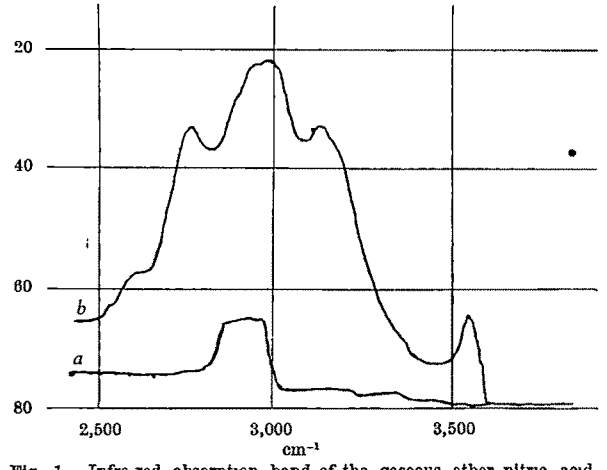


Fig. 1. Infra-red absorption band of the gaseous ether–nitric acid hydrogen-bonded complex. *a*, Spectrum of deutero dimethyl ether, *b*, spectrum of mixture of deutero dimethyl ether and nitric acid.

The spectrum in Fig. 1 shows a central peak at 2,950 cm^{-1} with subsidiary bands at approximately 3,125 and 2,760 cm^{-1}, and with a weaker shoulder at approximately 2,600 cm^{-1}. The high frequency band is displaced by about 175 cm^{-1}, and the low frequency band by 190 cm^{-1}, from the central peak. The weak shoulder is displaced by about 350 cm^{-1}.

Carlson, Witkowski and Fateley have observed a band in the far infra-red spectra of mixtures of nitric acid and dimethyl ether at 175 cm^{-1} and suggest assigning it to the stretching vibration of the hydrogen bond. Comparison can now be made with the sum and difference band interpretation of the infra-red structure of the band centred at 2950 cm^{-1}. The sum and difference band displacements are in fact not far removed from the suggested hydrogen bond stretching frequency. Agreement would be expected to be best for the difference band, but the accuracy of measurement is not high, as can be seen from Fig. 1, because of the broadening of the individual bands which make up the structure; this is particularly noticeable for the central band. The shapes of these constituent bands, like the origin of their breadth, is unknown. These could conceivably influence the observed separation between the maxima and lead to some discrepancy.

The far infra-red investigation of the hydrogen chloride–ether systems and of methanol–trimethylamine revealed no band which could be attributed to the hydrogen bond stretching vibration[2]. It is perhaps understandable that of the systems investigated only the nitric acid–ether system showed such a band, for the infra-red study of the hydrogen stretching bands indicates that the concentration of complex is most favourable in this case.

We thank Drs. Fateley, Carlson and Witkowski for sending us a copy of their paper[4] before publication, and the Gulbenkian Foundation for the award of a studentship (L. A.).

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Oxygen Reduction on Titanium Nitride in Alkaline Electrolyte

THE nitrides and carbides of the metals in groups IV*B* and V*B* of the Periodic Table have been studied during the course of investigations with a view to developing fuel cell catalysts for oxygen (O_2) reduction in alkaline electrolyte. In general, the carbides show considerably more oxygen reduction activity than do the pure metals. By simple atomic consideration this can be attributed to the presence of carbon, which *per se* is a good catalyst for the reduction of oxygen to hydrogen peroxide. The nitrides exhibit more activity than does the pure metal, but to a lesser degree than the carbides. Of special interest is the nitride of titanium, which shows considerably more activity for oxygen reduction than does pure titanium. In contrast to other metals titanium nitride is more active than titanium carbide.

For these experiments the potentiostatic current-potential measurements were made on rotating disk electrodes at 75° C in 2 M potassium hydroxide saturated with nitrogen or oxygen. Each specimen, after mounting

and polishing, was introduced into a solution of potassium hydroxide saturated with nitrogen at a preset 1,200 mV and rotated at a constant 600 r.p.m. throughout the tests. (All potentials were measured against a reversible hydrogen electrode in the same solution.) A potential sweep was initiated immediately at a rate 50 mV/min scanning from 1,200 mV to 0 mV and back again to 1,200 mV. This curve indicated the corrosion behaviour of the electrode. The oxygen reduction activity was studied by repolishing the electrode and repeating the potential scan in a solution saturated with oxygen.

The $i(E)$ curves obtained for titanium (Fig. 1*a*) show detectable corrosion in the whole range of potentials; oxygen reduction begins only at less than $E = 300$ mV. Although this potential seems to be very low (900 mV overvoltage), the activity of titanium for oxygen reduction is higher than that of zirconium or hafnium, which in the range of potentials studied show no appreciable activity under similar conditions.

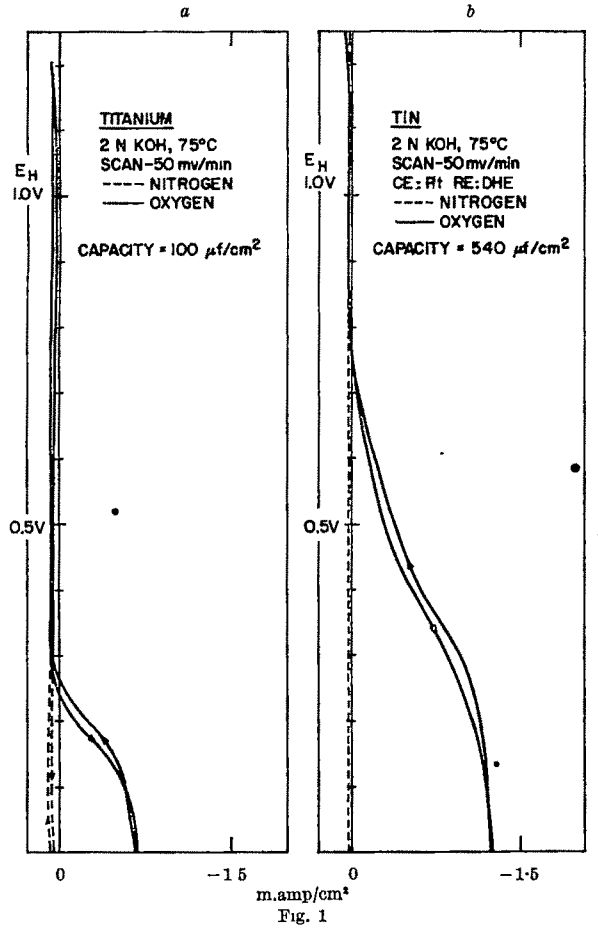


Fig. 1

The $i(E)$ curves for titanium nitride (Fig. 1*b*) show appreciably less corrosion than those of pure titanium but, on the other hand, considerably higher activity for oxygen reduction. As already mentioned, no current for oxygen reduction was observed above $E = 300$ mV with titanium, while titanium nitride showed oxygen reduction in the vicinity of 800 mV. In addition, the observed limiting current for titanium nitride is almost twice that of titanium, possibly indicating a higher efficiency for oxygen reduction (less hydrogen peroxide formation). Double layer capacity measurements show that the surface of the titanium nitride electrode is five times larger than that of the titanium electrode. Because the corrosion current is higher with titanium than with titanium nitride, however, it seems unlikely that the increase in oxygen activity is solely an effect of increased surface. Also, a five-fold increase in the surface area does not adequately account for a potential shift of 500 mV in the oxygen reduction potential.

A more plausible explanation is the reduction, by nitridation, of the rate of electrochemical oxidation and thus of the thickness of the oxide layer on titanium. This would mean that oxygen is reduced on titanium oxide and that a decrease in the thickness and/or the composition of this oxide by nitridation causes an increase in activity for oxygen reduction. (Oxynitrides of titanium are known[1].)

The increase in oxygen reduction activity on titanium nitride is of considerable interest because it could not be anticipated from atomic considerations as with titanium carbide (titanium carbide, as well as carbides of the other metals of the group, approaches graphite in activity for oxygen reduction). The results obtained with titanium nitride suggest that nitridation of such metals as iron, nickel, and cobalt—which have limitations similar to titanium for oxygen reduction—may help to increase their activity. In consequence, experiments with the nitrides and carbides of iron, cobalt, titanium and nickel are now in progress.

This work was sponsored by the National Aeronautics and Space Administration. We thank Mr. E. M. Cohn for directing our attention to nitrides and carbides as possible oxygen electrodes.

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Synthesis of Substituted Phenyl-β-D-xylopyranosides (II)

IN a recent communication we reported the synthesis of a series of mono-substituted phenyl-β-D-xylosideacetates through the Helferich condensation and that of the corresponding β-D-xylosides by catalytic deacetylation with sodium methoxide[1].

This communication reports on the preparation of a further series of these β-D-xylosides by the same method.

Tetra-*O*-acetyl-β-D-xylose was prepared according to Vogel[2]. The purity of the products was tested by thin-layer chromatography on silica gel using acetic acid–water–ethylacetate (1/1/3; v/v) for xylosides, and ethylacetate–benzene (3/7; v/v) for the acetates. The spray reagent used was 5 per cent sulphuric acid in ethanol (10 min at 120° C). The xylosetetraacetate (0·1 mole) and the appropriate phenol (0·4 mole) were fused together and the molten mixture then treated with a solution of *para*-toluenesulphonic acid (0·6 g) in a mixture of acetic anhydride–acetic acid (5/95; v/v) (50 ml.). The melt was heated under reduced pressure at 100° C for 1 h. After cooling, the resulting syrup was dissolved in chloroform (200 ml.), thoroughly washed with ice cold N sodium hydroxide and water, dried (using sodium sulphate) and evaporated *in vacuo*. When necessary, solutions of benzene were run through a column of alumina and eluted with more benzene in order to remove dark condensation products which were insoluble in alkali. The resulting syrup was then crystallized from the appropriate solvent to constant melting point and optical rotation.

Table 1. ACETYLATED β-D-XYLOPYRANOSIDES

	Melting point (° C) and crystal solvent	$(\alpha)_D^{22}$ chloroform	Yield	Analytic value	C	H
Para-methoxyphenyl	150°–151°	−43° 0	57	f*	56 5	5 8
$C_{18}H_{22}O_9$	Ethanol	c, 2		c	56 5	5·7
Para-ethoxyphenyl	108°–109°	−40° 5	30	f	57 5	6·4
$C_{19}H_{24}O_9$	Ethanol	c, 2		c	57 6	6·1
Para-benzoxyphenyl	121°–122°	−34° 8	36	f	62·8	5·7
$C_{24}H_{26}O_9$	Ethanol	c, 2		c	62 9	5 7
Ortho-methoxyphenyl	141°–142°	−64° 7	34	f	56·3	5 9
$C_{18}H_{22}O_9$	Methanol	c, 2		c	56·5	5·8
Para-ter-butyl-phenyl	168°–169°	−39° 8	37	f	61 7	6 9
$C_{21}H_{28}O_8$	Ethanol	c, 2		c	61·8	6 8
Para-diphenyl	156°–157°	−35° 7	10	f	64·3	5 7
$C_{23}H_{24}O_8$	Water/ethanol 3/1	c, 2		c	64 5	5 6

* f, Found; c, calculated.

Table 2. β-D-XYLOSIDES

	Melting point (° C) and crystal solvent	$(\alpha)_D^{22}$	Yield	Analytic value	C	H
Para-methoxyphenyl	157°–158°	−39° 2	70	f	56·2	6·1
$C_{12}H_{16}O_6$	Water	c, 2; CH_3OH		c	56·3	6·3
Para-ethoxyphenyl	151°–152°	−36° 2	87	f	57·7	6·8
$C_{13}H_{18}O_6$	Ethylacetate	c, 2; CH_3OH		c	57 8	6·7
Para-benzoxyphenyl	177°–179°	−30° 3	88	f	64·9	6 1
$C_{18}H_{20}O_6$	Water/dioxan 20/5	c, 2; dioxan		c	65 1	6 0
Ortho-methoxyphenyl	175°–176°	−60° 7	71	f	56·3	6·2
$C_{12}H_{16}O_6$	Water	c, 1; CH_3OH		c	56·3	6·3
Para-ter-butyl-phenyl	186°–187°	−33° 7	88	f	63·6	7 6
$C_{15}H_{22}O_5$	Ethanol/water 1/4	c, 2; CH_3OH		c	63·8	7·8
Para-diphenyl	221°–222°	−33° 4	86	f	67·5	5·9
$C_{17}H_{18}O_5$	Ethanol/water 1/3	c, 1; pyridine		c	67·6	5 9

The tri-*O*-acetyl-β-D-xylopyranosides shown in Table 1 were prepared in this way.

The *ortho*-substituted phenols, *ortho*-tert-butyl-phenol and *ortho*-isopropyl-phenol failed to react even at high temperatures. Catalytic deacetylation of the acetates according to Thompson and Wolfrom[3] yielded the phenyl-β-D-xylosides shown in Table 2.

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Interpretation of Electrolyte Invasion Data for Ion-exchange Polymers

IN recent years two quite independent theories have been developed to account for the observed electrolyte invasion properties of ion-exchange polymers. Scatchard and Freeman[1] suggest that in the application of the Donnan law (1), deviations are best interpreted in terms of the mean activity coefficient of the electrolyte in the exchanger phase, $\bar{\gamma}_\pm$.

$$(\nu_c.\bar{m})^{\nu c}\,(M + \bar{m}.\nu_g)^{\nu_g \nu c}\bar{\gamma}_\pm^{\nu} = k(\nu_c.m)^{\nu c}\,(\nu_g.m)^{\nu_g.\nu}\gamma_\pm^{\nu} \qquad (1)$$

where ν_c is the number of co-ions in a molecule of electrolyte; ν_g is the number of counter or gegen ions in a molecule of electrolyte, $\nu = \nu_c + \nu_g$; M is the observed counter-ion concentration; m, the concentration of external electrolyte; $\bar{m}$, the concentration of imbibed electrolyte; k, a constant; $\bar{\gamma}_\pm$ and $\gamma_\pm$ are the mean internal and external activity coefficients of the electrolyte, respectively. In accordance with the theory of specific ion interactions[2], an expression has been derived (2), in which a and b are constants, to account for variations in $\bar{\gamma}_\pm$ for ion-exchangers based on styrene-divinylbenzene copolymers. This relationship has also been applied to polymethacrylic acid ion-exchangers[3].

$$\log \bar{\gamma}_\pm = a + b\,\bar{m} \qquad (2)$$

It is assumed in this treatment that any deviations resulting from non-uniformity of the exchanger matrix are either negligible or can be eliminated by a small correction to the observed results.

The alternative view is that proposed by Glueckauf[4], in which departures from the ideal law are explained in terms of the heterogeneity of the ion-exchange matrix. A modified power law (3) for invasion by 1 : 1 electrolytes is introduced, on the assumption that the ratio $\bar{\gamma}_\pm/\gamma_\pm$ is approximately constant and that $M \gg \bar{m}$. The parameter z is characteristic of the heterogeneity of the ion-exchanger. This approach has also been applied to a series of solvent modified styrene-divinyl-benzene resins, with a wide range of heterogeneity[5].

$$\bar{m} = k'\,m^{(2-z)}, \text{ where } 0 > z > 1 \qquad (3)$$

Interpretation of electrolyte invasion results is particularly difficult as most investigations have been over short ranges of concentration and therefore do not provide an adequate test of these theories. Notable exceptions are the diffusion studies of invasion in ion-exchange membranes by Glueckauf, Watts and Crabtree[6,7], and more recently the work of Freeman, Patel and Buchanan[8], in which the resin and external aqueous phases are not separated. This report is concerned particularly with the interpretation of the data obtained in the latter investigation, which the authors have treated exclusively in terms of the mean activity coefficient in the exchanger phase, $\bar{\gamma}_\pm$. The results from this work are plotted as log m against log $\bar{m}$ in Fig. 1. Applying the Donnan law (1) to the three systems considered, the appropriate power law relationships are as follows:

for the system (*a*) $\bar{R}$-K^+, K^+Cl^-, H_2O, $\bar{m} = k_1.m^2$

(*b*) $2\bar{R}$-Mg^{2+} $Mg^{2+}2Cl^-$, H_2O $\bar{m} = k_2.m^{1.5}$

(*c*) $\bar{R}^+Cl^-$, $Mg^{2+}2Cl^-$, H_2O $\bar{m} = k_3.m^3$.

The materials used in this study were $AG50W \times 2$ (cation) and $AG1 \times 2$ (anion) exchangers supplied by Biorad Laboratories. Present-day theories on styrene-divinylbenzene copolymerization[9] suggest that resins of this type, with low divinylbenzene contents, will be very evenly cross-linked and therefore provide ion-exchangers of low heterogeneity. In consequence, if the heterogeneity theory is correct, much smaller deviations would be expected for these resins than those observed with materials of higher divinylbenzene content. From the plot for system (*a*) two significant factors are apparent: (1) with decreasing external concentration m, the gradient increases; (2) in the region $m < 10^{-1}$ molar the gradient is a very reasonable approximation to the value of two required by the ideal law. Both these effects were predicted by the heterogeneity theory. The results for the other systems, involving the magnesium ion, are somewhat ambiguous. System (*c*), for which the Donnan law requires an exponent of three, also has a gradient of about two and therefore would appear to support the conclusion of Glueckauf, from his work[7] with the lanthanum ion, La^{3+}, that equation (3) is generally applicable, immaterial of the valencies of the ions involved. The gradient for system (*b*) shows a similar increase with decreasing external concentration m, but even for $m < 10^{-2}$ molar the value is only 1·5; from which it might be concluded that it is approaching the exponent required by the ideal law and not the value of two expected for a homogeneous ion-exchanger from

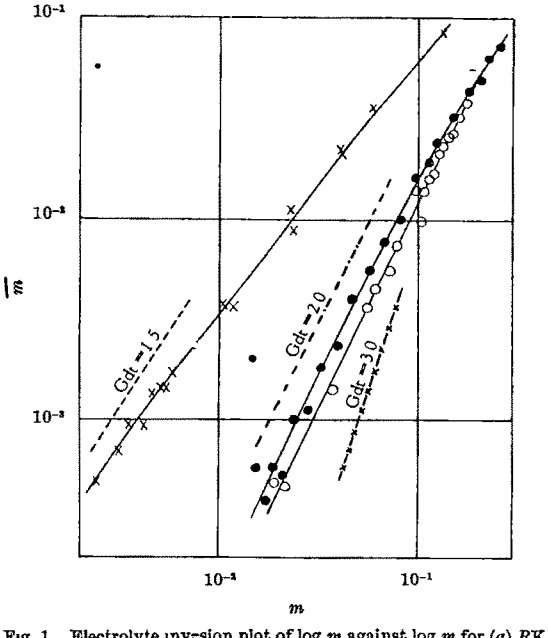


Fig. 1. Electrolyte invasion plot of log m against log m for (*a*) $\bar{R}K$, K^+ (●); (*b*) $\bar{R}_2Mg$, $MgCl_2$ (×); (*c*) $\bar{R}Cl$, $MgCl_2$ (○).

equation (3). From equation (3) for systems (*a*) and (*c*) the parameter $z \simeq 0$, but for the system (*b*) $z \simeq 0{\cdot}5$. On this basis the results for (*a*) and (*b*) do not give a consistent measure of the heterogeneity of the cation-exchanger $AG50W \times 2$.

In conclusion, the results for system (*a*) are clearly consistent with a theory based on heterogeneity. The results for systems (*b*) and (*c*) raise doubts as to the applicability of equation (3) to systems involving polyvalent ions, although in this case there is still a need for a comprehensive study.

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Role of Neutral Salts on the Denaturation Temperature of Biopolymers

IN recent years, a great deal of attention has been devoted to the study of the effect of salts on the denaturation of biopolymers, both in the bulk state (for example, shrinkage of fibrous collagen) and in dilute solutions (for example, helix-coil transitions)[1,2]. Recent work in this laboratory[3] has demonstrated that two main factors must be considered in describing the effects of both salt type and salt concentration on the shrinkage of collagen at neutral *p*H (when polyelectrolyte effects are absent). These are: the diluent and the specific effect. The former effect is predominant for salting-out agents, such as potassium chloride and magnesium sulphate, whereas the latter occurs with salting-in agents, such as potassium thiocyanate and lithium bromide. The diluent effect is satisfactorily described by the conventional melting point depression theory developed for binary polymer-diluent systems

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \frac{R}{\Delta H_u}\,(V_u/V_1)\,(\zeta_1 - \chi_1\zeta_1^2) \qquad (1)$$

where T_m is the melting temperature of the polymer in the presence of the diluent, T_m^0 the melting temperature of the undiluted polymer, ΔH_u is the heat of fusion per mole of repeating unit, V_u the molar volume of the repeating unit, V_1 the molar volume of the diluent, ζ_1 the volume fraction of diluent, and χ_1 represents a polymer–diluent interaction parameter which can be interpreted[3] in terms of conventional salting-out theories[4].

Considerably more debatable is the nature of the specific effect. One possible way to explain it is to consider a disproportion of the salt–water mixture resulting in ion binding to the protein[1,3]. As in the classical case of melting point depression, accompanied by the formation of a complex in the amorphous state[5], the large salting-in suggests that binding occurs mainly in the denatured state. Assuming that binding occurs only in the latter state, Schellmann, Flory and Mandelkern[1] have shown that an additional term should be added to the right-hand side of equation (1). This is

$$(RN_a/\Delta H_u)\ln(1 + kA_s)$$

where N_a is the mole fraction of available sites, k is the binding constant, and A_s denotes the activity of the salt. An alternative way of describing the specific effect is to postulate an indirect interaction by which the salt solutions affect the protein stability. This indirect mechanism could be related to such properties of the solution as its degree of ice-likeness[2].

Although present evidence favours the ion binding hypothesis[3], the critical experiment of determining whether a binding of salt actually occurs for salting-in agents, particularly at the denaturation temperature, has not previously been reported. Here we shall describe some preliminary results which unequivocally demonstrate that a removal of ions from solution does accompany the thermal shrinkage of fibrous collagen in potassium thiocyanate solutions.

We have equilibrated initially dry collagen tendons, cross-linked with *p*-benzoquinone[3], in a solution of carbon-14 labelled potassium thiocyanate of known thiocyanate concentration, C_s^i, and determined the thiocyanate concentration, C_s^f, after equilibration with the collagen[6], both below and above the shrinkage temperature (72° C for these samples in pure water). The *p*H of the solution was about 7, which is within the isoelectric plateau of the protein[3] (the isoionic *p*H of the solubilized tendons at 40° C was found to be 7·1). Concentrations of thiocyanate solutions were determined by liquid scintillation counting of equal portions of the solution in toluene–ethanol–2,5-diphenyl oxazole–1,4-*bis*-2-(5-phenyloxazolyl)-benzene scintillator using a Packard 314X liquid scintillation spectrometer. In Fig. 1*A* and *B* is shown the change in length and volume, respectively, which occurs during the shrinkage[7], and *C* shows the corresponding changes in the difference between C_s^i and C_s^f. The increase in C_s^i–C_s^f which corresponds to the

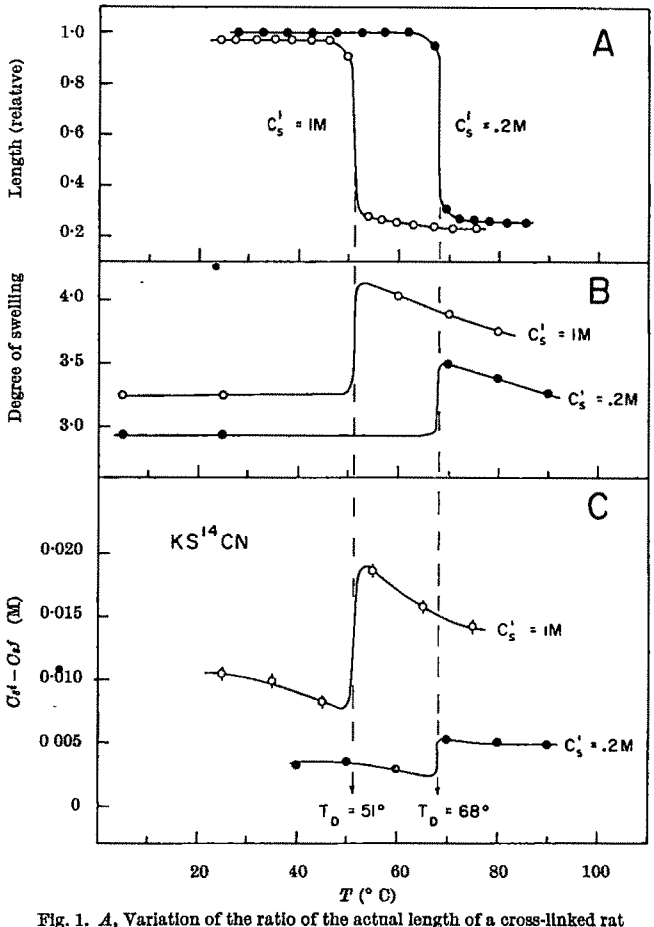


Fig. 1. *A*, Variation of the ratio of the actual length of a cross-linked rat tail tendon to the length in the native swollen state, with temperature (ref. 7). Tendons immersed in potassium thiocyanate solutions of the indicated concentrations. *B*, Corresponding variations of the ratio of the actual volume of tendons to the volume in the native, dry state, with temperature (ref. 7). *C*, Apparent uptake of carbon-14 labelled potassium thiocyanate by initially dry, cross-linked tendons as a function of temperature. Collagen concentration is about 25 mg/ml.

shrinkage temperature, T_D, indicates that shrinkage or denaturation can indeed be associated with an increase in the degree of binding.

The fact that $C_3'-C_3^I$ is greater than zero below T_D shows that some binding does occur even for the native protein. Because X-ray diffraction[7] does not reveal any alteration of the lattice spacings for native collagen in equilibrium with potassium thiocyanate solutions below T_D, it is likely that binding in the native state occurs mainly within regions of poor structural organization.

At present we are unable to define explicitly the type of association between the ions and the protein. Considerations reported elsewhere[3] indicate that an interaction of the ions with the dipoles of the peptide bond is involved, and that some difference exists between the binding behaviour of anions and that of cations. Furthermore, the binding of ions is competitive with the binding of water, and this leads to an expression which is even more complex than equation (1) with the Schellmann–Flory–Mandelkern term[8].

We are now repeating these experiments using various salts at several concentrations in order to determine both the apparent binding constants and the number of binding sites along the chain backbone.

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Isotopic Exchange Chromatographic Separation applied to an Equilibrium Strontium-90 and Yttrium-90 Mixture

ANALYTICAL techniques based on precipitation, volatility, electrolysis, ion exchange, solvent extraction, chromatography, etc., with or without carrier, have been used to separate individual radionuclides and to separate groups of radionuclides. Less attention has been paid to radiochemical separation or determination involving isotopic or displacement exchange reactions. The amalgam exchange procedures[1-6] introduced by Meinke and collaborators appear to be unique and will find wider use in radiochemical separations. Isotopic exchange has also been applied to the separation of radiosilver[7] and of iodine-131 (ref. 8) by Sunderman and Meinke, and adaptation of isotopic exchange to the determination of mercury in a solvent extraction system has been worked out by Handley[9]. A displacement method for the separation of radio-iodide on a column of silver chloride granules has been reported by Arnott and Wells-Cole[10]. Americium has been separated from curium and other trivalent actinides and lanthanides with a column of calcium fluoride[11], and this column has also been used for the removal of radioactive actinides and lanthanides from aqueous solutions[12].

The exchange of strontium ions between a precipitate of strontium sulphate and a solution of strontium salt is very rapidly completed. Because of the low solubility of strontium sulphate, at equilibrium there is a very favourable ratio between strontium atoms in the precipitate and in the solution. In the work to be reported here, this fact has been used to develop a rapid chromatographic method, involving only a single step and based on isotopic exchange for the separation of carrier-free Y-90 from its parent Sr-90.

Commercially available crystal powder of strontium sulphate was treated with boiling 0·5 N sulphuric acid solution for 5 min, and the crystals thus activated were transferred with small amounts of 0·5 N sulphuric acid to a conventional chromatographic column, 1·1 cm in diameter and 5 cm in height, pulled to a tip at the outlet to facilitate the collection of the eluate. The crystals were supported by a sintered glass plate attached to the lower end of the column; the crystal bed was usually 1·3 cm long. About 20 μl. of an equilibrium mixture of Sr-90 and Y-90, converted to sulphate form and adjusted to 0·5 N in sulphuric acid, was applied to the top of the column. By means of a slight over-pressure, the strontium was allowed to pass down the column. The column was filled with 0·5 N sulphuric acid, and the pressure regulated so that the flow amounted to one drop (~0·05 ml.) per 15 sec. Every two drops of eluate were collected on counting trays and evaporated to dryness with an infra-red lamp. The radioactivity of the eluate was measured with a conventional end-window GM counter.

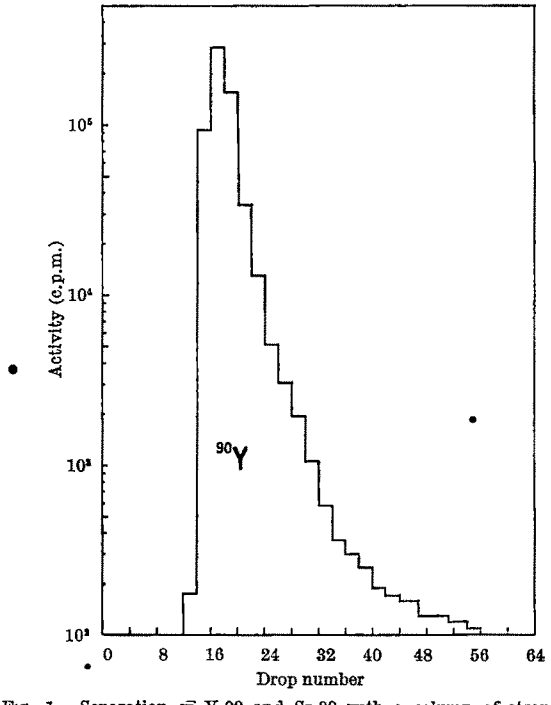


Fig. 1. Separation of Y-90 and Sr-90 with a column of strontium sulphate. Y-90 eluted with 0·5 N sulphuric acid. Strontium sulphate bed 1·3 cm long, Φ 1·1 cm (2·5 g).

A typical elution profile curve is illustrated in Fig. 1. No activity was found in the first twelve drops of the eluate. In later fractions, activity appeared rapidly, reaching a maximum at 16 drops, and then decreased rather gradually. It is apparent that there is a slight retention of the yttrium on the crystal column. This may be considered to be due partly to the similarity in ionic radius and electronegativity between Sr^{2+} and Y^{3+} ions, as indicated here:

	Sr^{2+}	Y^{3+}
Ionic radius (Å)	1·13	0·93
Electronegativity	125	160

The ionic radii are taken from Pauling (ref. 13) and the electronegativities from Povarennykh (ref. 14).

Several fractions, covering the entire elution profile, were used for decay rate measurement and γ-ray spectrometry. The decay curves consisted of two components corresponding to a β-activity with a half-life of 64·5 h and a long-lived nuclide emitting γ-rays. No sign of break-

through of Sr-90 was observed in the eluate. (A γ-ray spectrum of the long-lived activity revealed the presence of Cs-137 with a half-life of 30 yr deriving as impurity from the original Sr-90 and Y-90 mixture.) In this way the carrier-free Y-90 can be rapidly separated in only about 15 min from carrier-free parent Sr-90.

The same principle would be applicable to radiochemical separations, provided that insoluble salts exist and that exchange between the salt and its ions is rapid. Thus the separation of carrier-free Ce-144 and Pr-144 can be carried out on a short column of cerium (IV) iodate crystals. The bed was 0·8 cm long and was first treated with 6 N nitric acid. Pr-144 was recovered within 7 min, in the first 20 drops, by elution with 6 N nitric acid, but because of the increased solubility in elution with nitric acid, the contamination was rather high and averaged approximately 3 per cent.

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BIOCHEMISTRY

Examination of Isoenzymes of Several Dehydrogenases in Pure Cell Lines

MANY workers have demonstrated that lactic acid dehydrogenase (LDH) and malic acid dehydrogenase (MDH) exist in several isoenzyme forms[1–5]. Bands of different electrophoretic mobility have also been reported for 6-phosphogluconate dehydrogenase (6-PGDH) (ref. 6), but the kinetic properties of these isoenzymes have not been as clearly defined.

This report describes some preliminary results obtained on the characterization of several pure cell lines (as opposed to tissues) with respect to the isoenzymes of these three dehydrogenases. To do this, homogenate supernatants of the cells were fractionated by zone electrophoresis on starch gel, the isoenzymes being located as distinct blue formazan bands on the gel.

The following cell lines were examined:

(1) *L*5178*Y* lymphoblasts[7]: (*a*) obtained directly from *DBA*/2 mice with transplanted ascites tumour; (*b*) *radioresistant* and *radiosensitive* diploid and tetraploid cells carried through seventy-two generations *in vitro*[8]. Isoenzyme bands from *DBA*/2 mouse kidney and liver extracts were used as markers for cells which originate from mouse and hamster tissues. (Mobility of isoenzymes in the hamster is similar to that observed in the mouse.)

(2) Hamster kidney fibroblasts: (*a*) *BHK-C*13 normal[9] cells carried through fifty generations *in vitro*; (*b*) *BHK-TCY*[9] cells transformed with polyoma virus and carried through twenty-five generations *in vitro*.

(3) Rat sarcoma cells (induced by benzpyrene): (*a*) primary sarcoma tissue taken from a freshly killed hooded rat; (*b*) four clones of sarcoma cells grown *in vitro* for 8 months (about 500 generations), *S*6/1, *S*6/2, *S*6/3 and *S*6/4. Isoenzyme bands from rat kidney extracts were used as a marker.

(4) N_2 cells[10]—normal adult subcutaneous fibroblasts from *C3H* mice, grown *in vitro*.

The tissues were ground in 0·25 M 'Mannitol' (containing 0·1 mg/ml. ethylenediamine tetraacetic acid) *p*H 7·4 with a 'Teflon' pestle in a Potter–Elvehjem homogenizer, tough fibrous material being broken up first by grinding for 10 sec with an 'Ultra-Turrax': the final concentration was 150–200 mg/ml. Cells were washed twice with 'Mannitol' solution, resuspended to give a cell count of 20–25 × 10^6 cells/ml, and then broken up with the homogenizer. All homogenates were centrifuged for 30 min at 144,000*g*; the supernatants were used for the isoenzyme studies. A temperature of 0°–4° C was maintained throughout these procedures.

80–120 μl. of supernatant was applied to the gel with 2–3 pieces Whatman 3 *MM* paper (1·4 mm × 1 mm). The electrophoresis was performed at *p*H 7·0 using a phosphate citrate buffer[11] at 4° C, by applying a potential of 6 V/cm across the gel for 4 h. After electrophoresis, the gel was sliced into three longitudinal layers (9 × 16 × 3 mm), each layer being stained for a different dehydrogenase. The isoenzymes were visualized directly on the gel by coupling the reduction of dimethyl-thiazolyl diphenyl tetrazolium bromide (MTT) with reduction of coenzyme (in the presence of appropriate substrates) by using phenazonium methosulphate as an electron transporter. For LDH isoenzyme a slice of gel was incubated with 10 ml. of a 1 per cent agar overlay containing 8·8 ml. 0·1 M *tris*-hydrochloric acid (*p*H 8·0); 0·5 ml. 2 M D,L-lithium lactate (*p*H 8·0); 2·0 mg MTT; 0·1 ml. phenazonium methosulphate (5 mg/ml.); 0·6 ml. nicotinamide adenine dinucleotide (10 mg/ml.) for 15 min at 37° C in the dark. For MDH a similar medium was used except that 1 ml. 2 M D,L-sodium malate (*p*H 8·0) was substituted for lithium lactate, and the incubation time was lengthened to 30 min. For 6-PGDH, 2 mg nicotinamide adenine dinucleotide phosphate replaced the nicotinamide adenine dinucleotide, and 1 ml. 6-phosphogluconate (10 mg/ml.) was used as substrate instead of lactate. The incubation time here was 30 min. On all occasions the reaction was terminated by soaking the gel slice in a methanol/acetic acid/water (5 : 1 : 5) mixture, for 10 min.

Fig. 1 is a diagrammatic representation of the patterns observed. Column 3 illustrates the distribution of LDH, MDH and 6-PGDH obtained with all the various *L*5178*Y* cells examined. These results demonstrate the stability of the isoenzyme patterns during long term culturing and also the uniformity of the isoenzyme patterns in the three *L*5178*Y* mutants studied. Although mouse lymphoblasts and hamster kidney fibroblasts show a single LDH band, N_2 and sarcoma cells contain several. The M_4 LDH band was the most commonly observed isoenzyme; however, the N_2 cells exhibited a pattern which was distinct from that of the other cultured specimens. Only one band for MDH and 6PGDH was noted with the cell cultures, except for sarcoma cells (column 6) which displayed two bands for 6PGDH. The tissues appear to display several bands of each enzyme, under the conditions used here.

With rat sarcoma, cells grown *in vitro* show a different distribution in all three enzymes from that observed with the primary sarcoma.

Identical enzyme patterns were obtained with a normal and polyoma virus infected hamster kidney fibroblast. Caution should be exercised in the interpretation of these results. It is not known whether the difference observed between the cultured and *in vivo* cells (for example, sarcoma) results from a lack of cellular homogeneity of the fresh tissue or the result of culturing[12,13]. In addi-

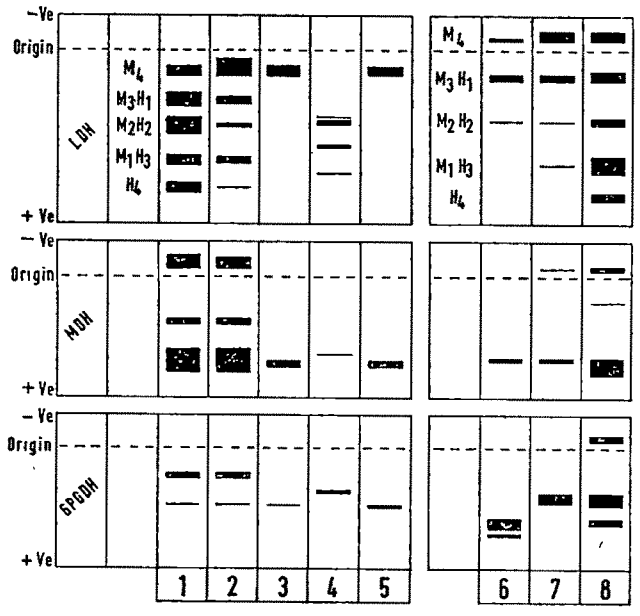


Fig. 1. Summary of the isoenzyme patterns obtained with tissue extracts and cultured cells: (1) *DBA*/2 mouse kidney; (2) *DBA*/2 mouse liver; (3) lymphoblasts directly from a tumour-bearing *DBA*/2 mouse, *radioresistant* and *radiosensitive* diploid lymphoblasts examined after forty-four, fifty-eight and seventy-two generations *in vitro*, and tetraploid cells after seventy-two generations *in vitro*; (4) N_2 cells *in vitro*; (5) *BHK-C*13 and *BHK-TCY* hamster kidney fibroblasts examined after fifty generations *in vitro*, (6) *S6*/1, *S6*/2, *S6*/3 and *S6*/4 sarcoma cells examined after 500 generations *in vitro*, (7) primary sarcoma directly from a hooded rat; (8) hooded rat kidney.

tion, periodic assessments of enzyme distribution during culture should be carried out with the kidney hamster cells, because it is possible that changes may be initially produced by infection with polyoma virus which are lost during culturing.

To ascertain whether enzyme patterns observed are an inherent characteristic of the cell, further investigations should be carried out to determine both the influence of long term culturing and tissue culture media used on the isoenzyme patterns of cells.

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Interactions of Protein (Antibodies) with the Deoxyribonucleic Acid Molecule

IN view of the current hypothesis on the protein nature of genetic repressors it is of prime importance to know whether certain proteins exist capable of "recognizing" and interacting with regions of the DNA molecule differing in base composition or configuration.

Antibodies to DNA were first discovered by Seligman[1] in 1957 in the serum of patients with systemic lupus erythematodes (SLE). Unexpectedly, however, it was found that these antibodies interacted similarly with DNAs of various origins and which differed significantly in their base composition. Levine *et al.*[2] have demonstrated that antibodies to DNA found in serum of patients with systemic lupus erythematodes react preferentially with denatured DNA. Using the method of passive haemagglutination—one of the most sensitive immunological reactions—we have found that antibodies to DNA occur fairly frequently. The presence of these antibodies was demonstrated in the blood serum of 60 per cent of the healthy individuals investigated and in various experimental animals[3].

The purpose of this investigation was to study the ability of antibodies present in different sera to interact with apurine and apyrimidine DNAs and DNA lacking a secondary structure. By this means we hoped to obtain some information on the nature of the regions of the DNA molecule with which the antibodies could interact.

In order to follow the interaction of antibodies with different preparations of DNA the antibody neutralization reaction based on passive haemagglutination was used. The details of both methods have been published elsewhere[4]. The reaction was carried out utilizing ram erythrocytes treated with formalin and tannin and sensitized at *p*H 5·5 with denatured DNA. By means of an antibody neutralization reaction we determined the minimal amount of DNA required to neutralize the antibodies contained in a given volume of various sera. The following preparations of DNA were used for neutralization : (1) native DNA ; (2) denatured DNA (obtained by heating DNA for 10 min at 100° C); (3) DNA denatured in the presence of formaldehyde (DNA solution (400 μg/ml.) was heated for 10 min at 100° C in a 1·3 per cent solution of formaldehyde). As was shown by Grossman[5] formaldehyde prevents the formation of helices in separated strands of DNA; (4) apurinic DNA obtained by the method of Tamm *et al.*[6] ; (5) apyrimidinic DNA, obtained by hydrazinolysis according to Habermann's[7] technique. The absence of purine or pyrimidine bases in these preparations was demonstrated by paper chromatography.

The results of the antibody neutralization reactions using different sera and preparations of DNA are summarized in Table I.

The analysis of the data given in Table 1 shows that different amounts of the diverse preparations of DNA are necessary for the neutralization of antibodies present in different sera. Three groups of antibodies may be differentiated on the basis of their ability to interact with different preparations of DNA. The first group are the antibodies which mainly interact with single-stranded (DNA which had been treated with formalin). To neutralize this type of antibody very much more denatured DNA (60–250×) is required. These antibodies are found in guinea-pigs, in some patients (sera 6 to 8) and in a patient with systemic lupus erythematodes during the early stages of the disease (serum 5, duration of disease 4 months). The second group comprises the antibodies which interact mainly with single-stranded DNA. Furthermore, their affinity for denatured DNA is much higher in that of the first group of antibodies because the amount of denatured DNA required for their neutralization is four to fifteen times as great as the amount of formalin treated DNA for the same purpose. Antibodies of this kind were found in the serum of most patients with

Table 1. AMOUNT OF DNA REQUIRED FOR THE NEUTRALIZATION OF UNITS OF SERUM ANTIBODIES CONTAINED IN DIFFERENT SERA*

N/N	Source of serum	Diagnosis	The neutralizing dose of fDNA (γ)	Ratio of neutralizing doses of different preparations of DNA to neutralizing dose of formalin-treated DNA			
				nDNA / fDNA	dDNA / fDNA	apurDNA / fDNA	apyrDNA / fDNA
1	Guinea-pig	Normal	0 002	> 1,000	125	—	—
2	,,	,,	0 002	> 1,000	125	—	—
3	,,	,,	0 001	> 1,000	250	—	—
4	,,	,,	0 004	> 1,000	250	—	—
5	Patient K.	SLE	0 008	> 1,000	66	No*	No*
6	,, M.	Discoid LE	0·008	> 1,000	125	—	—
7	,, E.	Scrofuloderma	0 004	> 1,000	250	—	—
8	,, P.	Werlhof's disease	0·008	> 1,000	250	—	—
9	,, Ku.	SLE	0 008	160	4	1,400	No*
10	,, D.	,,	0 002	160	4	2,700	54,000
11	,, Ko.	,,	0·008	160	4	40	No*
12	,, Mo.	,,	0 004	80	4	80	130,000
13	,, C.	,,	0 004	—	4	400	No*
14	,, Da.	,,	0 004	—	4	900	,,
15	,, A.	,,	0·004	—	15	100	,,
16	,, Sa.	,,	0 004	—	4	100	,,
17	,, S.	,,	0·004	—	4	50	,,
18	,, B.	,,	0·004	130	4	50	,,
19	,, R.	,,	0 004	500	4	2,000	,,
20	,, Ri	,,	0 004	700	8	2,000	,,
21	,, Bo.	,,	0·004	250	4	37	,,
22	,, Ba	,,	0 03	10	1	1,100	,,
23	,, Bar.	,,	1 0	0 037	0·12	No*	,,
24	,, Ch.	,,	0·16	0 25	0 5	—	—
25	,, Cu.	,,	0 1	0 1	0 2	No*	No*

Abbreviations. SLE, systemic lupus erythematodes; nDNA, native DNA; dDNA, denatured DNA; fDNA, formalin-treated DNA; apurDNA, apurinic DNA; apyrDNA, apyrimidinic DNA.
* One serum unit is the minimal amount of serum containing antibodies capable of agglutinating 0·05 ml. of a 2 5 per cent suspension of DNA-sensibilized erythrocytes.
No*, not neutralized by dose of 1 mg.

systemic lupus erythematodes (sera 9 to 21). The fact that different amounts of denatured DNA are required to neutralize the antibodies from different sera suggests that the length of the region of the single-stranded molecule of DNA with which the antibodies of the second group can interact is less than the corresponding region of polynucleotide chain which will interact with antibodies of the first group. The third group is composed of those antibodies which are equally capable of interacting with native, denatured and formalin-treated DNAs (sera 22 to 25). These antibodies were found in patients suffering from a severe form of systemic lupus erythematodes (three of the patients died shortly afterwards). It should be noted that it is this type of antibody which is able to inhibit the activity of DNA as primer in the RNA polymerase reaction and furthermore influences DNA translating activity[8]. Prolonged storage of sera containing these antibodies results in a considerable reduction in their ability to interact with single-stranded and denatured DNA.

Recently, it was shown that the antinuclear factors present in the blood serum of different people may exhibit variable properties. Thus Bardawil *et al.*[9] have found that the antinuclear factor present in the blood serum of normal people interacts with deoxyribonucleoprotein at low ionic strengths only. On the other hand, the antinuclear factor from serum of patients with severe forms of systemic lupus erythematodes can react with deoxyribonucleoprotein at higher salt concentrations.

Data presented in Table 1 show that apurinic acid is also able to interact with antibodies, the amounts of this DNA required to neutralize the antibodies being variable and depending on the serum under test. These values are, however, always higher than that for the amount of single-stranded DNA necessary for the neutralization of the same quantity of antibody. This finding suggests that purines may be an important constituent of the sites of interaction in the DNA molecule. There is another explanation which is also plausible ; treatment of DNA with hydrochloric acid—used for the preparation of apyrinic DNA—is known to degrade DNA and it is possible that this may also lead to a significant degradation of the site of antibody interaction in the DNA molecule. In fact we found that the incubation of DNA with DNase considerably decreased the ability of the DNA to interact with antibodies.

In contrast with apurinic DNA, DNA lacking the pyrimidine bases is usually unable to interact with antibodies. Thus pyrimidine bases may be directly involved in the formation of the sites in the DNA molecule which will interact with the antibodies.

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Electrophoretic Determination of Albumin/Globulin Ratios in Mice

INFORMATION on the serum protein patterns of normal mice is necessary if these animals are to be used for studies in cellular immunity. However, except for Albritton's report[1], in which no indication was made of the animal's age, sex and strain, such published data are not available. This communication, therefore, provides some of this essential information; we have found that there are differences in serum protein patterns in different groups of mice, and that these patterns are related to age and sex.

All mice, with the exception of the pathogen-free ones, were outbred Swiss–Webster supplied by the University vivarium. They were housed at 22° ± 1° C under conditions calculated to minimize infection by microbes. They were fed a diet of 'Purina' rat chow and were given as much water as they wanted to drink. The following groups of mice were studied. (1) Normal: these were of known sex and age and at no time appeared ill or debilitated. (2) Immune: normal males, 10 weeks of age at the start of our injection schedule, received 1·0 mg intraperitoneally of bovine plasma albumin three times a week. They were bled 3 days after the seventh injection.

(3) Hyperimmune: normal males, 9 weeks old, received three subcutaneous injections of 2·0 mg of alum precipitated bovine serum albumin (BSA) which caused them to become immunologically paralysed. Once an anti-BSA haemagglutinin titre could be demonstrated, indicating an escape from paralysis, the animals were immediately bled. (4) Pathogen-free: 9-week-old males were obtained from the Charles River Mouse Farms. They had been maintained under aseptic conditions and were found to be serologically negative for common mouse viral and bacterial infections. They were bled immediately on receipt to ensure that their stipulated pathogen-free condition prevailed. (5) Newborn: 2-day-old suckling mice were bled and immediately killed (and hence were of undetermined sex). (6) Infected: normal 9-week-old females were injected intraperitoneally with approximately 1×10^7 coagulase positive *Staphylococcus aureus*.

All mice were bled from the retrobulbar plexus into sterile capillary tubes. Each serum was separated from the clotted blood by centrifugation and electrophoresed immediately, as poor reproducibility was obtained when frozen sera were used. A human control serum accompanied each series of test sera. In studies requiring frequent bleeding of the same mouse, we alternated plexus on successive bleedings.

All electrophoretic analyses were carried out with a microzone electrophoresis cell (Model *R*-101, Beckman Instruments, Inc.). The only changes from that procedure were: (1) that the time of electrophoresis was increased from 20 to 30 min, thus effecting better separation of serum proteins; and (2) that the *B*-2 buffer was changed after the second analysis rather than after the fourth, thus preventing precipitation of the buffer at the electrodes. When electrophoresis was completed, the cellulose acetate membranes were developed, dried, and scanned in a Beckman model *RB* 'Analytrol' with model *R*-102 'Microzone' scanning attachment.

Fig. 1 shows the wide range of albumin/globulin (A/G) ratios observed in the various groups of normal and experimental mice. The serum protein patterns of normal males differed greatly from those of females, as can be seen in Fig. 2. The male A/G ratios were not only less variable, but also displayed smaller standard deviations. The alterations of A/G ratios which occur temporarily in infected mice are shown in Fig. 3. Bacteriaemias persisted for 25–27 days, but blood samples analysed after 28 days were negative.

Our interpretation of these results is that the serum protein pattern of 13-week-old male mice remain more

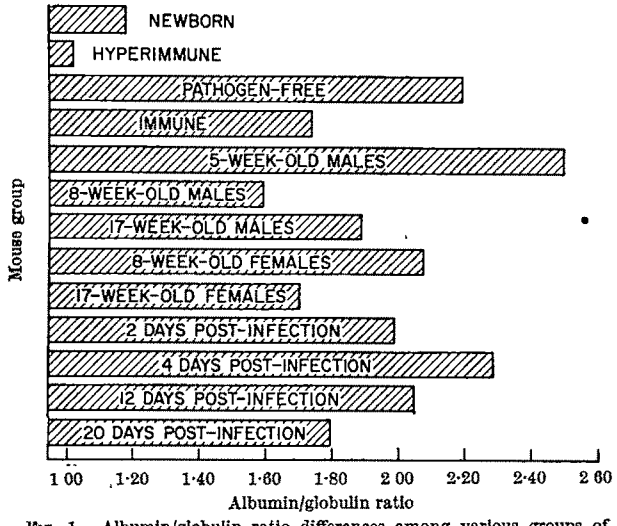


Fig. 1. Albumin/globulin ratio differences among various groups of mice. The infected animals were 8-week-old females at the time of infection. All groups except for the hyperimmune mice (four animals) contained at least ten animals.

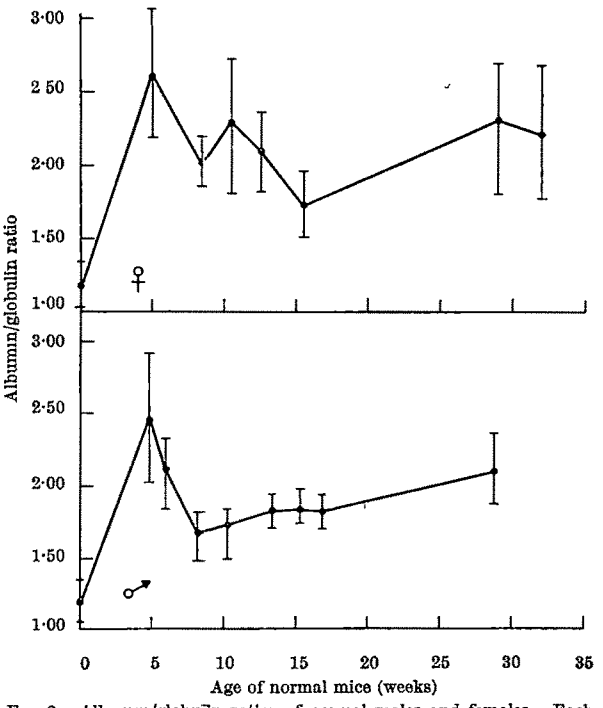


Fig. 2. Albumin/globulin ratios of normal males and females. Each point represents the mean value for at least ten mice. The line bisecting any given point represents the standard deviation for that age group.

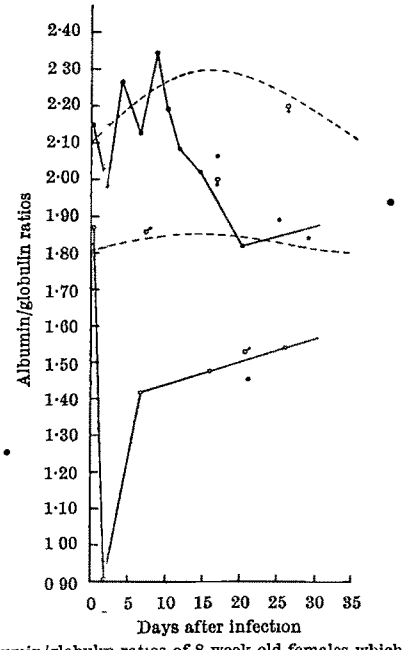


Fig. 3. Albumin/globulin ratios of 8-week-old females which were each injected intraperitoneally with 1×10^7 *S. aureus* compared with those of 13-week-old males which were similarly infected. The dotted lines represent the A/G ratios of uninfected animals.

stable and reproducible for at least 4 weeks than do those of other mouse groups. Serum protein patterns change dramatically with the age of the mice. The low A/G ratios found in newborn mice is attributable to colostrum-transferred maternal antibodies, and the very high A/G ratio found in 8-week-old mice denotes the immature state of their reticulo-endothelial systems. As the mice reach immunological maturity (at about 8 weeks of age) the A/G ratio reached a new, relatively stationary level. The fluctuation shown by the A/G ratios of normal females is believed to be related to oestrous cycle or other hormonal changes, or both. No attempt was made to

determine which group of animals elicits the highest antibody titre to any given antigen. It might well be that some group other than 13-week-old males should be used if the quantity of antibody produced is important. It is possible that the undulations in the A/G ratios exhibited by *S. aureus* infected females during the first 6–8 days after infection are caused by the focalization and subsequent breakdown of foci resulting in reinfection of the mice.

We thank Dr. Eli Sercarz for providing us with the hyperimmune mice used in these experiments. This investigation was supported by the National Science Foundation.

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Response to Insulin by Guinea-pig Taenia Coli

THE stimulating action of insulin on the metabolism of heart and striated muscle is well known, but the extent of its influence on smooth muscle is less clear and is variously assessed[1–6]. Preparations so far studied—such as aortic muscle[5–7] and intestinal segments[4]—suffer from the disadvantage that they are damaged during preparation or, like uterine muscle[1–3], have a highly individual endocrine response. Because of its ease of dissection and less specialized function, guinea-pig taenia coli muscle seemed an obvious tissue preparation with which to investigate the possible *in vitro* effects of insulin.

Insulin can without doubt enhance uptake of glucose by strips of this tissue *in vitro*, its retention of glucogen, the accumulation of the unutilized amino-acid, aminoisobutyrate, and the incorporation of carbon-14 labelled glycine into protein (Table 1). The figures given in Table 1, however, showing clear stimulating effects of insulin, are selected observations, and a large number of experiments were recorded in which a consistent stimulus by the hormone was not observed. The explanation for this is uncertain. For undeterminable reasons there seemed to be a much larger variation between strips from different animals than would be found with, for example, rat diaphragm muscle, and in different experiments the absolute values of glucose utilization and glycogen deposition moved between wider than usual limits. Axelsson, Bueding and Bülbring[9] have previously noted such fluctuations in the glycogen content, and they did not find an effect of insulin on glycogen retention. In most of the experiments in Table 1 the taenia preparations rested freely in a small quantity of incubation medium and consequently remained contracted. In other experiments the muscle strips have been tied to weights and placed under a tension of about 2 g. Under these conditions, the stimulating effect of insulin on the incorporation of ^{14}C-glycine was still observed (Table 1).

Table 1. EFFECT OF INSULIN ON THE METABOLIC ACTIVITY OF TAENIA COLI *in vitro*

	Effect of insulin: No insulin added	Insulin added (0·1 unit/ml.)	*P*
Unstretched tissue			
Uptake of glucose (mg/g tissue/h)	(6) 0·53 ± 0·08	0·95 ± 0·13	< 0·05
Glycogen content after incubation (mg glucose/g tissue)	(10) 0·78 ± 0·03	0·95 ± 0·06	< 0·05
Accumulation of carbon-14 labelled-aminoisobutyrate $\left(\frac{\text{(c.p.m./g tissue)}}{\text{(c p m./ml. medium)}}\right)$	(6) 5·3 ± 0·60	7·4 ± 0·69	< 0·05
Incorporation of carbon-14 labelled-glycine into protein (c p m / mg protein)	(9) 403 ± 28	632 ± 58	< 0·01
Tissue under tension			
Incorporation of carbon-14 labelled-glycine into protein (c p.m./ mg protein)	(6) 587 ± 51	657 ± 43 Difference 70 ± 19	< 0·02

Each figure is the mean ± *S.E.* of the mean of the number of observations in parentheses. For methods see refs. 23 and 24. For the experiments with unstretched tissue, two strips of taenia coli were removed from each guinea-pig (of either sex and of weight 200–400 g) and each strip cut into two approximately equal pieces. Medium consisted of Krebs-Ringer bicarbonate, gassed with 95 per cent O_2/5 per cent CO_2 and contained glucose (1 mg/ml.). Incubation was in 1 ml for 2 h at 37° C with shaking. Carbon-14 labelled-glycine was added at a concentration of about 25 μM and 0·15 μc./ml. Carbon-14 labelled-aminoisobutyrate was added at a concentration of about 67 μM and 0·15 μc./ml. When the tissue was incubated under tension, the two ends of a single strip were tied together to a 5 g weight and the loop hooked around a stainless steel frame in 10 ml. of medium containing no glucose.

In a study of glucose metabolism by aortic muscle, Urratia, Beavan and Cahill[5] have also commented on the wide variability in behaviour between pieces of this tissue. They concluded that most of the apparent response to insulin of pieces of aortic muscle was due to the presence of adventitious tissue, and when the muscle was meticulously stripped, response to the hormone disappeared. Thus it is possible that with taenia some of the inter-animal variation results from varying degrees of contamination of the strips with adipose tissue, though it is debatable whether the amount adhering would be sufficient to account for all the insulin effects observed. On the other hand, guinea-pig adipose tissue is not very sensitive to insulin[10,11] and it is possible that the guinea-pig is just less sensitive to ox insulin than are some other species. Because the amino-acid sequence of guinea-pig insulin shows so many variations from other mammalian insulins[12], its use might be advantageous, although the available evidence[10] does not indicate differential sensitivity.

A point of contrast with heart or diaphragm muscle is the greater degree of concentration of aminoisobutyrate by taenia muscle than by these other tissues[13,14]. This may be related to the greater turnover of cellular sodium and potassium ions in smooth muscle as compared with striated muscle[15] and to the probable linkage of amino-acid uptake with the movements of ions[16]. Others have commented on the low oxidative metabolism of smooth muscle[5,6] and its high production of lactate. In our experiments, taenia produced an average of about 10 μmoles of lactate for each gram in a period of 2 h, which indicates the anaerobic glycolysis of most of the glucose taken up. The amount of lactate produced was not affected by insulin.

Various workers have questioned whether with smooth muscle there is as clear a distinction between intracellular and extracellular fluid as with striated muscle[15,17], and Goodford[18] has commented on variations in estimates of extracellular fluid in taenia according to the agents used for its determination. However, figures obtainable with sucrose and sorbitol (Table 2) agree well with Goodford's estimates with lithium and ethylsulphate, although they are higher than those found with inulin or polyglucose[19]. Differences between values found with inulin and other agents in various tissues are not unusual (Table 2). Yalcin and Winegrad[7] found a glucose space in aortic segments greater than the inulin and raffinose spaces and concluded that transport of glucose into the cell may not be the step limiting the rate of glucose utilization in that tissue. Any response to insulin under such circumstances would be of great interest. Our estimates, however, of the glucose space in taenia—24·9 ± 1·4 in the absence of insulin, and 27·5 ± 3·1 in its presence—do not suggest much intracellular accumulation. A preliminary report by Timms, Salama and Engstrom[20] suggests that the glucose space is increased by insulin.

Table 2. COMPARISON OF INULIN, SUCROSE AND SORBITOL SPACES IN VARIOUS MUSCLE PREPARATIONS

	Space (ml /100 g) measured with: Sorbitol	Sucrose	Inulin
Guinea-pig taenia coli	40·2	42·3	22·5
Rat intact diaphragm	24·5	23·8	23·5
Rat isolated hemidiaphragm	47·6	53·3	34·3
Rat extensor digitorum longus	35·8	34·4	19·2

Carbon-14 labelled sorbitol was added at a concentration of about 180 μM and 0·15 μc /ml. Inulin was added at a concentration of 0·6 per cent and sucrose at 1·25 per cent. All were extracted from the tissue after incubation by boiling and the sucrose and inulin estimated after hydrolysis to fructose (ref. 25).

There is thus some evidence that the taenia smooth muscle of the guinea-pig responds to insulin, though the quantitative significance seems to be variable. We may have to contend with the possibility that we cannot categorize all tissues as simply responsive or unresponsive to insulin, however, but that their response varies according to (as yet undetermined) conditions—a situation possibly akin to the limited response of brain slices[21,22].

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Epidermal Keratin and Epidermal Prekeratin

PROGRESS in understanding keratinization of the epidermis has been hampered by the difficulty of preparing a reproducible keratinous protein from the epidermal tissue[1,2]. Several laboratories[1–5] have obtained an alkali- or urea-extractable protein from human, mouse, and cow's snout epidermis which precipitates from solutions at *p*H 5·5. This protein is present in large amounts in fully keratinized human epidermal tissue (callus or stratum corneum)[1,2] and is believed to represent epidermal "keratin". Matoltsy[6–8] has isolated and characterized a similar protein from cow's snout epidermis which is soluble in acetic acid. This protein is termed "prekeratin" by virtue of its ready solubility from whole epidermis which includes incompletely keratinized cells (full-thickness cow's snout epidermis). This report compares human callus keratin (protein precipitating at *p*H 5·5) with the prekeratin of Matoltsy. Their similarities heighten optimism concerning the definition of an epidermal keratin sub-unit.

Human plantar callus keratin was obtained by alkaline solubilization[1,2] (100 ml. of 0·02 M sodium hydroxide (solvent) per gram of dried neutral-buffer washed ground callus, 22° C, 24 h) and by precipitation at *p*H 5·5 by the dropwise addition of dilute hydrochloric acid with *p*H monitoring. The protein was redissolved and precipitated four times.

The approximate molecular weight of protein dissolved out at *p*H 5·5 by 0·02 N sodium hydroxide was determined by comparing its mobility with that of proteins of known molecular weight utilizing 'Sephadex' thin-layer gel filtration, modified by Andrews[9]. 'Sephadex' (*G*-100 superfine, Pharmacia) was equilibrated for 72 h with 0·02 M sodium hydroxide, and plates of 0·5 mm thickness were prepared with a spreader (Shandon Unoplan). Cytochrome *C* (horse heart, type II, Sigma Chemicals), chymotrypsinogen (thrice crystallized, Worthington Biochemical Corporation), γ-globulin (human, fraction II, Sigma Chemical Company), and callus protein which had been precipitated four times and lyophilized at *p*H 5·5 was dissolved in 0·02 M sodium hydroxide and applied to the thin-layer plate (5 μl. aliquots representing 20 μg of protein). The solvent (0·02 M sodium hydroxide) was allowed to flow horizontally for appropriate periods of time and the proteins were located by exposure to iodine vapour. The reference proteins migrated as single compounds for a distance proportional to the log of their molecular weight (Fig. 1). The approximate molecular weight of the protein dissolved out at *p*H 5·5 was estimated at about 50,000 from its comparative migration.

Peptide fingerprints were prepared from tryptic digests of *p*H 5·5 protein (5 mg dissolved in 1·0 ml. of 0·1 M ammonium carbonate and incubated at 37° C for 24 h with 0·1 ml. of 1 : 100 trypsin crystallized three times, Worthington Biochemical Corporation). Two-dimensional peptide maps (descending chromatography of aliquots representing 1 mg of the original protein in a butanol–acetic acid–water medium (4 : 1 : 5); electrophoresis at 3,000 V for 1 h in a formic acid–acetic acid–water medium (6 : 24 : 170) *p*H 2·0) revealed forty-five or forty-six peptides with a light ninhydrin stain. This is comparable to the number of peptides identified by Rothberg[5] in tryptic digests of *p*H 5·5 protein obtained from human callus and psoriatic and ichthyotic scales. With the usual assumptions that complete digestion occurs and that the action of trypsin is specific to peptide bonds formed from arginine and lysine, the *p*H 5·5 protein used in this study should contain forty-four or forty-five arginine and lysine residues.

Amino-acid composition (duplicate hydrolysates, 6 N hydrochloric acid, 24 and 72 h at 110° C in tubes sealed under vacuum) of the four times precipitated *p*H 5·5 protein was determined by automated column chromatography ('Spinco' model 120 amino-acid analyser) and corrected for destruction or delayed release of specific residues[1,2]. Based on the amounts of arginine and lysine present in these samples, a molecular weight which would contain forty-four or forty-five arginine and lysine

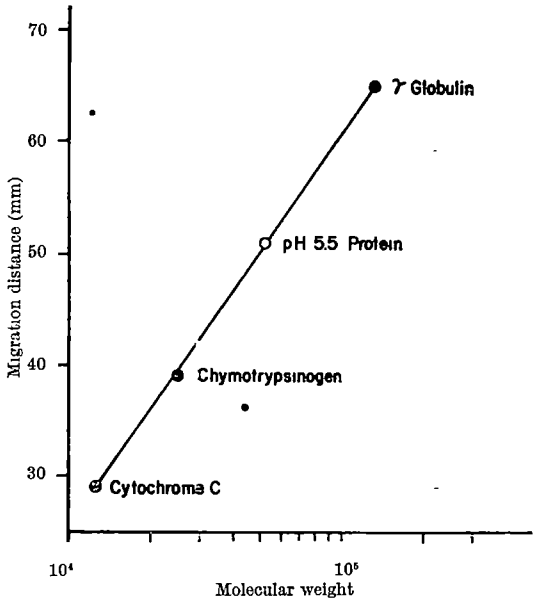


Fig. 1. Thin-layer gel filtration ('Sephadex *G*-100 Superfine' in 0·02 N sodium hydroxide at room temperature). Migration is plotted against (log) molecular weight (cytochrome *C*, 12,400; chymotrypsinogen, 25,000; γ-globulin, 160,000).

residues (and therefore agree with the results of tryptic peptide maps) was calculated. This estimate would require a molecular weight of approximately 50,000, which agrees with the estimate derived from thin-layer gel filtration.

Matoltsy[6–8] has estimated the molecular weight of epidermal prekeratin of the cow's snout to be 640,000 by the method of sedimentation equilibrium. Methionine was the amino-acid present in the least amounts (thirteen residues per molecular weight of 640,000). Because a protein must contain at least one whole residue of the least common amino-acid, the smallest possible molecular weight of prekeratin would be 1/13 of 640,000, or approximately 49,000 (the so-called minimal molecular weight). The number of arginine and lysine residues reported per molecular weight of 640,000 totalled 598. Thus the minimal molecular weight of 49,000 would contain 1/13 of this number, or 46. These two values are essentially identical to those determined for the *p*H 5·5 callus protein in alkaline solution.

Tryptic digests of prekeratin[8] were reported in a one-dimensional system and revealed only about fourteen peptides. Even if one assumes that a second-dimensional separation would reveal two or three times this number of peptides, the total number would be under fifty and therefore would be more compatible with the smaller (minimal) molecular weight. It would be reasonable to assume that a repeating sub-unit of molecular weight of about 50,000 might aggregate during sedimentation equilibrium, resulting in an apparent molecular weight of 640,000.

Thus, the suggested sub-unit molecular weight of 50,000 for prekeratin agrees well with the approximate estimate for *p*H 5·5 keratin (in alkaline solution) derived from gel filtration, amino-acid composition, and peptide maps. The amino-acid composition reported as residues per molecular weight of 50,000 for both *p*H 5·5 keratin and prekeratin is detailed in Table 1. Remarkable similarities are evident. The largest differences occur in those amino-acids (tyrosine, methionine, 1/2 cystine) which are technically most difficult. Both these and several smaller differences are as likely due to species differences as to preparative discrepancies. The presence of small amounts of lipids and less than 0·1 per cent of hexosamine in both preparations[8,10,11] are further points of similarity. Unfortunately, acetic acid does not dissolve protein from human callus or stratum corneum; for this reason, a direct comparison of the two proteins from these tissues is not feasible.

Table 1. AMINO-ACID RESIDUES PER MOLECULAR WEIGHT OF 50,000

	C5·5*	M†
Glycine	69	70
Glutamic	58	60
Serine	46	50
Aspartic	38	37
Leucine	36	37
Alanine	24	28
Arginine	23	25
Lysine	21	21
Valine	16	21
Isoleucine	17	17
Threonine	17	16
Phenylalanine	12	16
Tyrosine	14	9
Proline	6	7
Histidine	5	4
1/2 Cystine	2	6
Methionine	8	1

* C5·5, Crounse *p*H 5·5 human epidermal protein (keratin).
† M, Matoltsy cow's snout epidermal protein (prekeratin).

It is likely, therefore, that the more soluble prekeratin, obtained from whole epidermis including incompletely keratinized Malphigian and basal cells, and the less soluble keratin, obtained from fully keratinized callus tissue, differ primarily in their extractability. This interpretation is compatible with the proposed two-component theory of keratinization[12], which states that a low-sulphur fibrous keratin present in the early stages of ectodermal differentiation is stabilized (hence less extractable) by an amorphous protein synthesized at a later stage of keratinization. In the case of wool, the second "cementing" component is believed to be heavily cross-linked with disulphide bonds of cystine. In epidermis, no specific high-sulphur protein has yet been isolated and characterized. Inverse proportionality of cystine and glycine in hair and epidermal proteins has been suggested[13]. Reports of a polypeptide rich in glycine and histidine synthesized high in the epidermis[14,15] might relate to such a second component of epidermal keratinization. In any case, the remarkable similarities in composition of human callus *p*H 5·5 "keratin" and cow's snout epidermal "prekeratin" indicate hopeful progress in the definition of an epidermal keratin sub-unit.

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Monovalent Cation Requirement for the Aminoacyl Transfer Reaction in Protein Synthesis

IN this laboratory, previous investigations of the transfer of amino-acids from aminoacyl *s*RNA to polypeptides on ribosomes (the aminoacyl transfer reaction) were carried out with cell fractions prepared from rat liver with buffered solutions containing potassium[1–3]. The aminoacyl transfer reaction was dependent on the concentration of potassium ion in the incubation, and optimal incorporation was obtained when the concentration of potassium in the reaction mixture was between 0·075 and 0·1 M. Recent studies with preparations of *Escherichia coli* have shown that ammonium ions are more effective than potassium ions and that rubidium, caesium, sodium and lithium ions are much less active in promoting aminoacyl transfer[4,5]. Because similar results have been obtained with the rat liver system, solutions containing ammonium are at present being routinely used in this laboratory[6]. This communication presents a detailed investigation of the effective concentrations of various monovalent cations on polypeptide biosynthesis. The results reveal an interesting relationship between the relative biological activity of these ions and some of their physical and chemical thermodynamic properties.

The aminoacyl transfer reaction was carried out in the presence of varying concentrations of the chloride or nitrate salts of several monovalent cations, as shown in Fig. 1. Examination of the concentration optima for various cations indicates that ammonium ions are indeed superior in promoting the transfer of amino-acids from aminoacyl *s*RNA to polypeptides. Potassium is 60 per cent as effective as ammonium, and sodium, rubidium and thallium are only 20–25 per cent as active. Lithium

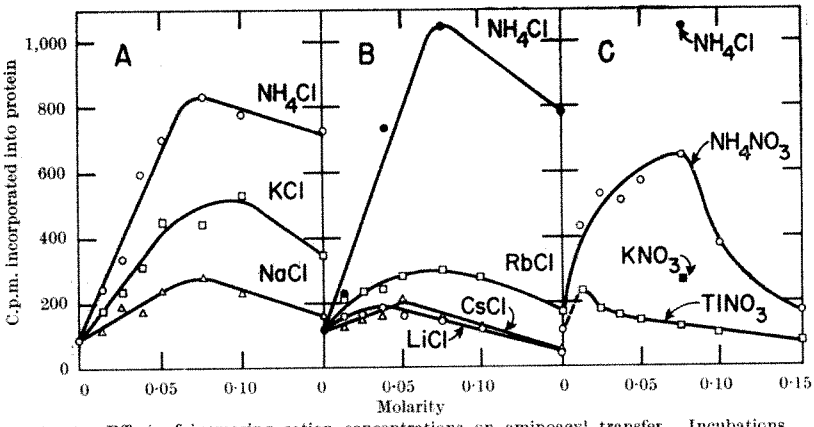


Fig. 1. Effect of increasing cation concentrations on aminoacyl transfer. Incubations, carried out at 37° C in a total volume of 2 ml., contained the following components: 0·6 mg of purified ribosomes, 0·4 μmoles of guanosine triphosphate, 12 μmoles of magnesium chloride, 40 μmoles of glutathione, 120 μmoles of *tris*-Cl or *tris*-NO_3 buffer, *p*H 7·4, 0·1 mg each of aminoacyl transferases I and II, 10,000 c.p.m. of ^{14}C-leucyl *s*RNA, and varying concentrations of the Cl^-- or NO_3^--salts of a monovalent cation as noted. The preparation of the reaction components, from rat liver, has already been described[6]. At the end of the incubation period, the hot 5 per cent trichloroacetic acid-insoluble fraction was prepared and collected on 'Millipore' filters for radioactivity determinations (ref. 6). The Cl^- salts of K^+ and Na^+ (*A*) were assayed in a separate series from those of Rb^+, Cs^+ and Li^+ (*B*). Ammonium chloride was run each time for comparison. Because thallium chloride was too insoluble to test, thallium nitrate (and ammonium nitrate for comparison) was tested in the system (*C*); also for comparison, values obtained with 0·075 M ammonium chloride (solid circle) and potassium nitrate (solid square) are included.

and caesium are the least active of those tested. Relatively high concentrations of all the compounds used, particularly the nitrate salts, led to decreased aminoacyl transfer. Salts of silver and copper could not be assayed because of their low solubility or tendency to precipitate other components of the incubation mixture.

A correlation between the relative activity of the monovalent cation in polypeptide biosynthesis and its crystal ionic radius, in Ångströms, or entropy of formation, at the reference temperature of 25° C, is presented in Fig. 2. The highest activity in the system in which amino-acids are incorporated is exhibited when the ionic radius is about 1·4 Å, or when the entropy value is near 27 calories; the biological activity falls off as the corresponding values deviate from these optima, particularly at the higher values. A similar pattern to that described here is also obtained when relative activities are plotted against the reciprocal of the radii of the hydrated ions. No obvious correlations were, however, found with respect to the standard heats of formation (ΔH_f^0), standard free energies of formation (ΔF_f^0) or oxidation potentials (E^0_{ox}). It is interesting to note that correlations have been suggested between the hydrated radii of various cations and their activity in the ATP : pyruvate phosphotransferase reaction[7] and in the binding of ^{14}C-chloramphenicol to ribosomes[8]. In these reactions, NH_4^+, K^+ and Rb^+ ions are active; their radii are similar but differ from those of Na^+, Cs^+ and Li^+ ions which are inactive.

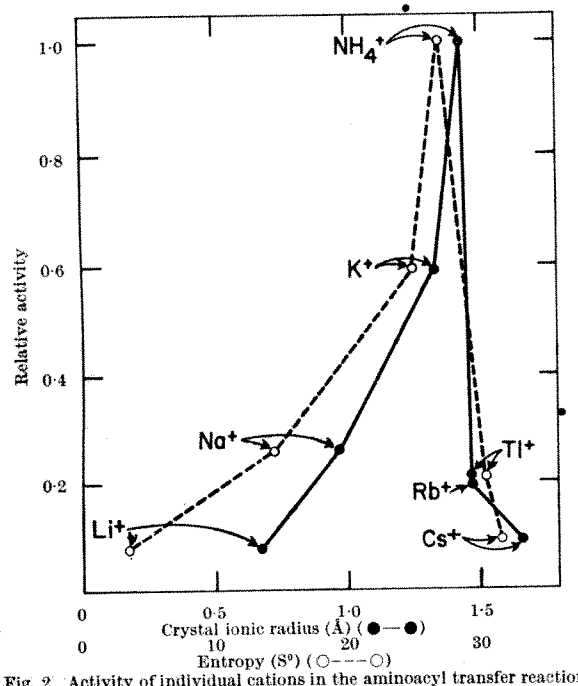


Fig. 2. Activity of individual cations in the aminoacyl transfer reaction as a function of ionic radius or of entropy. The most active cation, NH_4^+, is assigned a value of 1·0; all others are calculated from the percentage of activity at the optimal ion concentration, compared with NH_4^+. Values for Å and S° are taken from *Handbook of Chemistry and Physics* (forty-fifth ed., edit. by West, R. C., Selby, S. M., and Hodgmans, C. D.; The Chemical Rubber Co., Cleveland, Ohio, 1964–1965).

The significance of these physico-chemical characteristics in terms of the role of monovalent cations in protein synthesis remains to be determined. The role(s) of the monovalent cation in protein synthesis have not been firmly established. Studies on aminoacyl transfer in *E. coli* indicate that K^+ or NH_4^+ is involved in the binding of aminoacyl *s*RNA to polyribosomes, before peptide-bond synthesis[5,9]. Preliminary experiments in this laboratory with rat liver preparations, however, suggest that a monovalent cation is involved in an interaction between one of the aminoacyl-transferring enzymes, polyribosomes and guanosine triphosphate, before the participation of aminoacyl *s*RNA.

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Effect of Oestrogen on the Small Pigmented Spots in Hamsters

IN Syrian golden hamsters pigmented melanocytes of the skin are mainly restricted to the hair bulbs and occasional networks surrounding pilosebaceous units[1]. These melanocytic networks are seen as small pigmented spots in whole mounts of hamster skin. Because these spots bear some resemblance to melanotic naevi in man[2], and develop into melanomas when painted with chemical carcinogens[1,3] they have been the subject of extensive investigations in recent years.

It is well known that diverse endocrinological factors affect pigmentation in man and other animals. This, combined with the fact that small pigmented spots occur more frequently in the female than the male, led us to try the effect of oestrogen on these pigmented spots.

Eighteen male hamsters between twelve and fifteen months old were used in the experiment. The animals

were divided into nine pairs such that the difference in the age of the animals in each pair was less than 3 days. Two 15 mg implants of hexosterol were introduced subcutaneously in the scapular region of the experimental member of each pair. Three months later five pairs of animals in which the hair follicles were in telogen were killed and the skins collected.

On examination of whole mounts made from the skins of these animals it was obvious that the experimental procedure had produced an indubitable increase in both the number and size of the small pigmented spots. Figs. 1 and 2 illustrate this point. The mechanism by which oestrogenic stimulation produces an increase in the number of small pigmented spots is, however, obscure. Here also, as in the case of the increase seen with age[1], the question remains whether this results from the production and migration of new melanocytes or the accumulation of pigment in pre-existing ones.

Another interesting change observed in all experimental animals was that the costovertebral spot was much reduced in size (Figs. 1 and 2). This was found to result from atrophy of the large sebaceous glands normally found in this situation. The status of the melanocytes in this region could not be accurately determined by the methods used, but it is our impression that neither overt regression nor stimulation occurs.

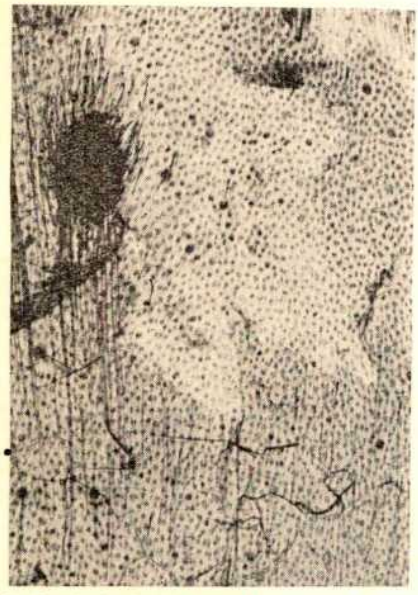


Fig. 1. Skin of control animal showing the left costovertebral spot and a few small pigmented spots.

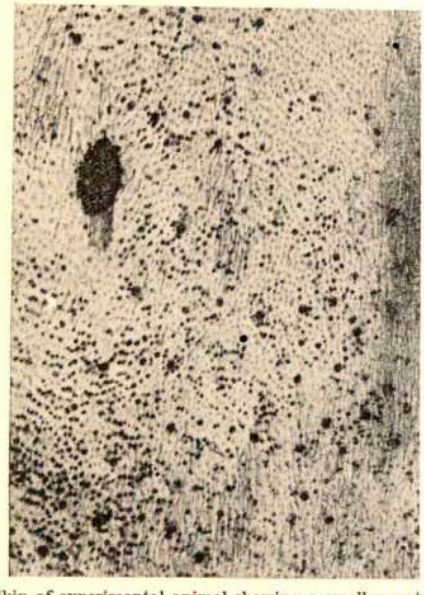


Fig. 2. Skin of experimental animal showing a smaller costovertebral spot but numerous small pigmented spots.

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Nuclear Magnetic Resonance Spectroscopic Studies of the Interaction of Phospholipids with Cholesterol

PHOSPHOLIPIDS are important constituents of cell membranes and constitute up to 40 per cent of the dry weight of the brain. We are at present studying the properties of these molecules, using a variety of physical techniques[1] including nuclear magnetic resonance spectroscopy. This is a particularly useful technique since broad line nuclear magnetic resonance spectroscopy can be used for studying molecular motion in the solid and liquid crystalline condition[2], and high resolution techniques[3] can be used to study the detailed interactions of these molecules.

An interaction of considerable biological importance is that of cholesterol with phospholipids. Cholesterol occurs in many membranes, particularly in the myelin sheath and red blood cell membranes, but its precise function and arrangement are not understood. The biochemical importance of the solubilization of cholesterol by phospholipid and its vehicular possibilities have been discussed[4]. Monolayer studies[5,6] have shown that cholesterol, when present in molar equivalents, has a marked effect on the expanded film obtained with unsaturated lecithins (phosphatidylcholines), producing a more condensed film and showing a decrease in the molar area per lecithin molecule.

We have now examined, with high resolution nuclear magnetic resonance spectroscopy, some aqueous systems containing 5 per cent by weight of egg yolk lecithin (EYL) and also a mixture containing 5 per cent by weight of EYL + 2·5 per cent by weight of cholesterol, both of which have been dispersed by ultrasonic radiation[7].

Egg yolk lecithin contains a mixture of saturated and unsaturated hydrocarbon chains. We have shown, by broad line nuclear magnetic resonance studies, that at the temperature at which the high resolution spectra were taken (33·5° C), the material is in a liquid crystalline phase and that the hydrocarbon chains are highly mobile. When the material is placed in water it swells to give myelin forms and tubes. It emulsifies by a simple fragmentation of the myelin forms[8]. These myelin forms can be broken down by the process of ultrasonification into smaller particles or aggregates of molecular weight about 5×10^6 which give optically clear solutions which remain stable for periods as long as a week. The high resolution nuclear magnetic resonance spectra of these aqueous systems (deuterium oxide) have been obtained with a Perkin–Elmer *R*.10 spectrometer at 33·5° C, using 3-(trimethylsilyl)-1-propane sulphonic acid sodium salt (TSS) as a reference material. The two spectra are shown in Fig. 1. The spectrum of EYL dispersed in water (Fig. 1*A*) shows peaks which can be assigned to the various proton groupings present in the molecule. The spectrum is quite similar to that which is observed when the material is dissolved in a solvent such as chloroform; for example, the CH_2 and CH_3 chain protons, the $\overset{+}{N}Me_3$, CHO, CH_2O and $CH_2-\overset{O}{\overset{\|}{C}}$ protons all show distinct peaks, the major difference in the spectrum being in the greater

width of the peak associated with the protons present in the hydrocarbon chain at τ 8·75 p.p.m. Also the peak corresponding to the $\overset{+}{N}(CH_3)_3$ protons is shifted by 0·17 p.p.m. to τ 6·77 p.p.m. from its observed position for EYL dissolved in chloroform[3].

The nuclear magnetic resonance spectra indicate that the nuclear magnetic interactions are being averaged out and that the lecithin molecules in this aqueous phase must have a high degree of mobility comparable with molecules in a liquid state. They further indicate that, while the CH_2 groups of the chains are highly mobile, the values of the spin–spin relaxation times T_2 in water and chloroform (which are related to the line widths) are significantly different, indicating that the water is inhibiting the chain motion in some way.

In the spectrum (Fig. 1*B*) of the 1 : 1 mixture of the lecithin with cholesterol, the major peak arising from the protons associated with the hydrocarbon chains of the lecithin has now disappeared. Only a broad peak is observed in this region, τ 9 p.p.m., which, because of its small area, could arise solely from the protons present in cholesterol. It is not possible to compare the spectrum directly with that of cholesterol in water because cholesterol cannot be dispersed alone. Cholesterol in chloroform, however, shows peaks in the same region as the broad peak referred to already. Further studies with a time averaging computer should provide information about this and the extent of fine structure from these protons. The peak associated with $\overset{+}{N}(CH_3)_3$ protons of the lecithin remains in the spectrum, it has the same chemical shift value and is only slightly broadened from its appearance in the spectrum of lecithin dispersed alone in water.

This demonstration of a selective change in the T_2 relaxation times of protons in different parts of the molecule, and hence a selective change in their correlation times[9], appears to be evidence for the direct interaction of a portion of the phospholipid, and particularly the hydrocarbon chains, with the cholesterol molecule and for the formation of a molecular complex between phospholipid and cholesterol. This is consistent with our recent comment[10] that, if the expanded nature of the phospholipid monomolecular films arises mainly as a consequence of the molecular motion of the hydrocarbon chains, a feature of the cholesterol interaction with phospholipids is to inhibit some of this chain motion. There are other alternative possibilities, however, which might also explain this phenomenon, such as an increase in solution viscosity or an increase in hydrocarbon chain interactions produced by the presence of the cholesterol which we are examining.

Although there have been various speculations[11,12], the exact nature of the interaction between phospholipids and cholesterol is still not known and the various models proposed differ considerably. We are carrying out further nuclear magnetic resonance studies on this phospholipid cholesterol system, including the measurement of relaxation times at differing temperatures and phosphorus-31 resonance spectra, which should help to clarify the nature of the interaction.

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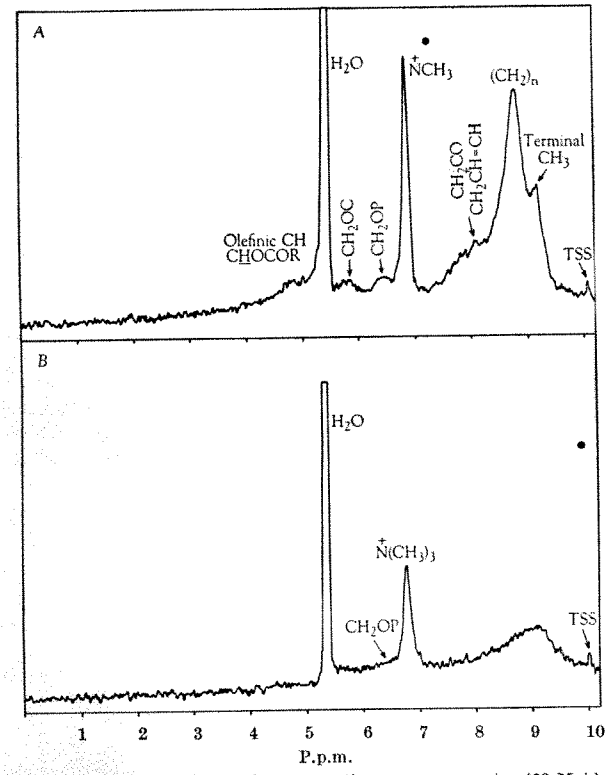


Fig. 1. High resolution nuclear magnetic resonance spectra (60 Mc/s). *A*, Egg yolk lecithin dispersed in deuterium oxide. *B*, A 1 : 1 molar egg yolk lecithin : cholesterol mixture dispersed in deuterium oxide.

HAEMATOLOGY

Chemical Characterization of Haemoglobin G_{St-I}

AMONG the genetic variants of adult human haemoglobin, some thirteen amino-acid substitutions have been described which occur along the primary sequence of the α-polypeptide chain. The most widespread and frequently encountered α-chain variant is the substitution of lysine for asparagine in the 68th residue of haemoglobin A (ref. 1). A structural mutation leading to the substitution of asparagine by lysine in a protein can be the direct result of a single nucleotide base alteration within the triplet code. Here an alteration in triplet AAU or AAC specifying asparagine could result in AAA or AAG which code for lysine[2]. Haemoglobin variants carrying a substitution of lysine for asparagine in the 68th residue of the polypeptide chain have been described in Negro families living in Philadelphia[1], Bristol[3], Azuokoli, St. Louis, Washington and Baltimore[4], and Knoxville[5].

The haemoglobin described in the present work was found in a member (J. D.) of a Negro family living in Marlin, Texas, and was first characterized electrophoretically and chromatographically by Schneider and Haggard[6]. These properties of the Texas variant were found to be identical with those of a variant found simultaneously by Lehmann *et al.* in a woman and her daughter in a West African Negro tribe and designated as Stanleyville I (ref. 7). Accordingly, the Texas variant was designated as G_{St-I}. The object of the present investigation was to identify the amino-acid substitution in Hb G_{St-I}. Fingerprints of haemoglobin G_{St-I} and A

Table 1. AMINO-ACID ANALYSES OF PEPTIDES αTVIII, IX AND αTI + II FROM HAEMOGLOBINS A AND GSt-I

Amino-acid	Haemoglobin A Residues/mole peptide		Haemoglobin GSt-I Residues/mole peptide			
	αTI–II	αTVIII–IX	αTIX	αTVIII–IXa*	αTIXb	αTI–II and VIII–IXa
Lysine	1·9	2	1	2·3	1·2	4·2
Histidine	–	3	3	–	3·3	–
Arginine	–	–	–	–	–	–
Aspartic acid	2·1	6	6	0·8	4·1	2·9
Threonine	1·0	1	1	0·6	–	1·6
Serine	1·0	2	2	0·4	2·0	1·4
Glutamic acid	0·1	–	–	–	–	–
Proline	1·2	1	1	0·1	1·3	1·3
Glycine	0·1	–	–	0·1	–	0·2
Alanine	1·1	7	7	2·0	5·1	3·1
Half cystine	–	–	–	–	–	–
Valine	1·8	3	3	0·8	2·2	2·6
Methionine	–	1	1	–	0·8	–
Isoleucine	–	–	–	–	–	–
Leucine	1·1	4	4	0·7	3·3	1·8
Tyrosine	–	–	–	–	–	–
Phenylalanine	–	–	–	–	–	–

* The amino-acid values for αTVIII + IXa were calculated by subtracting values of Column 1 from Column 4

Table 2. N-TERMINAL AMINO-ACIDS IN αTVIII, IX IN HAEMOGLOBINS A AND GSt-I

Haemoglobin A	DNP-derivative	$O.D._{340}$	Haemoglobin GSt-I	DNP-derivative	$O.D._{340}$
αT VIII, IX	Lysine	0·16	αT VIII, IXa, αT I, II, αT IXb	Lysine	0·15
αT I, II	Valine	0·19		Valine	0·17
				Alanine	0·25

revealed an alteration in three positions of the peptide patterns of G_{St-I} (Fig. 1). Peptide αTIX was absent from the neutral peptides on the fingerprint, a finding that was particularly conspicuous after staining with Pauly's reagent. Peptide αTIX of Hb G_{St-I} had split into two fragments, a and b. Close examination of the portion of the fingerprint containing peptide αTI, II revealed the presence of another peptide, αTVIII, IXa, in G_{St-I}. The tryptic peptides αIX and αVIII, IX are composed of residues 62–90 and 61–90, respectively, in the α-polypeptide chain. Because trypsin does not consistently cleave both lysyl residues, 60 and 61, both types of peptides, αVIII, IX (with an additional lysine) and αIX appear in fingerprints of haemoglobin digests. From the tryptic digest of G_{St-I} it was possible to obtain αTIXb free of contaminating peptides; however, αTVIII, IXa migrated on fingerprints in the same position as αTI, II. Thus, although N-terminal analyses and amino-acid analyses were performed on purified preparations of αTIXb, it proved necessary to perform the analyses on mixtures of αTI, II and αTVIII, IXa from G_{St-I}. Peptide αTI, II was analysed so that its amino-acid values could be subtracted from the amino-acid analysis of that area of G_{St-I} fingerprints containing both αTI, II and αTVIII, IXa (Table 1). One residue of asparagine, which was converted to aspartic acid during hydrolysis, has been replaced in G_{St-I} by a residue of lysine.

N-terminal amino-acids were identified by two-dimensional chromatography using toluene and phosphate as the solvents. Standard solutions of dinitrophenyl (DNP) derivatives were included on each chromatogram. N-terminal analyses of peptides αTVIII, IX of haemoglobin A and αTI, II plus αTVIII, IXa and TIXb of haemoglobin G_{St-I} are summarized in Table 2. Both αTVIII, IX of haemoglobin A and αTVIII, IXa of haemoglobin G_{St-I} have N-terminal lysine residues; however, αTIXb from G_{St-I} was shown to have an N-terminal alanine, which occurs as the 69th residue[9] immediately following the site of the amino-acid substitution of lysine in this variant. The cleavage by trypsin at the peptide bond between the 68th and 69th residue was responsible for the new peptide αTIXb the N-terminal of which was alanine.

Haemoglobin G_{St-I} is, therefore, identical with haemoglobin $G_{Philadelphia}$ and can be formulated $\alpha_2{}^{68}\,\text{lys}\,\beta_2\text{A}$.

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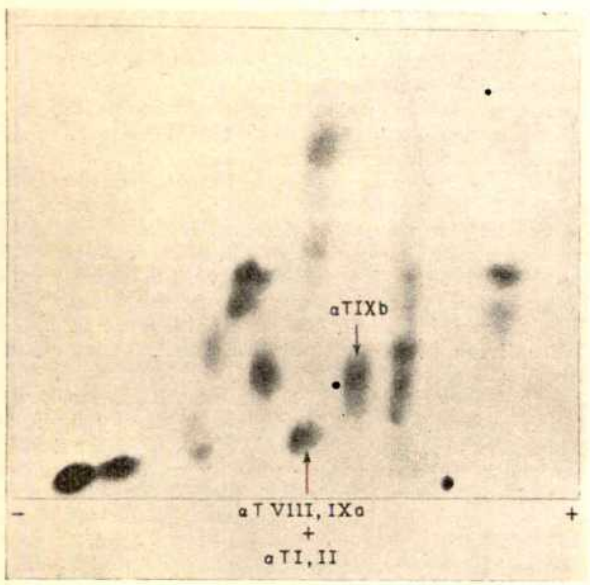


Fig. 1. Fingerprint of tryptic digest of Hb GSt-I. Electrophoresis at 3,000 V for 1 h at *p*H 6·5. Ascending chromatography in pyridine : isoamyl alcohol : water. Arrows denote peptides characteristic of Hb GSt-I.

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Importance of the Mechanisms of Blood Coagulation in the Breakdown of Adenosine Triphosphate in Blood

THE data presented in this communication demonstrate that the degradation of adenosine triphosphate (ATP) in whole blood, initiated by the damaged wall of a blood

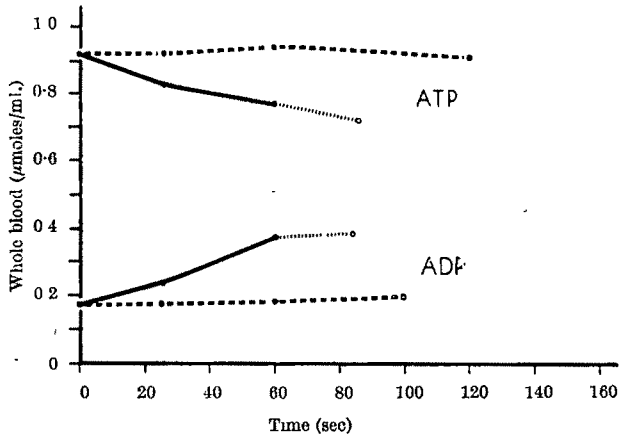


Fig. 1. The top pair of lines represent concentration of ATP obtained by separation of adenosine nucleotides by thin-layer chromatography, while the lower pair of lines represent concentrations of ADP obtained by the same means. The levels from a test-tube containing native whole blood are depicted by the broken line (control) and those from a test-tube containing native whole blood and pieces of guinea-pig aorta are depicted by the solid line. In four of the six experiments the blood in the test-tube containing the vessel wall clotted after 60 sec and no samples of blood could be taken which is indicated by the dotted portion of the solid line. Each point on the graph represents a mean value derived from six guinea-pigs.

vessel, can only take place in conjunction with activation of the coagulation mechanisms.

Ever since it became established that platelets are aggregated by either adenosine diphosphate (ADP) or thrombin there has been much speculation on which of these components takes a more prominent role in the mechanisms of haemostasis and thrombosis. In 1960 Bettex-Galland and Lüscher[1] showed that the addition of thrombin to washed, aged human platelets results in the degradation of ATP to ADP in the platelets, which suggests that thrombin may initiate the process. From this time on, however, the actual details of the mechanism by which platelets are aggregated by ADP received so much attention that the part played by the mechanisms of coagulation in haemostasis was almost disregarded. This point of view was strengthened by Haslam's[2] finding that the aggregation of washed platelets brought about by thrombin could be prevented or arrested when ADP was depleted by altering the equilibrium of several enzyme-substrate systems. Other supporting data were provided by Spaet and Zucker[3], who described the sequence of events leading to aggregation of platelets in contact with collagen fibres, in the omentum of platelets first adhered to the connective tissue and then to each other. As the latter process still occurred in the presence of heparin, these investigators concluded that thrombin does not have a role in the aggregation of platelets, and later they were able to isolate ADP from this system using paper chromatography. Marr, Barboriak and Johnson[4] have since demonstrated the simultaneous occurrence of two major changes appearing during bloodshed following the transection of small arterioles in the mesenteries of guinea-pigs. They found that most of the ATP in whole shed blood was broken down to ADP in 70 sec and in electron micrographs much fibrin could be seen adjacent to the bleeding end of the vessel a mere 30 sec after the haemorrhage had begun. Honour and Mitchell[5] showed that exogenous addition of extracts of red blood cells to vessels undergoing minor injuries resulted in the formation of platelet aggregates which blocked the vessels.

In our experiments 1 in. of aorta, dissected from a 400–450 g guinea-pig, was cut into five pieces and placed in a siliconized test-tube containing 2·0 ml. of iced, native, whole blood drawn by heart puncture from another large guinea-pig. A similar portion of blood served as control. 0·2 ml. samples of blood were drawn into pipettes and delivered into test-tubes containing 0·2 ml. of 10 per cent cold trichloroacetic acid and the adenosine nucleotides were separated by thin-layer chromatography according to Randerath[6], and quantitated by spectrophotometry. A detailed description of our use of these techniques has been published previously[4]. Standards of adenosine nucleotides were included in each experiment.

The results of ATP and ADP assays in native, whole blood at designated intervals after addition of injured vessel wall are shown in Fig. 1. It can be seen that the concentration of ATP fell while that of ADP increased proportionally for 60 sec. Only two values were obtained at 85 sec (indicated by the broken line) as the blood containing the pieces of artery clotted soon after 1 min while the blood in the control tube did not clot for more than 4 min. To obtain these results it was necessary to incubate a large amount of vessel wall with very little blood so the components from the injured vessel wall responsible for these alterations could bring about measurable changes in the concentrations of the adenosine nucleotides.

As can be seen in Fig. 2, 9 mg of ethylenediamine tetracetic acid (EDTA) added to 4 ml. of whole blood inhibit the degradation of ATP brought about by the injured arterial wall. No significant changes in the concentrations of ATP and ADP were observed when the pieces of artery were added to blood anticoagulated with EDTA and no coagulation occurred afterwards in either the blood in the experimental tube containing the blood vessel or the blood in the control tube.

EDTA not only acts as an anticoagulant by chelating sufficient calcium in the plasma to prevent activation of prothrombin but also removes so much calcium that ADP fails to aggregate the platelets—a reaction that requires only very low concentrations of calcium. A compound was therefore sought that would inhibit the activation of prothrombin but not the aggregation of platelets by ADP. Sodium citrate was tried but this compound was found to reduce greatly the total amounts of ATP and ADP in whole blood below those observed in all our previous experiments, perhaps by an inhibitory action on the metabolism of the red blood cell. Sodium oxalate was unsuitable because this compound has an inhibitory action on the activity of enzymes. Heparin was finally chosen, although its anticoagulant action is complicated.

As can be seen in Fig. 3, when heparin was added to whole blood, degradation of ATP brought about by the injured wall of the artery was inhibited and no further

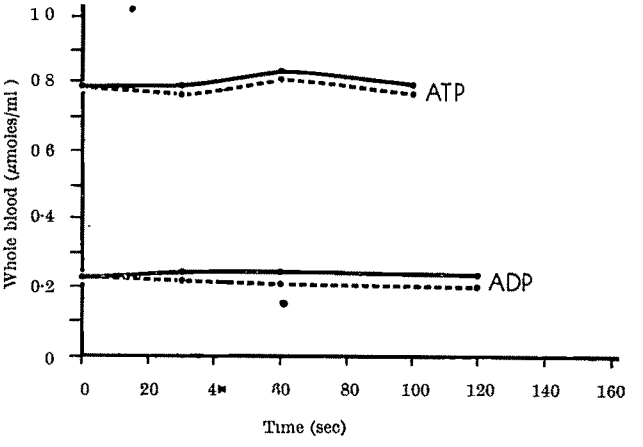


Fig. 2. The top pair of lines represent concentrations of ATP obtained by thin-layer chromatographic separation of adenosine nucleotides while the lower pair of lines represent concentrations of ADP obtained by the same means. The levels from a test-tube containing EDTA plus whole blood are depicted by the broken line (control) and those from a test-tube containing EDTA plus whole blood and pieces of guinea-pig aorta are depicted by the solid line. Each point represents six experiments.

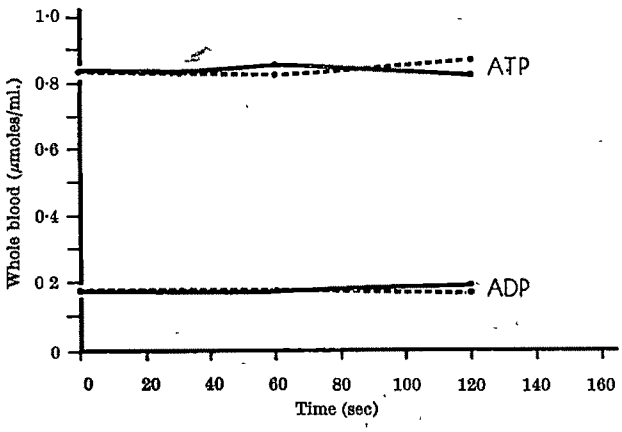


Fig. 3. The top pair of lines represent concentrations of ATP obtained by thin-layer chromatographic separation of adenosine nucleotides while the lower pair of lines represent concentrations of ADP obtained by the same means. The levels from a test-tube containing heparin plus whole blood are depicted by the broken line (control) and those from a test-tube containing heparin plus whole blood and pieces of guinea-pig aorta are depicted by the solid line. Each point represents six experiments.

increase in the concentration of ADP was observed. Similarly, no significant changes in the concentration of either ATP or ADP were observed when pieces of artery were added to blood anticoagulated with heparin. No coagulation was observed in the blood in either the experimental or control tubes.

It is evident that a long sequence of reactions must take place to achieve normal haemostasis. As any experiment can only elucidate a segment of the series it is not surprising that studies of the segment seem to be in conflict. Our findings appear to involve reactions similar to those studied by Bettex-Galland and Lüscher[1]. Spaet and Zucker[3] using a system devoid of red blood cells and containing collagen showed that ADP induced the aggregation of platelets even when the mechanisms of coagulation were depressed by heparin to such a degree that only traces of thrombin could be present. We have shown that the degradation of ATP to ADP in whole red blood cells initiated by the injured arterial wall is completely inhibited when the mechanisms of coagulation are depressed by EDTA or heparin ; this observation is not necessarily at variance with those of Spaet and Zucker as our system involved different components, namely, red blood cells.

Our previous work[4] demonstrated that most of the ADP formed in whole shed blood—in fact, 90 per cent—must come from red blood cells because our electron micrographs prepared 15–25 sec after injury to the vessel wall showed too few platelets, even concentrated in aggregates, to account for the extensive degradation of ATP observed. Thus the degradation of ATP both in platelets in the absence of collagen[1] and in red blood cells seems to require activation of the mechanisms of coagulation.

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IMMUNOLOGY

Effect of Thalidomide on the Graft versus Host Reaction

LYMPHOID cells from a highly inbred parental strain of rat injected into F_1 hybrids derived from that strain and another inbred strain provoke a reaction, characterized among other manifestations by a hyperplasia of the recipients' spleen and other lymphoid tissues[1]. A striking splenic enlargement can also be produced in the chick embryo by intravenous injection of adult chicken spleen cells, or peripheral white blood cells[2]. The splenomegaly in both instances is due to an attack by the inoculated immunologically competent cells directed against a host incapable of reacting against them[3–5]. The splenomegaly is part of a more generalized graft versus host (GVH) reaction and reduction of the degree of splenomegaly is therefore a measure of the inhibition of the GVH reaction[6,7].

We have investigated the effect of thalidomide on the GVH reaction in the chick embryo and in the rat (Tables 1, 2 and 3).

Chick embryo. Adult hybrids (*NIDR*) and Rhode Island Red (RIR) chickens served as donors. White Leghorn (WL) embryos were used as recipients throughout.

In determining the effect of recipient treatment, blood was obtained by cardiac puncture from untreated *NIDR* birds. The citrated blood was centrifuged, the "buffy coat" removed and resuspended in sufficient plasma to give a 5–10 per cent cell suspension. The recipients were 12 days old embryos, which had grids implanted on their chorio-allantoic membranes (CAM) 4 days previously. These grids localize solutions and facilitate daily treatment[8]. The leucocyte suspension (0·05 ml.) was placed in the meshes of the grid lying on the CAM in one experiment, and injected into a vein of the CAM in another. Whole citrated blood was used with the RIR/WL combination and 0·05 ml. was injected intravenously into 14 days old embryos 6 days after implanting the grids.

L-Thalidomide dissolved in dimethyl sulphoxide (DMSO) was dropped into the central "well" of the grids at the times and doses given in Table 1. Control embryos received similar applications of 0·2 ml. DMSO only. Embryos were killed at 19 days of incubation, their spleens removed and weighed. Table 1 summarizes the results. The greater splenomegaly obtained with RIR than with *NIDR* donors indicates a greater antigenic difference in the RIR/WL combination.

Embryos treated with L-thalidomide before being injected with RIR blood developed the usual splenomegaly (106 mg compared with 91 mg for controls). Splenomegaly was also not inhibited when thalidomide treatment was continued after injection of donor cells, although these embryos received a total of 20 mg of L-thalidomide.

Similar treatment of embryos inoculated with *NIDR* leucocytes, in which splenomegaly was weaker, also failed to influence the course of the reaction.

In a series of experiments to determine the effect of treatment of donor blood, citrated blood incubated with thalidomide was given intravenously to recipients, which were themselves otherwise untreated. About 10 ml. of blood was collected from the wing vein of one RIR donor. Portions 2·5 ml. in volume were centrifuged and 0·25 or 0·375 ml. of the plasma removed. The volume was then restored (to 2·5 ml.) with DMSO (controls) or a solution of D,L-, L- or D-thalidomide in DMSO (treated). The final concentration of thalidomide was 7·5–10 mg/ml. and that of DMSO, 10–15 per cent. The solutions were added dropwise to the cell suspensions which were then incubated at room temperature for approximately 1·5 h. After incubation, the cell suspensions were injected (0·1 ml.) into groups of 14-days-old embryos. Embryos were killed 6 days after injection, their spleens removed and weighed. The results are summarized in Table 2.

Table 1. EFFECT OF L-THALIDOMIDE ON SPLENOMEGALY (GVH REACTION) IN THE CHICK EMBRYO. TREATMENT OF RECIPIENTS ONLY

Donor	Cells	Route	TH treatment	Daily dose (mg)	Survival	Mean spleen wt. ± *S.E.*
NIDR	5 per cent suspension "Buffy coat" cells	CAM-(grid) at 12th day	At 8th, then from 12th to 16th day	0·5	9/11	51 ± 30
				1·0	11/13	37 ± 15
				DMSO	8/10	45 ± 32
NIDR	10 per cent suspension "Buffy coat" cells	CAM-(intravenous) at 12th day	From 12th to 17th day	2 0	12/13	39 ± 22
				DMSO	12/14	39 ± 27
RIR	Whole blood	Intravenous at 14th day	From 9th to 13th day	2·0	11/14	106 ± 89
			From 9th to 19th day	2·0	11/15	119 ± 65
			From 9th to 19th day	DMSO	13/14	91 ± 54

S.E., Standard error. No significant difference between treated and controls in any group.

Table 2. EFFECT OF THALIDOMIDE ON SPLENOMEGALY (GVH REACTION) IN THE CHICK EMBRYO. TREATMENT OF DONOR CELLS ONLY

Donor blood Thalidomide	DMSO (per cent)	Host chick embryo Survival	Mean spleen wt. (mg) ± *S.E.*	*P**
None	10	11/14	99 ± 38	
None	15	18/26	114 ± 47	
7·5 mg D,L/ml.	10	10/11	103 ± 16	*N.S.*
7·5 mg D,L/ml.	15	7/15	61 ± 66	0·05 > 0·02
10 mg D,L/ml.	15	10/29	56 ± 53	⩽ 0·01
10 mg L/ml.	15	30/63	79 ± 58	0·05 > 0·02
10 mg D/ml.	15	5/11	105 ± 39	*N.S.*

* *P*, Significance of difference (*t* test) from corresponding control value. *S.E.*, Standard error. *N.S.*, Not significant.

Table 3. EFFECT OF D,L-THALIDOMIDE ON THE SPLENOMEGALY INDUCED IN THE F_1 HYBRID RAT FOLLOWING INJECTION OF PARENTAL STRAIN SPLEEN CELLS

Experiment	Donors	F_1 Recipients	Donor/recipient	F_1 Recipients Mean spleen wt. (mg)†	
	Treatment*		Route	± standard error (6 rats/group)	*P*‡
1	Thalidomide	Thalidomide	Oral	953 ± 72	⩽ 0·01
	Thalidomide	None	—	1,519 ± 63	
2	Thalidomide	Thalidomide	Oral	1,452 ± 38	< 0·01
	None	Thalidomide	Oral	1,607 ± 96	0·04
	None	CMC	Oral	1,940 ± 73	
3	None	Thalidomide	Oral	1,729 ± 324	*N.S.*
	None	CMC	Oral	1,883 ± 67	
	None	CMC	Intraperitoneal	1,903 ± 118	
	None	None	—	1,893 ± 131	
4	Thalidomide	Thalidomide	Intraperitoneal	1,134 ± 51	< 0·01
	None	Thalidomide	Intraperitoneal	1,072 ± 65	< 0·01
	Thalidomide	None	—	1,519 ± 63	
	None	None	—	1,621 ± 107	
5	None	Thalidomide	Intraperitoneal	1,922 ± 112	*N.S.*
	None	CMC	Intraperitoneal	1,684 ± 127	

* Thalidomide 1·25 mg/g daily, suspended in 0 5 per cent carboxy-methyl cellulose (CMC) in physiological saline. Donors and recipients treated for 4 days before spleen cell transfer; recipients for 5 days after.

† Normal range: 450–550 mg.

‡ Significance of difference (*t* test) from the corresponding control value. *N.S*, Not significant ($P > 0·10$).

Incubation of donor blood with D,L-thalidomide at 10 mg/ml. considerably reduced the splenomegaly. The reduction was highly significant ($t = 2·995$; $P \ll 0·01$) despite the wide range of spleen weights in the control and treated embryos. Inhibition of splenomegaly also occurred after incubation with 7·5 mg/ml. D,L-thalidomide, although this was barely significant ($t = 2·2564$; $0·05 > P > 0·02$) as was the inhibition produced with L-thalidomide at 10 mg/ml. ($t = 2·1718$; $0·05 > P > 0·02$). No inhibition of splenomegaly was observed with 7·5 mg/ml. D,L-thalidomide when the DMSO level was reduced to 10 per cent.

By contrast with D,L- and L-thalidomide, incubation of blood with 10 mg/ml. D-thalidomide failed to have any effect on the splenomegaly produced.

Rat. Adult Marshall rats were the donors and (Marshall × August) F_1 hybrids the recipients. Hybrids were treated with D,L-thalidomide (1·25 g/kg body-weight) daily for 4 days before and 5 days after the intraperitoneal injection of paternal (Marshall) strain spleen cells. The thalidomide was administered either orally or intraperitoneally, as a suspension in 0·5 per cent carboxymethylcellulose (CMC) in physiological saline. Control hybrids received either CMC solution alone, or no treatment. Donor rats were given thalidomide, in the same dosage schedule, intraperitoneally for four days before transplantation of their spleens or were untreated. Spleen cell suspensions were prepared by mincing freshly excised spleens with scissors, straining through 150μ copper mesh and suspending the dispersed cells in Ringer's solution. Each hybrid received the equivalent of half a spleen. Nine or ten days after injection of the cell suspension, the hybrids were killed, their spleens excised and weighed. The hybrids weighed 130–170 g and the Marshall rats 160–180 g, but within any one experiment the hybrids (6 per group) were approximately matched for size, as were the Marshall rats. With the strain combination used here the size of the hybrid's spleen on the ninth or tenth day is related linearly to the number of parental strain lymphoid cells injected and is independent of the weight of the animal within the range specified[1]. With this combination of strains there is also no sex difference with respect either to the effectiveness of spleen cells or to response; data for both sexes have therefore been pooled[9].

Table 3 summarizes the results of five experiments. There was a highly significant ($P \ll 0·1$) reduction in splenomegaly in hybrids treated with thalidomide by the oral route, when the lymphoid cell donors had also been pre-treated with the drug (experiments 1 and 2). When donors had not been pre-treated, a less significant ($P = 0·04$) reduction in spleen size was obtained in one experiment and no reduction in another (experiments 2 and 3 respectively). Treatment of the hybrids with the solvent (CMC) alone whether orally or intraperitoneally had no effect on the severity of the GVH reaction (experiment 3).

Less consistent results were obtained when thalidomide was given by the intraperitoneal route. In experiment 4 a very significant ($P < 0·01$) reduction in splenomegaly was produced, but pre-treatment of donors with thalidomide had no effect on the response either of treated or of untreated hybrids. In experiment 5, however, thalidomide apparently failed to diminish the GVH reaction. This variability may be due to non-specific immunological effects of repeated intraperitoneal injections of particulate material.

Interpretation of the results of oral administration of thalidomide would appear to be less ambiguous. Nevertheless, it may be argued that pre-treatment of donor rats intraperitoneally could reduce the potency of their lymphoid cells. In experiment 2, however, a significant reduction in splenomegaly was obtained even when donors had not been pre-treated. Moreover, in experiment 4 pre-treatment of donors was shown to be without effect in untreated hybrids. These results therefore indicate that when thalidomide is given orally to F_1 hybrid rats the GVH reaction evoked by challenge with parental strain lymphoid cells is significantly reduced, and that this effect is enhanced if the lymphoid cell donors are also pre-treated with the drug.

It is evident, therefore, that thalidomide is able to reduce significantly the severity of the GVH reaction in the chick embryo and the rat, though not to suppress it completely. This is in line with findings on the rejection of skin homografts[10] and on the development following a skin homograft of immunoblasts in the draining lymph node[11].

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Immunological Unresponsiveness in Adult Rats to the Nematode *Nippostrongylus brasiliensis* induced by Infection in Early Life

ALTHOUGH it is known that mature animals may develop a marked degree of acquired immunity to infection with many species of parasitic nematodes[1,2] the occurrence of immunological unresponsiveness in adult animals, either under field or experimental conditions, has not been definitely established. Observations resulting from experiments with *Mesistocirrus digitatus* in calves[3] and *Haemonchus contortus* in sheep[4] indicated the possible occurrence of tolerance in these infections and formed the basis for a series of experiments with *Nippostrongylus brasiliensis*—a parasite of rats.

Adult rats infected subcutaneously with five hundred or more *N. brasiliensis* larvae acquire a striking immunity to this parasite during the course of the infection[5]. This is manifested towards the end of the second week by a "self-cure" reaction, during which there is a sharp fall in egg production by the parasites, followed over a period of a few days by expulsion of most of the worms from the intestine of the host (see Table 1). The animals are subsequently immune to re-infection.

Table 1. WORMS IN INTESTINE OF BABY AND ADULT RATS INFECTED WITH FIVE HUNDRED *N. brasiliensis* LARVAE

Age at Infection	Days after infection 10 Mean	*S.E.*	26 Mean	*S.E.*
3–4 weeks	177	20	215	28
3–6 weeks	195	23	210	35
Adult	118	31	33	6

In the course of a series of experiments with baby rats, we have found that an infection of *N. brasiliensis* acquired during the first 6 weeks of life is not eliminated in this characteristic fashion and persists well into adult life. In experiment 1, two groups of baby rats were infected subcutaneously with five hundred larvae. These groups were aged 3–4 weeks and 5–6 weeks respectively. The results of worm counts at autopsy on days 10 and 26 are listed in Table 1 and show that the infection had not been eliminated by day 26.

In the second experiment, which was designed to ascertain the duration of the primary infection in baby rats, five litters aged between 2–3 weeks received five hundred larvae per rat. Table 2 shows the results of worm counts of the rats autopsied on days 52, 67 and 87 after infection. Even on these dates it is apparent that the rats had still not undergone the typical self-cure reaction; autopsies of comparably infected adult rats on day 52 yielded a range of 0–5 worms. In view of the fact that a very strong immune response is normally developed within 2 weeks by rats 4–5 weeks younger than those on the date of last autopsy, these results cannot be attributed to immunological immaturity.

Table 2. WORMS IN INTESTINES OF RATS INFECTED AT 2–3 WEEKS OF AGE WITH FIVE HUNDRED *N. brasiliensis* LARVAE

	Days after infection 52	67	87
Mean	143	140	83
S.E.	12	15	18

On day 52 of the infection, a group of these rats was challenged with an infection of 3,000 larvae together with a control group of uninfected rats of the same age. Both groups appeared to be equally susceptible to infection in that the faecal egg counts rose to similar peaks 8 days after infection; on day 10, autopsies of both groups showed similar numbers of worms. Subsequently both groups underwent a typical self-cure reaction. The results of this challenge infection showed that the first infection given when young had not stimulated the high degree of immunity to re-infection characteristic of adult rats. On the other hand, the immunological unresponsiveness which allowed the persistence of the primary infection did not interfere with the immune response to the challenge infection and this occurred in a manner similar to that of rats which are experiencing a primary infection. A superficially similar situation is known to exist in hamsters infected neonatally with the oncogenic *SV* 40 virus[6].

The persistence of the primary infection is unlikely to result from failure of an immunologically mature rat to experience larval antigen since rats of 8–10 weeks which are infected by laparotomy with an adult worm population approximate to that in our unresponsive rats undergo a characteristic self-cure within 2 weeks[7].

Because the ability to self-cure and protect against re-infection can be transmitted with circulating antibody obtained from adult rats which have undergone self-cure, it is necessary, when considering a mechanism for the induction of unresponsiveness, to determine the immunizing ability of sera from infected baby rats. This is being investigated but it would be surprising if these contained protective antibody. We do know, however, that reaginic antibodies as described by Ogilvie[8] appear in the serum of these baby rats 3–4 weeks after infection and that the titre subsequently rises to as high a level as is found in adults following a similar infection. Also intestinal anaphylaxis can be induced in baby rats 3–4 weeks after infection by the intravenous injection of *N. brasiliensis* antigen, this reaction being as marked as that seen in adult rats[9]. Although it is possible that the persistence of this nematode infection during the adult life of the rats is due to acquired immunological tolerance in the classical sense, that is, non-recognition of antigen[10], this seems an improbable explanation in view of the high reaginic titre and the development of a typical immune response after a heavy challenge. Because a number of studies in different species have suggested that the earliest response of the young animal involves macroglobulins[11,12], we have considered it possible that the immune response of the young rat is perhaps initially confined to the production of a limited type of globulin, including reagins. If this is so, one might speculate that these antibodies could subsequently combine with antigen produced by the persisting adult infection in such a way that it is either neutralized or used solely for the stimulation of further synthesis of reagins. This state might cease and immunological competence be established when the rats are heavily challenged because relatively massive amounts of antigen would become available to the host in excess of that capable of reacting with reagin-type antibodies.

The unfixed antigen would then be available for stimulation of a normal immunological response.

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PATHOLOGY

Adrenal Necrosis induced by 7-Hydroxymethyl-12-methylbenz(a)anthracene and its Prevention

THIS communication reports our investigation of the action of 7-hydroxymethyl-12-methylbenz(*a*)anthracene (7-OHM-MBA) on the adrenal glands of normal rats and of rats treated by methods that protect the adrenal glands against 7,12-dimethylbenz(*a*)anthracene (9,10-dimethyl-1,2-benzanthracene, or DMBA)[1,2].

After treatment of rats with large doses of DMBA, the zona fasciculata and the zona reticularis are destroyed[3] Recent experiments[2,4] have demonstrated that rats with impaired liver function are protected from the adrenocorticolytic effect of DMBA, and it has been postulated that a metabolite of DMBA, and not DMBA itself, is responsible for adrenal necrosis. The metabolism of DMBA has been investigated and hydroxymethyl compounds identified as metabolites[5]. Synthetic derivatives of DMBA have been tested for their effect on the adrenal glands: while 12-hydroxymethyl-7-methylbenz(*a*)anthracene has no adrenocorticolytic property, 7-OHM-MBA is more potent than DMBA in producing adrenal necrosis[6].

Accredited female Sprague–Dawley rats bred from a specific pathogen-free stock were used; they were obtained from Oxford Laboratory Animal Colonies. Their diet was Thompson rat cake containing 14 per cent skimmed milk supplied by North-Eastern Agricultural Co-operative Society, Ltd., Aberdeen. 7-OHM-MBA was dissolved in arachis oil (10 mg/ml.), and administered by stomach tube. Rats were killed 3 days after treatment; adrenal glands were weighed and fixed in 4 per cent neutral buffered formaldehyde. One gland of each pair was embedded in paraffin wax and 5μ sections were stained with haematoxylin and eosin. Liver sections stained with haematoxylin and eosin were also prepared.

Methods previously shown[1,2] to protect the adrenal glands of rats from DMBA were used: (*a*) Carbon tetrachloride: Rats were treated with 0·3 ml. of a 50 per cent (v/v) solution in olive oil intraperitoneally 24 h before challenge with DMBA or 7-OHM-MBA. (*b*) Partial hepatectomy: Two-thirds of the liver was removed as in previous studies 24 h before challenge with DMBA or 7-OHM-MBA. (*c*) 2-Methyl-1,2-*bis*-(3-pyridyl)-1-propanone ('Metopirone' pure base, Ciba): Rats were given three doses of 10 mg 'Metopirone' in 0·2 ml. olive oil intraperitoneally at 4-h intervals[7] and the challenge of 7-OHM-MBA or DMBA given 4 h after the final dose. Later, rats were given either 15 mg 7-OHM-MBA intragastrically or 3 mg DMBA intravenously (these have been found to produce comparable degrees of adrenal damage) and killed 3 days later.

Action of 7-OHM-MBA on normal intact 50-day Sprague–Dawley rats. Doses of from 5 to 20 mg were given intragastrically to rats of 50 days of age (Table 1). Fifteen milligrams of 7-OHM-MBA intragastrically has the same effect on the adrenal glands as a dose of 35–40 mg DMBA intragastrically or 3·0–3·5 mg DMBA intravenously: thus 7-OHM-MBA is two to three times more potent than DMBA. This is slightly less than previously reported[6] and may result from differences in the stock of rats and/or diet. Damage to the adrenal glands occurs first in the zona reticularis after treatment with DMBA, whereas with 7-OHM-MBA early changes are frequently seen throughout the whole of the zona fasciculata and zona reticularis. The zona glomerulosa may show congestion with both compounds but necrosis has not been seen in this zone with either of them. Rats given 7-OHM-MBA, even up to a dose of 20 mg, appeared healthy, maintained body weight and did not have diarrhoea: this compound is much less toxic than the parent compound, DMBA.

Table 1. INCIDENCE OF ADRENAL NECROSIS IN 50-DAY SPRAGUE–DAWLEY FEMALE RATS TREATED WITH 7-OHM-MBA

Dose (mg)	No. of rats	No. sick	Incidence of severe adrenal necrosis	Weight of adrenal glands (mg) (±S.D.)
5	9	0/9	0/9	21·3±3·4
10	10	0/10	8/10	29·6±9·6
15	10	0/10	9/10	31·1±6·1
20	5	1/5	5/5	33·4±3·4

7-OHM-MBA treatment of "protected" rats. (*a*) *Carbon tetrachloride:* In view of our previous finding[2], which showed the importance of normal liver function for the development of adrenal necrosis after DMBA treatment, we have "protected" rats with carbon tetrachloride and treated them with 7-OHM-MBA or DMBA. The results are shown in Table 2: as would be expected from our previous studies[2], the incidence of severe adrenal necrosis is low in rats treated with DMBA, but the incidence with 7-OHM-MBA is the same as in the control rats given 15 mg derivative (see Table 1). (*b*) *Partial hepatectomy.* This also protects rats from adrenal necrosis induced by DMBA administration[2]. The effects of operation 24 h before treatments with DMBA and 7-OHM-MBA are compared in Table 2. Again, the incidence of severe adrenal necrosis remained high in rats given the derivative while partial protection was achieved in rats given DMBA. (*c*) '*Metopirone*': This compound completely protects adrenal glands against 7-OHM-MBA (Table 3), and is also effective against DMBA given intravenously (Table 3).

7-OHM-MBA is two to three times more potent than DMBA in its adrenocorticolytic action when given in oil intragastrically. Although rats with impaired liver function are protected from the adrenocorticolytic effect of DMBA, they remain susceptible to adrenal necrosis induced by 7-OHM-MBA: the action of 7-OHM-MBA seems to be independent of liver function. This is additional evidence in support of the hypothesis that DMBA must be metabolized by the liver to an active adrenocorticolytic derivative—which may well be 7-OHM-MBA.

'Metopirone' gives complete protection of adrenal glands against 7-OHM-MBA and almost complete protection against DMBA given either intravenously, as here re-

Table 2. EFFECT OF IMPAIRED LIVER FUNCTION ON ADRENAL NECROSIS INDUCED BY 7-OHM-MBA OR BY DMBA

Pretreatment (24 h pre-)	Treatment	No. of rats	No. rats surviving to day +3	No. rats with massive adrenal necrosis
None	15 mg 7-OHM-MBA intragastric	10	10	9/10
	3 mg DMBA intravenous	10	10	8/10
Carbon tetrachloride	15 mg 7-OHM-MBA intragastric	11	10	9/10
	3 mg DMBA intravenous	13	10	3/10*
Partial hepatectomy	15 mg 7-OHM-MBA intragastric	15	15	15/15
	3 mg DMBA intravenous	15	15	7/15†

* $P < 0.025$.
† $P < 0.005$.

Table 3. INCIDENCE OF ADRENAL NECROSIS AFTER TREATMENT WITH 'METOPIRONE'

Pretreatment	Treatment	No. of rats	No. rats assessed day +3	No. rats with massive adrenal necrosis
3 × 10 mg 'Metopirone' in olive oil intraperit. at 4 h intervals	15 mg 7-OHM-MBA	14	13	0/13*
Oil ×3	15 mg 7-OHM-MBA	14	14	10/14*
3 × 10 mg 'Metopirone' in olive oil intraperit. at 4 h intervals	3 mg DMBA intravenous	15	15	1/15†
Oil ×3	3 mg DMBA intravenous	15	13	13/13†

* $P < 0{\cdot}001$.
† $P \ll 0{\cdot}001$.

ported, or intragastrically[1]. This incomplete protection against DMBA may result from the production of adrenocorticolytic metabolite after 'Metopirone' has ceased to exert its maximal inhibitory action on steroid synthesis. 'Metopirone' inhibits 11β hydroxylation and therefore interferes with the synthesis of corticosterone and cortisol[8]. It has been suggested[3] that the adrenocorticolytic action of DMBA is the result of similarity between its structure and that of corticosterone or cortisol. We have clearly established, however, that DMBA itself is not adrenocorticolytic, but it seems likely from our experiments that 7-OHM-MBA will induce adrenocortical necrosis only if there is active synthesis of corticosterone or related steroid hormones that are hydroxylated at C11.

Wong and Warner[9] have reported protection against DMBA-induced adrenal necrosis by a C17 α-hydroxylase inhibitor *SU* 9055. It will be of interest to investigate the action of this inhibitor in 7-OHM-MBA-induced necrosis.

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PHARMACOLOGY

Combined Effects of Chlordiazepoxide and Dexamphetamine on Activity of Rats in an Unfamiliar Environment

IN previous experiments we found that, over a wide range of doses, amphetamine and amylobarbitone affected relatively little the spontaneous activity of rats in an unfamiliar environment, but that some mixtures of these two drugs greatly increased it. This somewhat surprising effect could be regarded as a result of true mutual potentiation[1,2].

Chlordiazepoxide ('Librium', Roche) is a comparatively new drug[3] which, like amylobarbitone, is widely used in the treatment of anxiety. Its effects on the activities of animals seem, in general, to be in the direction of depression; but with relatively small doses stimulation has been reported (refs. 4 and 5), and Maxwell, D. R., and Christmas, A. J., (personal communication), and this again is similar to amylobarbitone. In order to pursue the comparison, dexamphetamine was added to chlordiazepoxide in the present experiments and the effects were analysed of various mixtures on the behaviour of rats in an unfamiliar environment similar to that previously used.

Naive hooded female rats, about 120–130 days old and reared under standard conditions, were injected subcutaneously in the flank with either various doses of chlordiazepoxide alone, or with these same doses mixed with 1·18 mg/kg dexamphetamine, or with this dose of dexamphetamine alone, or with saline as control. The dose of dexamphetamine had previously been found particularly effective in combination with amylobarbitone[2]. Thirty-five minutes later each rat was tested singly for 5 min in a symmetrical Y-shaped runway[1]. Rats were used once only.

The results given here are the mean number of times the rats entered the arms of the Y with all four feet during the 5 min trial; this is a measure of locomotion, and is typically greatest when an animal is first exposed to an unfamiliar environment. Over the middle range of doses (3·3–100 mg/kg chlordiazepoxide) the mixture consistently induced more activity than the constituents alone. Maximal activity occurred with 25 mg/kg chlordiazepoxide + 1·18 mg/kg dexamphetamine (mean entries 30·7, *S.D.* 5·7), and this was significantly more than with the ingredient dose of chlordiazepoxide alone (mean 17·5, *S.D.* 7·4; $P < 0{\cdot}01$), and also more than with dexamphetamine alone (mean 18·1, *S.D.* 6·6; $P < 0{\cdot}01$), and with saline (mean 15·6, *S.D.* 6·5; $P < 0{\cdot}001$). The last three means did not differ significantly from each other.

Fig. 1 shows these results broken down into successive minutes. The most striking contrast is between chlordiazepoxide alone and the mixture. Rats treated with chlordiazepoxide were highly active during the first 2 or 3 min, but then they became immobile in a characteristic crouching position and without even moving their whiskers, though their bodies did not seem flaccid and their eyes were open. This "trance"-like state[6], interrupted by only occasional movements, usually lasted to the end of the trial and beyond, but if the runway was gently

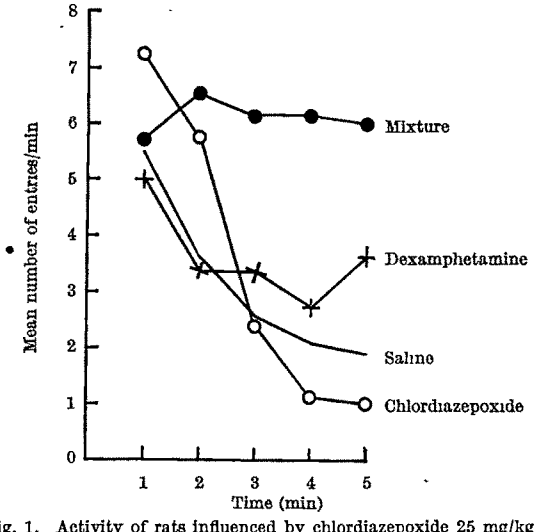


Fig. 1. Activity of rats influenced by chlordiazepoxide 25 mg/kg and dexamphetamine 1·18 mg/kg separately and in combination. The ordinate represents the mean number of entries into the arms of an unfamiliar Y-shaped runway during successive minutes of a 5 min trial by four different groups of rats: ○, chlordiazepoxide ($n = 8$); +, dexamphetamine ($n = 8$); ●, mixture ($n = 7$); —, saline ($n = 19$).

lifted away the rat would, after a few moments, "come to" and spontaneously run around the floor of the laboratory. If the rat was put back into the Y the cycle was repeated. The effect was not dependent on any sudden change in the time course of the action of the drug, because it occurred equally if rats were tested at various times other than 35 min after injection. The activity of the mixture rats, on the other hand, was high throughout the trial, and no "trances" were observed. As a measure of change in the amount of activity, the mean number of entries in the fifth minute was subtracted from the mean in the first minute. The mean change with chlordiazepoxide alone was 6·3 entries, compared with −0·3 with the mixture ($P < 0.001$), 1·4 with dexamphetamine ($P < 0.01$) and 3·6 with saline ($P < 0.01$). Similar trends were found over the whole range of doses studied except at the extremes.

Amphetamine is increasingly being found able to interact in a variety of ways with different centrally acting drugs depending on the doses and the kind of behaviour studied[1,2,7–12]. In the present case dexamphetamine prevented the sharp decline in activity which normally occurred with chlordiazepoxide and so activity was maintained evenly throughout the trial. It is unlikely that a new compound was formed by giving the two drugs as a mixture because both are strong bases. With dexamphetamine-barbiturate mixtures activity was also evenly maintained[2], and in this respect the two kinds of mixture are similar. But the decline in activity with amylobarbitone alone was much more gradual than with chlordiazepoxide, and the rats appeared sedated and sluggish and not in a "trance". The abrupt change from high activity to immobility which occurred with chlordiazepoxide and which seemed to depend mainly on length of exposure to an unfamiliar environment was unlike anything observed with amylobarbitone and is altogether behaviourally striking.

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PHYSIOLOGY

Effects of Temperature on Photo-sensitivity of Spermatozoa

ADVERSE effects of light on the movement and life-span of spermatozoa have repeatedly been demonstrated[1–7]. Norman, Goldberg and Porterfield[7] were unable to detect any relationship between light effects and temperature in a few experiments by means of simple visual observational methods. A definite dependence of photo-sensitivity on temperature has now been demonstrated in specific investigations using Rikmenspoel's photo-electric method[3–6,8–11].

In nineteen series of experiments, using sixty-seven ejaculates from ten different bulls of the Dutch–Friesian and Meuse–Rhine–Yssel breeds, sub-samples of each diluted ejaculate were incubated in glass tubes placed in water baths kept at different temperatures, ranging from 5°–40° C, with 5° C intervals. Dilutions (1 : 70) were made with Rikmenspoel's ultramicroscopically clear egg-yolk–citrate medium[12], *p*H 6·75 ± 0·05 (measured at 22°–23° C, *p*H change 0·03 per cent/°C[13]).

A constant illumination of 983 ± 3 lux was maintained, using a battery of incandescent bulbs (220 V, 150 W) with internal mirrors as the light sources. The dark controls were kept in small tubes of the size of the experimental specimens, but made of brown glass, painted black, wrapped in aluminium foil and pressed into a white tube of soft plastic to avoid rise of temperature due to the heating effect of the light. The control tubes were placed alongside the unprotected glass tubes in the same water baths. All the tubes in the water baths were gently agitated by a single agitator in order to prevent sedimentation of the sperms during the treatment. A cooling tank, operated with running cold water, was placed between the water baths and the battery of lamps. The whole equipment was installed in a thermostatically controlled room kept at 5° C.

Drops were taken at regular intervals from the test-tubes and placed in the photo-electric equipment[3–6]. All recordings were made at a temperature of 37·0° C. The recordings were analysed for rates of velocity decrease with time ($-\partial\bar{v}/\partial t$) and for the rate constants k of the decrease in numbers of normally moving spermatozoa ($k_N = \mathrm{d}(\ln N)/\mathrm{d}t$) and of migration rates, the latter being defined as the product of N with the mean velocity $\bar{v}$. Over periods exceeding those of the present experiments both N and $N\bar{v}$ decrease exponentially with time

$$y(t) = y_0.\exp(-kt) \qquad (1)$$

where $y = N$ or $N.\bar{v}$, respectively[3,12]. The rate constants k_N and $K_{N\bar{v}}$ were obtained from the slopes of the regression lines on a semi-logarithmic plot.

The relationship between these three rate constants and temperature was found to fit the Arrhenius equation for temperature-dependence of the rate constants of chemical reactions

$$k = A.\exp(-E_A/RT) \qquad (2)$$

where A is a constant representing the probability that a reaction occurs, E_A, the apparent energy of activation, R, the gas constant (1·987 cal/mole degree), and T, the absolute temperature. Consequently, a linear relationship is obtained by plotting log ($-\partial\bar{v}/\partial t$), log k_N or log $k_{N\bar{v}}$ as a function of $1/T$(Figs. 1 and 2). The apparent energies of activation can be derived from the slopes of these plots. This comes out to be 18,400 ± 250 cal/mole for the samples kept in darkness and only 9,300 ± 150 cal/mole for the samples under illumination. This result clearly demonstrates the accelerating effect of light on the rate of deterioration of bovine spermatozoa.

Photo-sensitivity can now be defined quantitatively by the factor with which the rate of deterioration is increased by a constant level of illumination, I. This means that it is measured by

$$Y_I = (k_y)_I/(k_y)_{I=0} \qquad (3)$$

where k_y represents either $-\partial\bar{v}/\partial t$, k_N or $k_{N\bar{v}}$ (ref. 13).

The value of Y_I is independent of which of these parameters is chosen, and the individual values can therefore be averaged to combine the total result into a single relationship (Fig. 3). Obviously photo-sensitivity decreases with increasing temperature, vanishing at ~48° C, where $Y_I = 1$. The apparent energy of activation of the photochemical process is −3,532 cal/mole, the negative sign appearing as an indication of the light acting itself as an activator or catalyst. The apparent temperature coefficients (Q_{10}) for different regions of the temperature range are shown in Table 1.

The very low Q_{10} values of photo-sensitivity show clearly that its temperature dependence is mainly due

Table 1

Temperature range °C	Q_{10} of rate constants Darkness	Light	Q_{10} of light sensitivity
0–10	3 27	1 83	0 80
10–20	3 06	1 76	0 81
20–30	2 84	1 69	0 82
30–40	2 66	1·64	0 83

to the photochemical process itself and not to secondary thermal reactions.

Norman *et al.*[7] chose the period of time after the beginning of their observations at which movements stopped

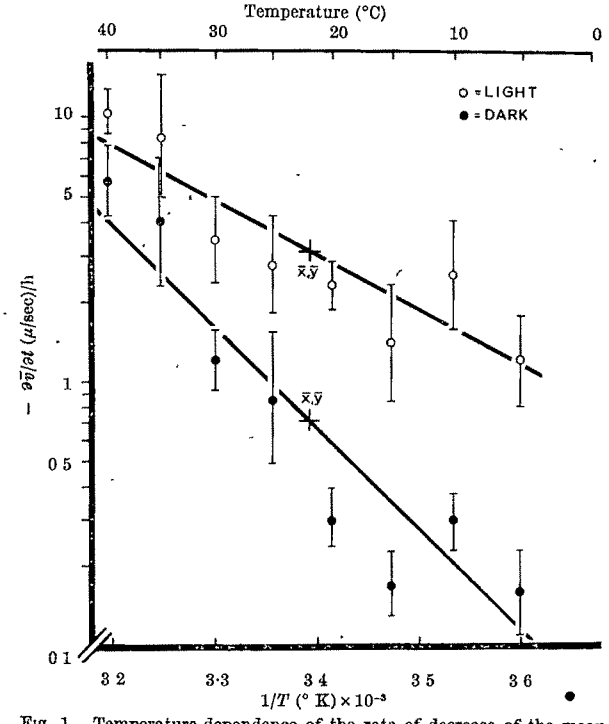


Fig. 1. Temperature-dependence of the rate of decrease of the mean velocity in (μ/sec)/h of storage of bull spermatozoa, illustrating the applicability of the Arrhenius equation (2). Ordinate logarithmic Corr. $1/T \times \log(-\partial\bar{v}/\partial t)$ (in darkness, $r = -0\,94$, under illumination at 983 ± 3 lux, $r = -0\,90$).

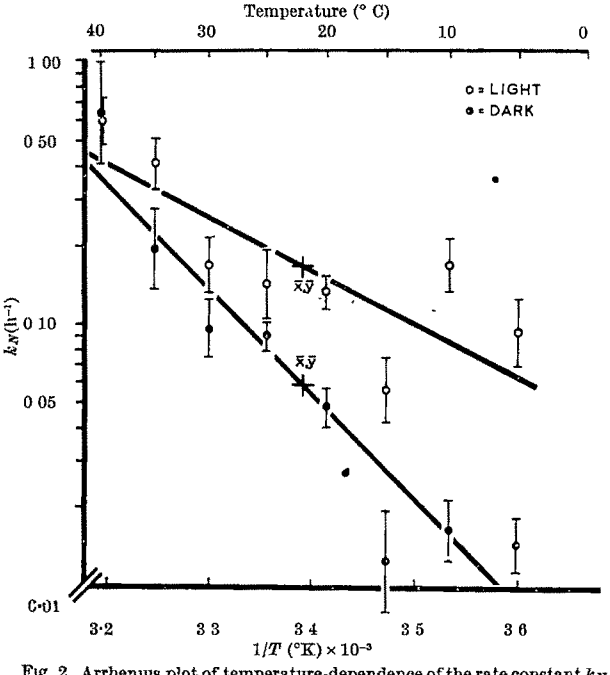


Fig 2 Arrhenius plot of temperature-dependence of the rate constant k_N of decrease of numbers of normally moving bull spermatozoa with time, under illumination at 983 ± 3 lux and in darkness Ordinate logarithmic. Corr. $1/T \times \log k_N$. (In darkness, $r = -0\,95$, under illumination, $r = -0\,78$.)

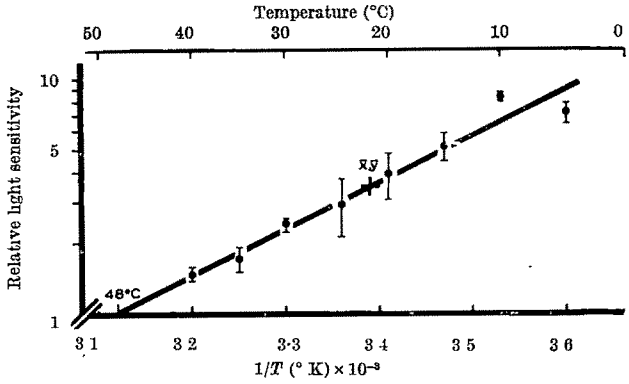


Fig 3. Arrhenius plot of temperature-dependence of photo-sensitivity of bull spermatozoa, defined as the factor by which deterioration is accelerated under a constant level of illumination of 983 ± 3 lux. Ordinate logarithmic. ($r = 0\,98$; $10^{-5} > P > 10^{-6}$.)

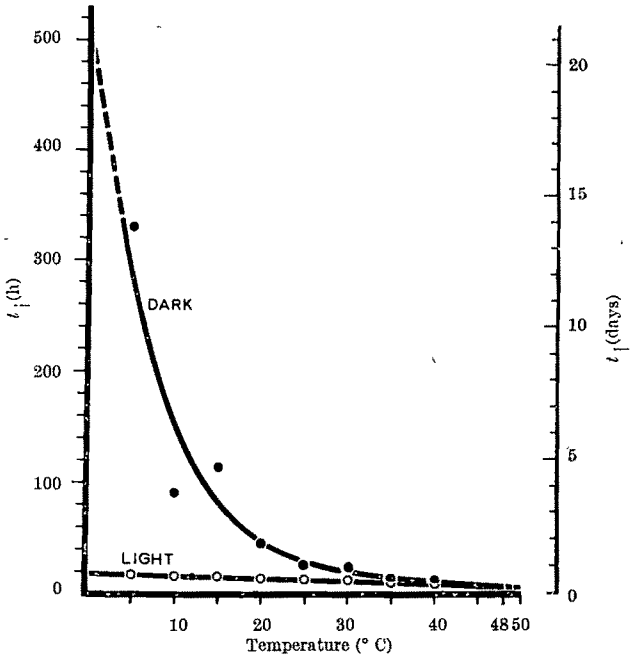


Fig. 4. Temperature-dependence of the time $t_\dagger$, after the beginning of the incubations, at which no more normally moving spermatozoa are encountered. Upper curve, darkness, lower curve, constant illumination at 983 ± 3 lux Relative photo-sensitivity is represented at each temperature by the distance between the two curves. The curves coincide at ~48° C, at this temperature spermatozoa are obviously killed by heat alone without further contribution of light absorption The curves as drawn are the most probable ones calculated by functional analysis of the data.

as a parameter for the process of deterioration. This has the important drawback that this moment $t_\dagger$ can only be determined with reasonable accuracy in cases where life-spans are short enough to allow close and continuous observation of the process of deterioration. Even then, the statistical error is large, since one or two sperms that have a markedly longer life-span, or have not yet received a sufficient number of hits, can influence the result considerably. It can be shown that the relationship between the swimming velocities of spermatozoa and time can be described empirically by the linear equation[13]

$$\log\,[(\bar{v}_0 - \bar{v}(t)]/\bar{v}_0 = k_v\,(\log t - \log t_\dagger) \qquad (4)$$

Hence, from the plot of $\log\,[\bar{v}_0 - \bar{v}(t)]/\bar{v}_0$ against $\log\,t$, the value of $\log\,t_\dagger$ can be read by extrapolating the linear regression line of all data to the point where $\bar{v}(t) = 0$. Although this indirect method is more reliable than direct observation, $t_\dagger$ is still more uncertain than are the rate constants and useful results could only be obtained from the pooled data. Furthermore, the ratio of $t_\dagger$ values obtained under different conditions is not a linear measure

of a biological effect. The total result obtained in the present series of experiments, however, is again a convincing demonstration of the temperature-dependence of photo-sensitivity (Fig. 4).

Because of these effects of temperature on photo-sensitivity of spermatozoa it can be inferred from photochemistry and target theory that this photo-sensitivity depends on either a one-target multiple-hit, or a multiple-target process. Then it follows that at certain levels of illumination deviations from Bunsen and Roscoe's reciprocity law must occur (Schwarzschild effect), and an intermittency effect can also be expected[14].

These investigations are being continued. A detailed account will be presented elsewhere.

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Critical Flicker Fusion Perimetry and the Adaptation Level of the Eye

MOST scientists concerned with the physiology of vision agree that the temporal resolution of the cone system is greater than that of the rod system. In only a few of the experimental studies, however, is the evidence convincing that exclusively rods or exclusively cones were indeed functioning over some appropriate range of stimulus environments. Colour visibility, small stimulus fields, and widely discrete levels of adaptation have been tried with only indifferent success. Moreover, the cone system is reported only to be generally faster, not always so. Many of the studies find that the rods sometimes resolve light pulses at higher frequencies than do the cones. Our predictive knowledge of this whole problem is poor. We can probably safely assert only that a small dim stimulus in the retinal periphery will be less adequately resolved than a similar stimulus in or near the fovea, while, on the contrary, a large bright stimulus will be better resolved in the periphery. There is some additional evidence for an annulus of high resolution at about 10° from the fovea

Every supraliminal stimulus presented to the eye affects its adaptation level, as was particularly emphasized by DeLange[1]. While the stimulus level can be used to control the adaptation level[1], so also can the level of the surrounding intensity[2]. Furthermore, it is possible to determine the increase in intensity (ΔI) above a constantly monitored instantaneous threshold (τ) required to resolve a constant flicker[3], and to measure the flicker resolution for a stimulus presented at some constant multiple ($\varkappa$) of the instantaneous threshold[4,5].

The relative integrity of physical units depends on the constancy of the physical zero and the fact that it is possible to specify it. The same is true for physiological units. We would like to suggest that whenever possible physiological intensities should be specified with respect to the physiological zero (threshold) then in force. In the more traditional constant-response method of Shikano[8], though his data could be replotted with respect to the physiological zero, this is difficult to do. It can be done automatically by the methods of DeLange and Hecht[1,2]; however, as usually used, the τ-coefficient ($\varkappa$) is restricted to unity. In essence, these methods consider the response of "fusion" to be their constant-response, as Shikano considered the response of "resolution" to be his.

Although Hecht and his colleagues have applied their methods extensively to the "duplexity" problem (that is, the simultaneous operation of rods and cones), their results show only that the eye functions more slowly in dim light; they have not shown how the rods or the cones function by themselves. Those workers using the DeLange sine-wave methods have not yet systematically studied the duplexity problem. We have used the method of constant $\varkappa$, feeling that functional differences between the rods and the cones might more readily appear with such constant physiological stimuli than with the usual physically constant ones. Unfortunately, large supra-threshold stimuli are necessary because the flicker resolution of the eye is very erratic near τ. Thus contamination of τ cannot be avoided. It is therefore necessary to obtain some measure of the degree of distortion in the threshold curve brought about by the presentation of the flashes of the critical flicker fusion test at some $\varkappa\tau$ well above threshold.

Our experiment consisted of obtaining uncontaminated dark-adaptation curves on the test eye in separate sessions, and taking experimental measurements of critical flicker fusions and of thresholds at alternate minutes in the same eye. Otherwise, identical conditions were used throughout the experiment. After exploration, a $\varkappa = 10$ was chosen because it did not seriously contaminate τ yet still allowed reliable critical flicker fusions to be taken.

The apparatus consisted of a Sylvania *R*1131*C* glow modulator lamp connected to a Grason–Stadler flicker fusion apparatus (model *E*622), interconnected by a system of lenses and filters to a 60 cm diameter perimeter The light was focused on a ground-glass screen at an exit-port at the centre of the perimeter. The flicker source produced a square wave white light pulse (50 per cent on time—thus 127 per cent modulation depth—DeLange[1]) with a continuously variable frequency of from 1 to 125 c/s. The colour of the light was acceptably neutral over 2 log units of intensity by way of current control, and four additional log units by way of neutral density step-filters.

The peak output of the light under steady conditions was 0·024 apparent ft.-candles (0·082 candles/m²). Using a 5 mm pupil and 1 sec bursts (to avoid the Troxler effect), we thus had 2·05 photopic-troland-seconds at the retina. The relative brightness of the lamp was independent of the frequency. A small red fixation light was placed just above the stimulus. The observer's pupil was dilated with 1 per cent mydriacyl. All observations were preceded by light adaptation for 3 min to about 2,642 candles/m² of white light.

As noted, two types of measurements were made: a 30 min run of alternating flicker (at $\varkappa\tau$) and light threshold readings, and a 30 min control run of pure threshold readings. The $\varkappa\tau$ was obtained by sliding a 1 log density filter in and out of the beam during the approximately 40 sec mechanical lag between the flicker and the threshold readings. The results are means of at least five runs, and are reported in Fig. 1 and Fig. 2. The legends describe the specific conditions.

In Fig. 1 the threshold curves do not have alpha flex points and they have high final values. On the basis of accepted principles in vision, we may take them to be pure cone curves. Also, on this basis, we may observe that there is a clear and steady fall in flicker resolution with time in the dark despite the fact that there is no evidence at all for flicker alpha points (thus no rod intrusion). This suggests that a gradual decrease in

lateral inhibition has occurred within the cone system and/or an increase in neural convergence, and that at least part of the drop in τ itself may be neural and not photochemical in nature.

Our crucial finding is shown in Fig. 2: the flex point in the curves for critical flicker fusion. We believe that this is the first time such flex points have been shown conclusively. For both observers this is delayed from the threshold alpha by the approximate time needed for the intensity of the critical flicker fusion stimulus to fall to the level at which the threshold alpha occurred. Because the alternate method necessitated placing the critical flicker fusion stimulus one log unit above the threshold, the flickering stimulus did not reach the rod environment until a few minutes later than the τ alpha. (The early flex point (at 8 min) is an experimental artefact arising from the sudden deceleration in the critical flicker fusion stimulus intensity at the τ-alpha point.)

As an aside, we may note that although the critical flicker fusion stimulus itself was one log above the threshold, the experimental "contaminated" threshold curves were never contaminated to this degree. Possibly this

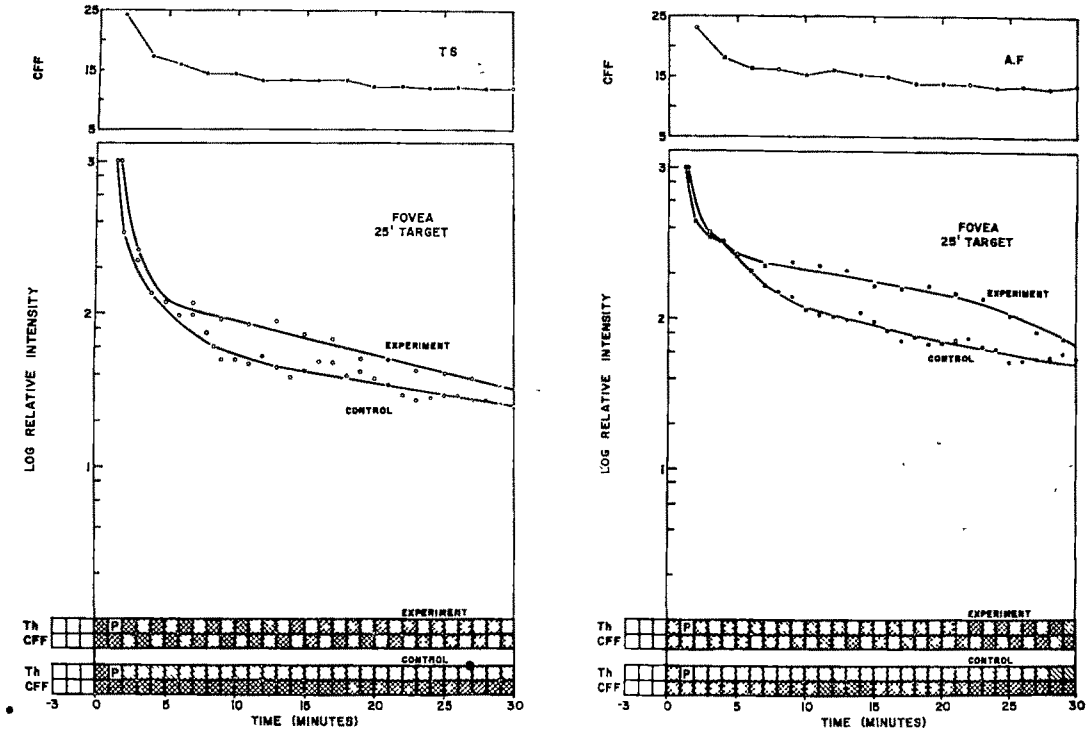


Fig. 1. A pure rod-free curve obtained with a 25′ central field.

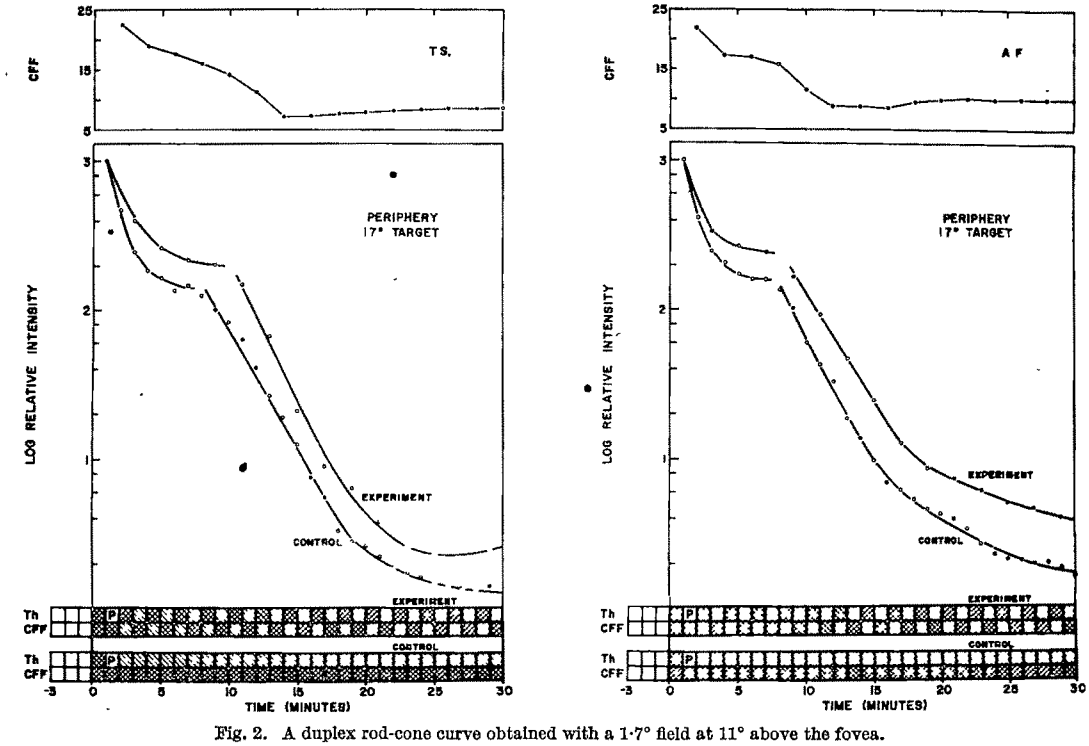


Fig. 2. A duplex rod-cone curve obtained with a 1·7° field at 11° above the fovea.

Table 1

Obs.	25′ at 0°	1·7° at 11°	t-test
A. F.	13 7 c/s	10 0 c/s	2·77
T. S.	11·9 c/s	8 65 c/s	9 56

results from the time delay between the last critical flicker fusion burst presentation and the subsequent alternate threshold measurement. In any event, the two threshold dark-adaptation curves parallel each other rather well—this is true for both observers under both conditions of test—and give a concise picture of the logic of the experiment.

Finally, in comparing the two figures, we may consider the last critical flicker fusion values after dark-adaptation for 30 min, as in Table 1.

The underlined value of the t-test ($N = 5$) is significant (at P less than 0·01), the other is nearly so. This means that the critical flicker fusion of the final pure cone is significantly higher than the final pure rod value, which is another way of saying that the cones have greater temporal resolution.

The results seem conclusive as to the superior temporal resolution of the cone system over that of the rod system. These flicker dark-adaptation curves show this functional duplexity in a clearer fashion than it has been shown heretofore because the alternate threshold monitoring procedure clearly informs us when we are in a pure cone (whole of Fig. 1) or a pure rod (latter part of Fig. 2) environment. We cannot yet say definitely why the curves for critical flicker fusion fall rapidly to alpha (or just fall, as in Fig. 1), or why they then rise slowly. There is an experimental flaw which might account for the fall: the fact is that κ is undoubtedly more than 10 during the fast falling cone portion of the threshold curve—actually, it appeared brighter here than later on—yet we, in default, must take it as 10. There are more attractive physiological speculations, however, as we have mentioned: the horizontal inhibitory interactions between the cones may decrease with time in the dark so that cone resiliency also decreases. This hypothesis would also permit us to explain the slight increase in rod resolution as a result of a slight increase in inhibitory interactions. A final decision on this awaits further investigation.

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Activation of "Secondary" Impulse Trigger Sites in Hippocampal Neurones

Intracellularly recorded spike potentials of hippocampal neurones occasionally exhibit fast prepotentials superimposed on spontaneous slow depolarizations and excitatory postsynaptic potentials evoked by stimulation of the subiculum[1]. Although the origin and nature of fast prepotentials have not been determined, available data suggest that they represent electrotonic reflexions of partial spikes evoked in dendrites by pathways arising in the subiculum[1]. Two investigations which are summarized here have permitted further analysis of fast prepotentials as well as the definition of some of the factors conditioning their development in hippocampal neurones. The results are illustrated in contrast with findings in elements which did not exhibit fast prepotentials during a long period of spontaneous activity or in response to low frequency (1/sec) stimulation of the perforant pathway in the subiculum.

Intracellular recordings from hippocampal pyramidal neurones of *encéphale isolé* cats were obtained with micropipettes filled with 2 M potassium citrate inserted as closely as possible to a microelectrode filled with saline and located at the cell body layer and used for registering of extracellular activity. In one study stimulation of the fimbria or fornix was used to evoke inhibitory postsynaptic potentials of short latency period (Fig. 1*A* 3) in a neurone which also exhibited 3–4 msec latency excitatory postsynaptic potentials and spike discharges in response to 1/sec stimulation of the subiculum (*B* 3) (ref. 2). It was noted that many neurones also showed a brief delayed depolarizing potential on the falling phase of the spike evoked by stimulation of the subiculum (*D* 2*b*) as well as a succeeding prolonged inhibitory postsynaptic potential (*B* 3). Spontaneous spikes exhibited prominent depolarizing after-potentials[3].

Inhibitory postsynaptic potentials produced by conditioning stimulation of the fimbria effectively blocked spike discharges associated with the early excitatory postsynaptic potentials evoked by the subiculum (*D* 2*b*). Under these conditions a fast prepotential appeared (at arrow in *D*3) on the crest of the early excitatory postsynaptic potential. Cell discharge was secured by the fast prepotential which was temporally related to the delayed depolarizing potential in the unconditioned response evoked by subiculum stimulation (*D* 2*b*). Variations in timing or magnitude of conditioning inhibitory postsynaptic potentials as well as alterations in early excitatory postsynaptic potentials evoked by the subiculum stimulation or previous spontaneous activity frequently resulted in failure of the fast prepotential to secure cell discharge (*D* 4 and *D* 5).

Marked changes were observed in focal activity recorded from the cell body layer during conditioning by the fimbria of responses evoked by the subiculum stimulation. The unconditioned response consisted of a prominent slow positivity with a superposed negative spike signalling cell body discharges (*B*, *C* 2). Appearance of the fast prepotential during the fimbrial-evoked inhibitory postsynaptic potential was associated with an extracellularly recorded diphasic positive–negative spike (*C* 3) the early positive component of which corresponded to the negative spike of the unconditioned focal response evoked by stimulation of the subiculum (*C* 2). The positive–negative spike recorded at the cell body layer during the appearance of the fast prepotential is consistent with a remote or dendritic origin of the latter and development of a dendrosomatic spike sequence in a relatively large population of cells.

Activation of fast prepotentials was produced in a second type of study illustrated in Fig. 1*E*. In this experiment the frequency of subiculum stimulation was increased to 25/sec for several seconds. During the early phases of repetitive stimulation the excitatory postsynaptic potentials increased in latency and amplitude and cell discharge was obtained on the crest of the excitatory postsynaptic potentials (*E* 2). The brief delayed depolarizing potential and the succeeding inhibitory postsynaptic potential evoked by 1/sec stimulation of the subiculum (*E* 1) were not observed during 25/sec stimulation. The excitatory postsynaptic potentials were depressed during continued repetitive stimulation of the subiculum, and many failed to elicit spike discharges (*E* 3). This depression of evoked excitatory postsynaptic potentials was accompanied by minimal depolarization of the membrane (6–8 mV) (ref. 4). When repetitive stimulation was stopped two varieties of spike prepotentials were observed. Spontaneous discharges arose from slow depolarizing prepotentials (excitatory postsynaptic potentials) (*E* 4*a*), whereas spikes evoked by 1/sec stimulation of the subiculum were triggered by fast prepotentials (*E* 4*b*). A small negative component preceded the fast prepotentials in some instances (*E* 4*b*) in the period immediately following activation, whereas later this component was absent and the fast prepotentials

appeared to arise directly from the baseline or from a small slow depolarizing potential (*E* 5). The relationship between the small negative component preceding the fast prepotentials in *E* 4*b* and field activity of spikes in distal dendritic regions following repetitive stimulation of the perforant pathway has been considered elsewhere[4].

The studies summarized here indicate that hyperpolarization of the membrane produced by inhibitory postsynaptic potentials generated in, or close to, the soma of hippocampal neurones[5] as well as repetitive activation of axodendritic excitatory synaptic pathways may facilitate the appearance of partial spikes of fast prepotentials remote from the cell body, presumably in some part of the dendritic system[1]. Other aspects of this investigation have revealed spikes arising directly from the baseline in hippocampal neurones during seizure activity initiated by repetitive stimulation of the subiculum[4]. Evidently, fast prepotentials represent a potentially explosive response of the hippocampal dendrites which, under appropriate conditions, may lead to all-or-one propagated activity in these elements. Conditions favouring the appearance of fast prepotentials are associated with depression of early excitatory postsynaptic potentials evoked by stimulation of the subiculum, suggesting that this factor is probably also of importance in the development of fast prepotentials. It is of interest to note that depression of axosomatic excitatory postsynaptic potentials by inhibitory postsynaptic potentials and repetitive stimulation of axodendritic synapses may also condition the development of partial and conducted spikes in dendrites of immature neocortical neurones[6,7]. This suggests that many of the properties of immature neocortical neurones which are not encountered in the vast majority of neocortical neurones in adult animals[8,9] are "retained" in mature hippocampal neurones. Probably in both immature neocortical neurones and hippocampal neurones secondary impulse "trigger" sites in dendrites not only increase the synaptic security of axodendritic pathways[10,11] but may also serve to facilitate dendritic input channels during selective inhibition or depression of excitatory axosomatic synaptic pathways as demonstrated here.

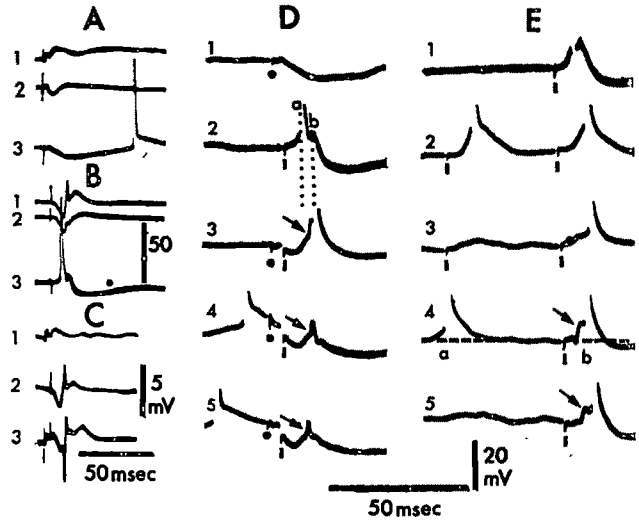


Fig. 1. *A*, Intracellular and extracellular responses to stimulation of the fimbria. Negativity upwards in records of the extracellular and ventricular surfaces. (1) Focal, extracellular response recorded from cell body layer; (2) ventricular surface evoked potential; and (3) short latency inhibitory postsynaptic potential and succeeding 75 mV spontaneous spike discharge with a prominent depolarizing after-potential. *B*, Recordings as in *A*. Responses evoked by single shock subiculum stimulation. (1) Focal response from cell body layer exhibits a slow positivity and superimposed negative spike and late negativity; (2) the spike is attenuated in the ventricular surface response, (3) subiculum stimulation elicits an excitatory postsynaptic potential and spike discharge which is followed by a small delayed depolarizing potential and an inhibitory postsynaptic potential. *C*, Extracellular responses from cell body layer (1) Response to fimbrial stimulation; (2) unconditioned subiculum evoked response; (3) combined fimbrial-subiculum stimulation. Fimbrial stimulation leads by 5 msec. *D*, Expanded intracellular records from same neurone as in *A* and *B*. Fast components of spikes not reproduced. Black dots indicate stimulation of the fimbria and bars indicate stimulation of the subiculum. *D* 1: Inhibitory postsynaptic potential evoked by fimbrial stimulation. *D* 2 response to subiculum stimulation. Spike take-off from early excitatory postsynaptic potential is indicated by dotted line, *a*. Delayed depolarizing potential preceding inhibitory postsynaptic potential is labelled *b*. *D* 3: conditioning fimbrial stimulation as in *C* 3 Note at arrow development of fast prepotential and late spike discharge. *D* 4 and *D* 5: Fast prepotential in isolation during blockade of spike, inhibitory postsynaptic potential shortens time course of fast prepotential. *E*, As with *D*. (1) response as in *D* 2, single shock stimulation of the subiculum, (2) during 25/sec stimulation of the subiculum —excitatory postsynaptic potentials increase in latency and amplitude, (3) few seconds after (2) excitatory postsynaptic potentials are depressed and occasionally fail to trigger late spikes; (4) after cessation of 25/sec stimulation (*a*, spontaneous spike arises from slow depolarizing prepotential, *b*, subiculum evoked spike arises from fast prepotential (at arrow)); (5) several seconds after *E* 4; fast prepotential exhibits slower time course. Calibration in *B* (50 mV) applies to intracellular records *A* 3 and *B* 3. 5 mV for *C*, and 20 mV for *D* and *E*.

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Renal Production of Erythropoietin and Renin after Experimental Kidney Infarction

IN previous experiments we have shown that injection of plastic microspheres into a renal artery results in production of erythropoietin and reticulocytosis[1] and development of hypertension[2].

In the present investigations we have attempted to elucidate further these phenomena by asking the following questions. Is the hypertension related to renin secretion? If renin is released by the kidney, is the time course of release the same as that for erythropoietin? Both questions have a bearing on the possible identity of these hormones and the nature of the stimulus regulating their release.

All experiments were carried out on trained, unanaesthetized mongrel dogs. Before training, a silicon-rubber catheter (silastic) was surgically implanted in one carotid artery. The dogs were then trained to stand quietly while their blood pressure was measured directly using a mercury manometer attached to the carotid catheter. Arterial blood samples were obtained on several successive days for determination on haematocrit, reticulocyte percentage renin and erythropoietin activity. In one dog renal venous blood was also sampled from a chronically implanted catheter[1] placed in the renal vein at the time of injecting the microspheres. Following control determinations the dogs were anaesthetized and about 200,000 plastic microspheres of 30–170μ diameter were injected into the left renal artery. Periodically, blood was sampled for analysis. Erythropoietin was assayed using 48 h iron-59 uptake in the polycythaemic mouse[1]. Plasma renin concentrations were determined indirectly by a modification of the method of Bouchor *et al.*[3].

The results for the dog in which renal venous blood was collected are shown in Fig. 1. Table 1 presents the data obtained from three other dogs. In all animals the injection of microspheres resulted in increased arterial concentrations of erythropoietin and renin reticulocyte percentage and mean arterial blood pressure (MABP). Peak

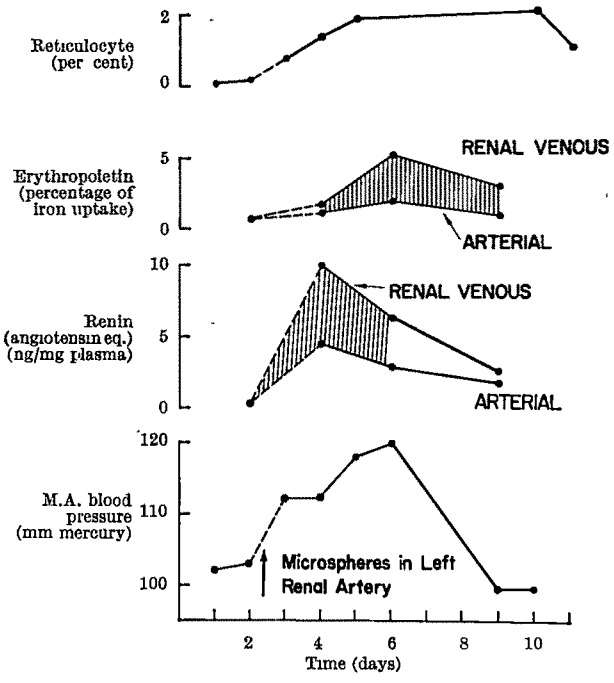


Fig. 1. Alterations in renin, erythropoietin, reticulocyte percentage and blood pressure following renal arterial injection of microspheres in dog Number 1.

reticulocyte percentage always occurred after the maximum in erythropoietin and peak blood pressure after the renin maximum. There was no constant temporal relationship between erythropoietin and renin plasma levels in the dogs. In one dog the renin maximum occurred first, in one dog erythropoietin preceded renin, and in one dog the renin and erythropoietin maximum coincided. In dog 4 the timing of the samples was such that the relation between erythropoietin and renin maxima could not be accurately determined.

These data indicate that the kidney of the unanaesthetized dog produces both an erythropoietic stimulating factor and a hypertensive factor following injection of microspheres into the renal artery, and that the two factors are separate substances. Although the response in terms of the liberation of hormones and changes in the percentage of reticulocyte and in the blood pressure is transient, increased release of hormones persists for several days. Thus it seems probable that the increased hormone levels represent actual production rather than release of stored hormones. These results indicate that the same stimulus can result in the production of both renin and erythropoietin. The present experiments are in agreement with the clinical findings of Luke *et al.*[4], who reported co-existing hypertension and polycythaemia in a patient with unilateral renal arterial stenosis. Red cell volume returned to normal and blood pressure to near normal after unilateral nephrectomy. Unfortunately, no erythropoietin or renin studies were carried out on the patient.

Table 1. Blood Pressure, Percentage of Reticulocyte, and Plasma Hormone Assays after Microsphere Injection

Time in days	MABP (mm Hg)	Renin (ang. eq.) (ng/ml. plasma)	Erythropoietin (per cent uptake of iron-59)	Reticulocyte (per cent)	Haematocrit
Dog 2					
0	114	1·0	2·6	0·3	28
1	130				
2	140	10·6	6 8	0 8	27
3	142	7·6	1·0	1·4	27
4	120	8·1	2 6	1·1	21
7	116	2·3	1 1	1·0	28
8	117			0 8	
9	110	2·4	2 3	1 1	27
Dog 3					
0	105	9·6	7 0	Rare	33
1				Rare	31
2	113	8·8	11 5	0 3	32
3	121	14 6	10 2	0 4	29
4	130	12·0	5 7	0 4	29
7	110	9 5	4 4	0 7	31
8	115	4 1		0 8	26
Dog 4					
0	110	3 8	3 2	Rare	27
1	115	9 8		Rare	25
2	133	7 8	9 5	0 2	26
6	115		5·0	0 8	

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Thyrocalcitonin: Failure to demonstrate a Parathyroid Releasing Factor

Thyrocalcitonin[1] is the plasma calcium lowering hormone secreted by the thyroid in response to a high blood calcium stimulus[2–5]. The hormone is identical to calcitonin which was originally thought to be of parathyroid origin[6]. The possible interrelated roles of the thyroid and parathyroids have been studied by Gittes and Irvin[7]. Their results suggested that a parathyroid factor might be responsible for the release of the hormone from the thyroid: parathyroidectomized rats were found less able to counteract the hypercalcaemia induced by exogenous parathyroid hormone than intact rats. The studies of these workers have been repeated, in part, to investigate the possibility of a parathyroid releasing factor by assessing the ability of parathyroidectomized animals to withstand an intraperitoneal challenge of calcium chloride.

Thirteen intact and sixteen parathyroidectomized male Wistar hooded rats weighing between 120 and 150 g were used. Parathyroidectomy was performed by electrocautery and only animals with plasma calcium of 3 m.equiv./l. or lower 1 week after the operation were included in the investigation. To prevent hypocalcaemia in the parathyroidectomized group, all animals were maintained on a high calcium–low phosphate diet[8] for 3 weeks. Before they were challenged with calcium, blood samples were collected in heparinized tubes by intraorbital puncture from each rat. 5 mg calcium chloride/100 g of rat weight were given by intraperitoneal injection and further blood samples were obtained after 1 and 3 h by the same technique. Plasma from all blood specimens was separated within 1 h and calcium determined by flame spectrophotometry[9].

The results are summarized in Fig. 1. In 1 h plasma calcium rose in the intact rats to 9·97 ± 0·14 m.equiv./l. and to 8·94 ± 0·17 m.equiv./l. in the parathyroidectomized rats. After 3 h plasma calcium fell to near equal levels in both groups (7·21 ± 0·09 m.equiv./l. for intact rats and 7·11 ± 0·14 m.equiv./l. for parathyroidectomized animals.)

The presence of the parathyroids in the intact animals provided no additional protection against exogenously induced hypercalcaemia. The thyroids in parathyroidectomized rats appeared to secrete thyrocalcitonin as efficiently as they did in intact animals. Similar intraperitoneal challenge to thyro-parathyroidectomized animals produced more marked hypercalcaemia[10]. Cumulative evidence unequivocally favours a thyroid origin for the calcium-lowering hormone. The data presented do not support the existence of a parathyroid releasing factor for thyrocalcitonin.

This work was done during the tenure of an Advanced Research Fellowship of the American Heart Association.

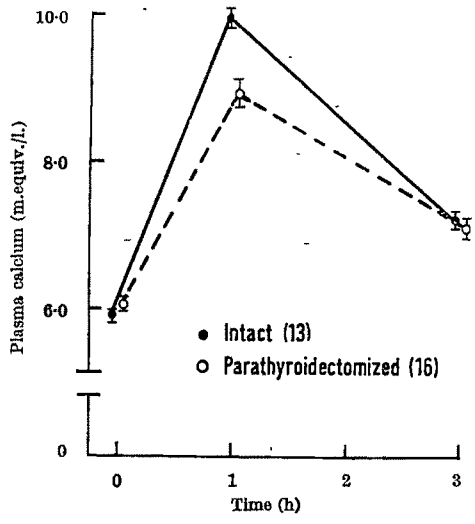


Fig. 1. Effect on plasma calcium of intraperitoneal calcium chloride (5 mg/100 g) given to intact and parathyroidectomized rats. Number of animals in each group is shown in parenthesis. The vertical bars indicate the standard error of the mean for each value.

I thank Miss Lynette Luck for performing the calcium determinations.

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BIOLOGY

Responses of some Anomuran and Brachyuran Crustaceans to High-speed Centrifugation

As part of a wider investigation of Crustacea in this laboratory[1], we have been seeking a method for removing hermit crabs from their "host" shells without damaging them. The generally recommended methods—application of heat, immersion in deaerated water, exposure to fresh water, etc.—have not proved particularly satisfactory for our purposes.

It seemed possible that mild centrifuging might disturb the crab sufficiently to cause it to vacate its shell and move toward an area of lesser centrifugal force. Preliminary trials with a hand-operated centrifuge were notably unsuccessful. Experiments were then carried out on an International Model *CS* Electric Centrifuge using head No. 242 with 250 ml. bottles. Initial runs with four *Pagurus floridanus* Benedict spun at 200 r.p.m. (8·5*g*) for 5 min did not appear to discomfit the animals in any way. (All relative gravity values given in this report were obtained from the nomograph supplied with the centrifuge.) They were active when the centrifuge was stopped and immediately showed "normal" responses (fright reactions, attempts to turn shells over when placed upside down). Further tests on these animals with maximum acceleration to and deceleration from speeds as high as 2,000 r.p.m. (800*g*) also apparently did not disturb normal behaviour patterns.

Because an adequate supply of *P. floridanus* was not readily available, tests were run on another hermit crab, *Clibanarius vittatus* (Bosc), which we were able to obtain in large quantities. Several hundred of these, collected along the beach at Ship Island, Mississippi, were sorted into rough size categories based on maximum dimension of the occupied gastropod shell. Shells of two gastropod genera (*Thais* and *Polinices*) were represented in this series.

Nine tests were run, using thirty-five hermit crabs selected from the various size groups. Crabs of several sizes in both types of shell and crabs without shell were exposed for varying periods to centrifugal forces in the range 800–2,700*g*. Table 1 presents test conditions and results. In all cases, acceleration and deceleration were the maximum obtainable with the centrifuge and electric brake. Acceleration times ranged from 15 sec to 26 sec, and deceleration times from 13 sec to 25 sec. Heads Nos. 240 and 242, with 50 ml. and 250 ml. tubes, respectively, were used throughout the investigation.

As Table 1 shows, maximum mortality in any single experiment was 50 per cent at termination of the experiment (22–26 h after centrifugation). Further, overall survival was twenty-nine crabs out of thirty-five, all of which had been exposed to centrifugal forces of at least 800*g*. The death of one animal (Experiment 9) was probably not related to centrifugation; it climbed from its beaker during the night, fell to the floor, and was found desiccated, but alive, 15·5 h after centrifugation.

To test the possibility that the observed resistance to centrifugal force might be a trait peculiar to the Anomura, we performed similar experiments on two genera of brachyuran crustaceans (*Sesarma reticulatum* (Say) and *Uca* sp.) (Table 2). It is apparent that these forms are considerably less resistant than the anomurans to high centrifugal forces. Some individuals of both species,

Table 1. RESPONSES OF HERMIT CRABS (*Clibanarius vittatus* (BOSC)) TO HIGH-SPEED CENTRIFUGATION

Experiment No.	No. of animals	Maximum force (relative gravities)	Exposure to maximum force (min)	Results following centrifugation
In shells				
1	4	1,600	0*	All active within 10 min; one dead at 19 h; three active at 26 h
2	4	1,600	0*	All active at 25 h
3	4	2,700	0*	All active at 25 h
4	4	2,700	3	All active at 24·5 h
5	4	800	30	Two alive and active at 24 h
Removed from shells				
6	3	800	1	All active at 23·5 h
7	4	2,700	1	All active at 22·5 h
8	4	2,700	3	Two alive and active at 22·5 h
9	4	2,700	3	All active at 15·5 h; three alive and active at 22 h

* Zero exposure time represents acceleration to maximum speed followed by immediate deceleration with braking.

Table 2. RESPONSES OF BRACHYURAN CRUSTACEANS TO HIGH-SPEED CENTRIFUGATION

Experiment No.	No. of animals	Maximum force (relative gravities)	Exposure to maximum force (min)	Results following centrifugation
Sesarma reticulatum (Say)				
1	4	2,700	0*	Two dead at 140 min, two active at 250 min
2	4	800	1	All active at 235 min
3	4	2,700	3	Two dead at 65 min; two active at 220 min
4	2	2,700	0*	One alive and active at 80 min
5	2	800	30	One moribund and one active at 90 min
Uca sp.				
6	4	2,700	0*	All dead at 140 min.
7	4	800	1	Three alive and active at 130 min
8	4	2,700	3	All dead at 112 min
9	2	2,700	0*	One alive and active at 80 min
10	2	800	30	One moribund and one active at 90 min

* Zero exposure time represents acceleration to maximum speed followed by immediate deceleration with braking.

however, were able to survive (short-term) after exposure to more than 800*g*.

Waterman[2] makes no reference to forces greater than 2·2*g* in his 1961 work, *The Physiology of Crustacea*. A search of abstracts since 1960 reveals only one pertinent reference. Eberly *et al.*[3] studied the effects of centrifugal force on nymphs of the grasshopper *Melanoplus differentialis* (the size of nymphs was not specified). They reported 50 per cent mortalities in 9 days at 9*g* and in 20 min at 10,000*g*. We were not able to obtain nymphs of *Melanoplus* locally at this season, but were able to capture two half-grown (40–50 mm) nymphs of lubber grasshoppers (*Romalea* sp.). Centrifugation at 800*g* for 10 min eviscerated and killed one nymph; centrifugation at 2,700*g* for 1 min reduced the remaining nymph to mush. Thus, we very much doubt the validity of the 10,000*g* value.

In view of the tolerance of anomuran crustaceans to extremely high centrifugal forces and because of their availability in quantity, hermit crabs could well be used as experimental organisms for extraterrestrial investigations. The use of hermit crabs in instrumented shells or simulated shells seems feasible. Furthermore, detailed physiological and biochemical investigations of the resistance mechanism could well provide valuable guidance in the design of life support systems for higher animals.

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Feeding Habits of the Squid, *Illex illecebrosus*

OBSERVATIONS have occasionally been made on the method of attack on prey by *Illex* in the inshore zone where it feeds mainly on small fishes[1–3]. This communication suggests a way in which the squid is able to capture smaller food organisms—mainly small crustaceans (Euphausiids)[4]—when it is schooling in the offshore zone.

It is generally agreed that fish such as herring, mackerel, etc., when swimming in schools, feed on the small crustaceans (copepods, etc.) which are abundant in the oceans. These they take into their mouths together with the water used to perfuse the gills in respiration and strain them from this water by means of gill rakers. During such feeding the school of fish remains intact. With the squid, *Illex*, this method of feeding is impossible not only because of its anatomy but also because of the way the individuals orientate themselves when schooling. When swimming in a school they generally orientate themselves with their tail forward. In this position the animal is propelled by successive and continuous jets from the siphon which is now at the rear end. Although the head is also at the rear end in this position, vision forwards or backwards is unimpeded because the eyes are appreciably protruded. When moving in well-formed, evenly spaced schools, the squid maintains its arms and tentacles compactly together and the siphon is held straight. When moving through swarms of small crustaceans, however, individual squids are able to trap some of their prey in their own wake by expanding their arms and thus creating a turbulence. The mantle intake and siphon ejection of water which effect propulsion also add to the turbulence in their wake. The prey pulled into this turbulence is easily captured by the suction cups on the arms or tentacles and then transferred to the mouth. This method of feeding is accomplished without breaking up the formation of the school. Fewer squids appear to fall prey to their fellows in the offshore zone where this type of feeding in schools is generally predominant[4].

It is probably not always possible for a squid to swim past a small active fish sufficiently close or fast to capture it in the turbulent wake set up by expanding its arms. Observations of the capture of fish by *Illex* reveal that although the squid swims past the fish or lure, it then turns and darts with arms forward and grabs its prey[1–3]. This invariably causes the schooling pattern to break up. It is quite probable that there is a connexion between break-up of schools and the not uncommon finding of squids in stomachs of inshore squids[4,5]. The change from feeding mainly on small crustaceans to feeding mostly on fish may in part be responsible for this. It would seem that squids have to learn this method of feeding because in the first seasonal occurrence of squid in the inshore zone there is a period in which fish-shaped lures do not attract them (the period during which fishermen consider the squids to be "blind"). Lures with dangling hooks designed in the shape of euphausiids would take into account the feeding method of squids and could well be used in the offshore zone and possibly also during the period in which they learn to feed on fish.

The observations referred to in this communication were made in inshore waters off Newfoundland at Holyrood where the squids are present from July–October, and offshore on the south-west edge of the Grand Banks where they are commonly present early in June.

I thank M. C. Mercer for help in preparing this manuscript.

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Corpus Allatum and Sexual Receptivity in Female *Drosophila melanogaster*

IN a number of insects the hormone secreted by the corpus allatum is known to affect reproductive behaviour. In female insects so far studied the hormone operates in one of two ways. It may control pheromone secretion and thus determine whether a female attracts courtship from males, for example, the cockroach *Byrsotria*. Here, Barth[1] showed that there is no effect on behaviour itself because if males are induced to court allatectomized females by adding pheromone artificially, the latter respond normally and mate. Alternatively, the hormone may affect the sexual receptivity of females, as in the grasshopper *Gomphocerus*. In this case pheromones are not important and Loher[2] has shown that allatectomized females, although courted normally, do not respond. Implanting mature corpora allata induced the return of receptivity within 7 days.

Few *Drosophila melanogaster* females kept on a 12 h : 12 h light/dark cycle are receptive to males on the day of eclosion (day 0) or on day 1, but almost all are fully receptive by the morning of day 2. This change in behaviour runs parallel to the growth of the corpus allatum[3] which is small at eclosion. Feedback from the ovaries

which grow under the influence of its hormone is known not to affect receptivity[4]. Accordingly, the effect on injecting mature corpora allata into pupae just before eclosion has been tested.

Corpora allata, together with the associated complex of fused corpus cardiacum and hypocerebral ganglion, were dissected from 6–12 day old virgin females and briefly stored in *Drosophila* Ringer's solution[5]. Single glands, together with a little Ringer's solution, were injected into the abdomens of pupae, 17–19 h before eclosion, through a glass needle using the technique of Ephrussi and Beadle[5]. Control pupae received a small piece of the aorta dissected from the same donor flies. Mortality was about 50 per cent, but with two exceptions all flies which survived day 0 went on to mate and lay fertile eggs.

Injected flies were tested for receptivity on the morning of day 1. Each was placed with a single male in a 'Perspex' observation cell[6] 1 in. in diameter. Males court females of all ages with equal persistence and will continue courting unreceptive virgin females for several hours. In practice, 30 min without acceptance is a suitable criterion because more than 90 per cent of receptive females accept in less than 10 min. Forced matings are not uncommon and the female can be seen struggling to push the male off, but unless she succeeded in doing so within a few minutes she was recorded as receptive.

Table 1. RECEPTIVITY OF *D. melanogaster* FEMALES MEASURED ON DAY 1

	Receptive on day 1	Unreceptive on day 1	Total
Corpus allatum injected	20	0*	20
Control, aorta injected	7	16†	23
Control, not injected	7	18	25

The difference between the two injected groups is highly significant (χ^2 with Yates's correction = 19·23, $P < 0{\cdot}001$).

* Two flies injected with allata were unreceptive on day 1 but died on day 2.

† These flies were tested for the normal appearance of receptivity on days 2 or 3, and all were receptive.

The results are given in Table 1 and strongly suggest that the presence of a mature corpus allatum in young flies induces the precocious onset of receptivity. This could result from the direct action of its hormone on some receptivity mechanism in the central nervous system. Alternatively, as Highnam[7] points out, the hormone may facilitate the release of stored neurosecretory products and these may affect behaviour. Preliminary experiments indicate that, given at this stage, the hormone requires some time to take effect and that females with implanted glands are not receptive on day 0.

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Impairment of Oxidative Phosphorylation by D-threo- and L-threo-Chloramphenicol

WE reported earlier that high concentrations of chloramphenicol impair certain energy-linked functions of mitochondria[1,2]. The experiments were carried out with the D-threo isomer (the antibiotic) which effectively blocks protein synthesis in bacteria[3]. Of principal interest is the suppression of respiration and oxidative phosphorylation. If these effects are produced *in vivo*, it cannot be assumed that responses produced in tissue by D-threo-chloramphenicol are necessarily related to inhibition of protein synthesis. This is not to deny that protein synthesis can be blocked; but it becomes essential that the investigator establishes that impairment of phosphorylation is not also, or even primarily, involved whenever concentrations of the order of 0·5 mg/ml. or greater are used.

One way of avoiding this difficulty is to use L-threo-chloramphenicol as a control treatment. The L-threo isomer is relatively ineffective in inhibiting protein synthesis[3]. Ellis[4] has reported that both the D-threo and L-threo isomers are effective in inhibition of ion accumulation, and he suggests that there is not necessarily any connexion between the uptake of ions and the synthesis of protein. Recently, Billet *et al.*[5] have used the L-threo isomer as a control to establish that the effects of the D-threo isomer on the development of the chick embryo result from inhibition of protein synthesis. The D-isomer was effective in arresting development while the L-isomer was not. There is thus support for the postulate that the antibiotic impairs development through inhibition of protein synthesis.

Table 1. IMPAIRMENT OF OXIDATION AND PHOSPHORYLATION BY D-THREO AND L-THREO-CHLORAMPHENICOL

Chloramphenicol	$Qo_2(N)$	P/O
none (0·1 ml. ethanol/vessel)	2443	1·70
D-threo (1 mg/ml.)	1102	0·98
L-threo (1 mg/ml.)	717	0·46

R. J. Ellis and W. Landauer have questioned (personal communications) whether L-threo-chloramphenicol will also suppress oxidative phosphorylation. In order to answer this point we carried out experiments with mitochondria from 3 day etiolated corn shoots as previously described except that 40 μmoles each of pyruvate and malate were used as substrate instead of pyruvate and succinate. The compounds were dissolved in absolute ethanol, with equivalent ethanol added to the control. The ethanol tends to have an inhibitory effect on oxidative phosphorylation. A typical result is given in Table 1. This shows that the L-threo isomer effectively suppresses oxidation and phosphorylation; indeed, it is consistently more effective than the D-threo isomer. This result can be correlated with the slightly greater inhibitory effect of root growth with the L-threo isomer[6].

Thus when only the D-threo isomer is effective on tissue (for example, Billet *et al.*[5]) there is reason to believe that protein synthesis is the process being inhibited. Where both the D and L compounds are effective (for example, Ellis[4] and Ronnicke[6]), the impairment of phosphorylation is probably involved. The mechanism responsible for the impairment of phosphorylation is still unknown. As reported previously[2], the antibiotic does not act like a true uncoupler; however, it does exhibit certain similarities to the respiration-suppressing phase of uncoupler action. More recent work has shown that chloramphenicol increases the rate and extent of mitochondrial swelling[7], which suggests that the compound acts by damaging the mitochondrial membrane rather than by specifically interfering with a phosphorylated intermediate.

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Specific Inhibition by Uracil Derivatives of the Mechanism of Dormancy Release in Light-sensitive Lettuce Seeds

RECENT studies have shown that pyrimidine analogues are effective in preventing the inhibition of *Lepidium*[1], *Eragrostis*[2], *Brassica*[3], and *Lactuca*[4] seeds. Investigations made in this laboratory of the effects on germination of a wide range of inhibitors of the synthesis of nucleic acids and proteins, including purine, pyrimidine and amino-acid analogues, have indicated that inhibition by uracil derivatives is intimately connected with the processes responsible for the release from seed dormancy.

The experiments reported here concern the effects of 2-thiouracil, 5-fluorouracil, and 5-bromouracil on the germination of intact seeds and "half seeds" of lettuce (*Lactuca sativa* L.), var. 'Attractie'. The failure of light sensitive lettuce seeds to germinate in the dark is dependent on the presence of intact seed covers (endosperm plus pericarp); removing the seed covers or simply cutting the seed in half allows 100 per cent germination in the dark[5]. Similarly, irradiation of the intact seed with red light of low energy (660 mμ), treatment with gibberellic acid or thiourea, or incubation at a relatively low temperature, enables the embryo to overcome the resistance of the seed covers and to germinate. It is significant that with each of the three uracil derivatives, a higher concentration is required for the inhibition of half seeds than for the inhibition of intact seeds.

Lettuce seeds (var. 'Attractie') obtained from Thompson and Morgan (Ipswich), Ltd., were incubated on Whatman seed test papers in 4 ml. of solution in 9 cm Petri dishes at 25 ± 0·5° C. Intact seeds were either incubated completely in the dark or irradiated with red light at an intensity of 5,800 ergs $cm^{-2}.sec^{-1}$ for 10 min, 2 h after inhibition, percentage germination being recorded 20 h later. Half seeds were prepared by cutting whole seeds transversely into two equal pieces; the halves containing the embryos were incubated in the dark as above. Observable geotropic curvature of the radicle was taken to indicate germination of half seeds. The 5-fluorouracil was a gift from Dr. M. Black, of Queen Elizabeth College, London.

2-Thiouracil, 5-fluorouracil and 5-bromouracil all inhibit the germination of the intact seeds, but at widely different concentrations, 5-fluorouracil being the most inhibitory and 5-bromouracil the least so (Figs. 1, 2 and 3). In the case of 2-thiouracil and 5-fluorouracil, intact seeds which had been maintained in the dark or irradiated with red light were inhibited at concentrations seven to twenty times smaller than those required to inhibit the germination of half seeds. Inhibition of half seed germination was not detected at any of the concentrations of 5-bromouracil used.

Although other analogues of nucleic acid precursors—for example, azaadenine and azaguanine—inhibited

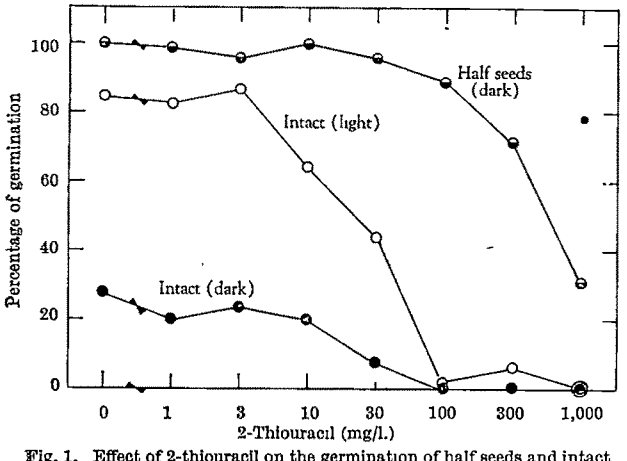


Fig. 1. Effect of 2-thiouracil on the germination of half seeds and intact seeds.

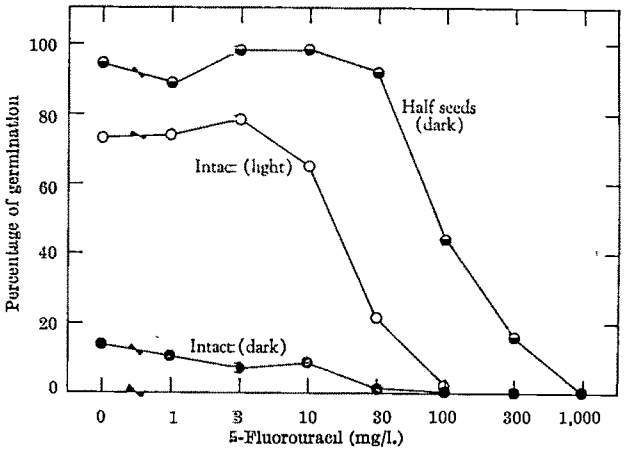


Fig 2. Effect of 5-fluorouracil on the germination of half seeds and intact seeds.

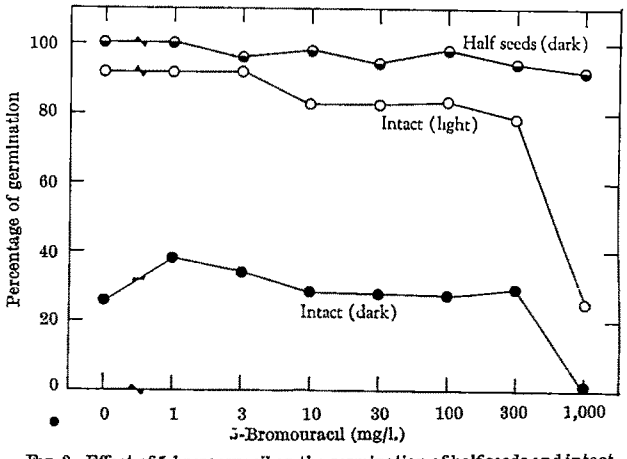


Fig. 3. Effect of 5-bromouracil on the germination of half seeds and intact seeds.

germination, there was no significant difference between the concentrations required for 50 per cent inhibition of intact seeds and of half seeds. Similarly, other experiments with inhibitors of protein synthesis (chloramphenicol, puromycin and *cyclo*heximide) and analogues of amino-acids (4-fluorophenylalanine) have shown that intact seeds and half seeds do not respond differently[6].

On the basis of these preliminary observations, it would seem reasonable to suggest that the uracil derivatives specifically inhibit the mechanism responsible for the ability of the embryo to overcome the restriction of the seed covers. Since 5-fluorouracil, 5-bromouracil, and 2-thiouracil are commonly held to be inhibitors of nucleic acid synthesis, it is tempting to suggest that they are acting in such a manner here. As yet, however, there is no evidence for such a conclusion because other inhibitors of nucleic acid synthesis do not have a similar differential effect on the germination of intact seeds and half seeds. If the inhibitors act on nucleic acid synthesis, it would seem that the uracil derivatives inhibit a facet of nucleic acid metabolism specifically related to the ability of the embryo to overcome the restriction of the seed coat. Experiments are at present being conducted to test for reversal of the inhibition with nucleic acid precursors, and to determine the effect of the inhibitors on the rate of incorporation of isotopic uridine into ribonucleic acid.

It is tempting to conclude that the uracil derivatives specifically inhibit the mechanism catalysed by phytochrome which results in the release from dormancy. It remains, however, to be seen whether this inhibition is directly related to the photo-morphogenetic process. Nevertheless, further investigation of pyrimidine and

nucleic acid metabolism should assist in elucidating the mechanism of dormancy release in light-sensitive seeds.

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Presence of Adenosine Triphosphate in the Skin Venom of Amphibia

PRUSOFF'S discovery of adenosine triphosphate (ATP) and 5-hydroxytryptamine (5-HT) in enterochromaffin cell granula[1] stimulated us to an attempt to extend this observation to other sections of Erspamer's enterochromaffin cell system[2,3]. In a preliminary investigation[4,5] we demonstrated the existence of ATP in "venom" squeezed manually from the parotoid glands of *Bufo vulgaris* and *viridis*, while we were unable to observe ATP in venom from *Salamandra maculosa* and *Salamandra atra* with the procedure used at that time.

This communication reports the results of investigations carried out on twenty-two species of Amphibia. The venom if possible was squeezed manually from the parotoids, otherwise ether stimulation was used[6].

The presence of ATP was usually demonstrated by the method of Strehler and Totter[7] using diluted Sigma 'Buffered Firefly Extract'. Light emission was evaluated subjectively, or objectively by photographic and photometric means as well as by direct reaction with paper chromatograms. The photo-emission reaction was detected with a Photovolt photometer 520 coupled with a photomultiplier "1*P*21" and recorded with a Microcord 44 Record (Photovolt). The sample was placed in a 'Plexiglas' cylinder sealed at one end to a glass plate with chloroform and adapted to a lightproof apparatus connected by means of 'Teflon' micro tubes with two syringes which allowed the introduction of reagents. The apparatus was tightly screwed to the photomultiplier assembly. Although the instruments and the procedures used fulfil the requirements for a quantitative evaluation, we nevertheless intend to attribute only qualitative or roughly semi-quantitative values to our data because the material we were dealing with was not homogeneous. This limitation must be taken into consideration particularly when considering the "venom" collected by ether stimulation, as such "venom" contains the secretions of both granulous and mucous glands in proportions which cannot be evaluated. We also carried out investigations using paper chromatography and electrophoresis in order to demonstrate the possible linkage between oxyindolic derivatives and ATP[8]. As a result of our findings we were able to confirm and substantiate the lucipherin–lucipherase reaction.

ATP was demonstrated in the skin venom in all the species examined except for *Bombinator pachypus*. Notably, we succeeded in finding ATP in the venom from *Salamandra maculosa* and *S. atra*; techniques used on previous occasions had given negative results for these two species. From our data we therefore conclude that ATP is of usual occurrence in the "venoms" of Amphibia. Of especial interest is the observation that ATP is present in species where the "venom" does not contain 5-HT, but where other oxyindolic derivatives are present or where indolic substances have not previously been found[9,10]. The absence of ATP in *Bombinator pachypus*—a species with a high concentration of 5-HT in the venom[9,11]—may be explained because the 5-HT exists in a free form, or is bound to an unknown compound which may resemble ATP in being a nucleotide. The hypothesis that there are substances in this "venom" which inhibit the common reaction may be discarded in view of the finding that the addition of small amounts (20γ) of ATP to the reaction mixture will rapidly produce photoemission by the "venom" of *Bombinator*.

In the species we examined variable quantities of ATP were estimated. In summary we may conclude that: (1) there is no constant relationship between ATP and 5-HT. A high concentration of ATP does not always correspond to high concentration of 5-HT and vice-versa; (2) apart from the possible relationship between ATP and 5-HT, ATP may be present in very high concentration in some species, while in others it is scarcely detectable.

The finding of ATP in yet another section of Erspamer's enterochromaffin cell system raises the possibility of being able to demonstrate the presence of ATP in other sections. Our observations are, in our opinion, particularly interesting, in view of the large variation in the composition of the granulous venom of Amphibia[9]. Such variability is not comparable with other sections of the system. Any interpretation of the functional significance of the ATP–5-HT linkage in the structures of the enterochromaffin cell system, however, must take into account the differences in the function of 5-HT which occur in different sections of the system.

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GENETICS

Age and Chromosomes

RECENTLY, I carried out chromosome analyses and sex chromatin determinations on ten patients—five females and five males—between 63 and 91 years old. The organic status of these individuals was variable, and all were ill in some way. Nevertheless, the results obtained in this investigation were reasonably uniform; their chromosomes and Barr bodies appeared normal and manifested the characteristics of the corresponding sex (Table 1).

These observations do not agree with those of Jacobs *et al.*[1], who stated that the number of aneuploid cells in blood culture increases with the age of the donor, particularly above the age of 60 years. It was further claimed that these aneuploid cells lacked the sex chromosome corresponding to their sex: *X* in females and *Y* in males.

As shown in Table 2, the chromosome counts were established for 564 cells and the percentage of each was calculated. The distribution around the modal diploid number, forty-six, is similar to that found in normal cultured cells[2–4]. Of the 564 cells, thirty-five were karyo-

Table 1

Case No.	Sex	Age	Sex chromatin
1	♂	68	Negative
2	♀	65	Positive
3	♂	81	Negative
4	♀	79	Positive
5	♂	62	Negative
6	♀	71	Positive
7	♀	66	Positive
8	♀	91	Positive
9	♂	77	Negative
10	♂	70	Negative

Table 2

Case	Total No. of cells counted	Chromosome No. −45	45	46	47	+47
1	67	2	1	62	2	—
2	45	1	—	44	—	—
3	56	—	4	51	1	—
4	53	—	3	50	—	1
5	60	4	—	54	1	1
6	65	—	4	58	3	—
7	58	1	1	56	—	—
8	45	2	—	43	—	—
9	60	1	2	55	2	1
10	55	—	6	48	1	—
	564	11	21	521	10	3
		1·9 per cent	3·6 per cent	92·3 per cent	1·7 per cent	0·5 per cent

typed and eight cells had only forty-five chromosomes and there was no consistency in the identity of the missing chromosome (Table 3). In five out of these eight instances, a chromosome of the 6–12–*X* series was missing. The missing chromosomes were particularized as follows: in two instances, either number 7 or number 8 was absent; in another two instances, number 6 or *X* was missing, and in another case, number 9. It is worth emphasizing that two of these karoytypes were obtained from male patients. In the remaining three instances where only forty-five chromosomes were present, the chromosomes that were lost were two from the 13–15 and one from the 19–20 groups. It is possible, as suggested by Miller *et al.*[5] and Morishima *et al.*[6], that during metaphase, chromosome distribution does not occur randomly and that sex chromosomes may have a particular tendency to move towards the periphery. If this is so, when the cell membrane is disrupted, there is a possibility that one of these peripheral chromosomes may be lost from the cell rather than one in the centre.

Bearing in mind the possibility of a greater chance of a chromosome loss from the 6–12–*X* group than in the others, Therkelsen[7] analysed a total of 177 metaphases from cultures of peripheral blood from seven females

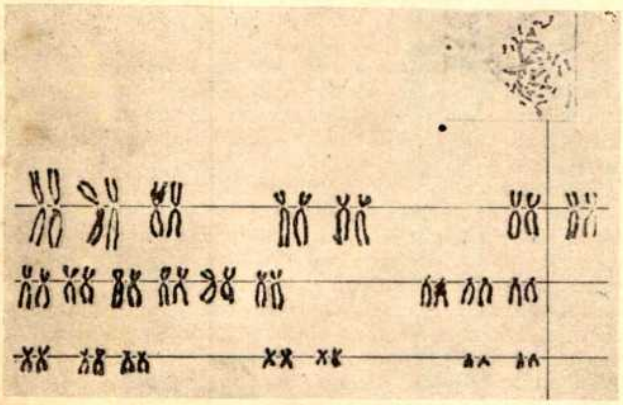


Fig. 1. Normal male karyotype (case No. 5).

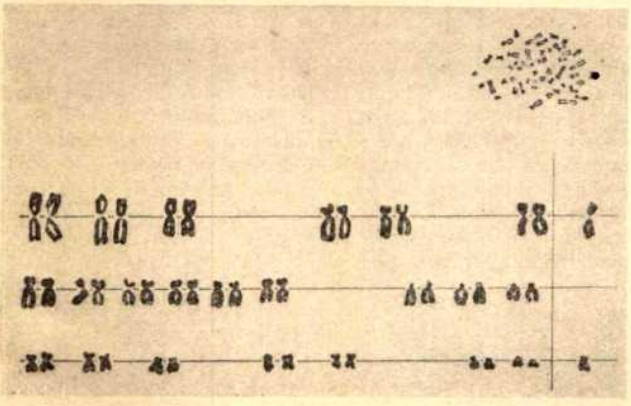


Fig. 2. Normal female karyotype (case No. 7).

Table 3. ANALYSES OF EIGHT CELLS WITH FORTY-FIVE CHROMOSOMES

Corresponding to case No.	Sex	Presumed missing chromosome
1	♂	7–8
1	♂	13–15
3	♂	19–20
4	♂	6–X
4	♀	7–8
6	♀	2
8	♀	9
9	♂	6–X

aged between 18 and 24 years. He found that of thirty-one chromosomes which were missing, eighteen belonged to the 6–12–*X* group.

It is quite possible that in my present investigations selective forces were operating in the cell culture to eliminate most of the cells with less than forty-six chromosomes and any with more than this number. No true increase in forty-five chromosomes was found, however, in any of the elderly patients investigated. Only autoradiography will show whether the lost chromosome (in cells with forty-five chromosomes) is an *X* or *Y*. The possible absence of the *X* is open to question in my investigations, but it is certain that it was never the *Y* chromosome that was missing. Furthermore, if there was any fault in cell division in aged people resulting in cells with forty-five chromosomes, about the same proportion of cells with forty-seven chromosomes could also be expected.

This investigation was carried out at the Institute for Medical Genetics, Uppsala, Sweden, during the tenure of a fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas Buenos Aires, Argentina.

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Argentina.

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SOIL SCIENCE

A Circuit for Integration of Temperature Duration greater than Preset Values

A SIMPLE instrument for measuring soil temperatures greater than pre-selected values has been developed for studies of biological processes in the soil[1]. Mean temperatures can be measured by using a thermistor in a circuit with a voltameter[2]. The basic form has been extended to measure the integral of temperature × time above the pre-selected value by building the thermistor, the voltameter and a diode into a wheatstone bridge circuit.

In the circuit shown in Fig. 1, the diode controls the direction of current flow through the central arm, allowing current to pass only when temperatures rise above the set value. By reversing the diode, temperatures below the pre-selected point could be measured. The resistor values depend on the power requirements and the resistance of the thermistor and in our work included a fixed resistor of 500 ohm and two variable resistors of 1 kΩ and 5 kΩ. The resistance of the thermistor was about 1·4 kΩ at 100° F. Copper wire of high purity was found necessary for the electrodes and the voltameter was made up with 8 s.w.g. electrolytic grade copper wire in a 10 per cent solution of copper sulphate. In measuring the copper transferred, the average of the weight changes of both electrodes was determined because, as a result of the low current density, the weight lost by one did not always equal the weight gained by the other.

Table 1. COMPARISON OF TEMPERATURE INTEGRALS OBSERVED FROM VOLTAMETER WEIGHT CHANGES AND CALCULATED FROM TEMPERATURE–TIME CURVES

	Circuit No.	Calculated (° F h)	Observed (° F h)	Difference (per cent)
1st week	1	226	229	+1·3
	2	143	153	+7·0
	3	190	184	−3·2
2nd week	1	24	26	+7·7
	2	65	70	+7·7
	3	112	122	+8·9
3rd week	1	290	296	+2·1
	2	369	340	−7·8
	3	433	407	−6·0
4th week	1	533	554	+3·9
	2	460	454	−1·3
	3	466	460	−1·3

By calibration, the relationship between the temperature of the thermistor and the current that passed through the central arm was shown to be linear (see Fig. 2). Small variations in voltage supply from the battery and large variations in the ambient air temperature around the circuits made little difference to their performance (Fig. 2).

The chief difference between the calibration curves of the circuits results from differences in the temperature-resistance characteristics of the thermistors. The calibration curves are related to each other by the formula xy = constant, where x is the slope and y the factor for that circuit. The differences are not important because either the resistor R_2 can be set initially (to adjust the current in the central arm to a standard value) so that one calibration curve fits many circuits, or the individual factors can be used when converting from weight change of the electrodes to degree-hours. (Here "degree-hours" means the integral of time × temperature above a particular value; for example, 103° F for 9 h with a base temperature of 95° F is 72° F-hours.)

The circuits were initially tested with the thermistor at constant temperature for fixed periods of time. Agreement between observed and calculated values of degree-hours was good; in 80 per cent of the figures the variation

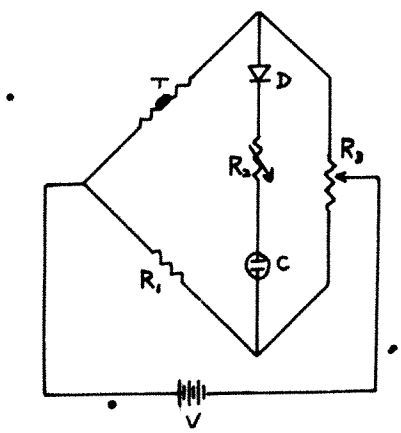


Fig. 1. Integrating circuit. R_1, 500 Ω; R_2, 1 k Ω variable; R_3, 5 k Ω variable; D, Philips diode $OA9$; T, Philips thermistor $928/3K$; C, copper voltameter; V, 12 V car battery.

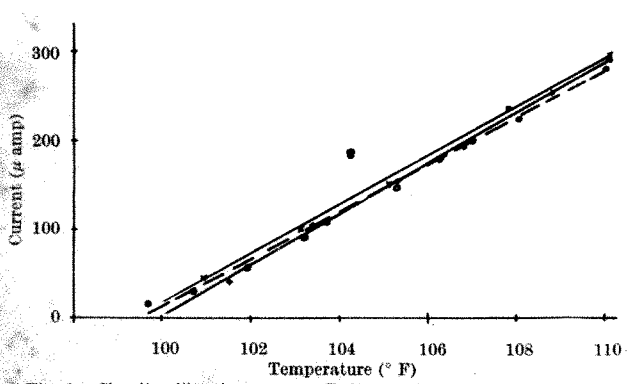


Fig. 2. Circuit calibration curves. Battery voltage and ambient temperature: ×——×, 12 V and 85°–90° F; +——+, 12·1 V and 60°–65° F; ○— —○, 11·8 V, 65° and 85° F.

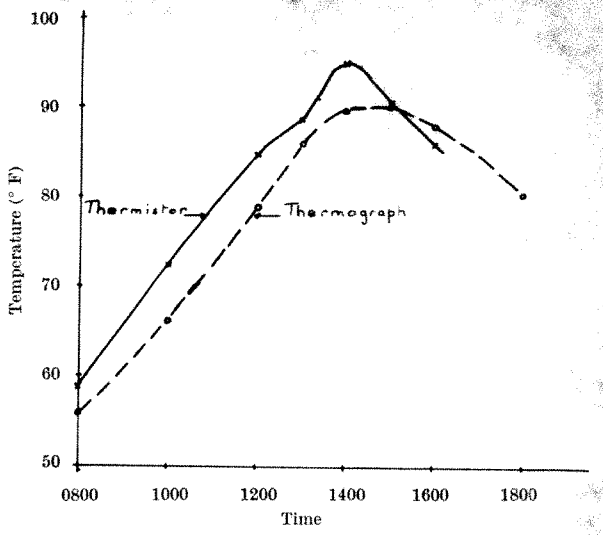


Fig. 3. Soil temperature records at 2 in. depth. August 25, 1965.

was less than 4 per cent and the remainder were less than 10 per cent. To check circuit performance under simulated field conditions the thermistors were placed in pots of plants in a greenhouse when temperature fluctuations were sufficient to obtain a fair proportion of time above the cut-in temperature—here 99° F. Each week differences between the temperatures of the three thermistors were obtained by changing the pots and their positions in the greenhouse. Hourly readings of the soil temperature were taken using the thermistors in the circuits and these were recorded graphically. The temperature–time integral was calculated from the graphs and compared with values from the voltameters (Table 1). Differences between the calculated values and the observed values over the 5-day periods were sufficiently low as to make the method useful in field studies.

The results obtained with thermistors were compared with thermograph records by taking hourly readings with thermistors placed alongside the thermograph elements in the field. Both had been calibrated against the same thermometer. Typical results are shown in Fig. 3 for elements 2 in. below the soil surface on a sunny day. The thermistor registered a temperature integral of 21 degree-hours above the cut-in point of 85° F as compared with thermograph record of 17 degree-hours. The low heat capacity of the thermistor made it the more sensitive instrument for measuring short periods at high temperatures, and higher results were always obtained with the thermistor circuits than thermographs in the field.

The circuit described has been used for one season in the field to record soil temperature integrals above selected values at many points in a soyabean crop[3]. A bank of fifty-four circuits connected in parallel were run from a 12 V battery. Sixty yard lengths of multi-core cable connected the thermistors with the remainder of the circuits which were housed in an adjacent shed. The battery required recharging every second day but during that period voltage changes were negligible.

We found that a modified form of the circuit could be operated from a.c. (220 V) mains by incorporating a rectifier. This necessitated a fixed resistor of 820 Ω for R_1 and the use of two fixed resistors of 27 kΩ and 56 kΩ with a variable one of 46 kΩ in place of R_3 in Fig. 1. The current–temperature ratio was higher but still linear.

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D. T. COKER

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Marandellas, Rhodesia.

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[3] Willatt, S. T., *Rhodesia, Zambia and Malawi J. Agric. Res.*, **4** No. 2 (1966).

FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Monday, September 19—Friday, September 23

INSTITUTION OF ELECTRICAL ENGINEERS, POWER DIVISION (in the Renold Building of the University of Manchester Institute of Science and Technology, Manchester)—Conference on "High Voltage D.C. Transmission".

Tuesday, September 20

SOCIETY FOR ANALYTICAL CHEMISTRY, SPECIAL TECHNIQUES GROUP (at the Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.1), at 3 p.m.—Mr. R. C. Chirnside: "Special Techniques, Past, Present and Future".

Tuesday, September 20—Thursday, September 22

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (joint meeting with the Plastics Institute and the Institution of the Rubber Industry, at the Institution of Electrical Engineers, Savoy Place, London, W.C.2)—Conference on "Advances in Polymer Science and Technology".

Wednesday, September 21

MINERALOGICAL SOCIETY (at the Research and Development Laboratories, Pilkington Bros. Ltd., Lathom, near Ormskirk, Lancashire), at 10 a.m.—Symposium on "Reflected Light Methods in the Study of Refractories and Ceramics".

SOCIETY FOR ANALYTICAL CHEMISTRY, MIDLANDS SECTION, AND THE BIRMINGHAM AND MIDLANDS SECTION OF THE SOCIETY OF CHEMICAL INDUSTRY (in the Haworth Lecture Theatre, Chemistry Department, The University, Edgbaston, Birmingham)—at 3 p.m.—Symposium on "Determination of Organic Acids".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP (at the School of Pharmacy, University of London, Brunswick Square, London, W.C.1), at 6 p.m.—Dr. J. R. Vickery (C.S.I.R.O., Australia): "The Scope and Status of Food Science" (First Food Group International Lecture).

Wednesday, September 21—Thursday, September 22

CHEMICAL SOCIETY (at the University of Sussex, Brighton), at 9.20 a.m. daily—Autumn Meeting.

Thursday, September 22

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Address by Mr. Michael T. Halbouty (American Association of Petroleum Geologists).

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN THE SUB-DEPARTMENT OF COMPUTATION—The Registrar, The University, Hull (September 23).

ASSISTANT LECTURER (preferably candidate who has worked in the field of insect physiology) IN ZOOLOGY—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (September 26).

ASSISTANT LECTURER or LECTURER in either: (*a*) SOCIAL PSYCHOLOGY or (*b*) SOCIOLOGICAL ANALYSIS—The Registrar, The University, Keele, Staffordshire (September 28).

LECTURER IN BIOCHEMISTRY IN RELATION TO VETERINARY STUDIES—The Secretary of the University Court, The University, Glasgow (September 28).

ASSISTANT EXPERIMENTAL OFFICER (graduate) IN THE ENTOMOLOGY DEPARTMENT to work on the ecology of soil animals—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/92 (September 30).

ASSISTANT LECTURER IN PHYSICAL CHEMISTRY—The Deputy College Secretary, Westfield College (University of London), Kidderpore Avenue, Hampstead, London, N.W.3 (September 30).

EXPERIMENTAL OFFICER (with a degree, H.N.C. or equivalent qualification) IN THE PEDOLOGY DEPARTMENT to assist in research on soils—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1052/91 (September 30).

GEOLOGIST, GRADE 2 (with a university degree with geology as a major subject, and preferably some experience), for geological investigations throughout Western Australia—The Public Service Commission, 184 St. George's Terrace, Perth, Western Australia (September 30).

HEAD (with a good honours degree and experience of research on methods of weed control, preferably in the field of horticulture) OF THE HORTICULTURAL SECTION, for work on the uses of herbicides in horticultural crops—The Secretary, A.R.C. Weed Research Organization, Begbroke Hill, Kidlington, Oxford (September 30).

LECTURER IN THE SCHOOL OF ELECTRICAL ENGINEERING (in the field of automatic control systems), University of New South Wales, Australia—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 30).

SENIOR LIBRARY ASSISTANTS (2) (with experience of classification and cataloguing, and competence in Russian would be a strong recommendation for one of the posts)—The Librarian, University of Essex, Wivenhoe Park, Colchester, Essex (September 30).

UNIVERSITY LECTURER (or possibly, initially, a Departmental Demonstrator) IN PALAEONTOLOGY IN THE DEPARTMENT OF GEOLOGY AND MINERALOGY—The Professor of Geology, Department of Geology and Mineralogy, University of Oxford, Parks Road, Oxford (September 30).

ASSISTANT LECTURER (preferably with interests in historical geography) IN THE DEPARTMENT OF GEOGRAPHY—The Secretary, University College, Gower Street, London, W.C.1 (October 1).

ENTOMOLOGIST (with a good honours degree or equivalent qualification, preferably in zoology, with specialization in entomology) for research on, and development of, biological control—The Secretary, Glasshouse Crops Research Institute, Worthing Road, Rustington, Littlehampton, Sussex, quoting Ref. N/22 (October 1).

JUNIOR RESEARCH FELLOW IN GEOGRAPHY—The Registrar, The University, Sheffield (October 1).

SENIOR LECTURER IN BIOTECHNOLOGY in the Faculty of Food Science and Biotechnology, Massey University of Manawatu, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mal, London, S.W.1 (New Zealand and London, October 1).

CHAIR OF MECHANICAL ENGINEERING—The Secretary, Heriot-Watt University, Edinburgh (October 3).

SENIOR LECTURER, LECTURER (Grade I or II) or ASSISTANT LECTURER IN EDUCATION with special reference if possible, to the psychology of education at the University College of Rhodesia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 3).

RESEARCH ASSISTANT (graduate in a biological science) to Dr. J. D. Dodge in the Department of Botany, to collect and culture marine dinoflagellates and examine them by light and electron microscopy—The Assistant Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1 (October 7).

EXPERIMENTAL OFFICER (with a degree or considerable experience of preparative organic chemistry) IN THE DEPARTMENT OF CHEMISTRY IN THE FACULTY OF SCIENCE to be attached to the Polymer Group—The Registrar, The University, Manchester, 13, quoting Ref. 206/66 (October 8).

CHAIR OF SURGERY AT UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 10).

CHAIR OF ANIMAL HEALTH AT MASSEY UNIVERSITY OF MANAWATU, Palmerston North, New Zealand—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (New Zealand and London, October 15).

CHIEF TECHNICIAN OR LABORATORY SUPERINTENDENT IN THE DEPARTMENT OF CHEMICAL PATHOLOGY—Prof. G. H. Lathe, Department of Chemical Pathology, School of Medicine, The University, Leeds, 2 (October 15).

LECTURERS (3) IN COMPUTING (for one post a knowledge of numerical analysis would be an advantage)—The Assistant Registrar (Science and Engineering), The University of Birmingham, P.O. Box 363, Birmingham, 15 (October 15).

PROFESSOR (preferably with specialist interests in civil or mining engineering); and SENIOR LECTURERS or LECTURERS in the School of Engineering, University of Zambia—The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 15).

READER IN CHEMICAL ENGINEERING—The Secretary, Royal College of Advanced Technology, Salford, 5, Lancs, quoting Ref. No. CH/49 (October 17).

SENIOR LECTURER or LECTURER in VETERINARY HISTOLOGY IN THE DEPARTMENT OF VETERINARY ANATOMY, University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 22).

LECTURER IN HYDRAULICS or HYDROLOGY IN THE DEPARTMENT OF CIVIL ENGINEERING—The Deputy Secretary, The University, Southampton (October 24).

SENIOR LECTURER/LECTURER IN PHARMACOLOGY at the University of Hong Kong—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Hong Kong and London, October 31).

PROFESSOR OF PHYSIOLOGY (Zoology) IN THE INSTITUTE OF BIOLOGY—The Dean of the Faculty of Science, The Hebrew University of Jerusalem, Jerusalem, Israel (January 1).

ASSISTANT PROFESSOR IN BIOCHEMICAL PHARMACOLOGY or BIOCHEMISTRY—The Dean, Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada.

BASIC GRADE BIOCHEMIST (with an honours degree in biochemistry, chemistry or A.R.I.C.) for the hospital laboratories—The Secretary, Dudley Road Hospital, Birmingham, 18.

HEAD OF THE DEPARTMENT OF MATHEMATICS AND PHYSICS; and HEAD OF THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary, Wolverhampton College of Technology, Wolverhampton.

PHYSIOLOGIST/BIOPHYSICIST to study physiological response to controlled changes in environmental conditions—D. C. Page, Head of Personnel Services, The Electricity Council, Research Centre, Capenhurst, Chester, quoting Ref. N/28.

PROFESSOR and HEAD OF THE DEPARTMENT OF OPHTHALMIC OPTICS—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff.

PROGRAMMER IN THE COMPUTER UNIT—The Acting Registrar, University College of Wales, Aberystwyth.

RESEARCH ASSISTANT (graduate, preferably with experience of Atlas Autocode and ABL) IN THE DEPARTMENT OF SOCIAL AND PREVENTIVE MEDICINE, to take part in the further development of a compiler for analysing surveys—The Registrar, The University, Manchester, 13, quoting Ref. 202/66.

RESEARCH ASSISTANT (honours graduate in electrical engineering) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING for a project concerned with a study of the distribution of electromagnetic fields in conducting media—The Academic Registrar, Loughborough University of Technology, Loughborough, Leicestershire, quoting Ref. 18A/F.

RESEARCH ASSISTANT to work with Prof. A. G. Gaydon on spectroscopic study of excitation processes in shock-heated gases—Prof. A. G. Gaydon, Chemical Engineering and Chemical Technology Department, Imperial College of Science and Technology, London, S.W.7.

RESEARH ASSISTANT (with a good honours degree in geology or chemistry and an interest in crystalline rocks and/or phase equilibrium studies) IN THE HIGH PRESS/HIGH TEMPERATURE EXPERIMENTAL UNIT, GEOLOGY DEPARTMENT—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire.

RESEARCH FELLOW or RESEARCH ASSISTANT (with a good mathematics degree, or a degree with a high mathematics content, and preferably some knowledge of computer programming and statistical analysis techniques) IN THE DEPARTMENT OF CIVIL ENGINEERING, to take part in basic research in the design of materials for mechanical properties—The Deputy Secretary, The University, Southampton.

SCIENTIFIC OFFICER (with an honours degree, or equivalent, and preferably some research experience) to undertake research on reproductive physiology—The Secretary, Institute of Animal Physiology, Agricultural Research Council, Babraham, Cambridge.

SENIOR LECTURER or LECTURER to teach Zoology full-time—The Registrar, Regional College of Technology, P.O. Box 143, Leicester.

SENIOR PHYSICIST to take charge of the Physics Department, Radiotherapy Centre, St. William's Hospital, Rochester—The Group Secretary, Medway and Gravesend Hospital Management Committee, Medway Hospital, Gillingham, Kent.

TECHNICIAN (with H.N.C. in electronics and preferably experienced in radio frequency techniques) IN THE DEPARTMENT OF CHEMISTRY to work on research and development work—The Personnel Adviser, University of Birmingham, P.O. Box 363, Edgbaston, Birmingham, 15.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Bulletin of the British Museum (Natural History). Entomology. Vol. 18, No. 7: Revision of the Genus *Orosius* Distant (Homoptera: Cicadelloidea). By M. S. K. Ghauri. Pp. 229–252. (London: British Museum (Natural History), 1966.) 10*s.* [147

Science Museum. The Machine Tool Collection: Catalogue of Exhibits with Historical Introduction. By K. R. Gilbert. Pp. v+111+32 plates. (London: H.M. Stationery Office, 1966.) 11*s.* 6*d.* net. [157

British Drug Houses, Ltd. Chemical Testing of Petroleum Products. Pp. 22. (Poole, Dorset: British Drug Houses, Ltd., 1966.) [157

Institute of Personnel Management. Selecting and Training the Training Officer. By Nancy Taylor. Pp. 90. 15*s.* The Computer in Personnel Work. By Edgar Wille. Pp 55. 7*s.* 6*d.* (London: Institute of Personnel Management, 1966.) [157

Royal Observatory Bulletins. No. 110: Fundamental Data for Southern Stars (Sixth List). By David S. Evans. Pp. E185–E202 3*s.* net. No. 111: Proper Motions in the Field of NGC 6522. By S. V. M. Clube. Pp. E203–E238. 5*s.* net. No 112: Photoelectric Photometry of RR Lyrae Stars. By D. H. P. Jones. Pp. E239–E255. 3*s.* 6*d.* net. No. 113: Time and Latitude Service 1965, April–June. Pp. B25–B40. 3*s.* net. (London: H.M. Stationery Office, 1966.) [187

Office of Health Economics. Medical Manpower. Pp. 32. (London: Office of Health Economics, 1966.) 2*s.* 6*d.* [187

The World Land Use Survey. Occasional Papers, No. 6: Uboma—a Socio-Economic and Nutritional Survey of a Rural Community in Eastern Nigeria. By H. A. Oluwasanmi, I. S. Dema and others. Pp. xi+116 (35 plates). (Bude, Cornwall: Geographical Publications, Ltd., 1966.) 25*s.* [187

The Edinburgh School of Agriculture—The Edinburgh and East of Scotland College of Agriculture. Calendar for 1966–1967. Pp. 66. (Edinburgh: The Edinburgh School of Agriculture, 1966.) [197

Department of Education and Science; Ministry of Technology—Committee on Manpower Resources for Science and Technology. Education and Training Requirements for the Electrical and Mechanical Manufacturing Industries. Pp. v+34+6 plates. (London: H.M. Stationery Office, 1966.) 4*s.* 6*d.* net. [217

Council for National Academic Awards. Statement No. 4: Conditions for the Award of Higher Degrees. Pp. 26. (London: Council for National Academic Awards, 1966.) [217

Building Research Station Digest 72 (Second Series): Home-grown Softwoods for Building. Pp. 6. (London: H.M. Stationery Office, 1966.) 4*d.* [217

Proceedings of the A.I.Ch.E.—I.Chem.E. Joint Meeting, London, June 1965. Symposium No. 2: Chemical Engineering Under Extreme Conditions. Pp. 117. Symposium No. 3: Reaction Kinetics in Product and Process Design. Pp. 50. Symposium No. 4: Application of Mathematical Models in Chemical Engineering Research, Design, and Production. Pp. 135. (London: The Institution of Chemical Engineers, 1965.) [217

Other Countries

Fonds National de la Recherche Scientifique. Trent-huitième Rapport Annuel, 1964–1965. Vol. 1: Pp. 289. Vol. 2: Pp. 149. (Bruxelles: Fonds National de la Recherche Scientifique, 1966.) [137

Population Reference Bureau, Inc. *Population Bulletin*, Vol. 22, No. 2 (June 1966): California: After 19 Million, What? Pp. 29–60. (Washington, D.C.: Population Reference Bureau, Inc., 1966.) [137

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Food Preservation, 1964–65. Pp. vii+73. (Sydney: Commonwealth Scientific and Industrial Research Organization, 1965.) [137

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 64–54: Geology of Canadian Tin Occurrences (Report and Map 32–1964). By R. Mulligan. Pp. 22. 75 cents. Paper 66–13: Drilling for Scientific Purposes—Report of the International Upper Symposium, Ottawa, 2–3 September, 1965. Edited by D. C. Findlay and C. H. Smith. Pp. 264. 75 cents. (Ottawa: Queen's Printer, 1966.) [157

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Australia: Commonwealth Scientific and Industrial Research Organization. Programme of Work of the Division of Forest Products, 1966–67. Pp. 120. (South Melbourne: Commonwealth Scientific and Industrial Research Organization, 1966.) [157

Report of the Department of Agriculture, New South Wales, for the year ended 30th June, 1965. Pp. 143. (Sydney: Government Printer, 1966.) 1.80 A. dollars. [157

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Geological Maps. Map 1158A: Groundwater Probability in Surfacial Deposits, Rosetown, West of Third Meridian, Saskatchewan. Geological Maps—Preliminary Series. Map 3–1966: Bonaparte River, British Columbia. Map 11–1965: Burnt Timber Creek, Alberta. (Ottawa: Director, Geological Survey of Canada, 1966.) [157

Records of the Queen Victoria Museum, Launceston. No. 21 (26 March, 1966): Flora of the Nut with Brief Account of Botanical Investigation in the Circular Head District. By H. J. Willis. Pp. 12+8 photographs. No 22 (4 May, 1966) Notes on Lesser Long-Eared Bat *Nyctophilus geoffroyi* in Northern Tasmania. By R. H. Green. Pp. 4+4 photographs. (Launceston, Tasmania: Queen Victoria Museum, 1966.) [157

Institut Royal Météorologique de Belgique. Bulletin Mensuel. Observations Ionosphériques, Mai 1966. Pp. 26. (Bruxelles: Institut Royal Météorologique, 1966.) [157

Food and Agriculture Organization of the United Nations. Fruits and Vegetables in West Africa. By H. D. Tindall. With a chapter by Florence A. Sai. Pp. ix+259. (Rome: Food and Agriculture Organization of the United Nations; London: H.M. Stationery Office, 1965.) 12*s.* 6*d.*; 2.50 U.S. dollars. [187

The Plant Sciences, Now and in the Coming Decade. (A Report on the Status, and Requirements of Plant Sciences in the United States.) Pp. x+167. (Washington, D.C.: National Academy of Sciences—National Research Council, 1966.) 5 U.S. dollars. [187

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WHO STAYS HOME?

THE most recent fuss about the emigration of British doctors, chiefly to North America, has raised again a question which can never be far beneath the surface, but which is inevitably painfully obtrusive at times of economic crisis—is it possible, and is it even desirable, to try to prevent the drift of technically skilled people towards the countries in which they and their kind are already most plentiful and best used? The fact that the doctors are the centre of public attention in Britain just now is probably more a consequence of the row the medical profession is having with the British Government over the terms of service in the National Health Service than a sign that there has been a recent acceleration in the exodus of newly qualified doctors. Indeed, the scanty statistics which exist suggest that the loss has persisted for the best part of a decade. And what is true of doctors is likely to be true of other technical people in Britain and, to a smaller extent, from other countries in Western Europe.

The first thing to be determined, however, is the cause of this substantial exodus. Otherwise, attempts at a solution must necessarily be exercises in pot luck. The trouble is that there is no single reason why technical people emigrate, and often a temptation for them and those who watch them go to hide from the most obvious but most unpalatable truths. This, no doubt, accounts for the frequency with which departing scientists proclaim that they are going "not for the money, but because I shall have more scope in my new job". Of course there is a lot in what they say, but there is also something in the less rosy view that money and the things it brings can be a powerful attraction. Newly qualified people are particularly open to influence on grounds like these. Given similar electron microscopes, or equal chances of working with a big bubble chamber on a particle accelerator, people are likely to be influenced by the ease with which houses, or even motor cars, are to be had. To think otherwise would be to pretend that technical people are not human.

It follows from this that money by itself could do a lot to cure some of the problems of emigration. Certainly it would be a great advantage if some of the problems of emigration were translated into money terms. The doctors are an easy case to start with, for it is a fair guess that, by adding something like £20 million a year, or roughly 20 per cent, to the annual cost of paying British doctors, emigration would be reduced to a mere trickle. With care, and chiefly by seeing that younger doctors were well looked after, it might be possible to achieve the same result with less expenditure. To make this kind of calculation does not imply that the British Government should promptly foot the bill, even though it may only be a small proportion of the whole cost of running the National Health Service, but it is the kind of yardstick which it ought to keep in mind. The point is that the purely financial incentives to emigration could be extinguished at a fairly manageable cost, at least where doctors are concerned. It is obviously not necessary to go all the way to match salaries and other conditions in countries elsewhere, for even skilled people do not lightly pull up their roots. But it is necessary that governments should go in for more hard-headed calculations of the cost of keeping potential emigrants at home. Sometimes they might decide that it is cheaper to let the errants go. Sometimes they might have to reconcile themselves to the apparent paradox that comparatively poor countries may often have to pay disproportionately more to those skilled people whose services they wish more urgently to retain.

To say all this does not imply that the flow of emigrants from countries such as Britain is entirely a function of the cash rewards available in various countries, but merely that this is a much neglected feature of the process. The kind of career which a young man can look forward to is also an important consideration, in academic life as well as in industry. One of the attractions of the United States in recent years has been the way in which young people look forward to early responsibility, and the sense of freedom that accompanies it. American industry is fond of giving young people "their heads", as the saying goes. Then American universities are often more able to let a young person free in charge of his own work, partly because of the machinery for getting financial support, partly because of the technical assistance which is available, and partly because of the comparatively flexible organization of university departments. In industry and universities, there is an impression that American scientists move from one place to another more freely, so that promotion can be rapid—and the frustrations of marking time correspondingly painful. To take the edge off this kind of temptation to emigrate, of course, nothing but simple imitation will suffice, but imitation is necessary on other grounds than those of limiting emigration. Industry and universities in Western Europe, and certainly in Britain, would be better institutions if they were able to give young recruits a greater sense of freedom and opportunity. They would be much healthier and more effective if their technical people were helped along by technical and other assistance as good as any to be had. There is no point in keeping cadres of scientists in half-employment for lack of equipment or other necessary facilities. Better to let them emigrate than that.

No amount of organizational imitation will entirely halt the emigration of scientists from countries such as Britain, however. There are many occasions on which people of all ages decide that they will move from one

country to another because they will then be able to work in fields which are, to them, more exciting. No doubt the space programme in the United States has served to lure some scientists from Western Europe, though its influence has probably been exaggerated. The plentiful supply of particle accelerators has certainly been a magnet for nuclear physicists. And, of course, a young man wishing to work on aircraft development and construction will be attracted almost irresistibly towards the great aircraft plants of the United States. So does it then follow that smaller countries than the United States must enlarge their space programmes and aircraft industries? Arguments like these are common but invalid. Emigration is often the lesser evil, for there is no reason to believe that the numbers of people attracted away from smaller countries by temptations like these are in any way substantial. The costs of keeping them would usually far outweigh the potential benefits.

In any case, of course, emigration is not the simple and distasteful scourge which it is often supposed to be. For one thing, emigration works in both directions. In Britain, for example, there is probably one Irish doctor working for every Englishman recently flown overseas. Britain is probably making still greater demands on the potential medical resources of India and Pakistan—both of them countries much more in need of doctors. But, in the long run, these transfers of skill and training from one country to another are beneficial and, up to some point, are to be encouraged. For emigration is not always permanent. British medicine has probably as much to gain from a better working knowledge of American practices as India, in the long run, will benefit from the return of a proportion of the Indian doctors now working in Britain. In academic science and in industry, these comings and goings are even more to be welcomed. Moving a person from one place to another is, after all, the most effective way of letting one place know what the other is doing. In this sense, emigration can be the life-blood of scholarship. No country can tolerate indefinitely a steady outward flow of bright young people, but by seeing that the conditions of work for technical people, in universities and elsewhere, are those which the criteria of good management in any case suggest, the inward and outward flows of migrants would be reasonably in balance. That, and not demographic isolation, should be the goal.

PUGWASH AFTER PUGWASH

THE Pugwash organization has long since become familiar and even respectable. It has survived the overexcited ballyhoo which attended its creation a decade ago, and which tended to imply that the goodwill and innocence of a handful of scientists would create sweetness and light throughout the world, and especially in foreign ministries. If Pugwash is now less ambitious—and less innocent—than it used to be, it is also more useful. There is good reason to think that the meetings which now take place are modestly valuable and informal means of exchanging information on matters related to arms control between the military powers. The only tangible achievement in this field consists of the work of the informal meetings which preceded the signing of the present test-ban treaty in 1962 and 1963, and which were of some value at least to officials in the United States, but there is also continuing value in the opportunities provided by the larger Pugwash meetings for letting nationals of one country learn more about the policies of others on matters of disarmament. In other words, the value—or the potential value—of Pugwash is not that it can make the problems of disarmament melt away, but rather that it can enable its participants to appreciate why even apparently trivial measures of arms control can be exceedingly difficult to implement. Though its participants would not always appreciate this, Pugwash should be as much a means of ventilating disagreement as of winning agreement.

From this point of view, unfortunately, the published statements of the Pugwash meetings are usually a great disappointment. With respectability, Pugwash has also acquired a kind of folk-lore of received doctrines which cloud its judgment on the affairs with which it wishes chiefly to concern itself. The formal communiqué still contains proposals for arms control which have no relevance to contemporary problems—or even to the problems of a decade ago, when they first made their appearance. The non-aggression pacts and the nuclear-free zones which once more make their appearance may sometimes—though not always—be ways in which security might be increased, but they are no longer central problems. The assertion that the main obstacles to a comprehensive ban on nuclear tests are "political rather than technical" has turned up again, though this time it is accompanied by a fuller discussion than usual of how the technical obstacles—by no means unreal—might be overcome. Then there is some wide-eyed complaint about the remoteness of "General and Complete Disarmament" without much evidence that the participants are aware of the size of the windmill at which they are tilting. This is where the proceedings of Pugwash are most open to criticism. Seeking as they do to influence public opinion, the participants in Pugwash would be much more effective if their pronouncements could give the technicalities of disarmament some hard-headed perspective. The Pugwash communiqué, in other words, should aim to convince those who read it that somebody has made a thorough technical examination of the problems, and has hit on what promises to be a practical solution. To ask all this is possibly to ask that Pugwash should give continuing attention to problems in between major conferences, and there is some encouraging news of attempts to set up permanent working groups. Evidently there is still a great deal to be done.

NEWS AND VIEWS

Small Comfort about Particles

THERE is more relief than disappointment about the news that CERN has failed to confirm an announcement from the United States in June this year that the laws of particle physics are invariant under charge conjugation. To be sure, after the principle of parity conservation was abandoned a decade ago, nothing has seemed inviolate. Three years ago a hint that there might be something wrong with the common assumption of invariance under charge conjugation was provided by some anomalous observation of the decay of K mesons. Among all the troubles which beset the theoreticians, however, there is nothing to suggest that life would be simpler or more reasonable if the laws of physics dealt differently with particles and anti-particles—electrons and positrons, protons and anti-protons. This is why there was consternation in some quarters when a group from Columbia University, working with a hydrogen bubble chamber at the 30 GeV accelerator at Brookhaven, announced evidence that the principle of charge conjugation is invalid. Among a gigantic collection of photographs from the bubble chamber it had been possible to find 1,441 representing the decay of the neutral eta meson into three pi mesons, and statistical analysis suggested that the decay process was unsymmetrical with respect to the electrical charge of the particles. Briefly, the positive pi meson has a statistically greater velocity than the negatively charged pi meson. Since this was precisely the behaviour that would have been expected if the trouble about the K meson decay is indeed a consequence of the invalidity of charge conjugation, it is perhaps not surprising that the Columbia group tended to throw its hat a little in the air (*Phys. Rev. Letters*, **16**, 1224; 1966).

The CERN experiment has also been concerned with the decay of eta mesons, but the technique has been somewhat different from that used at Brookhaven. Instead of having to hunt for significant information in a welter of bubble chamber pictures, the CERN group has used a counter technique and has therefore been able to accumulate significant information quickly. The conclusion that there is no significant asymmetry in the decay of neutral eta mesons is based on seven times as many events as the Columbia group had to work with. Though it will obviously be some time before the matter is fully resolved, the chances are that the CERN view will eventually prevail. Meanwhile, those who may be sensitive to the charge, sometimes heard in recent years, that CERN is always second, will now be quoting the old fable about the tortoise and the hare. Those who want to see even bigger accelerators built quickly will be urging the advantages of duplication in this field. There remains the problem of the K meson and its anomalous decay.

Pugwash in Poland

THE sixteenth Pugwash Conference held in Sopot, Poland, last week seems to have been chiefly concerned with problems of disarmament. In this respect the sixteenth conference differs from its immediate predecessor, when matters concerned with the use of science in developing countries took up a great deal of time. In Poland, four groups of people seem to have devoted themselves to problems of disarmament and the politics of disarmament. On the proposal that there should be a freeze on nuclear weapons in central Europe, the Pugwash committee emphasized the difficulty of controlling the movement of small nuclear weapons and delivery systems, and said that a treaty on non-proliferation of nuclear weapons would help to pave the way for an agreement on the freeze. The same section of the conference was in favour of a reduction of conventional troops in Europe in present circumstances. The traditional Pugwash assertion that a non-aggression pact between the NATO and Warsaw Treaty powers would be a boon was accompanied this time by a statement that some participants saw this as a step towards German reunification and that others considered that the problem of the German Democratic Republic and its legal status could be overcome by the same devices as those which enabled it to adhere to the Partial Test Ban Treaty. Another of the working groups agreed that the issue of recognition for the German Democratic Republic should not prevent the growth of collaboration between the two Germanies in economic, scientific and cultural affairs. In the same spirit the Pugwash statement urges more collaboration between European states on matters such as the exploitation of water resources, energy, transport and the like. The group seems also to have considered that educational collaboration might eventually lead to "common textbooks" in international relations, international law and other fields.

This year's Pugwash seems to have had little to say about general and complete disarmament except to recognize, perhaps more realistically than before, that this is a difficult problem. On more particular schemes for arms limitation, the conference was apparently in agreement that the nuclear test ban treaty should be extended to cover underground tests, though the statement also adds its previous view that the obstacles to a comprehensive test ban treaty are "political rather than technical". The conference suggested that a move towards a comprehensive test ban treaty might be brought about by a treaty incorporating a threshold below which nuclear explosions would be permitted, on the understanding that the threshold would be gradually lowered. To this device could be added commitments undertaken by particular states to refrain from testing nuclear weapons except under various circumstances, the provision of unmanned seismic detection stations and even on-site inspection under a "request and invitation" procedure. In this connexion the statement commends the proposal that there should be a "detection club" of countries willing to collect and to publish seismic data on the basis of which underground explosions might be identified.

Among other less carefully worked out proposals in the statement is a commendation of the non-proliferation treaty, the establishment of nuclear-free zones in places such as South America, Africa and the Middle East, and proposals to refrain from deploying anti-ballistic missile systems and the like. Pugwash commends the efforts of the International Atomic Energy Authority to control the uses made of plutonium and other fissile materials arising from civil uses of nuclear power.

More Discord about Concord

THE Committee of Public Accounts of the House of Commons has added to the public criticism, in Britain and in France, of the Anglo-French project to develop the supersonic aircraft called Concord. In a report now published (*Second Report of the Committee of Public Accounts*, H.M.S.O., 3*s*.) the committee draws attention to the way in which the estimated cost of the Concord project has risen from between £150 millions and £170 millions in 1962 to £275 millions in May 1964 and has now reached the present approximation of £500 millions to the final cost. The total cost is to be shared equally by the two governments. Increases of cost in the past four years have been necessary because of substantial changes of design, primarily intended to increase the range of the aircraft, but the general upward drift of industrial costs has also played some part. It seems plain that the Public Accounts Committee, which is one of the most persistent of the public bodies empowered to inquire into the spending of public money in Britain, intends to press a reluctant Treasury to agree that there should now be a thorough review of the procedures for forward estimating in collaborative aircraft projects, chiefly because the committee has decided that "the Concord project provides a severe test of the Ministry's system for controlling research and development projects".

The committee's report has directed public attention to a number of important features of the Concord contract. In the first place, the sum of £500 millions now estimated as the final cost includes £80 millions for the development work which, it is expected, will continue after a certificate of airworthiness has been granted to the prototype machines two years from now. (There is also a sum of £50 millions labelled "contingencies".) It also appears that the contracts which are being negotiated between the governments and the aircraft manufacturers will make it possible for the governments to recover some of the initial outlay on research and development. Even on an "admittedly optimistic forecast of the number of aircraft to be sold", however, it is apparently considered improbable that as much as a third of the initial cost will be returned to the treasuries of Britain and France by means of levies.

In line with its reputation as a watchdog over public expenditure, the Public Accounts Committee is understandably anxious that there should be much better arrangements for negotiating contracts for the development of aircraft. One of the most worrying features of the present position is that so far only £90 millions of the total expenditure has been incurred. Obviously there is plenty of time yet for the cost to rise still further. It is small wonder that the Public Accounts Committee promises to return to this theme.

Laboratory Work Study

THE report of the Public Accounts Committee which drew attention to the financing of the Concord aircraft (see above) has also raised a more modest protest about the way in which the Ministry of Aviation—soon to be abolished—has apparently resisted for sixteen years the attempts of the British Treasury to see that research scientists in government laboratories are being used to the full. Arrangements were apparently made in 1949 to see that each department of government should be responsible for inspecting the use made of its own staff, and for seeing that it is employed efficiently. When the Treasury pointed out, in 1963, that the Ministry of Aviation had not sent its inspectors into the laboratories, the Ministry replied that it was short of inspectors. By 1965, however, research staff in some though not all of the Ministry's laboratories were inspected, and it was apparently decided that of 160 scientific posts at the Radio Department of the Royal Aircraft Establishment, Farnborough, 24 were in some way open to criticism and 7 could actually be abolished. (The same inspectors also approved the creation of three additional posts in the same department.) To judge from what the Public Accounts Committee has to say, the Ministry is hoping to decide, on the basis of inspections at several establishments, whether the game is worth the candle. Everyone seems to agree "that there are limitations on the value of inspection in the scientific field compared with its value in the clerical and office field", but here again the Public Accounts Committee promises—or threatens—that it will not let the matter drop.

Industrialized Building

ON-SITE battery production of the basic units of large buildings, such as blocks of flats, has been used extensively in Europe and especially in the Soviet Union. The system offers considerable savings in manpower and costs, but has not been widely used in Britain, where the building trade is still very much dominated by a traditional approach. In co-operation with the Building Research Station, the Edmonton Borough Council undertook in 1964 to build a 17-storey block of flats using the industrialized system and casting walls and floors on site. The results of the experiment are reported in *Building Research 1965* (H.M.S.O., London, 1966).

The internal structural units were produced by vertical casting between concrete panels, the moulds being in groups of up to sixteen units. The external cladding panels had to be produced by horizontal casting and needed far more labour. The total labour required for the erection of the shell of the building was 570 man-hours—it is hoped to reduce this substantially in the future when more experience with the technique has been gained. Among the problems to be overcome is the battery casting of the cladding panels, and simpler panels have now been designed which can be cast in this way.

The technique has aroused considerable interest and is now being used by a number of firms. The report expresses concern, however, that the relative ease with which the process can be adopted should not lead to a deterioration in the standards of design. If past experience is anything to go by, this fear is only too well founded.

Synchronized Training

THE Council of Engineering Institutions, comprising the twelve British engineering institutions and the Royal Aeronautical Society, has finally designed a single examination for all engineers to replace the individual examinations previously administered by each engineering institution separately. The new examination will be conducted at degree level and, like the previous individual exams, will provide another

avenue to engineering qualification equivalent to a university degree or sandwich course.

Mr. F. E. Warner, chairman of the education and training committee responsible for the change, feels that the majority opinion among engineers is in favour of a comprehensive engineering education. It was only when setting the papers that various engineers discovered, to their surprise, the similarity of their problems. The examination is in two parts, the first consisting of one paper in each of six subjects—properties of materials, applied thermodynamics, fluid mechanics, applied mechanics, applied electricity and mathematics—corresponding to a level half-way through a degree course, and the second including one compulsory paper on "the engineer in society" and five others from a choice of thirty-seven. The first paper covers such areas as government, economics and business management; it is not expected to encompass a full business course but, Mr. Warner said, "to make engineers aware of the properties of men and money and their interaction with engineering". This new system will become mandatory by January 1974, but timetables for incorporating all or part of the examination will differ for various groups.

The committee has also worked to revise the other membership requirements of all the institutions, and there is a growing belief that there is a need for engineers and engineering administrators to do a stint in the universities so as further to broaden their outlook—and that of university students. While the CEI is justly proud of the new examination and feels it is an important step away from overspecialization, it is also aware that it will become less important as more and more students enrol in full-time and sandwich courses. The great increase in the numbers of technical and training colleges has meant that fewer students are studying part-time while holding down jobs as well. The examination may, however, remain important as an internationally acceptable standard.

Demographic Revolution

A POPULATION Conference sponsored by the Council of Europe was held in Strasbourg at the beginning of September. The justification of yet another conference so soon after that organized by the United Nations in Belgrade last year is that Europe is the only area now reaching the third and final stage of the 'demographic revolution'. The fertility rate is declining to the point at which it just offsets a very low death rate—a state characterized by controlled fertility and mortality. In his opening address to the conference M. Jean Bourgeois-Pichat, chairman of the organizing committee, said that "it is in Europe that solutions will first have to be found to the economic and cultural problems which the end of the demographic revolution will bring. Perhaps it is no exaggeration to say that it is in Europe that the future of the human race will be decided".

By bringing together 174 participants from 25 countries the conference attempted to emphasize the increasing need for co-operation in research and data collection by all European nations. Besides holding general meetings on such subjects as "developments in mortality", "developments in fertility" and "trends in population structure and their consequences", the conference adopted a series of recommendations—on comparative research into fertility, studies of the changing needs of elderly people, the training and housing of migrant workers, the standardization of population statistics, and the international aspects of regional planning, particularly in areas close to national frontiers.

Fish for Food

WHAT can the intensive production of freshwater fish contribute towards the world dietary deficiency of protein? This was discussed at an international meeting of 160 fishery biologists at the University of Reading during the first week of September. The meeting was convened to survey the biological basis of freshwater fish production and to prepare a handbook of methods for use in the Productivity of Freshwaters section of the international Biological Programme—a document which should be both authoritative and up to date and an invaluable aid to further research on a world-wide basis.

The first stage in evaluating a fishery is to determine the size of the exploited population, its age structure and rates of mortality and recruitment. It was clear that methods for estimating these vital statistics are now well advanced, although populations in tropical waters, where the seasonal rhythm of growth and reproduction is not well defined, present special difficulties. For the efficient management of production it is also necessary to know the food consumption of the fish and the efficiency with which the food is being converted to flesh. In this field much more work is needed, for though it is relatively easy to determine the efficiency of food utilization of fish reared on artificial foods in small ponds, it is not yet possible to calculate with any certainty the food consumption of natural populations. An important Russian school favours indirect calculations based on measurements of metabolic rates, while in North America the emphasis is on extrapolation from feeding experiments in aquaria. The influence of behaviour patterns on food consumption and hence production has more recently been appreciated—among species which are territorial in habit, for example, the number of individuals an area will support is limited by interspecific competition for territory, so that the production of a particular stretch of stream remains approximately constant whatever the density of stocking above a certain figure.

Those concerned with practical aspects of fishery management tend all too readily to assume that predators are undesirable and should be exterminated. The meeting at Reading was warned that natural predators may serve a useful purpose, for example by removing weak or deformed individuals from the breeding stock. It was also told about techniques for the management of freshwater fisheries in ponds and in natural habitats. It appears that the most impressive yields are to be obtained from ponds, since these afford the greatest degree of control at all stages of the production process. In many parts of the world the potential of an acre of fresh water for the production of high grade protein is far greater than that of an acre of land, so that a policy of controlled flooding might well be advantageous. A speaker from FAO, after presenting a rough protein budget for the year 2000, suggested that it might be possible to increase the production of freshwater fish in ponds by a factor of fifteen to thirty. Increasing production from the

land, already heavily exploited, might well be more difficult.

Physical Research in Africa

DR. B. N. C. AGU of the Department of Physics, the University of Ibadan, Nigeria, writes in retrospect of the problems discussed at the eighth annual conference of the Science Association of Nigeria earlier this year:

Most indigenous scientists, including those who graduate at home, tend to do their postgraduate research abroad for two main reasons. One is because of the limited facilities in most parts of Africa for research in the branches of physical sciences in which the students wish to work, including nuclear physics, electronics, metallurgy, solid-state physics, health physics and radio-astronomy. Another group of postgraduate students goes abroad in search of a doctorate from a reputable university in the United Kingdom or North America. Many of these will eventually return to take up teaching assignments in the local universities.

Most of the scientists who take postgraduate degrees abroad soon find in Africa that the convenient set-up on which they worked in the older universities abroad is no longer available to them. Should they attempt to maintain a tenuous umbilical cord with their mother universities abroad in order to continue research, or try to make a fresh start in African universities?

This latter procedure requires years before any progress is made and large sums of money which are not usually readily available. The purchase, replacement and repair of research equipment can be very frustrating. Orders for equipment take several months to arrive and the replacement of defunct parts takes even longer. The problem of getting and retaining competent technicians is almost insurmountable. Without enough local experience the repair and maintenance of research equipment become a major operation which can hold up important work for months.

In recent years, several countries and international organizations have been showing interest in developing research in Africa. However, a quick survey of the fields in which research is supported by these bodies shows a bias towards agriculture and veterinary science, African studies, population problems, child health, medicine and malnutrition problems in the developing countries. For the five year period 1961–1965 the distribution of foreign benefactions to the various departments of the University of Ibadan and its Teaching Hospital was as follows:

Medicine and Child Health	£483,361
Agriculture	£250,810
Education and African Studies ..	£246,910
Chemistry	£32,100
Physics	£7,650

The share of the physical sciences in these funds is meagre. One explanation may be that since these benefactions come from highly industrialized and developed countries, none of the organizations is interested in encouraging basic physical science research in developing countries. Except during the International Geophysical Year and the International Year of the Quiet Sun, when some equipment and limited funds were provided for collecting data from outstations in Africa, all the burden of research in the physical sciences has had to be borne by the resources of the particular developing country. Given the need to provide social amenities, very little money will in fact be available from the governments for any form of research. The very few industries in these countries are usually operated by foreign companies which have their headquarters in the developed countries. Most of their research can usually be done better in their home countries and therefore they will have little interest in encouraging research in the universities of the country in which they are operating.

The Quality of Radiation

A REPORT is about to be published by the International Atomic Energy Authority, Vienna, of an international panel meeting held in Vienna last year to discuss the biophysical effects of ionizing radiations of various kinds and properties (Technical Report Series No. 58, *Biophysical Aspects of Radiation Quality*, I.A.E.A., Vienna, 1966). The subject is important not merely because of its practical value in assessing the hazards due to radiation which is not simple in character, but also because of its interest in fundamental radiobiological research. One of the conclusions of the panel at Vienna was that there is a need of more systematic measurement of energy dissipation in biological material by ionizing radiation of various kinds. The intention is that the activity of the group shall be continued within the framework of a research programme co-ordinated by the international agency, and it is expected that the programme will be more clearly defined at a meeting to be held under the auspices of the agency in 1967.

Much of the discussion of the technical panel was concerned with the survival curves for cells of various kinds, and with the interpretation of the available experimental evidence. On one model, the results were interpreted in terms of the amount of energy required to produce critical molecular damage in small regions of the nucleus from a mammalian cell possibly not greater than 50 Å in diameter. In the same vein, a model of genetic damage supposing that critical effects are produced by one ionization in each of the very small structures situated more than 100 Å apart, showed that more heavily ionizing atomic particles have a greater probability of doing damage. Other interpretations of cell survival curves described at the meeting of the panel consisted of a model in which the damage done by alpha-particle radiation can be accounted for by the dissipation of between 350 and 500 eV in a small volume of a mammalian cell. (From this discussion it also emerged that the proportion of single-hit killing by X-rays in kidney cells is between 10 and 20 per cent.) Other interpretations of the effects of ionizing radiation on single cells were based on various stochastic models.

Replant Problem

THE "replant problem" is only one of many which face fruit growers, but solving it could lead to a spectacular increase of yield on many farms. It turns up when fruit trees are planted in ground previously planted with trees of the same species—for example, when apple trees are planted in old apple orchards. The cause is not yet clear, but the result can be a considerable retardation of growth in the first year of the life of a tree and proportional losses throughout. Clearly

something in the soil has an effect on the roots, which have a characteristic stunted appearance and cover a much smaller volume than normal roots.

The consequences of the replant problem have now been convincingly demonstrated by a series of experiments at the East Malling Research Station. Apple trees planted in an old apple orchard are small and poorly developed compared with similar trees planted after treating the soil with chloropicrin. This substance obviously gets rid of whatever it is that stunts growth, but it is dangerous, expensive and unpleasant to handle. Acidifying the soil is also effective but is much more expensive. Research is continuing at the root laboratory of the station to identify the cause—organism or substance. Although nematodes and viruses have been more or less ruled out, bacteria may be the culprits. In any event, the work has produced interesting new results on the growth of fruit tree roots and on the habits of the nematodes which prey on them.

Weizmann Institute

THE Annual Report of the Weizmann Institute shows that the establishment of the institute at the end of 1965 consisted of 300 scientists and engineers. At the same time there were 89 visiting scientists in residence. During the year £952,000 was contributed to the institute's budget by agencies, foundations and other organizations in Israel and elsewhere, and supported roughly a quarter of the work being done.

Index to Food Safety

THE vigour of the Food and Drug Administration has given the United States a fine reputation on food safety. The National Academy of Sciences has now done its bit to help by the publication of a comprehensive definition of standards of identity and purity for some 500 food additives now in use in the United States (*Food Chemicals Codex*, National Academy of Sciences—National Research Council, $25.00). The volume is the result of five years of work by a Committee on Specifications under Dr. Henry Fischbach, of the Food and Drug Administration. Draft specifications prepared by the committee, and with the help of an advisory panel under Mr. R. B. Smith, President of the Medical College of Virginia, were criticized by interested parties in industry, research and government before being cast in final form. The principal value of the new publication will be the way in which it will enable food manufacturers in the United States to interpret and abide by the necessarily laconic regulations which embody the ways in which the U.S. Government has sought to control the use in American foodstuffs of the 575 chemicals considered to be safe.

Professor E. A. Guggenheim

THE University of Reading is about to lose Professor E. A. Guggenheim, who retires on September 30. He has been head of the Department of Chemistry at Reading since 1946, and during this period has made his principal contributions to thermodynamics and statistical mechanics. During the same period he has built up a vigorous School of Physical Chemistry at the University. He will be succeeded as head of the Department by Professor G. W. A. Fowles, at present Professor of Inorganic Chemistry. Guggenheim has been nominated Emeritus Professor.

University News: London

DR. J. M. HINTON, first assistant in the Academic Department of Psychiatry in the Middlesex Hospital Medical School, has been appointed to the chair of psychiatry tenable at that school.

Appointments

MR. W. J. BRAY has been appointed director of research at the Post Office in succession to Dr. G. H. Metson, who retired in August.

Announcements

THE United States National Academy of Sciences and the National Academy of Engineering has announced the appointment of John C. Kohl as executive secretary of the Division of Engineering of the National Research Council, effective September 15. The NRC is the operating agency for both Academies.

Mr. Kohl, a civil engineer with broad experience in the field of urban transportation, became executive vice-president of the American Transit Association in March this year, after serving since 1961 as assistant administrator (transportation) of the Federal Housing and Home Finance Agency, responsible for its urban transportation programme including capital grants and transit demonstration projects authorized under the Urban Mass Transportation Act of 1964. He held that position on leave from the University of Michigan, where he was Professor of Civil Engineering and Director of the Transportation Institute.

Mr. Kohl succeeds Louis Jordan, who is retiring as executive secretary of the division after 24 years as a member of the NRC staff. Mr. Jordan will continue to serve the division as special consultant to its chairman, John A. Hutcheson.

The Division of Engineering, one of eight NRC Divisions, includes in its membership representatives of more than 20 national professional organizations as well as members-at-large drawn from various engineering specialties; more than 1,200 engineers and scientists from the academic community, industry and government participate in the Division's activities

AWARDS for postdoctorate research on the biochemical and physiological mechanisms of fertility and early stages of reproduction in various forms of life are being offered by the Lalor Foundation, Delaware, U.S.A., for tenure during 1967. They are open to scientists of all nationalities and may range up to $8,000 per year depending on the scope and duration of the projects approved. Preference will be given to younger members of university and college faculty and staff with an upper age limit of 41 years. The work may be carried on at the applicant's own institution or elsewhere. The foundation will also grant postgraduate summer or short-term research awards at the Marine Biological Laboratory at Woods Hole, Massachusetts. These awards will normally not exceed $1,200 for a single man or a woman, $1,400 for a married man working at his home institution, and $1,600 for a married man with principal programme at another institution. Requests for information and for application forms should be directed to the Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware 19805.

THE International Association for Plant Physiology has prepared an International Directory of Plant Physiologists associated with its fifteen constituent national organizations. Copies may be purchased for $1.00 from the secretary-treasurer, Prof. Arthur W. Galston, Department of Biology, Yale University, New Haven, Connecticut.

THE journal *Respiration Physiology* (*Nature*, 211, 901; 1966) is published by the North-Holland Publishing Company.

WHAT NEXT AT BERKELEY?

THE University of California at Berkeley, one of the finest academic centres in the United States, was torn by a campus upheaval in the autumn of 1964 that was unprecedented in an era of campus conformity for the majority and Vietnam and civil rights protests by the vocal minority. Were they 'Reds' and 'Chinese communists'? Or were the participants in the Free Speech Movement at Berkeley intelligent students revolting against the impersonality of a university the size of Berkeley—27,000 students in 1965—and their own lack of motivation? Prompted in part by the unrest represented by the Free Speech Movement, the university's Emergency Executive Committee appointed in March 1965 a Select Committee on Education to examine in detail the problems of the university and present possible solutions. The committee, under the chairmanship of Prof. Charles Muscatine, has now submitted its report, and many of its recommendations should be implemented during the present academic year (*Education at Berkeley*, University of California Press and Cambridge University Press, March 1966).

The report does not present a broad, sweeping programme, nor does it attempt completely to revolutionize any large segment of the university; what it does try to do is establish an atmosphere of change and adaptation and at the same time to present a number of proposals aimed at specific problems. "Our ideal for the student is that he be provided with rich opportunities, generous guidance, and plenty of room for experiment, and that he be enabled to make for himself as many of the important decisions about his own education as possible".

The complaints that were levelled against Berkeley by the Free Speech Movement included claims that students were ignored and even deprived of their right to speak, that they were treated as IBM numbers rather than individuals, and that professors were more interested in research than teaching and had no contact with their students. The select committee admits that some of these charges are valid and has attempted to offer some solutions. To be sure, the committee does conclude, from a number of surveys among students, that most students consider they are getting a good education. In spite of this, the supporters of the Free Speech Movement were not simply a small minority but the hard core of intelligent and articulate students who wanted a cause with which they could identify.

But the problems at Berkeley stem not only from its size but from its composition. Berkeley students are as mobile as most Californians. Of the class entering as freshmen in 1961, only 50 per cent graduated by January 1966 or were still on the campus. Of the class that graduated in 1965 from the College of Letters and Science —the largest school in the university—38 per cent had completed their first two years elsewhere; only one-fifth of the graduate students come from Berkeley, so there are also more than 3,000 new students in the graduate schools each year.

An unstable, fragmented body of students, many of whom commute for between two and six hours each week, does not make for ease of contact in a large university, and many of the committee's proposals centre on this problem. It wishes to see the advising system, especially for freshmen, changed from an empty formality to a more personal though voluntary opportunity for students to seek out an adviser of their choice when they need help and not, as at present, merely to acquire the signature of a teacher who neither knows nor has time to care about what he is signing. The report says that the present system "is based on the doubtful assumption that a spontaneous and genuine relationship will develop between a student and a faculty member within the short periods officially required for advising twice a year. There are probably few men who are capable of forming meaningful relationships with dozens of students on short notice". To help new students the committee would like to see a summer orientation and guidance programme so that incoming students can learn what choices are available and map out a tentative schedule well before they register.

The complaint of many students at large universities that their professors have little interest in them and are more concerned with their research than with teaching can and has been made at Berkeley. At a university where more than one-third of the students are graduates, it would be very easy for teachers to neglect their undergraduate students, but, in fact, this does not seem to be generally the case. From a study of the distribution of faculty time-tables, it appears that the average teacher spends 24 hours on courses and lectures, 10 on undergraduate consultation and advising, 7 on graduate research instruction, and only 10 on individual research.

Size is a problem for classes in the lower division courses in the first two years, where classes average seventy. It seems clear, however, that the problem of student–faculty contact has not arisen from a neglect of teaching in favour of research on the part of the faculty. Even so, the committee recommends an eventual increase of the teaching staff with the student enrolment held constant. In the meantime, it considers that the proportion of lecture courses should be decreased in favour of discussion sections, seminars and tutorials, and that students be given more opportunity for supervised but independent study.

The report would also like Berkeley to reverse the increasing importance of course grades. Both students and faculty have often expressed dissatisfaction with a point-grading system—a student survey showed that 35 per cent of honours students believed that their grades did not reflect their actual knowledge and understanding of a subject—but the committee did not feel prepared to offer a comprehensive alternative. It does, however, propose that students should be allowed to take one course apart from their major each term on a pass or fail basis, and that some courses should be offered in a sequence of two or three quarters with a grade given only at the end.

Possibly the most important and far-reaching recommendation outlined in the report is for the establishment of a Board of Educational Development. As the committee says, "we believe it is important to establish machinery, not to implement any single curricular change, but to give continuing consideration, encouragement and financial support to all worthy proposals for educational development". The committee visualizes a permanent board of seven, each member serving for three years, to determine policy, implement projects, carry out studies and attempt to establish priorities for educational development.

None of these proposals will bring about any radical changes in education at Berkeley, but they do reflect and bow to the general unrest that has hamstrung the campus for the past few years. They betoken a willingness to experiment and to consider new concepts. Whether any innovations will be able to cope with the problems of size and composition that face such a State university remains to be seen, but Berkeley's pragmatic and gradual approach looks like making a practical start.

INSTEAD OF COSMOLOGY

By PROF. OSCAR KLEIN
Ringen 21, Stocksund, Sweden

THE attempt outlined in this article, to consider the origin and evolution of the system of galaxies—our metagalaxy—in a similar way to that of smaller stellar systems, is not a new cosmology but is essentially based on a somewhat detailed criticism of the various branches of cosmology of which some indications will be given below. On the other hand, it depends largely on the considerations developed in connexion with relativistic cosmology, the aim of which—apart from its philosophical foundations—has been real enough, namely, the interpretation of some remarkable results of astronomical observation, in the first place the characteristic red-shift of the spectral lines from other galaxies in its dependence on their distance, as discovered by Hubble; second, the tendency towards an even distribution of galaxies and galaxy clusters, when sufficiently large volumes are considered and, further, the recently discovered electromagnetic radiation apparently corresponding to a temperature equilibrium of a few degrees above absolute zero.

On the other hand, present day cosmology also has much in common with an age-old inclination of the human mind appearing in early creation myths and later giving rise to world systems based on *a priori* arguments, either theological—the universe being worthy of the wisdom of the Creator—or philosophical—so-called necessities of thought. Thus the system ascribed to Aristotle but also influenced by *The Bible* reigned on the whole, though not without modifications, throughout the Middle Ages.

In contrast to such grand world systems, physical science developed from a thorough study of certain simple classes of experience : the equilibrium conditions for solid bodies and liquids and the motion of falling and thrown bodies—not to mention the experience behind Euclidean geometry. This gradually led to the disclosure of general laws behind phenomena such as the principle of virtual work, the law of inertia and the principle of relativity, which in extended and modified form still belong to the foundations of physics. This search for general principles by means of well-chosen characteristic features of gradually more extended classes of experience is still the leading stimulus in physics, as illustrated by the development in the present century of relativity theory and quantum theory.

In earlier work of this kind, such as Newton's discovery of the law of general gravitation and his development of what we now call classical mechanics on the foundation laid by Galilei and his predecessors, the contrast with the "cosmological" trend was very conspicuous. This appears in Newton's general statements in his *Principia*, of which the claim of using the same explanation for analogous things was far from trivial, when things were the fall of earthly bodies and the motion of celestial planets. It may be said that the mentioned results of Newton's researches first gave substance to the concept of the universal laws of nature, which since then has been the leading idea behind physical investigation.

Although in formulating his law of general gravitation Newton had to rely on astronomical experience as embodied in the Keplerian laws, the later disclosure of general laws in electromagnetism and quantum physics has been mainly based on "earthly" experience. Conversely these laws have come to play an increasingly important part in astronomy the problems of which, apart from size and time, are well within the range of what can be studied in the laboratory. Scientists who wish to introduce new laws of nature in order to make the universe satisfy their *a priori* claims have, however, a certain advantage over Newton's contemporaries, who believed in the short time-scale of *The Bible*.

It is of interest in this connexion to consider the statement called "the cosmological postulate" by the defenders of the steady state cosmology : *that on the average the universe is the same everywhere and at all times.* Taken as the background for the hypothesis of a spontaneous creation of matter at a rate just sufficient to compensate for the rarefaction of the metagalaxy as a result of its observed expansion this is certainly an extreme example of cosmological thinking. On the other hand, the statement itself is a natural induction from astrophysical experience concerning the validity of the laws of gravitation, electromagnetism and atomic physics at the most distant places and early times so far accessible to observation. Thus, just as a sufficiently large sample of a population living under unchanging natural conditions and human legislation will show a constant distribution of ages and professions, we should expect that a sufficiently large region of the universe would present a constant distribution of stellar systems with respect to types and stages of evolution. Because the metagalaxy is in fact changing we conclude that—despite its enormous size—it is too small to be representative of the average situation in the universe. Moreover, we assume that it is just one of a multitude of similar systems in different stages of evolution, some of them expanding and some contracting.

On this assumption, our metagalaxy would not be a singular case but, like the smaller stellar systems, a regular occurrence in a very much larger—perhaps infinite—universe, very thinly populated by stellar systems, so as to avoid the well known paradox of infinite gravitational fields and infinite starlight illumination in the way suggested by Charlier and others. Such considerations lead us to assume that the metagalaxy once started as a very extended and very thin collection of the simplest stable particles—protons and electrons—which contracted by means of mutual gravitation.

As we shall see, these assumptions are further supported by a closer comparison of the foundation of Einstein's general relativity theory with the arguments behind his cosmology. Also, a tentative interpretation based on the view just mentioned of the origin of the metagalaxy of the relationship noticed by Eddington between the size and mass of an Einstein universe (adapted to the average density of the system of galaxies) to the so-called radius of the electron and the mass of the proton seems to offer some clue as to the manner of its transition from an earlier contraction to its present expanding state.

After some preliminary attempts on this basis it occurred to me that it would be extremely improbable for the huge

cloud under consideration not to have contained as many antiprotons as protons and as many positive electrons as ordinary electrons. The evidence obtained from experiment as well as theoretical studies points to an equal *a priori* probability of a particle and its antiparticle. This hypothesis of equal amounts of matter and antimatter in the original cloud made it necessary to assume that some natural mechanism of separation has been in action during the evolution of the metagalaxy. Questioned about this point, Professor Hannes Alfvén soon proposed some such mechanisms. This was most stimulating and led us to consider seriously the possible role of matter–antimatter annihilation as a hitherto neglected force in cosmical physics, the first result of which was a sketch of the evolution of the metagalaxy from its start to the present expanding state (published in a joint paper, where his separation mechanism was also briefly considered).

The great progress made during the past twenty years in interpreting the many new discoveries in elementary particle physics is based on the combination of quantum theory and special relativity theory. This has given rise to the opinion shared by many physicists that, while special relativity is one of the most fundamental principles in physics, the concepts of general relativity are limited to the realm of very large dimensions—gravitation being not only the weakest but also the least understood kind of interaction. I believe that this opinion has arisen from a misunderstanding of general relativity partly caused by Einstein himself in connexion with his cosmological considerations. Thus I believe that elementary particle physicists are right in assuming that the fundamental laws of physics are those found in the limiting case of negligible gravitation. But far from being contrary to the theory of general relativity, this view is a consequence of what Einstein called the "equivalence principle", the very cornerstone of his general theory.

It is of interest that the concepts on which this fundamental principle is based depend on experience well known since Newton's time and already known to Galilei —namely, that all bodies undergo the same acceleration in a gravitational field—and from which follows an explanation of the now well known weightlessness inside a satellite. In Newtonian theory, however, this applied only to mechanical phenomena. But Einstein—starting from special relativity, emanating in its new form from a thorough study of electromagnetism and optics—saw in this old fact a deep law of nature applicable to any physical phenomenon. Already in Newtonian mechanics the fact mentioned implies that an inertial frame of reference—that is, a frame in which the law of inertia is directly valid—can be introduced locally anywhere and at any time in any gravitational field. The word "locally" here means that such a frame can be used in the neighbourhood of a certain point during a certain time interval, sufficiently small for the variation of the gravitational field to be negligible. Mathematically this is related to the replacement of a small part of a curved surface by a bit of the tangent plane. But just as a lake—despite the curvature of the Earth's surface—can be considered as plane for most purposes, this kind of frame will in most cases have a practical validity within a quite large space–time region.

Einstein's principle of equivalence states that any physical law, when referred to an inertial frame, will hold in circumstances where gravitation may be neglected —that is, will be a law of special relativity theory. Conversely, any such sufficiently well known physical law can be generalized so as to describe the effects of gravitation on the phenomena in question.

Two important implications of this principle which are often forgotten will be emphasized here. The first is that this principle provides the physical interpretation of the mathematical formalism of general relativity theory; measurements of physical quantities—most directly those of length and time—being in principle referred to an inertial frame, that is, a frame freely falling in the local gravitational field and having zero velocity with respect to the practical rest frame at the time of the measurement. The other, which is a consequence of the first, is that there is no essential difference between so-called inertial forces—such as the centrifugal force—and "true" gravitational fields.

We shall now consider briefly the properties of an approximately isolated system—for example, its mass—from the point of view of the principle of equivalence. The use of an inertial frame implies the practical removal of all gravitational fields other than that due to the system itself. Thus, in order to measure the mass of the Earth by means of the gravitational field it produces, a frame has to be used in which the gravitational field from the Sun is removed as far as its lack of homogeneity permits, that is to say, a frame freely falling in the field of the Sun. This field is sufficiently strong to influence appreciably the direction of gravitation but is compensated by the inertial force as a result of the accelerated motion of the Earth around the Sun. As is seen, this compensation is automatically taken care of by using the aforementioned frame.

A similar case is provided by the Copernican frame, in which the centre of gravity of the solar system is at rest, and which is freely falling in the gravitational field due to the remainder of the galaxy. Here the average field is very weak, which means that this frame deviates very slightly from one at rest with respect to the sphere of fixed stars, a frame which was assumed to give a practical definition of Newton's concept of absolute space. In a frame of reference at rest or in uniform motion with respect to absolute space there would be no inertial forces. Referred to such a frame and using the corresponding concept of absolute time the true laws of physics would hold—in the first place the law of inertia.

These Newtonian concepts were strongly criticized by Mach, the famous physicist-philosopher, in his admirable book on the evolution of mechanics. Regarding them as metaphysical in the worst sense of the word, he took the view that only the motion of a body with respect to other bodies could be physically meaningful. He concluded that inertial forces must be gravitational effects resulting from accelerated motion with respect to the multitude of distant bodies in the universe. This idea appealed strongly to Einstein in his search for a generalization of special relativity and became—under the name of "Mach's principle"—one of the main incitements to his cosmology.

While Mach's apparently well founded criticism of Newton's concepts was actually based on preconceived ideas of the meaning of words like "body" and "empty" space, Einstein's principle of equivalence was, as we have already seen, an induction from empirical facts. Thus, it is not so surprising that this principle and not Mach's idea concerning inertia is in best agreement with relativistic quantum theory, where the words "matter" and "vacuum" have obtained a new and richer content, their everyday meaning being correspondingly limited.

We shall now summarize the relation between the equivalence principle and the Mach principle. According to the former the mass of a body—which could be anything from an elementary particle to a stellar system—is referred to an inertial frame, which is better defined the better the body is isolated, its surroundings approaching the physical definition of an empty space. In practice such a frame has no direct relation to the structure of the universe. With Mach's principle, on the other hand, a body would have zero mass in an empty universe, the mass being smaller the less matter there is.

As is well known, the deviations from Euclidean geometry—which are very small in the applications of general relativity to ordinary problems, such as the motions of the planets—play an important part in cosmology. Their general background is by no means mysterious, being the fact that the different points of a gravita-

tional field in general require different inertial frames. In this connexion Einstein's field equations which replace the Newtonian law determining the gravitational field arising from a given distribution of matter should be mentioned. Because there is no gravitational field in the limiting case of special relativity these could not be derived by means of the equivalence principle in its usual form. Einstein found them by means of the idea of "general covariance" with respect to the choice of the four co-ordinates used for the mapping of a finite region of space and time, together with the requirement of greatest possible simplicity. Because these equations have been used in connexion with speculation exceeding by far the range of our experience it should not be forgotten that their derivation is probably less fundamental than the part of the theory which is based on the principle of equivalence. None the less, they represent an important advance in several respects from Newtonian theory.

Einstein's original attempt to describe the average situation of the universe by the famous "closed world" solution of his somewhat modified field equations depended strongly on his hope of satisfying the Mach principle. Another important aspect was the avoidance of the boundary condition valid in the Newtonian case as well as in ordinary solutions of his own equations: *that the gravitational field vanishes asymptotically towards infinity*. As has already been shown, this condition (although, in practice, infinity here means distances from the system under consideration) is automatically fulfilled in the inertial frame, where any gravitational field other than that due to the system itself is removed. Einstein, however, regarded the use of such a frame as running against the claim of general covariance. Strangely enough, both these arguments are contrary to the spirit of the principle of equivalence discovered and used most ingeniously by him in the development of a theory of general relativity.

A third assumption—the static nature of the solution—was based on the belief of astronomers before Hubble's discovery that even distant stellar systems have very small velocities with respect to our galaxy. Later Einstein abandoned this assumption in favour of a class of expanding solutions discovered by Friedmann and later, independently, by Lemaître, who together with Robertson and others developed the relativistic cosmology mentioned in the introduction.

As was mentioned at the beginning of this article, the average mass density as obtained by counts of galaxies and galaxy clusters together with more or less reliable estimates of the average masses of such stellar systems tends to become spatially constant, when sufficiently large volumes are considered. There is a simple sub-class of the expanding solutions just mentioned which satisfies the condition of pressureless matter of spatially constant density. Moreover, these solutions give a generalized red-shift law, which for moderate distances agrees with Hubble's empirical law: *the red-shift of the spectral lines from a given galaxy proportional to its distance from our galaxy*. Thus as a result of the slowing down of the expansion because of gravitation, the coefficient of the Hubble law decreases in the course of time. Taking any point, for example our own galaxy—all points being equivalent—as the origin, the position of any other galaxy may be described by means of three time-independent co-ordinates, one radial co-ordinate η and two angles representing latitude and longitude. The distance of a point from the origin is then a function of η multiplied by a time-dependent factor a of dimension of length, η being chosen dimensionless. Thus a describes the expansion of the system, and its relative time-derivative $1/T$ is the Hubble law coefficient at the time under consideration. (T is frequently, but rather inadequately, referred to as the age of the universe.)

There are two subclasses of the solutions under consideration characterized by the value $+1$ and -1 respectively of a parameter ε; the first represents an infinite universe, the second a finite, closed universe of the Einstein type. In order to satisfy the gravitational field equations and the condition expressing the conservation of particles (doubtlessly well satisfied at the present time), a, T and the density μ have to fulfil two conditions which, because they are very simple we shall give explicitly, namely

$$\frac{8\pi}{3}\gamma\mu T^2 = \frac{a_0}{a_0 + \varepsilon a}, \quad \frac{4\pi}{3}\mu a^3 = \frac{c^2}{2\gamma}a_0 .$$

Here γ is the ordinary gravitational constant, a_0 an integration constant of the same dimension as a, and c the vacuum velocity of light. T is the reciprocal of the Hubble coefficient, and is well known for the present time; the estimates of μ, however, are rather less certain. Thus, $T = 1{\cdot}3 \times 10^{10}$ (thirteen thousand million) years and $\mu = 10^{-30}$ g cm^{-3} (that is, 1 g in a cube, the side of which is a hundred thousand kilometres) seem to be reasonable estimates This gives for the left hand side of the first formula a value considerably smaller than unity (0·09), which can, however, be reconciled with the right hand side only if $\varepsilon = +1$. Solving then for a and a_0 we obtain a value for a_0 of about 10^9 light-years and $a \sim 10\, a_0$.

Assuming that these estimates are not very far from the best fit that can be obtained between relativistic cosmology and our metagalaxy at the present stage of evolution, we shall now proceed to a model of the kind mentioned at the beginning of the article which represents a bounded expanding solution to Einstein's equations of spatially constant density, and which satisfies the ordinary boundary condition. Such a solution can be constructed from the cosmological solution stretching to infinity by allowing the matter distribution to end at a certain finite value η_0 of the radial co-ordinate η, the space outside of the expanding spherical surface, thus defined, being empty. This is the three-dimensional analogue to the cutting out of a circular cap around a point on a spherical surface. Thus we obtain a spherical expanding cloud of spatially constant density, which satisfies the foregoing equations —the boundary condition leading to the following relation for its total mass M:

$$M = \frac{4\pi}{3}\mu a^3\eta_0^3 = \frac{c^2}{2\gamma}a_0\eta_0^3$$

Thereby M is defined by means of the external gravitational field due to the system. It should be pointed out that the centre is now a meaningful point, and its distance from our galaxy is an observable quantity. Because η_0 together with a determines the radius of the system, it is possible that an estimate of η_0 could be obtained by a survey of the distribution of stellar systems of high red-shifts with respect to direction. Such a survey is still lacking but the highest red-shifts so far known correspond to about four-fifths of the velocity of light when interpreted as Doppler effects. This would correspond to an η-value of the source slightly greater than unity, if our galaxy (for this calculation) is put at $\eta = 0$. On the other hand, an upper limit for η_0 may be obtained by means of the so-called Schwarzschild limit applied to the system at the time when the observed light was emitted. For an expanding or contracting system such as that under consideration, this limit is the state where the velocity of the boundary is equal to that of light. For densities beyond that state there is no natural way of satisfying the boundary condition. In the present case this state is reached for $a = a_0\eta_0^2$, which means that this is the smallest value a could possibly have had according to the model. Bearing in mind that the present expanding state of the galaxy should, according to our programme, have been preceded by an earlier contracting state, this value of a is probably smaller than the smallest value for which the expansion was fully established. Now, a red-shift

corresponding to a Doppler effect velocity of 4/5c means that a at the time when the light was emitted was as large as one-third of the present value of a. This and the estimate of the η-value which belongs to such a red-shift would point to a value of η_0 in the neighbourhood of unity.

It is of interest to compare the solution under consideration with the corresponding solution of Newtonian theory. In the latter case we have a common time for the whole space, which in the present case corresponds to the time of the external inertial frame. An important difference between the two solutions is involved with the Schwarzschild limit, which does not exist in the Newtonian theory, where c is regarded as infinite. In the present solution—using the inertial frame time—a contracting solution will approach this limit asymptotically but never reach it. Apart from this the classical formulae are valid for $\eta_0 \ll 1$. In the classical case, $a\eta$ is the distance of a point from the centre, while in the relativistic case this is the first term of a series expansion in powers of η—the approximation being quite good up to η-values not much smaller than unity.

Now, in the classical case, $\varepsilon = +1$ means that there is a surplus of kinetic energy beyond that needed to bring the expansion to infinity, while $\varepsilon = -1$ means a corresponding deficit—a_0 being in that event the largest value which a can take. This is quite similar to the situation in the relativistic case, where Mc^2 is the total energy including the rest mass energy of the system, while M_0c^2, the sum of the energies of its sub-systems (galaxies and galaxy clusters), is equal to the volume integral of the density μ. Thus for $\varepsilon = +1$ $M > M_0$, while $M < M_0$ for $\varepsilon = -1$. Thereby the ratio M_0/M is a function of η_0, which for $\varepsilon = +1$ is given by

$$\frac{M_0}{M} = \frac{3}{2\eta_0^3}\left(\eta_0\sqrt{(1+\eta_0^2)} - \ln(\sqrt{(1+\eta_0^2)} + \eta_0)\right).$$

With $\eta_0 = 1$ this gives $M_0/M = 0{\cdot}8$, and the surplus energy $Mc^2 - M_0c^2$ is 20 per cent of the total energy.

Assuming that all these estimates, although necessarily uncertain, are reasonably realistic we shall consider the last result from the point of view of our basic hypothesis: *that the metagalaxy began as a very large and very thin cloud of practically zero temperature.* This means that its total energy $M'c^2$ (M' being the original mass of the cloud) was practically equal to the sum of the rest mass energies of its particles. Suppose now that during its evolution up to the present state an amount of energy $c^2\Delta M$ has been lost by radiation including neutrinos, together with the amount $c^2\Delta M_0$ of the rest mass energy. Then we shall have

$$M' = M + \Delta M = M_0 + \Delta M_0,$$

or

$$\frac{M - M_0}{M} = \frac{\Delta M_0 - \Delta M}{M}.$$

It thus follows from the above estimates that ΔM_0 must be more than 20 per cent of M; how much more depends on the amount of energy lost.

To gain some further clue as to how the transition from the original state of the metagalaxy to its present state occurred, we shall consider the strange empirical relations noticed by Eddington for the linear dimensions and the total rest mass of an Einstein universe adapted to the observationally estimated density. Their essence is the appearance of the large number N of the ratio between the electrical and gravitational attraction between a proton and an electron, when macroworld quantities are compared with their counterparts from the microworld. Instead of the Einstein universe, we shall consider the bounded solution with the value of a_0, already estimated, and the corresponding mass M.

With

$$N = \frac{e^2}{\gamma m_e m_p} = 2{\cdot}27 \times 10^{39},$$

e being the elementary electric charge, γ again the gravitational constant and m_e and m_p the masses of electron and proton respectively, the above estimate of a_0 fulfils almost exactly the relation

$$a_0 = 2Nd,$$

where

$$d = \frac{e^2}{m_e c^2} = 2{\cdot}82 \times 10^{-13}\ \text{cm}$$

is the so-called radius of the electron. Assuming $\eta_0 = 1$ it follows that

$$M = N^2 m_p.$$

Although the exact form of these relations should not be taken seriously, we shall see that their order of magnitude is probably meaningful.

Thus, considering the metagalaxy from the present point of view, it does not seem unreasonable that the mechanisms responsible for its evolution also determine its natural average size; it is feasible that in so large a system the relative fluctuations from this "natural average size" are quite small. If we tentatively assume that the turning of the original contraction into its present expansion resulted from radiation pressure on the electrons of a cloud by means of scattering according to the well known Thomson formula, well valid in the visible and infra-red part of the spectrum, the mean free path of a photon—determining the degree of opacity of the matter

—would be equal to $1 : \frac{8\pi d^2}{3} n_e$, where n_e is the number of electrons in each unit volume. It follows from the foregoing estimates that this length is of the same order of magnitude as the radius of the cloud in its densest state, where $a = a_0$ (the Schwarzschild limit), if n_e is comparable with the number of protons in each unit volume. The meaning of this is suggested by the fact that the contraction of the cloud to the density in question—about 10^{-27} g cm^{-3}—would lead to annihilation of a sensible fraction of its total mass.

The formation of electromagnetic radiation needed to break the motion during the contraction of the cloud is still far from clear; the possible sources would be collisions of the particles of the cloud with each other and with electrons as a result of annihilation, the increase of already present radiation through the process resulting in braking and, finally, synchrotron radiation as a result of the presence of magnetic fields. The last type of radiation has been studied by Alfvén *et al.* in an attempt to consider annihilation as the main energy source in the newly discovered so-called quasars. This process, however, would scarcely be important in the present connexion, because the relevant wavelengths would be very large compared with the average distance of the particles in the cloud, and this would mean high transparency because of the equal amount of positive and negative electrons.

Short of a detailed theory for the formation of radiation, it may be worth while to estimate its spectrum under the assumption that at this turning point the system is not too far from a temperature equilibrium. The temperature would then be determined by the energy available from the contraction of the system from its beginning state of very low density to a state approaching the Schwarzschild limit.

With a mass of the order of magnitude estimated here the temperature in question would be somewhere around 50° K, and practically all the thermal energy would then be in the form of electromagnetic radiation (the particles would contribute almost nothing, even if their temperature were many orders of magnitude higher). The average wavelength at that temperature (smaller than 1/10 mm) would be well within the region of validity of the Thomson formula and sufficiently small with respect to the average distance of the particles in the cloud (more than 100 mm).

Looking somewhat more closely at the possibility—suggested by the above estimates—of explaining the transition to the present state of the metagalaxy, it would seem that although the orders of magnitude are promising there is too little annihilation as well as opacity, if one makes the simplest assumptions : that the homogeneity of the cloud is conserved during the contraction and that the number of electrons is equal to that of the protons. There is no necessity, however, for the latter assumption—electrical neutrality being upheld by protons and antiprotons separately and positive and negative electrons separately—while a moderate increase of n_e (the number of electrons in each unit volume) would give sufficient opacity. The limit would probably be given by the highest permissible value of n_e in the present state of the system, taking into consideration the decrease of this quantity resulting from expansion and annihilation. Verification of the approximate temperature equilibrium as well as of the explanation of the expansion by means of radiation pressure can perhaps be seen in the observed low-temperature radiation mentioned at the beginning of the article. Thus, if the radiation expands in doing work on the electrons, the opacity being sufficient, its temperature would decrease approximately in the same proportion as the linear dimensions increase, that is, by about a factor of ten according to the estimates of a and a_0. Within the uncertainties this would agree well with the temperature (about 3° K) of the present radiation.

As to the other difficulty, it would be removed if a sufficient number of inner condensations appear inside the cloud during its contraction, their time scale being shorter than that of the general contraction, as a result of their higher density. Such an occurrence produced by inner instabilities seems highly probable. Assuming a mass of such a condensation of the order of magnitude of a galaxy, considerable annihilation would occur quite long before its opacity is sufficiently great to stop the contraction and very long before the Schwarzschild limit is reached.

Here also another point should be mentioned, namely the transfer of the momentum given to the electrons from the radiation to the protons of the cloud. According to Alfvén this would be brought about by the magnetic fields necessarily present in a thin plasma of electric particles.

Thus, as already mentioned, a considerably smaller mass would develop in a manner differing widely from that of a metagalaxy, the lack of sufficient opacity impeding largely the braking of the contraction by means of radiation pressure. Here one would expect that the magnetic fields—implying the Alfvén separation—would become important and lead to the development of a galaxy or a galaxy cluster but not to a metagalaxy. A much larger mass, on the other hand, would scarcely suffer any significant annihilation before inner condensations had appeared because the Schwarzschild limit density is inversely proportional to the square of the mass.

Further reading:
For orientation in cosmology:
North, J. D., *The Measure of the Universe. A History of Modern Cosmology* (Oxford University Press, 1965).
For the alternative:
Alfvén, H., and Klein, O., *Arkiv för Fysik*, **23**, 187 (1962)—where earlier work is quoted.
Klein, O., *Astrophys. Norveg.*, **9**, 161 (1964).
Klein, O., *Boundary Conditions and General Relativity, Preludes in Theoretical Physics*, 23 (North-Holland Publishing Company, Amsterdam, 1966).
Alfvén, H., *Rev. of Mod. Phys.*, **17**, 652 (1965).

BOOK REVIEWS

ORIGINS OF INVENTIONS

A Social History of Engineering

By W. H. B. Armytage. Second edition. Pp. 378+36 photographs. (London: Faber and Faber, Ltd., 1966.) 50*s*. net.

THE aim of this book is "to chart technological developments with special reference to Britain, to indicate how they have affected and been affected by social life at certain stages and to offer some clues as to the origins of innovations and motivations". This is a far from modest aim for a book of less than 400 pages which starts its story in 3000 B.C. and ends with the problems posed by space, high energy physics and the power revolution.

The book is one of a series designed for the student and professional scientist and technologist, and for such a public the treatment of technological developments and of the relevant scientific discoveries is sketched in with a fine sense of perspective and economy. It is not a book for the general reader, for whom inevitably, in a book of the size, there is not enough description of the inventions listed.

The treatment of how technological developments affected and were affected by social life is less satisfactory. The analysis of the interaction between technology and education, and also of the ways in which, from time to time, necessity has mothered inventions is well done. But in dealing with the effects of technological developments on social life Professor Armytage has been much less successful. If a third edition of this book is published it is to be hoped that Armytage will have more to say about the social impact of changing war technology, about the effects of changing technology on family life, social mobility, the location of industry, the distribution of income, the forms and concentration of economic and political power, and so on. Attention to such analysis, deriving from Marx, Weber, Sombart and Durkheim, should help to add to the engineer's understanding of the "social, political and philosophical implications and consequences of his work and influence in society at large".

The final aim of the book—to offer some clues as to the origins of innovations and institutions—is fairly well accomplished. In a book on the social history of engineering, however, it is a little disappointing not to find rather more discussion of the relations between science and engineering and, in terms of economic development, possible conflicts between them. How many countries have been successful in both together? Armytage touches on this problem in his treatment of Greece and Rome but does not take it much further. In contemporary Britain he simply treats research as the key to innovation, without much attempt to examine the problem of the best allocation of scarce scientific and engineering manpower between alternative uses. Here too I hope that the third edition of this book will include a discussion of the complex economics of research, development and innovation.

BRUCE WILLIAMS

ARCHAEOLOGICAL FINDINGS

The Annual of the British School at Athens

No. 60. Pp. 345+36 plates. (London: The British School at Athens, 1965.) 105*s*. net.

THE British School of Archaeology at Athens enjoys a high reputation in archaeological circles, not only for the general competence of its research and excavation organization but also for providing a wide range of papers

in its annual journal. The *Annual* for 1965 illustrates this scope of the School's interests in Aegean archaeology. Of some fifteen papers, more than half provide catalogues of regional or typological material; the range of subjects is varied, and includes bronze coinage in central Greece of A.D. 1,000, the provenance and identification of the Knossos sealings, and Late Helladic IIIA2 pottery from Mycenae. Also of importance is a paper on the geographical distribution of Minoan sites in western Crete. One excavation report, on work done at Palaikastro, VI, and one or two papers on specialized research subjects also find places in the *Annual*; of the latter, a detailed assessment of the *Battle of Oinoe* in the Stoa Poikile attempts to show how far Greek art and history can be reconciled in solving the problems raised by accepting that the battle of Oinoe occurred during the Pentekontaetea. Several difficulties cannot be resolved.

Although a majority of papers in the *Annual* deal with the typological and other problems of coinage, sealings and pottery from Greek and Cretan sites, two articles are concerned with the application of newer techniques of study and classification to material remains. The first of these is a study of composition patterns of Mycenean pictorial pottery from Cyprus, using optical emission spectroscopy, and following on earlier work on Mycenaean and Minoan pottery in the *Annual* for 1963. Almost all the twenty-five samples investigated correspond to a group previously shown to be of a fabric typical of much of the Peloponnese, and fourteenth and thirteenth century shipments of pottery from Peloponnese ports to Cyprus are therefore attested. Difficulties over sampling methods have apparently been eliminated.

Spectrographic analysis has also been used in the investigation of other materials from the Aegean, and a full report appears on obsidian, a material not particularly important to peoples living in Greece or the islands in the second or first millenia, but of considerable importance to their predecessors through some four or five thousand years. Obsidian as a raw material for the production of tools and weapons was traded extensively in the Mediterranean world, and every piece on the mainland of Greece had to be imported from regions where recent volcanic activity under certain conditions had produced obsidian that was glassy and could be fractured conchoidally. Only Melos, Antiparos and Giali in the Aegean possessed this material, although other areas to the west, north and east were also available. Spectroscopic examination and analysis have revealed various groups, with barium and zirconium important as trace elements, and the Melian obsidians are separated conclusively from those of Lipari in this way. The archaeological implications for this work, work which has been considerably extended since the paper was written, are varied. Perhaps the most striking is the very early use of Melian obsidian in Crete and Greece, in the late seventh millenium B.C. In fact, Melos was virtually the only supply of obsidian throughout the prehistoric period, and its trade and exchange in the area is particularly well attested in the late Neolithic and Early Bronze Age of the south Aegean. One of the interesting aspects of this study lies not in the material traded, but in its significance for early transport. Sea travel over great distances was clearly well within the capabilities of these people, although until recently it was assumed that only primitive forms of sea-going vessels would have been available. JOHN M. COLES

BINARY LOGIC

Logique Binaire et Commutation

Par J. Brunin. (Bibliothèque de l'Automaticien.) Pp. xvi+548. (Paris: Dunod, 1966.) 128 francs.

THIS book forms Volume 11 of a series of books by various authors (by no means all French) entitled "Bibliothèque de l'Automaticien" edited by Pierre Naslin, a well-known figure in the world of automatic control. The author, Jean Brunin, is a specialist in telephonic circuitry, and has published widely in the field of electromagnetic relay systems and the use of Boolean algebra in their analysis and synthesis. More recently he has devoted attention to the realization of such systems by electronic means. After a few examples on the use of truth tables for solving logical problems, including a particularly disarming one showing how a (quite exceptional) detective tracks down the perpetrator of some felony, the book thereafter clearly reflects the author's interest in relay circuits and their description in terms of Boolean functions. Thus the first ten chapters, occupying the first 450 pages, are exclusively devoted to such circuits and their synthesis from specified Boolean expressions, and only the last, admittedly longest, chapter treats electronic (that is, diode, transistor and ferrite) logic circuits, and most of these are associated with telephonic requirements.

Although the book is intended as a teaching manual, the enormous number of relay circuits, with their truth table representations and the all-too-brief explanations of their action, while forming a useful compendium for the practising telephone engineer, would leave a newcomer to the subject somewhat bewildered. Undoubtedly the inclusion of some questions would have been helpful.

While it may not be inappropriate in the present context, the treatment of electronic switching circuitry is cursory, no consideration being given to switching speeds, leakage currents, effects of loading, and so forth. The design, even the circuits, of practical logical elements are not given, and the use of such elements is restricted to the simulation of relay circuitry. Strangely enough, the diagrams in this section are noticeably inferior to those of the first ten chapters, and tend to be cramped, the resistors in particular being "hand drawn". A quaint feature, which I have never seen before, is the cross hatching of the base of non-conducting transistors.

G. DE VISME

COSMIC RAYS

Proceedings of the Ninth International Conference on Cosmic Rays

London, September 1965. Vol. 1: Pp. i–xiv+1–606. Vol. 2: Pp. xv–xxvi+607–1113. (London: The Institute of Physics and the Physical Society, 1966.) 210*s*. the two volumes.

THE past decade or so has witnessed an enormous expansion of activity in all branches of geophysics, and a measure of the current interest in cosmic rays is afforded by these proceedings of a recent international conference. The two volumes, totalling more than 1,100 pages, contain some 300 papers covering the whole field of cosmic rays. The papers fall into three categories. Fourteen major invited contributions cover the following topics: cosmic rays and the interplanetary field, the magnetic field in interplanetary space and the magnetosphere, the theory of cosmic ray modulation, magnetic fields in the galaxy, the Earth's radiation belt, the origin of cosmic rays, X-ray and γ-ray astronomy, quasi-stellar objects and radio galaxies, long period variations, and high energy interactions. Some 293 short papers are grouped according to subject: acceleration modulation, spectral composition, geophysical effects, isotopic distribution, extensive air showers, high energy interactions, muons and neutrinos, techniques. Seven other survey papers review the papers presented at the Conference on particular topics. The editors have also included verbatim reports of the discussions which took place at the meetings. These are sometimes very brief and contribute little, but other discussion reports include quite useful additional

points, and on the whole the inclusion of this material enhances the value of the volumes. With such an extensive field to cover, and with so many papers contributed, it is a commendable feature of the publication that all contributions, apart from the invited papers, are limited to about two thousand words.

This was the ninth of a series of international conferences organized since 1947 by the Cosmic Ray Commission of the International Union of Pure and Applied Physics and the first of the series to be held in the United Kingdom. In his preface to the volumes, Professor J. G. Wilson, chairman of the Organizing Committee, calls attention to this fact and rightly goes on to comment on how appropriate it was that the conference should meet at Imperial College in the month which marked the retirement of one of the great figures of the cosmic ray world, Prof. P. M. S. Blackett.

All too often a just criticism of the proceedings of scientific conferences is that their publication is delayed many months, sometimes even years, after the conclusion of the meeting and it is not unknown for papers presented at conferences to be outdated long before the proceedings appear. In the present case the editors and publishers are to be congratulated on achieving early publication of such a substantial body of material. The volumes are excellently produced by a photo-offset process and the price is reasonable. W. J. G. BEYNON

ORBIT THEORY

Orbital Hodograph Analysis

By Samuel P. Altman. (American Astronautical Society Science and Technology Series, Vol. 3.) Pp. 134. (Baltimore, Md.: American Astronautical Society, 1965. Distributed by Western Periodicals Co., North Hollywood, California.) $11.25.

CLASSICAL theories of celestial mechanics depend on expressing the properties of orbits in terms of orbital elements, for example, semi-major axis, eccentricity, etc., and then investigating the changes in the elements. An alternative approach, which is the subject of this book, is to make use of the hodograph, that is, to focus attention on the velocity or acceleration vector of the body in orbit. Chapter 1 is introductory and shows that the velocity hodograph of an ellipse, parabola or hyperbola is a circle, the origin being inside the circle if the orbit is an ellipse, on the circle for a parabola, and outside for a hyperbola: the hodograph thus allows changes from elliptic to hyperbolic orbits without the discontinuities which bedevil classical analysis. Other forms of hodograph are also defined. Possible analytical transformations are discussed in Chapter 2 and trajectories of ballistic missiles in Chapter 3. Orbital transfer and its optimization are the subjects of Chapter 4, and Chapter 5 is concerned with orbital rendezvous. Chapter 6, on lunar landing, shows how numerical solution by hodograph methods is just as good as by normal methods, despite the fact that the landing point, with zero velocity, becomes indeterminate in the hodograph formulation. A final chapter offers suggestions on the future uses of hodograph methods, including optimization problems and the n-body problem.

Dr. Altman has provided a useful introduction to a theoretical method which may well prove important in the future, and it is a pity that the book has several annoying faults. To begin with, symbols are usually left undefined in the main text, and the reader has to keep on referring to the list of symbols at the end of the chapter, where the definitions are not always immediately comprehensible. The book appears to be printed photographically from a typescript: unfortunately the print is very faint on many pages and part of page 69 was quite invisible in the review copy. Also on some pages the print extends to within 5 mm of the stitching on the spine and is unreadable unless the book is almost torn apart. Finally, the standard reviewer's complaint that the book is too expensive really does seem justified when it is so short and so poorly printed. D. G. KING-HELE

CARBONYL CHEMISTRY

The Chemistry of the Carbonyl Group

Edited by Saul Patai. (The Chemistry of Functional Groups.) Pp. xii+1027. (London and New York: Interscience Publishers, a Division of John Wiley and Sons, 1966.) 220*s*.

THIS is the second volume to appear in the series of large monographs on individual functional groups edited by Prof. Patai, and it follows very closely the pattern of its predecessor on the alkenes. The resemblance extends even to the fact that a salient chapter—in this case the one on equilibrium additions to carbonyl groups—is missing because the manuscript did not materialize on time.

There is no question but that this is a useful compilation, its seventeen chapters containing an abundance of information and a wealth of references. One does, however, wonder a little at the balance of space allocated to various topics: whether, for instance, the photochemistry of aldehydes and ketones merits the longest chapter in the book, and whether in the chapter on chemical and physical methods of analysis the former should receive as much emphasis as the latter. Multiple authorship makes it very difficult to maintain uniform standards, with the result that some chapters tend to become catalogues while others present a reasoned, and even elegant, review of their topic. Examples of the latter are the chapter on general and theoretical aspects of the carbonyl group, and the one on the basicity of carbonyl compounds, though it is only fair to say that their rather general subject matter assists in achieving a more spacious view.

The book includes extensive surveys of methods by which carbonyl groups may be formed as well as the reactions that they undergo, but there is no formal treatment of the effect of carbonyl on adjacent groups. This is not unreasonable except perhaps as it effects α-hydrogen, though there is some discussion of aldol and kindred reactions in Chapter 12.

The book's production standards are relatively mediocre and, large though it is, its price puts it beyond the reach of all but institutional buyers. PETER SYKES

ORGANIC REACTIONS

The Investigation of Organic Reactions

By Ross Stewart. (Prentice-Hall Foundations of Modern Organic Chemistry Series.) Pp. xii+125. (Englewood Cliffs, N.J., and London: Prentice-Hall Inc., 1966.) 20*s*. paper; 44*s*. cloth.

Reaction Mechanisms in Organic Chemistry

By Blanca Tchoubar. Pp. xiv+197. (London: Iliffe Books, Ltd.; New York: American Elsevier Publishing Company, Inc., 1966.) 45*s*. net.

A SOUND appreciation of the mechanisms of organic chemical reactions requires a reasonable background knowledge of how these mechanisms are determined and this *The Investigation of Organic Reactions* provides in a clear and concise manner. A detailed and satisfying survey of equilibria, reaction intermediates, transition states, reaction paths, and catalysis is given in the course of five chapters to each of which there are appended some half a dozen problems. The treatment is stimulating and the book can confidently be recommended both to undergraduates approaching the subject for the first time and to more experienced chemists who wish to renew their acquaintance with this field.

The second work is complementary to the first. Its purpose is to describe the fundamentals of electronic theory of chemical structure and of reaction mechanism over a wide field of organic chemistry. There are two sections. The first outlines the electronic theory of valency and includes a description of the transmission of polar effects in conjugated and non-conjugated systems, while the second and larger portion of the book deals with the various types of heterolytic and homolytic reactions and their occurrence in aliphatic and aromatic substitutions, in elimination and addition reactions, in prototropic processes, and finally, in reactions involving carbonyl groups. The book is clear and readable but its presentation is marred by numerous misspelt words and misleading statements. As an example of the former, there is written in connexion with Mannich bases, "... the case with which they de-animate . . . " (page 154). The second example results from Tchoubar's failure to distinguish between conjugative effects in the ground state (mesomeric effects) and those in the excited, transition states (electromeric effects). He remarks (page 175) that in aromatic nitration the steric effects of dimethyl groups in the *ortho* positions reduce or wholly suppress the deactivating and *meta* directing effects of nitro- and sulphonic-acid groups by suppressing the associated negative conjugative effects. This cannot be so, because only inductive effects can operate in such cases, and, indeed, in 1964, E. Baciocchi and G. Illuminiti showed that there was no reduction in the deactivating influence of these groups. In my opinion these and other blemishes seriously diminish the value of this book for the general reader.

E. ROTHSTEIN

BASIC ORGANIC CHEMISTRY

Modern Textbook of Organic Chemistry

By G. P. Ellis. Pp. xii+466. (London: Butterworth and Co. (Publishers), Ltd., 1966.) 57*s*. 6*d*.

IT is most pleasing to have a good, well-written book on basic organic chemistry by a British author. In this book, aimed at the first two years of an honours course, or equivalent, the variations in reactions of the functional group for aliphatic and aromatic compounds are usefully considered together. The first chapter, which introduces fundamental concepts, is, as is the whole book, simply and informatively written. Each chapter, sub-divided into well-enumerated sections, includes rigorous adherence to the IUPAC system of nomenclature, most importantly even for simple compounds. Reactions are classified according to their type, for example, electrophilic substitution, addition which, with the emphasis on actual reactions with yields as examples, should enable the student to appreciate the subject. A summary at the end of each chapter outlines the various methods of preparation of the class of compound being discussed while other useful assets are spectral characteristics, surveys of economically important compounds, and selected questions, to which answers are thoughtfully provided. The superb cross-referencing, a highlight of the book, together with an excellent index ensure that the student can rapidly find any particular topic regardless of the lecture course he may be following.

The treatment of some reaction mechanisms does not reach the high standard maintained in other aspects; some intermediate steps are omitted and little explanation of the driving force is given. This is accentuated by the omission or complete misplacement of arrows to designate electron pair shifts and will only serve to further confuse the student (pages 229, 237). Again, tautomerism is not an intramolecular hydride shift as depicted (page 14), and the proposed mechanism for osazone formation is quite wrong (page 389). The badly distorted chair forms of *cyclo*hexane with axial bonds at 90° to those in the ring are of no help in an understanding of stereochemistry.

I hope that considerable attention will be directed to the correct placement of dots, charges, arrows, and three-dimensional depictions in any revised edition, for the typographical errors mar what is otherwise a most useful text-book.

ROBERT D. H. MURRAY

FERTILIZATION

Fertilization

By C. R. Austin. (Foundations of Developmental Biology Series.) Pp. x+145. (Englewood Cliffs, N.J., and London: Prentice-Hall, 1965.) 21*s*. paper; 40*s*. cloth.

C. R. AUSTIN has been interested in the biology of the gametes for a long time and he has courageously undertaken to write this account of the cytological, physiological and behavioural mechanisms concerned with their union. Nor does he speak only as a compiler; his 20 years or more of research on this aspect of biology qualify him unusually well for the undertaking. The result is a notably significant book.

Too often in the past cytological studies of fertilization have been primarily descriptive with little attempt to evaluate the functional significance of the mechanisms involved. In this study the approach is from the functional rather than the purely morphological standpoint and much emphasis is placed on the method of experimentation. This book not only provides concrete information on gamete physiology, but also adopts a realistic attitude towards the larger problems and responsibilities growing out of our increased knowledge in this field. But although it is valuable to have our present knowledge of the subject accurately summarized and evaluated, a most important contribution of this slender volume is the attention it attracts to the problems that remain to be solved.

The first part of the book includes an introductory chapter with a concise account of the structure and function of the cell. This part then continues with a discussion of meiosis, mitosis and cell division. Then follow chapters which are devoted to a comprehensive and thorough discussion of the significance of fertilization, the form, differentiation, approximation, contact and fusion of the gametes, as well as the immediate consequences of fertilization. The final chapter describes unusual and abnormal forms of fertilization; this is a restrained and reasonable account which forms an effective conclusion to a book which is outstanding for its clarity and simplicity.

To make the book more readable each of the chapters has subheadings which are valuable in organizing the information. In addition references are not included in the body of the text. These have been covered by the larger treatises mentioned in the adequate bibliography at the end of the book, which also includes a comprehensive list of the chief monographs, reviews and original papers devoted to the subject of reproduction or more specifically fertilization. Excellent figures are included in the text as well as photomicrographs, many of which are original.

If any critical comment were called for, it would merely be that the text had brought to light disproportionately few new insights into mammalian fertilization. Austin would probably agree. From intent, however, the discussion of this aspect of the problem has been limited so as not to obscure the contributions made by the less commonly investigated species. Where there is a genuine paucity of evidence, thinking such as this, soundly based on what is known, is the one thing most likely to stimulate the research that will produce evidence, either for or against. No student interested in the physiology of the gametes can afford to be ignorant of this gem.

E. C. AMOROSO

MARINE BORERS

A Survey and Illustrated Catalogue of the Teredinidae (Mollusca : Bivalvia)

By Ruth D. Turner. Pp. vii+265 (64 plates). (Cambridge, Mass.: The Museum of Comparative Zoology, Harvard University, 1966.) $8.

FROM the moment that seafaring man started to use wood for his boats and piers, shipworms demanded attention on account of their unseen but devastating effects, and when later he became a scientist they aroused admiration because of their unique adaptive features. Yet the systematics of the family Teredinidae have remained in a state of confusion. This is partly a result of the erroneous creation of new species from fragments of moribund or dried remains found in drift-wood or ships from distant water, and of the fact that no comprehensive study of the family has until recently been undertaken. Dr. Turner has now marshalled information from libraries and museums over the world and examined all available material.

The results presented in the first part of the book include illustrated descriptions of the gross anatomy of the soft parts of fourteen genera of the family; these prove to be important in classification. There are also drawings and descriptions of the valves, tubes and pallets on which the diagnosis of both living and fossil species is based. The genera are redefined and a key is compiled for the identification of fresh or well-preserved adult specimens which is easy to use and will minimize one of the most formidable tasks of those working on teredine borers. Moreover, the second part of the book eases the systematist's problems, for it comprises a catalogue of all the generic and specific names which have been used in the family, with their original citation, type locality and the location of the type specimen; the present status of the name is indicated wherever possible.

The author points out that this is a preliminary survey and indicates a number of lines of research for the systematist, physiologist or ecologist who is interested in shipworms. Much-needed monographic studies of the various genera are now being planned. V. FRETTER

CYTOPHOTOMETRY AND INTERFERENCE MICROSCOPY

Methoden und Ergebnisse der Zytophotometrie und Interferenzmikroskopie

Herausgegeben von Walter Sandritter und Günter Kiefer. (Acta Histochemica: Zeitschrift für Histologische Topochemie, Supplementband 6. Herausgegeben von Hermann Voss, Gerhard E. Vogt und Joachim-Hermann Scharf.) Pp. 459. (Jena: Gustav Fischer Verlag, 1965.) 99.30 MDN.

THE collection of sixty-six papers presented at the ninth symposium of the very active Gesellschaft für Histochemie is a mine of interesting and stimulating information. The volume consists of two parts: the first deals with cytophotometry, the second with interference microscopy. Each part is subdivided into a description of instruments, their parts and arising problems, inevitable errors and results obtained in histochemistry. Each paper is supplemented with a bibliography and a critical discussion by the present participants.

Quantitative histochemistry is growing rapidly and instruments for physico-optical investigations were the prevailing topic of the symposium. Caspersson presented a new universal microspectrograph, where X-rays, ultra-violet, visible and infra-red light could be used. Unfortunately, instruments of this kind are very complicated and expensive; their equivalents in different laboratories are often inadequate. Caspersson suggested that laboratories should be specialized and planned on the national, or even international, scale. Sandritter assembled a list of chemical components present in cells, which could be localized and estimated quantitatively with physico-chemical methods. The list is supported with references and a description and bibliography of new instruments designed for this purpose. Thorell analysed microspectrographically the respiratory system of the cell and defined the metabolic state from an area of 1·5 μm diameter in a single intact cell. The problem of measuring the thickness of a section is important in quantitative investigations. Hallén re-examined this old subject and came to the sad conclusion: "The only way to avoid the error is to avoid sectioned material". Fluorescence microscopy combined with photometry was the subject of several papers (Ruch, West and others). The method is particularly useful, as the fluorescent spectrum is proportional with respect to concentration of the examined material within the living or fixed cell.

The complexity of physico-optical methods is often the source of errors. There are a few important papers dealing with this problem. Sandritter and Schlütter, for example, pointed out the significance of the depth of the field. Changing the depth or defocusing produces differences in value as much as up to 45–55 per cent. On the other hand, a comparison of results obtained from the literature dealing with interference microscopy, X-ray and chemical estimation showed that the values of measured dry mass differ by only about 15 per cent (Sandritter). Cytophotometry of DNA, RNA and proteins of normal and malignant cells stained with Feulgen, gallocyanin or other methods, is the subject of several other papers.

In a short review it is impossible to mention everything, particularly when the specification of the instruments is concerned. The book can be recommended for all interested in physical methods applied to histochemistry. J. KRUSZYNSKI

MICRO TO ULTRA-MICRO

Introduction to Quantitative Ultramicroanalysis

By I. M. Korenman. Translated by Scripta Technica, Inc. Translation edited by Ronald Belcher. Pp. ix+234. (New York: Academic Press Inc.; London: Academic Press, Inc. (London), Ltd., 1965.) $9.50.

Submicro Methods of Organic Analysis

By Ronald Belcher. Pp. ix+173. (Amsterdam, London and New York: Elsevier Publishing Company, 1966.) 55*s*.

BY title these two books are complementary and they do, indeed, give a good account of the present state of ultra-microanalysis. At first sight they appear to be quite different in approach and in the fields which they cover, but closer examination shows that this is more apparent than real. It is the result of the historical fact that although in the inorganic field methods which can be classed as ultra-microanalysis were used in the nineteenth century in inorganic chemistry, and have been increasingly studied since the early thirties, the much more exacting field of organic ultra-microanalysis has only been attacked relatively recently. The result is that Prof. Korenman's book, which in fact is inorganic, is an account of the work of a very large number of workers who have contributed considerably in important ways, and who include Korenman himself, Alimarin and Benedetti-Pichler.

Organic ultra-microanalysis, on the other hand, has mainly been developed by one school of analytical chemistry so that Prof. Belcher's book is in effect an account of one compact line of research, largely emanating from a single school. But it gives as fair an account of the present state of organic ultra-microanalysis as does Prof. Korenman's book in the inorganic field.

Probably one of the most telling arguments in favour of both books is that one realizes that ultra-microanalysis, whether in the inorganic or the organic field, has no longer that *noli me tangere* connotation that it used to have. Like microanalysis thirty years ago, it now has the right to be included in the equipment of any general analytical laboratory.

I cannot refrain from closing these reviews with an almost irrelevant comment. The attribution of "firsts", profitless though it may be, is intriguing to anyone with a historical bent, but must be handled with the greatest caution. Prof. Korenman, in saying that "as early as 1868, K. A. Timiryazev described some procedures for gas ultramicroanalysis", must surely be unaware that in 1855 Thomas Andrews described to the British Association the detection and measurement of hydrogen and oxygen from the electrolysis of water in amounts of approximately 10^{-4} cm^3. CECIL L. WILSON

The Origin and Evolution of the Universe

By Evry Schatzman. Translated from the French by Bernard and Annabel Pagel. Pp. 288+4 plates. (London: Hutchinson and Co. (Publishers), Ltd., 1966.) 84*s*. net.

THIS is a translation of a book which first appeared in French in 1955. Some of the developments in astronomy and cosmology since then have been mentioned in the English version, but the rapid advances and discoveries of the past few years (such as quasars) have not been included. There is a fairly extensive account of the state of knowledge of stellar astronomy, and there are sections dealing with theories of the origin and evolution of stars, stellar systems and the solar system. The title of the book is perhaps a little misleading; cosmology as such occupies a relatively small proportion of the contents.

The overall planning of the book and the choice of subject-matter are excellent. In general, the treatment of the individual topics is quite good; the discussion, which is mainly non-mathematical, is readable. Unfortunately the book contains a rather large number of careless errors. Names are mis-spelt and references are incorrect. There are also some inconsistencies of a more serious nature. To name one, on pages 36 and 37, short-period variables are assigned to the spherical component of the Galaxy without further comment; however, on pages 46 and 47 it is stated that the disk population includes *RR* Lyrae variables. In reality, only some short-period variables are of the *RR* Lyrae type; *RR* Lyrae variables are probably represented in both the disk and in the spherical components. J. B. ALEXANDER

Plasma Physics

(Lectures presented at the Seminar on Plasma Physics organized by and held at the International Centre for Theoretical Physics, Trieste, from 5–31 Oct., 1964.) Pp. 649. (Vienna: International Atomic Energy Agency; London: H.M.S.O., 1965.) 273 schillings; 78*s*.; $13.

APPROXIMATELY half of *Plasma Physics* is devoted to a series of introductory lectures. In these the important aspects of plasma physics are discussed by various authors both from the macroscopic points of view using the magnetohydrodynamic equations, and from the microscopic one. The latter includes a discussion of the self-consistent Vlasov approximation and of the transport corrections. These lectures are individually of a high standard and give a comprehensive introduction to plasma physics but inevitably lack continuity.

The other half of the book contains lectures given by experts discussing work in which they are actively engaged. Although these lectures were given nearly 2 years ago they contain much material which is still relevant.

One topic of current interest is the theory and experimental work on the stabilization of plasma to low-frequency (small compared to the ion cyclotron frequency) instabilities. A detailed discussion is presented of the stabilizing effects of Joffe bars, the concept of minimum B and its generalization to toroidal systems.

Another topic which receives considerable attention is the problem of plasma turbulence. In the limit of weak turbulence the basis of quasi-linear theory and its extension to include mode–mode coupling terms is described and the application of these theories to the problems of enhanced diffusion and the structure of collisionless shocks discussed.

Two lectures, one on fully developed turbulence and the other on the kinetic theory of weakly unstable systems, give a clear indication of the more fundamental aspects of plasma physics.

These lectures, particularly those in the second half of the book, provide a very stimulating exposition of some of the present-day problems of plasma physics. The printing is of a high quality which makes the book easy to read though, of course, not necessarily easy to understand. This collection of lectures is to be thoroughly recommended both as an introduction to plasma physics and as a reference book to the present-day problems in this field. G. ROWLANDS

Testing of Polymers

Vol. 1. Edited by John V. Schmitz. Pp. xiv+479. (New York and London: Interscience Publishers, a Division of John Wiley and Sons, 1965.) 147*s*.

IN materials science and technology, testing is an essential requirement: it provides an experimental description of the material as a means for the objective appraisal and comparative evaluation of its performance. As a result of the diversity and explosive growth of synthetic polymers and the limitless chemical possibilities of providing tailor-made polymeric products of desirable properties, a unification and co-ordination of the wide variety of testing procedures has become desirable. In this series of publications an attempt has been made to provide an authoritative discussion of all aspects of testing and in a form suitable for use by polymer chemists and physicists, material scientists, engineers and manufacturers, all of whom need to understand and apply test data.

Volume 1 of the series consists of fourteen chapters each of which covers some significant aspect of the testing of synthetic polymers. Most chapters review methods for evaluating individual properties, but other articles present background information or methods of evaluating individual properties, as well as service tests for end use purposes. The opening chapter discusses the purpose and the importance of standards, sources of standards, specification of materials and classification. This is followed by an account of the conditioning equipment for polymer testing. Chapters 3 and 4 which deal with the mechanical relationships in testing for the mechanical properties of polymers are concerned only with the basis of the tests and do not describe types and procedures for mechanical tests. The next five chapters (Chapters 5–9) cover in detail the wide subject of electrical property test methods and include d.c. dielectric conductance and conductivity, dielectric constant and loss measurements, electrical resistivity techniques and high voltage testing. The remaining chapters deal with specialized topics, testing for odour and taste, indentation and compression tests for floor coverings, cavitation erosion, and testing of gas and vapour permeation. The final chapter provides a useful literature coverage of selected references on sources of standards and tests for polymers.

This first volume is certainly a most valuable reference book and will be widely used by all interested in polymeric materials and their applications.

C. E. H. BAWN

MEETINGS

THE CELL NUCLEUS

DURING May 9–11, 1966, a meeting was held at the Radiobiological Institute of the Organization for Health Research TNO in Rijswijk, the Netherlands, on "The Cell Nucleus: Metabolism and Radiosensitivity".

J. Brachet opened the meeting with a paper on the crucial question of control of nuclear function. He pointed out that in many life forms the nucleus plays a key part in the production of RNA throughout life, but that the nucleus was, in fact, very sensitive to the activities of the cytoplasm and its function was intimately related to the cytoplasmic products. Brachet considered the type of cell substance that would influence the function of the nucleus, and pointed out the possibility that *s*RNA and possibly the derivative of *s*RNA without the -CCA terminus might be responsible for control of nuclear activity.

D. Mazia discussed the functions of cells in mitosis, and in particular the nature of the protein involved in the mitotic spindle and in mitotic contraction. He indicated that this protein contains a number of substructures of which at least two, the 4*S* and 2·5*S* units, are probably linked through disulphide bonds. He suggested that spherical elements of spindle fibres might be "popped out" of the strand, thereby producing shortening without thickening. J. H. Frenster discussed the hypothesis that heterochromatin (dense) and euchromatin (less dense) might be inactive and active forms of chromatin. He claimed that the euchromatin in the thymocyte is uncoiled and is therefore less dense.

In the next session, E. C. Henshaw discussed the isolation, purification and characterization of messenger RNA. An effort to isolate and purify this substance was undertaken with cytoplasmic RNA, particularly with liver polysomes. The rapidly labelled cytoplasmic RNA was studied and stimulatory activity for amino-acid incorporation into protein was found in the RNA fractions with sedimentation constants ranging from 6 to 14.

C. E. Sekeris dealt with the hormonal stimulation of *m*RNA synthesis. Much attention has recently been focused on the possibility of hormonal stimulation of *m*RNA synthesis, but it has yet to be shown that an increased amount of a specific type of messenger RNA does, in fact, produce a specific readout characteristic of a hormonal effect. Some hormones produce an increased RNA synthesis in some cells, but others do not.

M. Sluyser brought forward some evidence that the histones of the ventral prostate are different from the histones of other tissues. He suggested that hormones might combine effectively with histones and that this combination would produce changes in the activity of the genome. Cortisone may bind to acidic proteins and to histones in the cell and possibly more strongly to "arginine rich" (AR) histones. J. C. Sirlin reported labelling with ^{14}C-methionine of RNA components in *Smittia* larvae. He found that only the 4*S* peak of nucleolar RNA became labelled. This evidence, he suggested, supports the concept that the nucleolus synthesizes 4*S* RNA which has a transfer-RNA function.

M. Errera studied the DNA–RNA complexes of HeLa cells using a modified Sevag procedure and MAK chromatography. The DNA-linked RNase resistant RNA has a base composition similar but not identical to that of DNA. There is also present in this preparation an RNA which is of the poly *A* type although approximately 25 per cent quanylic acid was present after hydrolysis.

The second day of the meeting dealt with the control of gene function by histones and with the nucleolus. E. W. Johns reported that the new $f2a_1$ and $f2a_2$ fractions of the slightly lysine rich (SLR) histones made it possible to account for the histones of a given cell in five more or less equal fractions. He reviewed the evidence for the similarity and differences of histones of various species and pointed out that most animal histones are very similar, but those of plants differ considerably. Johns noted that the binding of DNA to histones varies considerably, and that the histones with the least affinity are the f_1 or very lysine rich (VLR) histones. The f_3 or "arginine-rich" (AR) histones have the highest affinity for DNA, but the histones that produce the most precipitation of DNA from a saturated solution are the VLR or very lysine rich histones.

G. J. M. Tonino discussed the yeast chromatin and found two species of histones present, one containing approximately 1·5 times as much lysine as the other (9·2 against 5·5 per cent). Further studies are being made on these fractions.

M. G. Ord showed that the f_3 "arginine rich" or AR histones contain some thiol groups but the other histones contain few or none. Efforts have been made to purify all these fractions to determine the amount of phosphate linked using labelled orthophosphate. Ord reported that the active, uncoiled chromatin or less dense chromatin contained less phosphate and the dense or coiled chromatin contained more labelled phosphate.

L. A. Stocken reported on the possibility that the thiol groups of nuclear proteins were responsive to X-irradiation. The effects of radiation on hexokinase, glucose-6-phosphate dehydrogenase and glutathione reductase were studied, but little effect was found on these sulphydryl containing proteins. Changes in conformation might, however, have occurred.

A. L. Dounce discussed the nativity of nucleoprotein gels. The methods for measuring the viscosity of nucleoproteins were discussed as a means of determining the stability of gels. Treatment of nucleoproteins with urea and dilute saline solutions was carried out to establish stability. Previous workers have reported that histones must be present with DNA in order to stabilize these gels. Dounce has found however, that DNA gels can be obtained, but these can be broken by sulphydryl reagents, such as dithioerythritol. Histones increased the rigidity of the gels, but a higher concentration of DNA also produces a rigid gel. The acidic proteins present in the chromatin or DNA preparation are probably essential to maintain rigid gels.

E. Harbers showed that the priming efficiency of chromatin from Ehrlich ascites cells was not affected by the presence of histones; moreover, X-rays, shearing and sonication did not alter the priming activity of the nucleoprotein preparations. Harbers reported that heterochromatin had a decreased binding capacity for histone in the presence of actinomycin and suggested that histones like actinomycin should be in the small groove of the DNA helix. In the liver and kidney 60–80 per cent of the chromatin occurred as euchromatin. One of the important questions about euchromatin is whether its presence is related to the activity of the DNA. Less than 5 per cent of the chromatin is probably active in most somatic cells, and the high values obtained by Harbers suggest that yet another mechanism must be involved. Frenster suggested that this may be the amount of single stranded DNA in the nucleus.

H. Busch has been concerned with isolation of nucleoli on a large scale in order to obtain sufficient material for satisfactory fractionation and characterization of nuclear components. Studies have been made on the 45*S* nucleolar RNA which is the precursor of 28*S* RNA of the ribosomes. Purification of 45*S* RNA by countercurrent distribution shows that much of the RNA belongs to a small number of species

W. J. Steele indicated that the turnover of RNA in the cytoplasm was increased in animals treated with thioacetamide. As a result, the synthesis of 55, 45 and 35*S* nucleolar RNA increases markedly, and along with this increment in synthesis there is both enlargement of the nucleolus and an increased amount of rapidly sedimenting RNA within the nucleolus. The lesion involved is not yet defined.

M. Muramatsu dealt with the order of biosynthetic events in the nucleolus and the lack of information of the biosynthesis of 18*S* RNA. Ultra-violet analyses of the base composition of nucleolar RNA have shown that 45, 35 and 28*S* nucleolar RNAs have the same base composition, but this differs from that of 18*S* RNA.

R. B. Hurlbert presented studies on the effects of histones on biosynthesis of RNA in the RNA polymerase system of the nucleolus. He indicated that various histone fractions apparently produce different changes in the base compositions of the newly synthesized RNA. Moreover, trypsin, which probably attacks the histones and presumably other proteins of the perinucleolar chromatin, produces marked changes in the base compositions of the newly synthesized RNAs.

T. Y. Wang showed that extracts of the nucleus provide nucleoprotein fractions which take up labelled amino-acids into protein without a requirement for *s*RNA or ribosomes. The synthesis of protein in this fraction may proceed by very different mechanisms from those described for cytoplasmic systems. Wang also reported procedures for fractionating the acidic nuclear proteins; the products formed differ primarily in their solubility at *p*H 6 and 5, and have a low content of glutamic and aspartic acids.

On the third morning of the meeting, G. Siebert discussed the energy metabolism of the nucleus. He indicated that a number of enzymes involved in oxidative phosphorylation are found in the liver nucleus but that a number of cytochromes and enzymes are not present. He pointed out the need to isolate nuclei under conditions in which the enzymes remain in the nucleus. R. Penniall showed that labelled orthophosphate was bound not only in pyrophosphate bonds to nucleosides and nucleotides in nuclear preparations, but also formed inorganic di- and tri-phosphates.

I. Betel presented evidence for the presence of a complete and functioning respiratory chain in isolated thymus nuclei. The inhibition of oxygen dependent nuclear ATP synthesis by carbon monoxide is light reversible, suggesting the involvement of cytochrome oxidase in this process. He also showed that thymus nuclei are capable of oxidative phosphorylation coupled to a respiratory chain.

D. Klouwen has studied the occurrence of nuclear oxidative phosphorylation in other cell types. Nuclear oxidative phosphorylation was demonstrated in various lymphosarcoma cells and a myeloid leukaemia cell. He brought forward evidence that the exhaustion of ATP synthesizing capacity can be regarded as a biochemical basis for early cell death after radiation (interphase death).

J. F. Scaife indicated that a primary lesion of radiation might be the effect on nucleohistones or deoxyribonucleoprotein. He suggested that there is some form of labilization of DNA followed by liberation of histones from these macromolecules.

H. Grunicke reported that there is a decrease in glycolysis following administration of alkylating agents which might be related to the effects on NAD, inhibited biosynthesis of enzymes or increased degradation enzymes or blocks in transfer reactions.

In the final paper of the meeting, K. Rees dealt with the effects of the carcinogens aflatoxin *B*, thioacetamide and dimethyl aminoazobenzene on the rat liver cell. Dimethyl aminoazobenzene interacts with the protein associated with DNA, while aflatoxin reacts directly with DNA in an analogous manner to actinomycin *D*.

H. BUSCH

OBITUARIES

James Anderson Scott Watson

THE death of Professor Sir James Anderson Scott Watson, C.B.E., M.C., on August 5 is a loss to agricultural science in Britain and elsewhere. Scott Watson was born in 1889 on his father's farm in Angus. After a year working on the farm he went up to the University of Edinburgh at a very young age and graduated D.Sc. in agriculture in 1908. He won nearly all the class medals and was awarded the Vans Dunlop postgraduate scholarship, which took him for one year to Berlin and the second to the Iowa State College of Agriculture.

He was appointed a lecturer in agriculture in the University of Edinburgh in 1910, and served in the Artillery during the First World War and was awarded the M.C. He succeeded Professor Robert Wallace in the chair of agriculture in Edinburgh in 1922. In 1925 he went to Oxford and followed Sir William Somerville in the Sibthorpian chair of rural economy and held that post until 1944.

The Government created, for the first time in 1942, a post of Agricultural Attaché to the British Embassy in Washington combined with agricultural adviser to the High Commissioner in Canada. Scott Watson was the first occupant and he soon won the respect of American and Canadian farmers.

It had been decided as the result of the Luxmoore Committee on agricultural advisory work to create a National Agricultural Advisory Service for England and Wales, and Scott Watson was asked in 1944 to return to London and inaugurate the service. Many will share the view that not the least of Scott Watson's contributions to British agriculture was that he achieved the preliminary work and launching of the N.A.A.S. in October 1946 so successfully. His reputation, ability, integrity and the universal respect in which he was held quietened many fears and smoothed out many difficulties —it is doubtful if anyone else at that time could have accomplished it so quickly. He was the first director of the Service, and when in 1948 he was appointed chief scientific and agricultural adviser to the Ministry of Agriculture, a new post of director general of the N.A.A.S. was created for him. He held both posts until he retired in 1954.

Scott Watson had a wide and intimate knowledge of farming and farming conditions and an equally wide knowledge of the sciences on which agriculture is based; he was an omnivorous reader and had a prodigious memory. By some of his colleagues he was regarded as the "universal provider". He was a "born" teacher, equally effective with undergraduates, scientific gatherings or meetings of practical farmers. His great gift was in being able to express his vast knowledge in simple, clear and fluent language suitable to his audience. He was also an effective broadcaster, and his talks in pre-war days on rural Britain, subsequently published in book form, were a joy to listen to and to read. Before he left Edinburgh he published with J. A. Moore, one of his former students and then his colleague, the first edition of *The Science and Practice of British Farming*, which has run into many editions and is the standard text book in many countries of the world. For some fifteen years he also acted as editor of the journal of the Royal Agricultural Society.

As a man he was a most lovable personality, always kind and considerate; if he was aware of his great gifts he carried them easily and they were never paraded. To any request for his services or for his help the answer was invariably yes if it was humanly possible. He hated to have to return no for an answer. He had a vast multitude of admirers and friends, and to the large group of those who had been his students at Edinburgh and Oxford was added a deep bond of affection—to them he remained to the end simply Jimmy Watson.

LASER RADAR PROBE FOR THE MEASUREMENT OF METASTABLE NITRIC OXIDE IN THE UPPER ATMOSPHERE

By L. J. NUGENT
Electro-Optical Systems, Inc., Pasadena, California

THE application of the pulsed gas laser in an optical radar probe for the remote determination of the location and concentration of molecular and ionic species in metastable state populations in the upper atmosphere has recently been proposed by Young[1]. In this technique the probe operation is in part similar to that of conventional *A*-scope radar. In addition, the gas laser emission comes from the same chemical species to be investigated in the atmosphere and it is necessary that the laser transition should terminate on an excited state which is metastable with respect to the ground state. The laser output pulse is transmitted into the upper atmosphere where a small fraction of the energy is absorbed by the species to be detected. The latter subsequently produces resonance emission with high efficiency in the low pressure region above 50 km, and this scattered radiation signal is detected at a receiver with an optical field of view aligned along the direction of the transmitted pulse. From an oscilloscope display of signal intensity against time, species concentration can be determined as a function of position in the atmosphere. In particular, such measurements of metastable state N_2 and N_2^+ by this type of laser radar are considered to be possible.

The detection of the concentration of nitric oxide (NO) in metastable $a^4\Pi_i$ state in the atmosphere appears to be impractical for the gas laser radar technique because of a difficulty inherent in the nitric oxide gas laser. An optical radar approach to this measurement could be attempted, however, if specially selected giant-pulse Raman laser sources[2] are used in place of the nitric oxide gas laser. Accordingly, the sensitivity of a metastable state NO* optical radar probe using the giant-pulse Raman laser was examined, and estimates of the minimum detectable NO* concentrations made as a function of altitude and sky radiation background conditions. The results indicated that this technique is feasible under certain background conditions and that it should provide the means of obtaining new information about metastable state nitric oxide in the upper atmosphere.

Several of Gilmore's[3] lower lying potential energy curves for nitric oxide are presented in Fig. 1. The metastable state of interest here is $a^4\Pi_i$ with lowest lying vibrational state quantum number $V'' = 0$. Transitions terminating on $a^4\Pi$, the allowed Ogawa $b^4\Sigma^- - a^4\Pi_i$ bands[4,5], and transitions originating from $a^4\Pi$, the forbidden $a^4\Pi - X^2\Pi$ bands[6,7], have been observed in emission. The lifetime for radiative decay from the lowest lying $a^4\Pi_{5/2}$ multiplet to the ground state has been measured to be 156 msec in a solid neon matrix at 4° K[7]. The effective radiative lifetime of $a^4\Pi$, however, may be somewhat shorter at the higher temperatures in the upper atmosphere because the slightly higher lying multiplets $a^4\Pi_{3/2}$, $a^4\Pi_{1/2}$, and $a^4\Pi_{-1/2}$, which have shorter expected lifetimes, could be significantly populated by molecular collisions. Nevertheless, the effective radiative lifetime in the environment of the upper atmosphere should be at least of the order of milliseconds, which is long enough to permit build-up of the $a^4\Pi$ state population, through photodissociation and molecular reactions, to many orders of magnitude greater than the populations of the other higher lying electronic states.

Absorption and resonance emission in the Ogawa bands provide the mechanism by which these metastable state populations could be investigated most easily in the upper atmosphere using the laser radar technique. The possibility of obtaining laser emission in the Ogawa bands or in any other bands of nitric oxide in a gas discharge has been discussed by Young[8], however, and it appears that the reaction $NO + N \rightarrow N_2 + O$ with rate coefficient $k \approx 2 \times 10^{-11}$ cm³/sec may considerably decrease, if not completely eliminate, the output that would otherwise be expected. Even if laser emission is obtained in the Ogawa bands, therefore, the intensity will most likely not be high enough for application in an atmospheric probe.

It is a fortunate coincidence that the Ogawa band heads expected to be strongest in absorption in the upper atmosphere, namely those corresponding to transitions involving initial vibrational state quantum number $V'' = 0$ and terminal state vibrational quantum numbers $V' = 2$ or 3, occur in spectral regions where detector sensitivity and atmospheric transmission are high and where giant-pulse Raman laser radiation can be efficiently generated. Optical radar probe operation in the spectral region 11,447–11,577 cm^{-1} for the band heads corresponding to $V' = 2$, and in the spectral region 12,595–12,719 cm^{-1} for the band heads corresponding to $V' = 3$, is therefore most suitable for the proposed nitric oxide measurements.

Giant-pulse Raman laser radiation at any narrow wavenumber-band in the above spectral regions can be obtained. First the means is provided for coarse-tuning the giant-pulse ruby laser radiation to within ± 14 cm^{-1} of a desired NO* Ogawa band head by

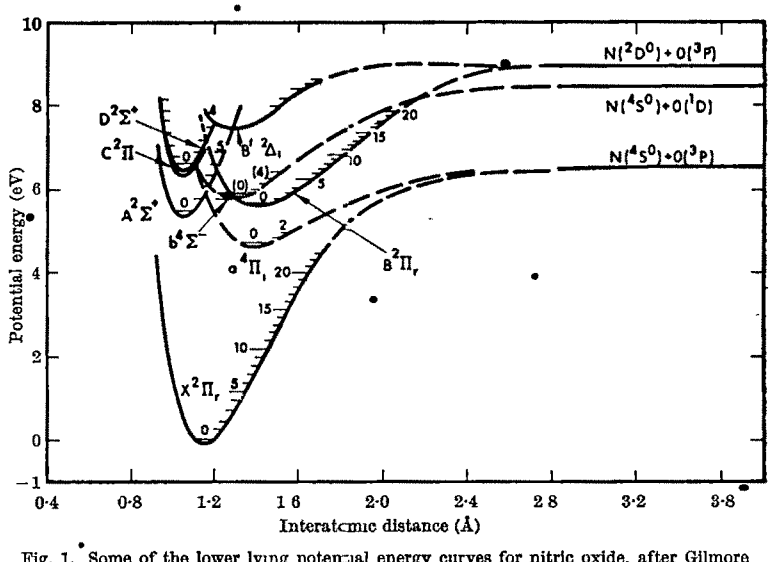


Fig. 1. Some of the lower lying potential energy curves for nitric oxide, after Gilmore (ref. 3). Estimated error: ———, < 0·02 eV; ± —, < 0·2 eV.

using the coherent Raman technique[9]. Then fine tuning is provided on a pulse by pulse basis by temperature tuning the ruby: ruby temperature control to within ±0·6° K over the range 77–357° K provides giant-pulse output selection over 14,418–14,391 cm^{-1} to within ±0·06 cm^{-1}. The coherent Raman technique for coarse tuning is more complex. The giant-pulse ruby radiation is Stokes-shifted, with about 10 per cent first-order conversion efficiency, by focusing it inside a cell containing a liquid, or a gas at pressures of 10 atmospheres or higher. The material is selected so that the frequencies and corresponding wavenumbers of totally symmetric molecular vibrations have the following restriction: the difference between the wavenumber of the ruby laser radiation and the wavenumber of the molecular vibration must be within temperature tuning range of a desired NO* absorption band head.

The following are examples of molecules with totally symmetric carbon–hydrogen (C—H) stretching frequencies such that they can be used to Stokes-shift the ruby radiation wavenumber to within temperature tuning range of the absorption region of some of the $V' = 2$ NO* band heads: CH_3I (gas) 2,969·8 cm^{-1}, CH_3Cl^{35} (gas) 2,966·2 cm^{-1}, CH_3F (gas) 2,964·5 cm^{-1}, CH_3Cl (liquid) 2,955 cm^{-1}, CH_4 (gas) 2,914·2 cm^{-1}, C_6H_6 (liquid) 2,899 cm^{-1}, C_2H_6 (liquid) 2,890 cm^{-1}, H_3CCN (liquid) 2,942 cm^{-1}, and CH_3COCH_3 (liquid) 2,925 cm^{-1}. Similarly, the following are a few examples of molecules that have totally symmetric carbonyl (C=O) stretching frequencies such that they can be used to Stokes-shift the ruby radiation wavenumber to within temperature tuning range of the absorption region of some of the $V' = 3$ NO* band heads: CH_3COH (liquid) 1,716 cm^{-1}, D_2CO (gas) 1,700 cm^{-1}, and H_2CO (gas) 1,744 cm^{-1}. In addition many other molecules fitting into either of these categories may be selected.

It is worth noting that giant-pulse coherent Raman emission has already been reported[10] for CH_4 at 11,488·9 ±0·5 cm^{-1} using room temperature ruby giant-pulse excitation, and this wavenumber exactly matches one of the band heads reported by Brook and Kaplan[4] for the Ogawa NO* system in emission. Although coherent Raman emission from the other substances above has not been reported yet, there is little doubt that it can be achieved with high efficiency in most cases. Typical output characteristics for the conventional giant-pulse ruby and for the corresponding giant-pulse Raman laser are presented in Table 1. It is safe to assume that lasers with the operating characteristics shown in this table can presently be made available for the NO* atmospheric probe.

Table 1. TYPICAL PARAMETERS REQUIRED FOR THE ESTIMATION OF THE MINIMUM DETECTABLE CONCENTRATION OF NO*

Source parameters	
Ruby laser	Raman laser
Output power, 33 MW	2–8 MW in first Stokes line
Output energy, 1 J	0·25–0·05 J
Pulse duration, 30 nsec	30 nsec
Bandwidth, < 0·1 cm^{-1}	< 0·5 cm^{-1} (liquid), 0 1 cm^{-1} (gas)
Beamwidth, ~ 1 mrad	~ 1 mrad
Repetition rate, ~ 15 cycles/min	~ 15 cycles/min

Detection parameters

Required voltage-signal-to-noise ratio = 5.
Number of times each measurement is repeated and the results integrated = 16.
Detection response time = 10^{-5} sec (resolution distance = 1·5 km).
Quantum efficiency of photodetector = 0 003 (*S*-1 photosurface with λ = 8,700Å).
Diameter of photon collector = 40 cm.
Estimated cross-section for absorption in the strongest Ogawa band heads of NO*, $\sigma = 10^{-13}$ to 10^{-14} cm^2.
Photodetector dark current = 1 8 × 10^{-14} amp (for *S*-1 photocathode cooled to −78° C and with a 2 cm^2 area).
Sky background irradiance,
$H\lambda$ = 6 × 10^5 photons/sec/cm^2/ster/Å (clear night).
$H\lambda$ = 10^9 photons/sec/cm^2/ster/Å (aurora at night)†.
$H\lambda$ = 4 × 10^{15} photons/sec/cm^2/ster/Å (day sky).
Detector bandwidth = 5 Å.
Detector field of view = 10^{-4} ster.

† Chamberlain, J. W., *Physics of the Aurora and Airglow*, 571 (Academic Press, New York, 1961).

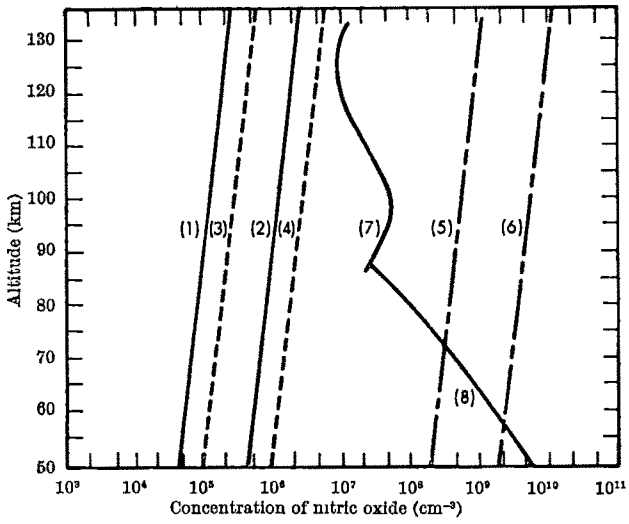


Fig. 2. Distribution of nitric oxide and calculated detectable concentrations of NO* at various altitudes in the atmosphere, where (1) and (2) are calculated using irradiance $H\lambda$ for a dark night sky and $\sigma = 10^{-13}$ and 10^{-14} cm^2, respectively. (3) and (4) are the same but refer to an auroral night sky. Similarly, (5) and (6) refer to the day sky. (7) is the distribution of total nitric oxide (refs. 12 and 13), and (8) the distribution of total nitric oxide (ref. 14). (1) to (6) are represented with sufficient accuracy as straight lines.

The sensitivity criterion for the NO* atmospheric probe is the minimum number density of NO* molecules that can be detected at a range h from the laser radar. Values of the parameters required for a sensitivity evaluation are listed in Table 1. Most of these numbers are accepted figures, but the estimated range for σ, the cross-section for absorption in the strongest Ogawa band heads of NO*, warrants special comment. Typical oscillator strengths for each vibrational band of formally allowed electronic transitions of nitric oxide lie between 0·002 and 0·02 (ref. 11). Because the Ogawa bands of nitric oxide are formally allowed and since it appears from the potential energy curves of Fig. 1 that the $V'' = 0 \rightarrow V' = 2$ and the $V'' = 0 \rightarrow V' = 3$ bands will have the strongest intensity, it is assumed that the oscillator strengths for these transitions lie at least within the above limits. Now there are six band heads in each Ogawa band and some of these overlap others. Furthermore, the Raman laser output has about the same bandwidth as each Ogawa band head and it can be tuned to overlap each band head. Therefore, it is reasonable to estimate the effective absorption cross-sections for each NO* band head as 10^{-14} to 10^{-13} cm^2, understanding that the absorption cross-section is proportional to oscillator strength and that an oscillator strength of unity corresponds to an absorption cross-section of 10^{-11} cm^2.

Values for the minimum detectable concentrations of NO* as a function of range for three representative background conditions and for the estimated factor-of-10 range in σ were calculated from the typical operating parameters listed in Table 1 (see ref. 1 for discussion of this type of calculation). These results are presented in Fig. 2, with recent estimates of the total nitric oxide concentration as a function of altitude above the Earth's surface, where the optical radar range is taken to be altitude. The total nitric oxide concentrations were taken from Nicolet[12] and normalized to agree with the measurements of Barth[13] for the region from 85 to 135 km. For the region between 50 and 85 km, they were taken from the *Handbook of Geophysics*[14]. It can be seen in Fig. 2 that for a night sky observation a signal would be obtained from an altitude of 50 km if the concentration of NO* is of the order of 10^{-4} of the total nitric oxide concentration, and that the corresponding fraction at 50 km for a day sky observation is of the order of 10^{-1}. Similarly, for an altitude of 135 km these numbers are 10^{-1} and 10^{2} respectively.

From this it appears that the chances for an NO* measurement of the day sky are not good; at best they are marginal at the stratopause and clearly out of the question at higher altitudes. The chances for an NO* observation of the dark night sky, the night sky with an aurora, or the twilight sky, however, appear to be very promising. Furthermore, the outlook for the future is even more promising because the probe sensitivity could be appreciably improved if a larger collecting mirror and/or the recently developed gigawatt-pulse ruby laser were used. Thus, the giant pulse Raman optical radar may become a tool for monitoring the concentration and chemical kinetics of metastable state nitric oxide in the upper atmosphere during the night or twilight hours, and will at least provide a means of setting upper limits on these unknown concentrations as a function of altitude and time.

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ELECTRON SPIN RESONANCE STUDIES OF THE REACTIONS OF NITRIC OXIDE AND NITROGEN DIOXIDE WITH POLYMETHYL METHACRYLATE

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AFTER exposure to nitric oxide, polymethyl methacrylate (PMMA) exhibits an electron spin resonance (ESR) spectrum, and its response to radiation is modified[1]. Ebert and Law[2] found the same ESR spectrum after exposing PMMA to nitrogen dioxide and inferred that the spectrum produced by each gas arises from its reaction with small amounts (of the order of 1 per cent) of methyl methacrylate monomer (MMA). Exposure of PMMA containing MMA to either gas inhibited the formation of the well-known 5 + 4 line ESR spectrum when the gas was pumped off and the PMMA irradiated in a vacuum. If purified PMMA is exposed to either gas, no ESR spectrum is developed and in the case of nitric oxide, subsequent irradiation of the PMMA in a vacuum produces a normal response.

This article describes an investigation of the effects of nitric oxide and nitrogen dioxide both on PMMA containing MMA, and on pure MMA, using the ESR technique. Various possible reaction schemes and mechanisms will be discussed.

PMMA was prepared from purified MMA by irradiation as described elsewhere[2] and was allowed to retain small amounts of MMA. Nitric oxide was obtained from cylinders supplied by Matheson and Co., Inc., and was purified by passing through aqueous potassium hydroxide and two cold traps of solid carbon dioxide and acetone. Nitrogen dioxide was prepared by heating lead nitrate ($Pb(NO_3)_2$); oxygen was present in the mixture of nitrogen dioxide and nitrogen peroxide in the proportion indicated by the reactions quoted below. A trace of nitric oxide may have also resulted from decomposition of nitrogen dioxide at high temperatures, but in the presence of oxygen this would have rapidly formed nitrogen dioxide at room temperature. Samples of PMMA for treatment were prepared by filing from a block of the polymer and evacuating the resulting filings with a diffusion pump for about 20 h to pressures of the order of 10^{-5} torr. The samples were prepared in glass tubes of the type shown in Fig. 1, and each sample contained approximately 0·1 g of PMMA filings in a gas volume of about 50 c.c. The volume of filings was more than sufficient to fill the part of the tube entering the resonant cavity of the ESR spectrometer. All experiments were done at room temperature.

The pressures of the gases on admission to the samples were measured with a mercury manometer. All pressures of nitrogen dioxide quoted are partial pressures of nitrogen dioxide in mm of mercury, not of dinitrogen tetroxide mixtures, and were calculated from the observed pressure of a mixture of dinitrogen tetroxide, nitrogen dioxide and oxygen on the assumption that lead nitrate decomposes on heating according to the reactions

$$2\,Pb(NO_3)_2 = 2\,PbO + 4\,NO_2 + O_2$$
$$2\,NO_2 \rightleftharpoons N_2O_4$$

The dissociation constant of dinitrogen tetroxide was taken to be 0·18 at 25° C.

Electron spin resonance signals were observed using a Hilger and Watts 'Microspin' *X* band spectrometer. All such signals described here appeared to be of the same shape (except where otherwise described in the text) and this shape did not alter on reduction of microwave power fed to the cavity. Relative intensities of such signals were measured from the peak-to-peak amplitude

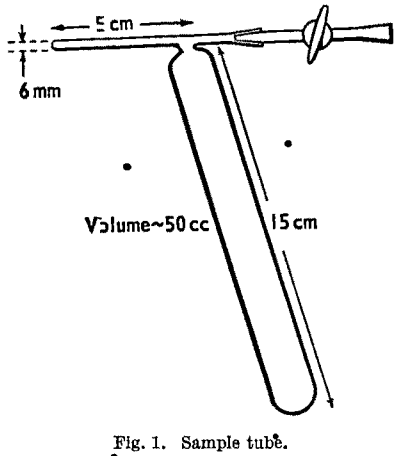


Fig. 1. Sample tube.

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of the main feature of the first derivative absorption signal.

Immediately after filing, the PMMA samples showed a weak asymmetric signal of the kind usually attributed to ROÓ, but this disappeared within a few minutes. Some samples were kept at 80° C for 24 h before exposure to nitric oxide or nitrogen dioxide, while others were kept in air for a similar interval of time before evacuation and exposure. These variations in initial treatment had no effect on their response to nitric oxide or nitrogen dioxide.

(*a*) Exposure of PMMA to nitrogen dioxide. Samples of PMMA filings continuously exposed to nitrogen dioxide at a pressure of 137 mm of mercury showed a very weak ESR signal within a few minutes of the start of the period of exposure. This signal decayed and disappeared within about an hour. Samples exposed to a lower pressure of nitrogen dioxide showed a larger signal which decayed more slowly. The signals were all of the form shown in Fig. 2, and had *g* factors close to two (ref. 2). Average curves for the growth and decay of the signals were plotted against time after admission of gas to the samples, for various gas pressures (Fig. 3). At the lower concentrations the gas was replenished at intervals of about 2 h by evacuation and immediate refilling, to maintain an approximately constant pressure. The gas remained in excess throughout each run at all concentrations. At all pressures a maximum was reached in the ESR signal intensity, after which the signal decays. Below 20 mm of mercury the decay rate was very low and at 9 mm of mercury it was not detectable. The relative values of these maxima are plotted in Fig. 4 against concentrations



Fig. 2. ESR signal from PMMA after exposure to nitric oxide or nitrogen dioxide.

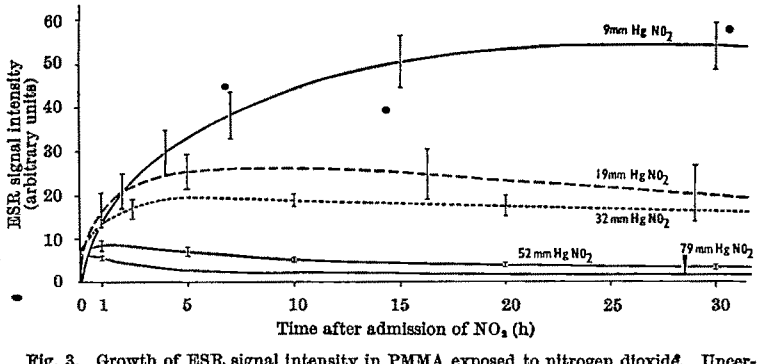


Fig. 3. Growth of ESR signal intensity in PMMA exposed to nitrogen dioxide. Uncertainties are standard deviations of means. Ordinate: ESR signal intensity (arbitrary units). Abscissa: time after admission of nitrogen dioxide.

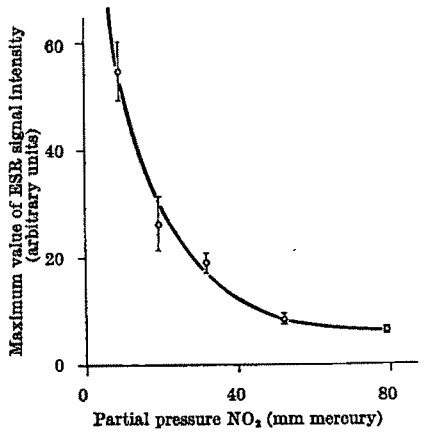


Fig. 4. Maxima of ESR signal intensities in PMMA exposed to nitrogen dioxide against partial pressure of nitrogen dioxide. Uncertainties are standard deviations of means. Ordinate: maximum value of ESR signal intensity (arbitrary units). Abscissa: partial pressure nitrogen dioxide.

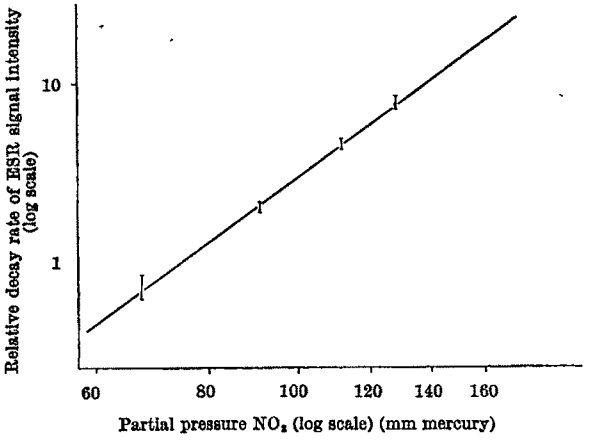


Fig. 5. Decay rate of ESR signal against partial pressure of nitrogen dioxide. Uncertainties are standard deviations of means. Ordinate: relative decay rate of ESR signal intensity (log scale). Abscissa: partial pressure nitrogen dioxide (log scale).

of nitrogen dioxide. If the gas was pumped off when the maximum signal intensity was reached, the signal was stable, in air or in vacuum, for several months. If samples which had been exposed to high concentrations of nitrogen dioxide for long enough for the ESR signal to disappear were then exposed to low concentrations of gas, no further signal was developed. If, on the other hand, samples which had first been exposed to low gas concentrations, and had developed strong signals, were then exposed to higher pressures of gas, the strong signals decayed within a few hours.

This procedure was adopted to investigate the rate of decay of the ESR signal in the presence of excess nitrogen dioxide. Several samples were first exposed to nitrogen dioxide at 9 mm for 48 h and then to nitrogen dioxide at pressures varying from 68 to 128 mm of mercury. Relative decay rates were plotted against concentration of nitrogen dioxide (Fig. 5).

(*b*) Exposure of PMMA to nitric oxide. Samples of PMMA exposed to nitric oxide developed ESR spectra apparently identical with those developed using nitrogen dioxide. Typical growth curves are shown in Fig. 6, where it will be seen that in the early stages the curve is concave upwards—that is, the growth rate of the signal increases. The

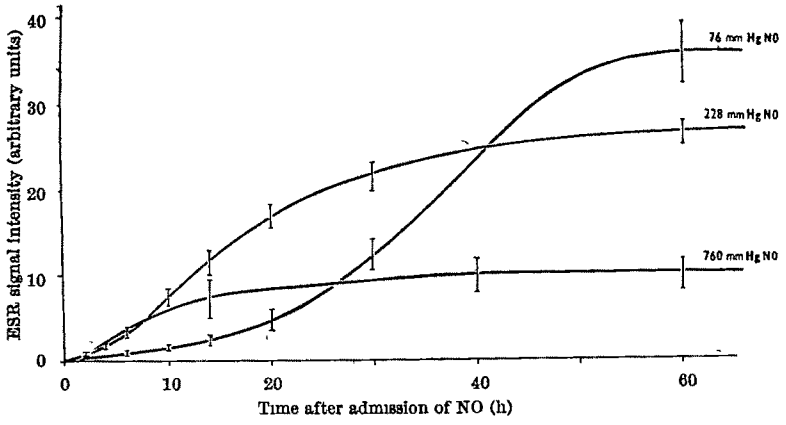


Fig 6. Growth of ESR signal intensity in PMMA exposed to nitric oxide. Uncertainties are standard deviations of means. Ordinate: ESR signal intensity (arbitrary units). Abscissa: time after admission of nitric oxide.

signal in all cases reached its final value within 60 h or less after admission of nitric oxide and then remained stable. At this time, a surplus of nitric oxide was still in contact with the PMMA at all concentrations. The signal was also stable in vacuum and in air for several months, as were signals produced by nitrogen dioxide. The relative intensity of this stable signal was plotted against concentrations of nitric oxide (Fig. 7).

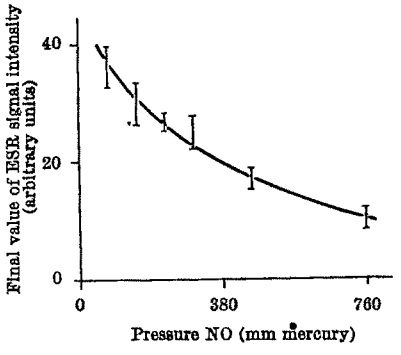
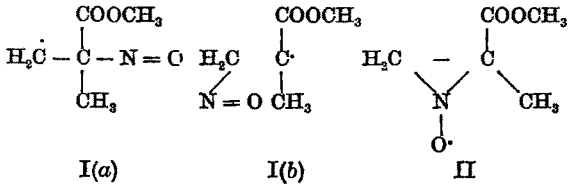


Fig. 7. Final values of ESR signal intensities in PMMA exposed to nitric oxide. Uncertainties are standard deviations of means. Ordinate: final values of ESR signal intensity (arbitrary units). Abscissa: pressure nitric oxide.

When samples are exposed to nitric oxide at atmospheric pressure and develop a stable ESR signal, they show no change in signal intensity if the nitric oxide pressure is later reduced. This is in line with the results from nitrogen dioxide. On the other hand, samples which are first exposed to nitric oxide at lower pressures and develop a larger signal show no reduction in that signal if the pressure of nitric oxide is subsequently increased, that is, the radicals are stable in nitric oxide at atmospheric pressure for periods of at least a week, in strong contrast to their rapid decay in nitrogen dioxide at 137 mm of mercury.

(c) Exposure of PMMA to nitric oxide and nitrogen dioxide alternately. Samples of PMMA were exposed to nitrogen dioxide at 137 mm of mercury for a few hours, until the ESR signal had disappeared. The gas mixture was then removed and nitric oxide at atmospheric pressure substituted. The sample turned blue but developed no ESR spectrum. Other samples of PMMA were exposed to nitric oxide at atmospheric pressure for 48 h until the ESR signal had reached a steady value. The nitric oxide was then removed and replaced by nitrogen dioxide at 137 mm of mercury. The samples lost their ESR signals and did not change colour. If nitrogen dioxide was used at 9 mm instead of 137 mm of mercury, no change in the ESR signal intensity was observed. Thus each gas attacks the same site

The two main aspects of this work which must now be considered are: (i) the chemical identity of the radicals, and (ii) the reaction systems giving rise to the kinetics described above. Ebert and Law[2] found that PMMA only showed an ESR spectrum after exposure to nitric oxide and nitrogen dioxide if it contained small amounts of MMA, regardless of the method used to prepare the PMMA. This result showed that the radicals arose from reactions involving only MMA and the gases, and it was suggested that the gases reacted with the C=C bond in the MMA. It was also established in the same paper that the nitrogen atom was closely associated with the orbit of the unpaired electron and little if any proton splitting was observed. This suggests radicals of type II rather than of type I.



After nitric oxide had been bubbled through pure MMA, and any PMMA which resulted had been filtered off, the radicals formed in the liquid could be extracted with ether; this suggests that the molecule exists as a monomer. In an attempt to convert these radicals to a more stable form, the reaction mixture was reduced by hydrogen over platinic oxide as catalyst, and the group $\begin{matrix} C-C \\ \diagdown\diagup \\ NH \end{matrix}$ looked for. It is known, for example, that di-methyl ethyleneimine has absorption bands at 425 and 303 mμ. The reduced radicals showed a band at 425 mμ but also an end absorption below 310 mμ, possibly from unreduced radicals, or unreacted MMA, which may have obscured the 303 mμ band. A Liebermann test gave a weak positive result for a secondary amine.

An attempt was made to isolate the resulting reduced compound by vacuum distillation. A viscous yellow residue resulted, which could not be distilled without decomposition at 120° C/3 mm of mercury. The residual liquid did not form a picrate, but gave a positive test for nitrogen. The infra-red absorption spectrum of the ether-extracted material contained a peak at 1530 cm^{-1}, suggesting nitroso groups. This peak disappeared on reduction of the radicals, but the spectrum of the reduced compound was difficult to interpret. Although no definite identification of the radicals can be made, the balance of evidence certainly favours type II.

An interpretation has been made of the kinetics of the reactions between PMMA and nitrogen dioxide. It must first be established that the kinetics are not limited by the rate at which the gases diffuse into the PMMA particles. The particle size in the PMMA samples was such that all the material was within 100μ of a surface. Powder samples of PMMA made up in the same way and irradiated in a vacuum developed the well known 5 + 4 line ESR signal which was removed with a time constant of a few minutes, or less, when either nitric oxide or nitrogen dioxide was subsequently admitted. Thus nitric oxide or nitrogen dioxide was available to all the material in the PMMA samples within a few minutes of admission of the gas, that is, in a time which was short compared with the time

scale of the growth curves (Figs. 3 and 6). A reaction model which is consistent with the observations in nitrogen dioxide can be put forward without the exact identity of the radicals requiring to be established first. Let us suppose that the reactions are of the general form

$$\text{MMA} + \text{NO}_2 \rightarrow X \quad (1)$$

$$X + \text{NO}_2 \rightarrow Y \quad (2)$$

where X is a radical (possibly, for example, Type II above) having the ESR spectrum of Fig. 2, and Y has no ESR spectrum. Either reaction may proceed by more than one stage. The weak component of the ESR signal, referred to by Ebert and Law[2], is disregarded in this analysis, as it was never responsible for more than 2 per cent of the observed signal intensity.

It is assumed that reaction (1) proceeds at a rate proportional to the concentration of nitrogen dioxide, N, which is constant in any single experiment, while reaction (2) proceeds at a rate proportional to some power α of N. (It may be seen later that if $\alpha = 1$ we should get a series of growth curves for different values of N which would have the same shape and would differ only in their time scale. This would conflict with the data in Figs. 3 and 4.)

If M is the concentration of reactive sites in MMA and x the concentration of radicals X at time t, then

$$\frac{dM}{dt} = -k_1 NM \quad (3)$$

$$\frac{dx}{dt} = k_1 NM - k_2 N^{\alpha} x \quad (4)$$

where k_1, k_2 and α are constants. k_1 and k_2 refer to reactions (1) and (2) respectively. The initial conditions governing these equations are

$$\text{at } t = 0,\ M = M_0$$
$$x = 0$$

The solution of the equation is then

$$x = M_0 \left(\frac{k_1 N}{k_1 N - k_2 N^{\alpha}}\right)(e^{-k_2 N^{\alpha} t} - e^{-k_1 N t})$$

On this model the maxima of the growth curves in Fig. 3, X_m, are related to the times at which they occur t_m and N by the equation

$$\log_e\left(\frac{X_m}{M_0}\right) + k_2 N^{\alpha} t_m = 0$$

If estimates of X_m and t_m based on the observations shown in Fig. 3 for various values of N are substituted in this equation, a least squares fit method yields a value for α of $2{\cdot}7 \pm 1{\cdot}0$. An estimate of α can also be made directly from the experimental results, and this provides an indication of the validity of the reaction model. In the experiments to investigate the rate of decay of the ESR signal in different concentrations of nitrogen dioxide reaction (1) has been virtually eliminated by allowing a small gas concentration to convert nearly all the available sites M to radicals X. This removes the $k_1 NM$ term from equation (4). If N is then increased suddenly to a relatively large value, only reaction (2) occurs to an appreciable extent since M is small. As a consequence of this we can, under these conditions, plot the rate of decay of x for various values of N, as in Fig. 5, and get a straight line of slope α. The experimental value of α obtained directly from Fig. 5 is $3{\cdot}5 \pm 0{\cdot}3$, which is in excellent agreement with the value of $2{\cdot}7 \pm 1{\cdot}0$ predicted on the basis of the reaction model. The best estimate of α to the nearest integer is three. This might correspond to reaction (2) requiring three nitrogen dioxide groups to convert each X group to Y. Since X and nitrogen dioxide are both radicals and Y is not, an odd integer might be expected for α rather than an even one. It is not clear why three nitrogen dioxide groups rather than one should be required, but this should be useful in the eventual identification of X and Y.

The results shown in Figs. 3–5 are thus consistent with a simple reaction scheme of the type outlined here. If the pressure of nitrogen dioxide in contact with PMMA is varied, from a low concentration to a high concentration, the reactions proceed to the degree corresponding to the higher concentration, whereas if a high concentration is used first, all the reactive sites in the MMA are filled by nitrogen dioxide (twice over) and further radical production in the powder does not occur. Similarly if nitric oxide is used following a high concentration of nitrogen dioxide no signal would be expected, while nitrogen dioxide after nitric oxide removes the ESR signal produced by the nitric oxide.

With nitric oxide no simple reaction model of the type suggested for nitrogen dioxide will explain the observations in Figs. 6 and 7, because the radicals X are stable in nitric oxide, that is, no decay reaction of type (2) occurs in nitric oxide. The ESR spectrum is the same after exposure to either gas, with each feature having the same g factor. Moreover, pure MMA, when exposed to either gas, shows the same triplet ESR spectrum at room temperature, and this transforms to the spectrum of Fig. 2 at 77° K (ref. 2). This implies that the same radical is responsible in each case, and suggests that nitric oxide may react with oxygen to form nitrogen dioxide which then reacts with MMA as previously described. The growth curves in nitric oxide (Fig. 6) are concave upwards in their early stages—that is, the rate of production of radicals is increasing during this period. This is consistent with a reaction which proceeds in two or more stages. It is difficult to suggest any likely source of oxygen in the PMMA samples after evacuation, however, or in the MMA which had been de-gassed by repeated freezing, evacuation and thawing. The results represented in Figs. 3, 4, 6 and 7 (in which the ordinate units are the same) show that the effects observed with nitric oxide could not be due to traces of nitrogen dioxide present as an impurity.

The results reported here with nitric oxide and nitrogen dioxide are qualitatively similar to some radiobiological results involving nitric oxide, and the possibility that they might arise from a similar mechanism is of some interest. It is often found that nitric oxide reduces radiation damage in dry biological systems and increases damage in wet systems as compared with radiation damage in nitrogen[3–7]. Dale *et al.*[5] found that if the pressure of nitric oxide in contact with *Shigella flexneri* during irradiation was varied, the effect of the gas on the radiosensitivity of this organism (measured as the reciprocal of the dose required to kill 90 per cent of the bacteria) showed a maximum at a nitric oxide pressure of 76 mm of mercury. Russell and Davies[8] found the same effect in *Escherichia coli* and Johansen and Howard-Flanders[7] have recently confirmed these results. No explanation of them has yet been reached but work on simple systems such as that described here on MMA and PMMA may prove a useful step towards such an explanation.

I thank Dr. M. Ebert of this laboratory for his interest and for many discussions in the course of this work, and Dr. B. W. Fox, also of this laboratory, for valuable suggestions and advice in attempting to identify the radicals.

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HIGH LOAD-PRESSURE MINERALOGICAL RELATIONS IN CHONDRITES

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An advance in the understanding of the mineralogical relationships in chondrites was made with the abandonment[1,2] of the hypothesis[3] that chondrites are formed at temperatures below 1,000° C and at high volatile pressures.

This had been based largely on the temperature relations of orthopyroxene at 1 atm. Recent data[4] show that the infrequent occurrence of clinoenstatite is no indication that the temperature of original crystallization was about 1,000° C because at load-pressures of more than 8 kbars those conditions necessary for the formation of clinoenstatite no longer exist. Although it is possible that a little iron in solid solution could affect these relations, this seems unlikely in chondrites. Boyd and England further show[4] that the field of stability of clinoenstatite lies in fact below 600°–700° C. It is, however, improbable that the clinoenstatite observed in chondrites is formed at such low temperatures, for clinoenstatite is most likely to occur by inversion from protoenstatite at load-pressures of less than 8 kbars and temperatures[2] of about 1,200°–1,300° C.

The presence of (100) lamellae in orthopyroxene (Figs. 1 and 2) may result simply from the exsolution of lime-rich portions from the orthopyroxene and can occur at any pressure; but the presence of (001) style lamellae indicates the former presence of a clinopyroxene, and therefore of load-pressure conditions of less than 8 kbars. Barwell, Bjurbole and Indarch chondrites are examples of the former. The clinopyroxene to orthopyroxene inversion textures are rarely seen in chondrites (for example, Chainpur chondrite[5]), and it seems likely that protoenstatite did not exist as a primary phase in the majority of chondrites. This implies that at the temperatures of 1,200°–1,300° C now suggested[2], the primary crystallization would have occurred at load-pressures of more than 5 kbars.

The term "load-pressure" is here used to indicate pressure resulting from the weight of a superincumbent load, in contrast to the pressure resulting from volatiles. Because pressure due to load, P_l (often misleadingly termed hydrostatic pressure), and pressure due to volatiles (or vapours), P_v, commonly have opposite effects on the temperature of crystallization of solids, it is desirable that the distinction be clearly made.

As a result of examining the hypersthene-chondrite which fell at Barwell, Leicestershire, recently[6], two aspects have aroused interest: (*i*) the effect of load-pressure on chondrites; (ii) the role of plagioclase and the significance of the platey and prismatic texture in many chondrules.

Various authors[7–9] have put forward examples in favour of the formation of chondritic material at high load-pressures, while others[2,10–12] prefer lower load-pressures. Although meteorites examined now show low load-pressure relationships, it does not eliminate the possibility that many meteorites may have initially existed in a high load-pressure regime but were subsequently equilibrated to lower pressures. There has been much discussion[2,9,11–14] of the recrystallizations said to occur in meteorites, and it is thought that in many cases recrystallization may be related to the changes of pressure suggested here. Fig. 3 illustrates an exception to this. The metamorphism described by Wood[13] implies processes associated with increases of temperature and perhaps of pressures (P_l or P_v)—that is, conventional geological metamorphism as well. In contrast, it is suggested here that many of the phase changes resulted from the release of load-pressure largely by adiabatic expansion, followed by a fall in temperature. The normal type of metamorphism may also occur, but to a lesser extent. This theory is related to Mason's[15] suggestion that chondrules are porphyroblasts formed by solid state thermal recrystallization.

It has been customary in the past to correlate load-pressure relationships observed in meteorites with depth below surface and thus with size of the parent body. Some[3,8,16] have inferred that this parent has lunar dimensions; others[10,12] prefer asteroidal or smaller dimensions. More recently, high pressures resulting from shock during impact in space or on landing such as Lipschutz and

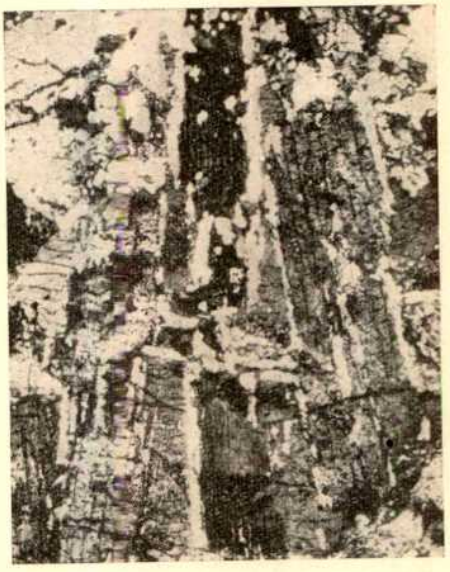


Fig. 1. Prismatic hypersthene crystals with (100) exsolution lamellae and rims of clinopyroxene. Chondrule in Barwell meteorite. (× c.35 crossed polars.)

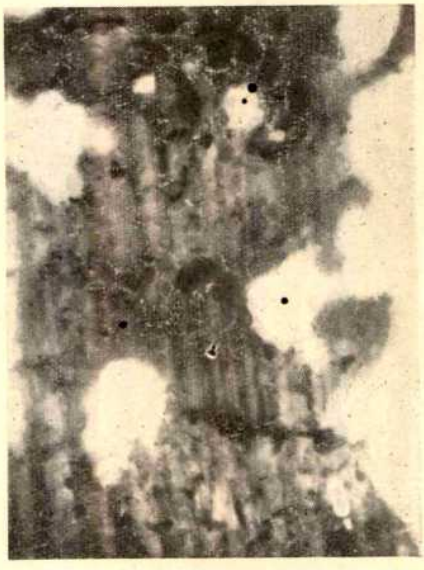


Fig. 2. Enlargement of portion of Fig. 1 showing the (100) exsolution lamellae in hypersthene. (× c. 720, crossed polars.)

Anders[17] have suggested for the origin of diamonds have been favoured[2,18] to account for the high load-pressure features.

It is difficult to reconcile existing ideas of processes involved in asteroidal or smaller bodies[2,19] with ideas which have been put forward to explain the relation between the various chondrites, achondrites and irons and their correlation with the structure of the Earth[1,9,20–23]. The latter ideas are currently accepted in one form or another by most mineralogists, and consequently theories must now be based on a real planetary model.

In subsequent paragraphs it is shown that load-pressures of 20 and 30 kbars may have been realized in the early history of chondrites. This would indicate on the above model a parent body or bodies of lunar or half lunar dimensions. (The central pressure in the Moon, radius 1,740 km, is about 47 kbars.) This raises the difficulty that, so far as is known, there is not enough asteroidal material to provide for one or more such large bodies.

The presence of plagioclase in chondrites has received scant attention[24], for plagioclase is often only represented by glasses and these need X-ray micro-probe methods to determine their composition[25]. In the Barwell meteorite, crystalline oligoclase plagioclase is present in the chondrules and in the matrix. It occupies a quarter of the volume of many chondrules, although it is absent from some which consist of orthopyroxene with exsolved clinopyroxene (Figs. 1 and 3). The plagioclase occasionally shows albite twinning (Figs. 4 and 5). This is unusual but not unknown elsewhere (for example, the Estacado chondrite[26]), and suggests that the Barwell meteorite has been more thoroughly re-equilibrated to lower temperatures than chondrites which show only cryptocrystalline plagioclase[14,24].

The examination of several chondrites under the polarizing microscope led me to recognize the similarity between the textures of barred and fibrous chondrules and the textures arising from exsolution seen in terrestrial pyroxenes. Grigoryev[27] has shown that chondrules occur in three simple textures: eccentrically radial, graticulate and porphyritic, and that they are related by recrystallization. Thus Harpum's comprehensive list[28] of twenty-five types of chondrules in Tanzanian chondrites can be reduced to Grigoryev's three classes.

These textures have been particularly well portrayed in the illustrations of the papers by Wood[19], Christophe-Michel-Levy[25] and Harpum[28]. Some examples of Barwell chondrules are shown in Figs. 1–9. Another textural

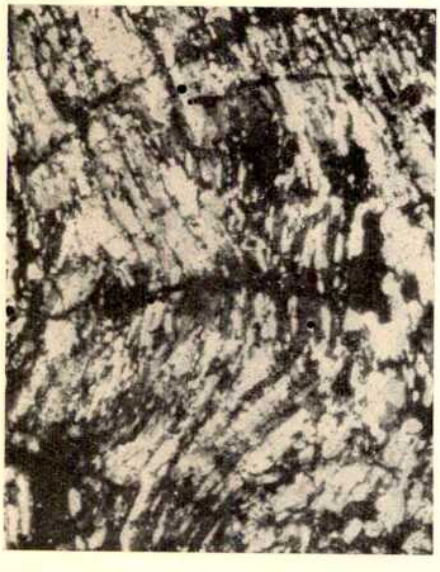


Fig. 3. Bent hypersthene prisms in fibrous hypersthene chondrule from the Barwell meteorite. Evidence of deformation at time of crystallization of the pyroxene. (× *c.*190, crossed polars.)

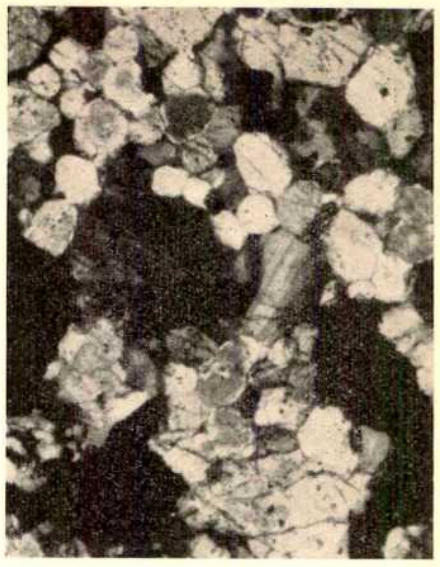


Fig. 4. Twinned oligoclase in part of a granular olivine-pyroxene-plagioclase chondrule from the Barwell meteorite. This chondrule is poorly defined and is texturally half-way between well defined chondrules and the matrix. (× *c.* 120, crossed polars.)



Fig. 5. Twinned oligoclase in olivine-plagioclase chondrule. Enlargement of portion of Fig. 6. (× *c.* 640, crossed polars.)

feature which has been noticed, particularly in the barred olivine-chondrules, is the polygonal outline (see Figs. 6 and 7) reminiscent of the shape of garnet crystals. A search of the literature showed this observation was not original, having been made by Sir Lewis Fermor[29] many years ago in the Khohar meteorite. He postulated[29] that garnet was converted to enstatite by a reduction in the pressure conditions. Later, he suggested[30] the existence of a new garnet called "khoharite" ($Mg_3Fe_2Si_3O_{12}$). The existence of "khoharite" was never substantiated, and Fermor's theory was abandoned.

The original observation of the polygonal outline of many chondrules can, however, be confirmed and needs to be explained. A modern interpretation might be obtained from the recent experimental work of Kushiro and Yoder[31], who have shown that garnet (pyrope : grossular = 2·1) stable at high load-pressures (> 20 kbars) and temperatures (1,200°–1,300° C) inverts on a reduction of the load-pressure to less than 8 kbars, and through intermediate phases of pyroxene, spinel and plagioclase, to a mixture of forsterite and anorthite plagioclase. They

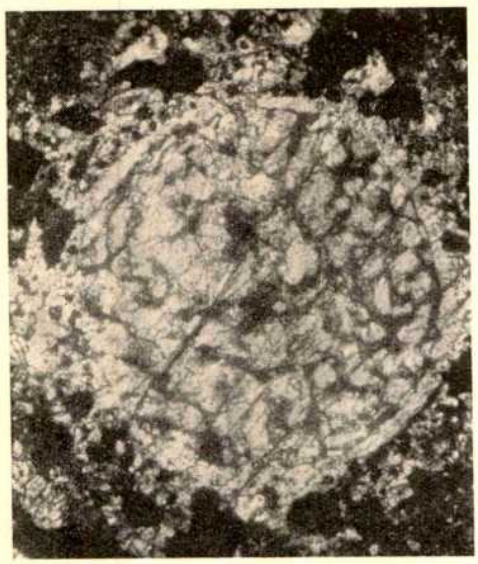


Fig. 6. Olivine-plagioclase chondrule with polygonal outline from the Barwell meteorite. The olivine is nearly all in optical continuity, but the plagioclase extinction is quite irregular. (× *c.* 75, crossed polars.)

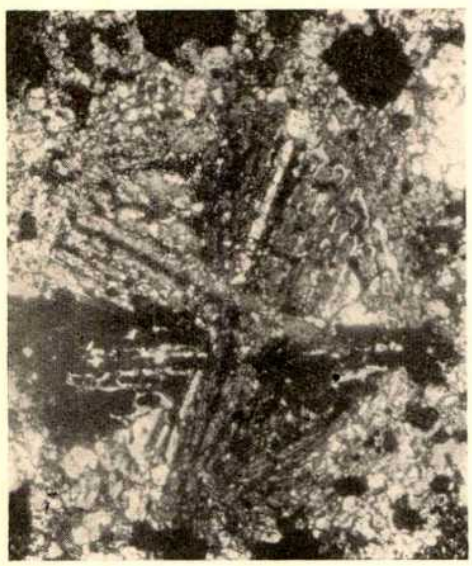


Fig. 7. Olivine-plagioclase chondrule from the Barwell meteorite with polygonal outline showing curious texture possibly related to the "hour-glass" structure. (× *c.* 45, crossed polars.)

have also shown[31] that similar garnet together with quartz at similar pressures and temperatures invert by way of a comparable sequence of events to enstatite and anorthite plagioclase. Because the plagioclase in chondrites is oligoclase, these experiments make the original presence of garnets in chondrites doubtful.

More recent experimental work by Kushiro[32] has shown that at 10 kbars load-pressure solid forsterite and liquid (albitic) co-exist at 1,400°–1,500° C, but that at load-pressures of 15–22 kbars at these temperatures, ortho-pyroxene, forsterite and liquid (albitic) become the stable phases. This assemblage bears a close resemblance to the minerals present in chondrules, even to the glassy representation of the albitic liquid. Kushiro also found[33] that at 22 kbars and 1,100° C, an albite–nepheline–forsterite composition glass crystallizes to give a jadeitic pyroxene. In other experiments, Yoder and Tilley[34] have shown that forsterite and jadeite co-exist at 33 kbars load-pressure and 1,250° C, and Bell and Davis[35] have shown that jadeite and diopside co-exist at 30 kbars load-pressure at 1,200°–1,300° C.

Coupled with these experimental data are the observations by Mason[24] and Wood[19] on the relative abundances of orthopyroxene and clinopyroxene in chondrites, and the fact that the absence of plagioclase can be correlated with the presence of lime-poor clinopyroxene (pigeonite). Together, these data suggest that jadeitic pyroxene with or without forsterite may have formed the primary material from which the chondrules formed. The jadeitic pyroxene would probably have been a pigeonite with a small amount of jadeite ($NaAlSi_2O_6$) in solid solution. Mason arrived at a similar conclusion from other considerations[24,26].

In some chondrules, a curious texture, as shown in Fig. 7, is seen. The angle between the adjacent sectors of the chondrule is about 75°. This is also the value of the angle between the x and z crystallographic axes of clinopyroxenes. Another chondrule (Fig. 8) shows a texture which is reminiscent of "herring bone" twinning,

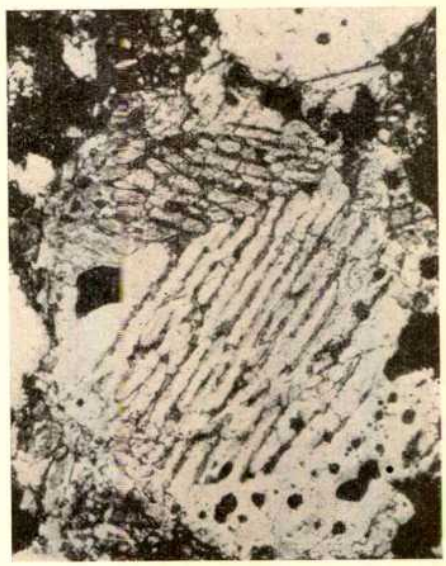


Fig. 8. Olivine-plagioclase chondrule from the Barwell meteorite showing texture related to "herring-bone" twinning. (× *c.* 120, crossed polars.)

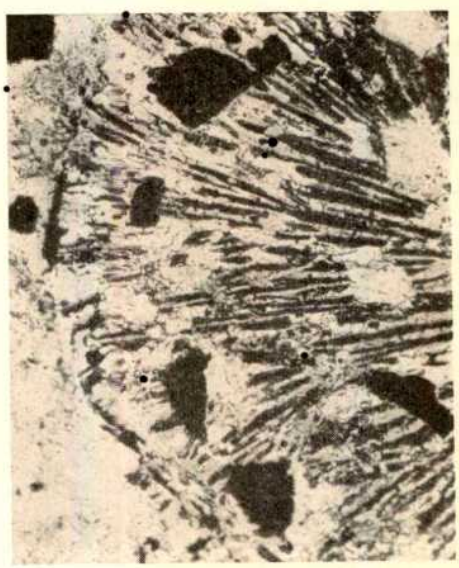
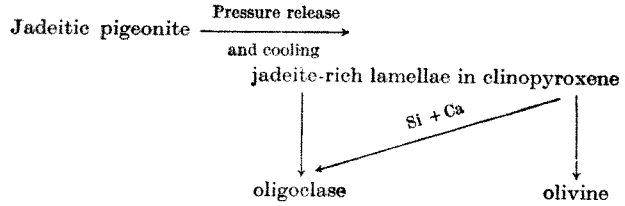


Fig. 9. Unusual chondrule from the Barwell meteorite formed of 50 per cent plagioclase, 25 per cent olivine and 25 per cent grey-brown turbid cryptocrystalline material (possibly pyroxene), and enclosing four crystals of unknown red-brown isometric mineral with high relief. (× *c.* 120, plane polarized light.)

a common feature of clinopyroxenes. It is considered that these textures are quite possibly crystallographic relics of a former clinopyroxene phase. The inversion stages could then be schematically represented as follows:



It is not yet possible to say whether or not individual chondrules may represent former pyroxene crystals. Too little is known of the habit and crystallography of jadeitic pigeonite, but jadeitic diopside (omphacite) commonly forms equidimensional crystals.

The data considered here suggest that it may be possible to distinguish chondrites formed at high load-pressure environments from those formed at lower load-pressures. In this way a model might be constructed of the original body or bodies. It would also contribute to the solution of the problem of whether or not the *H* and *L* chondrites[1] might have come from different bodies[16], possibly of different sizes, which came close enough to each other to cause their disintegration.

In conclusion, textures of chondrules in the Barwell and other meteorites are taken to indicate that some chondrites formerly existed in a high load-pressure environment in which dense minerals, such as jadeitic pigeonite, were stable. On release of the pressure, separation occurred to give the textures and mineralogy commonly seen in chondrules. It is suggested that the parent body or bodies may have been of lunar dimensions.

I thank Dr. M. H. Hey for permission to examine thin sections of meteorites in the British Museum (Natural History) collection, Dr. A. J. Meadows, Department of Astronomy, University of Leicester, for reading this note, and Mr. J. A. Dixon for assistance in the photomicrography.

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MECHANISM OF OXIDATION OF DILUTE NICKEL—CHROMIUM ALLOYS

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It is generally held that chromium additions to pure nickel progressively increase its high temperature oxidation rate until a concentration variously reported as 5–8 per cent is reached, whereupon further additions decrease the rate[1–6]. A widely quoted theory explaining this behaviour is that chromium in low concentrations increases the oxidation rate by increasing the number of cation vacancies in the NiO scale, whereas larger concentrations decrease it by also forming other phases of a more protective nature, namely $NiCr_2O_4$ or Cr_2O_3. The present communication describes results which prove that it is necessary to modify certain of these now classical views on the subject.

Electropolished and occasionally finely abraded specimens, 2·0 × 0·5 × 0·06 cm, of annealed pure nickel (99·97 per cent) and alloys containing respectively 1, 2, 3, 5, 7, 10, 15 and 27 per cent pure chromium were oxidized by immediate exposure at 1,000° or 1,200° C to pure dry, flowing oxygen in a silica spring thermobalance. After cooling relatively slowly for 10 min, they were prepared in cross-section by conventional metallography and examined in a Cambridge 'Microscan' Mark II electron probe microanalyser. Other scales from specimens of larger area were examined by X-ray diffraction.

Fig. 1(*a*) and (*b*) shows the kinetic growth curves for the whole range of alloys at 1,000° and 1,200° C respectively, plotted as the weight gain against the square root of time. Each curve was obtained by averaging the curves for several closely reproducible runs for each alloy. All the curves for alloys in the range 1–10 per cent chromium at 1,000° C and 2–10 per cent chromium at 1,200° C lie randomly within the narrow scatter bands indicated, there being no significant difference in oxidation rate in the composition ranges quoted. At 1,000° C, therefore, addition of 1–10 per cent chromium to nickel increases the parabolic rate constant from 2·93 × 10^{-10} to 1·73–2·13 × 10^{-9} g^2/cm^4/sec, precise adherence to the parabolic relationship being maintained for the full 25 h of the runs. The corresponding increase at 1,200° C is from 2·33 × 10^{-9} to 1·31–1·54 × 10^{-8} g^2/cm^4/sec for the first 8–10 hours. At this stage there is a positive deviation, scarcely detectable for pure nickel but pronounced for the alloys, probably because the alloy core is now very thin; localized thicker scale is observable at the ends, perhaps because of oxide disruption there. It is remarkable that at 1,200° C nickel containing 15 per cent chromium oxidizes as fast as the dilute alloys in the early stages and even at 1,000° C the initial rate is more rapid than for pure nickel.

Alloys of nickel with 15 and 27 per cent chromium eventually oxidize more slowly than the other alloys at both temperatures because of the development of a healing layer of Cr_2O_3 at the scale base[6]. Very small patches of similarly healed scale are observed with nickel–7 per cent and 10 per cent chromium at 1,000° C but have an insignificant influence on the growth curves. The curve for nickel containing 1 per cent chromium at 1,200° C is drawn for 5 h only because the growth kinetics for this alloy were highly irreproducible especially at long times. Indeed the curve shown represents only the average kinetics of the faster runs and even this is slightly lower than for alloys containing 2–10 per cent chromium. The general conclusion is therefore that there is no sharp maximum in oxidation rate, as previously reported. Instead, the rate increases with chromium content up to 1–2 per cent, whereupon it remains substantially constant with further chromium additions until scale healing by Cr_2O_3 occurs.

The morphology, structure and analysis of scales in section are summarized schematically in Fig. 2. Single quotation marks are used to denote single oxide phases containing low concentrations of the other element. On pure nickel, adherent, uniform single-layer scales were obtained at both temperatures, the oxide grain size increasing considerably towards the outer surface. On nickel with 1 per cent chromium at both temperatures and with 2 per cent chromium at 1,200° C, thicker, apparently single-layer scales of 'NiO', which lost adhesion to the alloy on cooling, were produced. A deeply penetrating layer of fine acicular 'Cr_2O_3' particles penetrated the alloy. Alloys containing 2–10 per cent chromium at 1,000° C and 3–10 per cent chromium at 1,200° C all developed scales with double layers in the sense that only the inner layer contained visible second-phase particles. On

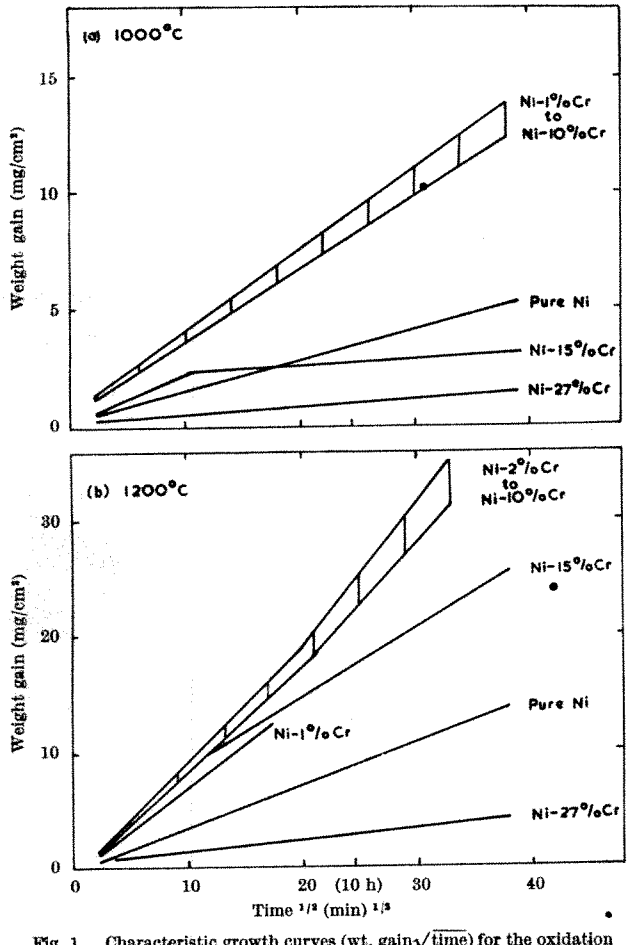


Fig. 1. Characteristic growth curves (wt. gain $\sqrt{\text{time}}$) for the oxidation of nickel–chromium alloys in oxygen at (*a*) 1,000° C, (*b*) 1,200° C.

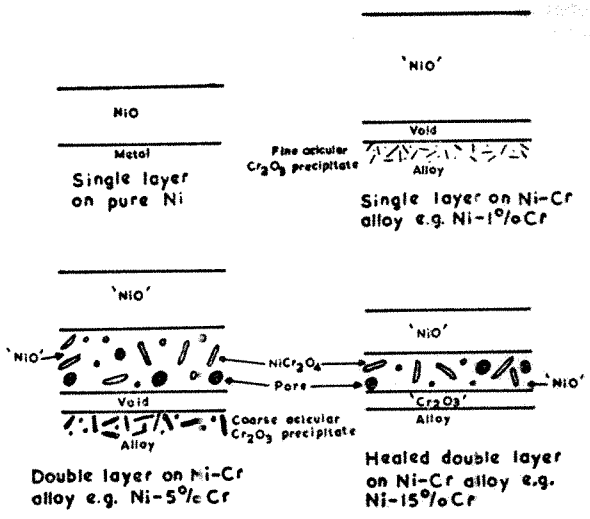


Fig. 2. Schematic diagrams of scales on dilute nickel–chromium alloys.

cooling, the two layers maintained excellent adhesion but the entire scale lifted from the alloy. The 'Cr_2O_3' internal oxide was of a coarser acicular nature, at least in the alloy range 2–5 per cent chromium, and penetrated less deeply into the alloy. The inner layer contained similarly shaped $NiCr_2O_4$ particles. There were also several large pores but it was difficult to decide whether a continuous porous network existed in the inner layer, especially as the 'pores' bore some resemblance merely to a relief-polishing effect when viewed in oblique illumination. The outer layer appeared completely compact, there being no obvious evidence of the pores developed in similar sulphide films by anisotropic dissociation[7]. It should also be emphasized that the demarcation between single- and double-layer growth was not clear-cut. Thus, although the alloy with 1 per cent chromium at 1,000° and 1,200° C and that with 2 per cent chromium at 1,200° C were adjudged from the lack of many visible pores and precipitate in the inner regions to have single layers, the large outer columnar grains ceased about halfway through the scale and traces of $NiCr_2O_4$ were detected by X-ray diffraction. Time appeared to have an influence on the structure. Scales on 15 per cent and 27 per cent chromium alloys adhered to the alloy because of the linking of the healing 'Cr_2O_3' layer to the alloy[8].

Concentration profiles through a characteristic single-layer scale formed on nickel-1 per cent chromium at 1,000° C and a double-layer scale on nickel–5 per cent chromium at 1,200° C are shown in Fig. 3(*a*) and (*b*) respectively. The curves are ultra-slow scanning traces corrected for absorption and fluorescence effects at the points indicated and confirmed by static probe measurements in vital locations. The apparent single layer on nickel–1 per cent chromium is clearly 'NiO' containing chromium, rising in concentration from the scarcely detectable level of 0·1 per cent or less at the scale/oxygen interface to 1·29 per cent at the alloy/oxide interface at 1,000° C; at 1,200° C the similar rise is typically from 0·2 or less to 2·10 per cent. Both values at the true inner interface should possibly be a little higher, but this is partly obscured by the voids. For nickel–1 per cent chromium at 1,200° C the chromium concentration near the alloy/oxide interface varied somewhat, possibly explaining the scatter in the kinetic curves. For nickel–1 per cent chromium at 1,000° C and nickel–2 per cent chromium at 1,200° C, a chromium plateau at about 1 per cent and 3 per cent respectively was sometimes achieved in the inner region, depending on the time. This suggests that the solubility of chromium in nickel oxide was being virtually satisfied there, so a few spinel particles could persist. Some lack of equilibrium due to the

slowness of diffusion of Cr^{3+} ions in 'NiO' may also have been involved.

The outer layers on nickel–5 per cent chromium show somewhat similar chromium concentration profiles, the values at the outer interface being the same as for the alloy with 1 per cent chromium. At 1,000° C the chromium concentration at the inner layer/outer layer interface is typically 1–2 per cent for this type of specimen and at 1,200° C it is consistently constant at 3·5–4·5 per cent for the whole range of alloy compositions (Fig. 3(*b*)). Since the latter value was also always found for the 'NiO' between the second-phase particles in the inner layer, it is considered to be the limit of solubility of chromium in NiO at this temperature. Spot analyses and X-ray diffraction showed the acicular second phase particles to be $NiCr_2O_4$, there being no 'Cr_2O_3' in the inner layer. Resolution of 'NiO' and $NiCr_2O_4$ was not possible at 1,000° C by microanalysis. Static probe microanalysis demonstrated that the internal oxide particles are 'Cr_2O_3' and additionally that the alloy between the particles, although much depleted in chromium, is by no means pure nickel. There is admittedly always some possibility of the X-ray source entering internal oxide particles buried beneath the surface but this would not always be expected to be the case, or alternatively some fluorescence of chromium radiation in the particles by nickel radiation excited in the matrix.

In explaining the results, it may be proposed that when the allow is exposed to oxygen, 'NiO' and 'Cr_2O_3' both form initially, but rapidly combine and coalesce, producing either an unsaturated or just saturated solution of chromium in 'NiO' for the very dilute alloys, or $NiCr_2O_4$ particles in a saturated solution of chromium in 'NiO' for alloys containing more than 1–2 per cent chromium. Partial dissociation of 'NiO' occurs at the alloy-oxide interface and oxygen diffuses into the alloy, provoking the precipitation by preferential oxidation of 'Cr_2O_3', thus causing considerable chromium depletion in the surrounding alloy. As the external scale develops, the 'Cr_2O_3' granules become incorporated into the inner layer where they, together with any chromium left in the oxidizing alloy, saturate the 'NiO' there with chromium and are converted into $NiCr_2O_4$ particles of a similar shape. For the very dilute alloys almost total dissolution of 'Cr_2O_3' in the 'NiO' tends to occur. Ni^{2+} ions diffuse from the alloy to build the outer layer at its outer surface. Cr^{3+} ions from the alloy or the 'NiO' in the inner layer can penetrate the outer layer but reach only a low concentration in it. This theory differs from that of Moreau and Bénard[9] in that the alloy between the internal oxide particles is apparently not almost pure nickel, presumably because equilibrium is never maintained in this region and chromium can still diffuse into it, and also because chromium ions can clearly diffuse well into the outer scale layer. Birks and Rickert[10] maintain that, in explaining the incorporation of the 'Cr_2O_3' internal oxide particles into the scale, it is necessary to postulate in addition that oxygen gas, obtained from dissociation of 'NiO' in voids in the inner layer, moves through a porous inner layer to form fresh oxide at the alloy/oxide interface. Without necessarily disputing this theory, the authors believe that it is still to be demonstrated that the inner layer does contain a complete network of pores. Consideration should also be given to the possibility of plastic 'NiO' relaxing around the 'Cr_2O_3' particles as the alloy is consumed, so detaching the 'Cr_2O_3' particle "markers". Evidently some stress is developed at the interface during the incorporation process, because of the lack of adhesion on cooling, but whether it is due to chromium reducing the plasticity of 'NiO' or directly to the internal oxide cannot yet be stated. Microhardness measurements at room temperature suggest that 'NiO' is harder than NiO.

It remains to be explained why all the dilute alloys generally oxidize at the same parabolic growth rate. Although the chromium is distributed across the scale in a complex manner, the concentrations in the 'NiO' at the alloy/oxide and oxide/oxygen interfaces are constant with time for nearly all the alloys at a given temperature. This presumably could mean that the concentration difference of cation vacancies across the film is constant with time, so a parabolic rate is maintained. The effect of chromium in increasing the rate ceases once the 'NiO' near the alloy is saturated with chromium and the profile through the 'NiO' in the film is established, any further additions being superfluous. As postulated by Hauffe[11], the chromium enrichment in the inner region of the scale may flatten the cation vacancy concentration profile across the scale rather than enhance it. The effect of chromium in increasing the rate must therefore be related to an increase in the total vacancy concentration rather than in the vacancy concentration difference. Additional problems in interpretation may exist because of present doubts about the defect structure of NiO and the applicability of the valency rules.

It is, however, a little surprising that the blocking effect of $NiCr_2O_4$ precipitate in the inner layer has an apparently negligible influence on the flux of outwardly moving ions. This would seem to suggest that oxygen flow through pores in the inner layer could be contributory, by the dissociative process[10], although this could not

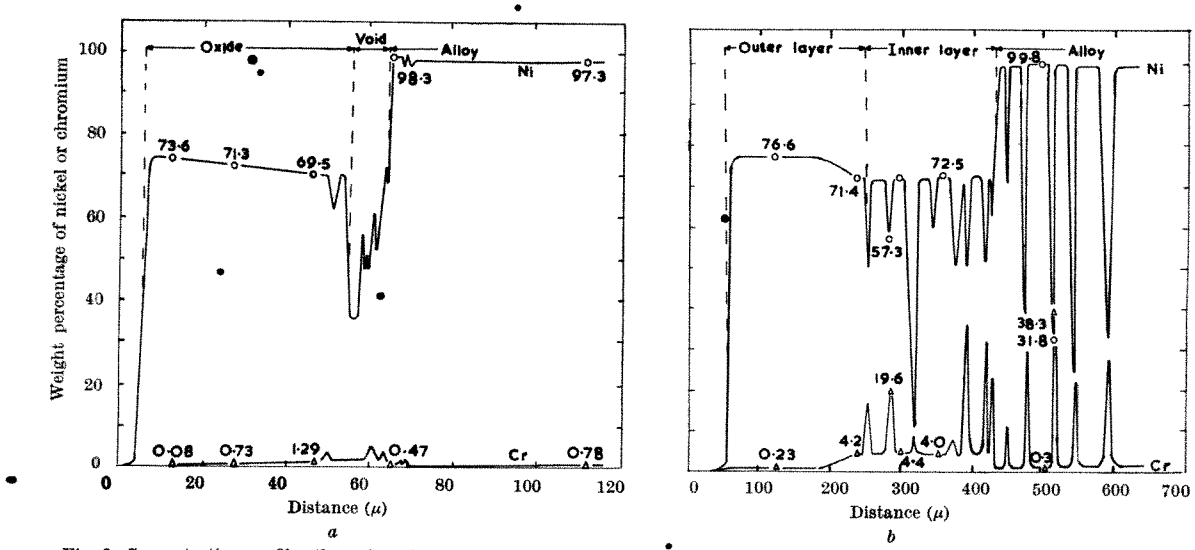


Fig. 3. Concentration profiles through scales and alloys after oxidation of dilute nickel–chromium alloys. (*a*) Nickel–1 per cent chromium for 5 h at 1,000° C; (*b*) nickel–5 per cent chromium for 25 h at 1,200° C.

hold if the inner layer does not exist on the very dilute alloys, and would be less likely if there is never a complete network of pores in the inner layer on other alloys. It might, therefore, also be argued that the outer 'NiO' layer (the entire layer for dilute alloys) is rate-determining, because the chromium concentration gradient across it is constant and the same for most alloys, the inner layer effectively being a short circuit. The authors will return to these matters elsewhere when additional data are available.

Rapp[12] has recently independently reported views tending in the same direction as ours, based on theoretical considerations, but has apparently not confirmed them experimentally.

We thank Prof. T. K. Ross for providing facilities, the International Nickel Company (Mond) Limited for the alloys and the Science Research Council for a maintenance grant to one of us (T. H.) and for providing the micro-analyser.

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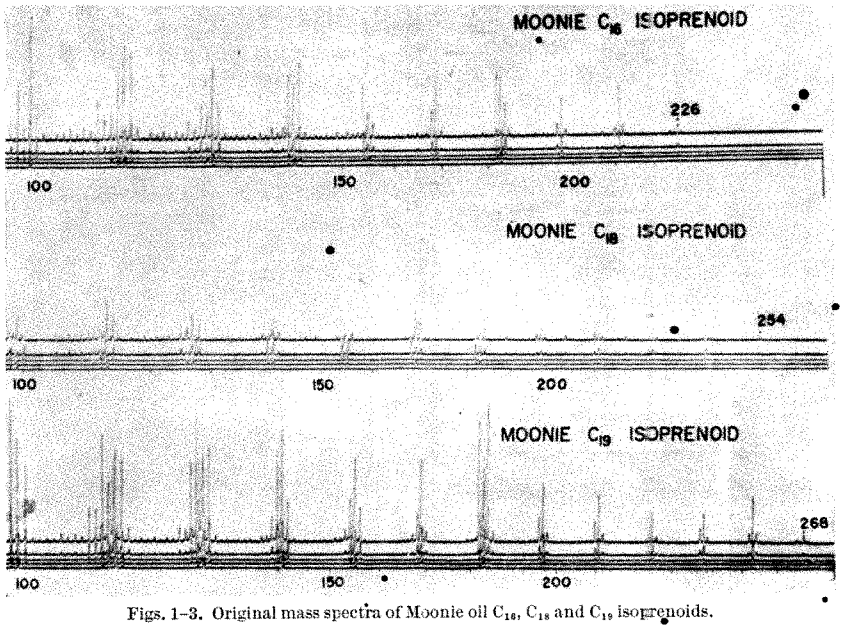
HYDROCARBONS FROM AUSTRALIAN OIL, TWO HUNDRED MILLION YEARS OLD

By WILLIAM VAN HOEVEN, PAT HAUG, A. L. BURLINGAME and PROF. MELVIN CALVIN
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IN an attempt to extend our investigation of the organic components of ancient sediments[1–4] to another continent and presumably a separate origin, we studied the hydrocarbons present in the Moonie crude oil from Queensland, Australia. It is believed that the source of the oil was in Permian rocks, probably sedimentary, and that later it migrated along an unconformity at the base of the reservoir basin into Jurassic and Triassic sandstone—dated on the basis of spores[5] (also A. S. Keller and R. C. McLaughlin, personal communications). A wide spectrum of analyses of sediments from various ages and from various places can be expected to provide data relevant to a solution to the question of when and how life evolved on earth. Such an approach may eventually shed some light on the ecology of a particular region at a given geological period.

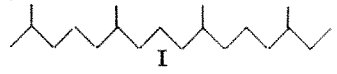
The experimental procedure, already explained in detail elsewhere[1], involved the use of column chromatography (thin layer chromatography with grade neutral alumina eluted with *n*-heptane) to separate the "total" hydrocarbon fraction (1·46 g) from 3·89 g of crude oil. Using this "total" fraction, the "normal" (straight chain) hydrocarbons were separated by means of a 5 Å molecular sieve, from the "branched-cyclic" hydrocarbons, which represented about 42 per cent of the "total" hydrocarbons. Individual compounds of the "branched-cyclic fraction" were separated on a 3 per cent *SE*-30 gas-liquid chromatography column (10 ft. × ¼ in.) and purified successively on two columns (25 ft. × ¼ in.) containing 2·5 per cent 7-ring metapolyphenylether (PPE) and 3 per cent tetracyanoethylated pentaerythritol chromatographic phases. A modified G.E.C. 103 mass spectrometer was used to elucidate the structures of isolated compounds. Figs. 1–3 show the original mass spectra of the first three compounds isolated from the Moonie oil. These are sample spectra illustrating the purity attained by the isolation procedures described and proper mass spectrometric handling techniques. In Figs. 5–7 the bar graphs drawn from these originals are presented. (Relative intensities for all peaks which are off scale because of the choice of the base peak are given in Table 1.) All spectra published in this and preceding papers have been handled in the same manner.

The gas chromatograms (packed column, 10 ft. × ⅛ in. 3 per cent *SE*-30 programme rate of 6°/min) of the "total", "branched-cyclic" and "normal" fractions are presented



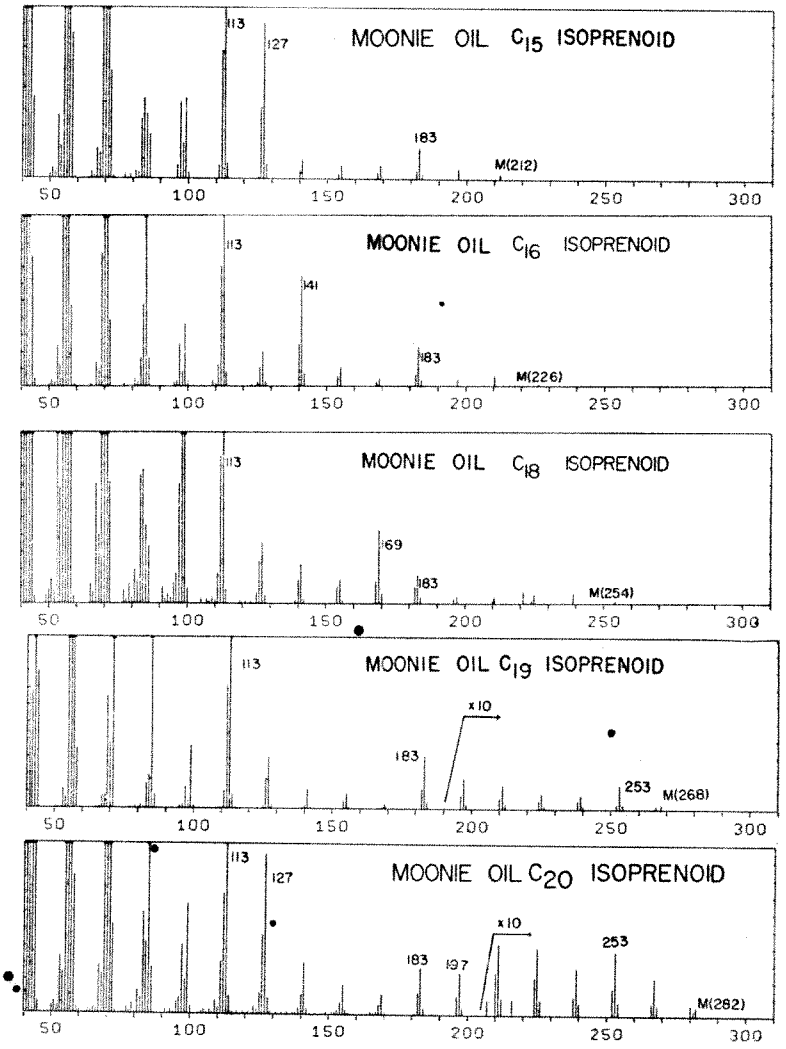
Figs. 1–3. Original mass spectra of Moonie oil C_{18}, C_{18} and C_{19} isoprenoids.

in Fig. 9. The normal alkanes can be seen to run from C_9 to C_{29} with maxima at C_{12} and C_{18} and negligible odd over even predominance. Particularly indicative of life processes are the isoprenoid alkanes, which possess a high degree of structural definition. The isolated C_{15}, C_{16}, C_{18}, C_{19} (pristane) and C_{20} (phytane) isoprenoids, the mass spectra of which are given in Figs. 4–8, appear to have a regular isoprenoid structure corresponding to that of phytane (Structure I).



I

Evidence was also obtained for the existence of a C_{21} isoprenoid but it could not be separated from other components. Also identified were representatives of three other homologies; Fig. 10 gives the mass spectrum of C_{15} *iso*-alkane (2-methyltetradecane), Fig. 11 that of C_{18} anteiso-alkane (3-methylheptadecane), and Figs. 12 and 13 those of C_{15} and C_{16} *n*-alkyl-*cyclo*hexane. Possible biological origins of these compounds have previously been discussed[4]. Figs. 14–17 give the mass spectra of four unidentified compounds X_1, X_2, Y_1 and Y_2, which are relatively pure by gas chromatographic as well as by mass spectrometric standards. Compounds X_1 and X_2 are

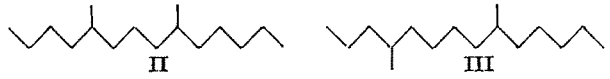


Figs. 4–8. Mass spectra of Moonie oil C_{15}, C_{16}, C_{18}, C_{19} and C_{20} isoprenoids.

Table 1. OFF-SCALE RELATIVE MASS SPECTRAL INTENSITIES

m/e	4	5	6	7	8	10	11	14	15	16	17	*m/e*
40			210	160	180	121	670	120	120	840		40
41	770	420	2,000	770	710	2,140	1,000	1,200	2,500	2,300	1,040	41
42	220	134	500	220	170	820	800	300	550	630	256	42
43		1,070	3,300	1,800	1,400	3,570	6,000	2,600	4,000	5,000	1,930	43
44			131	260	150	157	3,300		116	170		44
53			101									53
55	335	230	900	450	420	515	600	640	580	110	655	55
56	400	194	660	460	370	393	260	470	400		375	56
57		950	3,400	2,400	1,720	1,790	500	2,600	1,800	5,200	2,620	57
58			170					120		180	109	58
60							400					60
61							3,500					61
62							150					62
69	120		285	200	270	179	250	250	230	380	336	69
70	180	147	230	140	280	150	110	280	200	370	304	70
71		820	1,300	1,300	910	800	250	1,300	790	2,000	1,100	71
72										110		72
83							130	131	190	136		83
84									125			84
85		250		410	410	672	113	500			120	85
97							210	120	125	220	110	97
98			140					160		200	160	98
99			130			143	190		160			99

branched C_{16} alkanes and were collected from the region marked X in the gas chromatogram in Fig. 9; Y_1 and Y_2 are branched C_{17} alkanes and are from the region marked Y. An interpretation of the mass spectrum of compound X_1 suggests Structure II (5, 9-dimethyltetradecane).



II

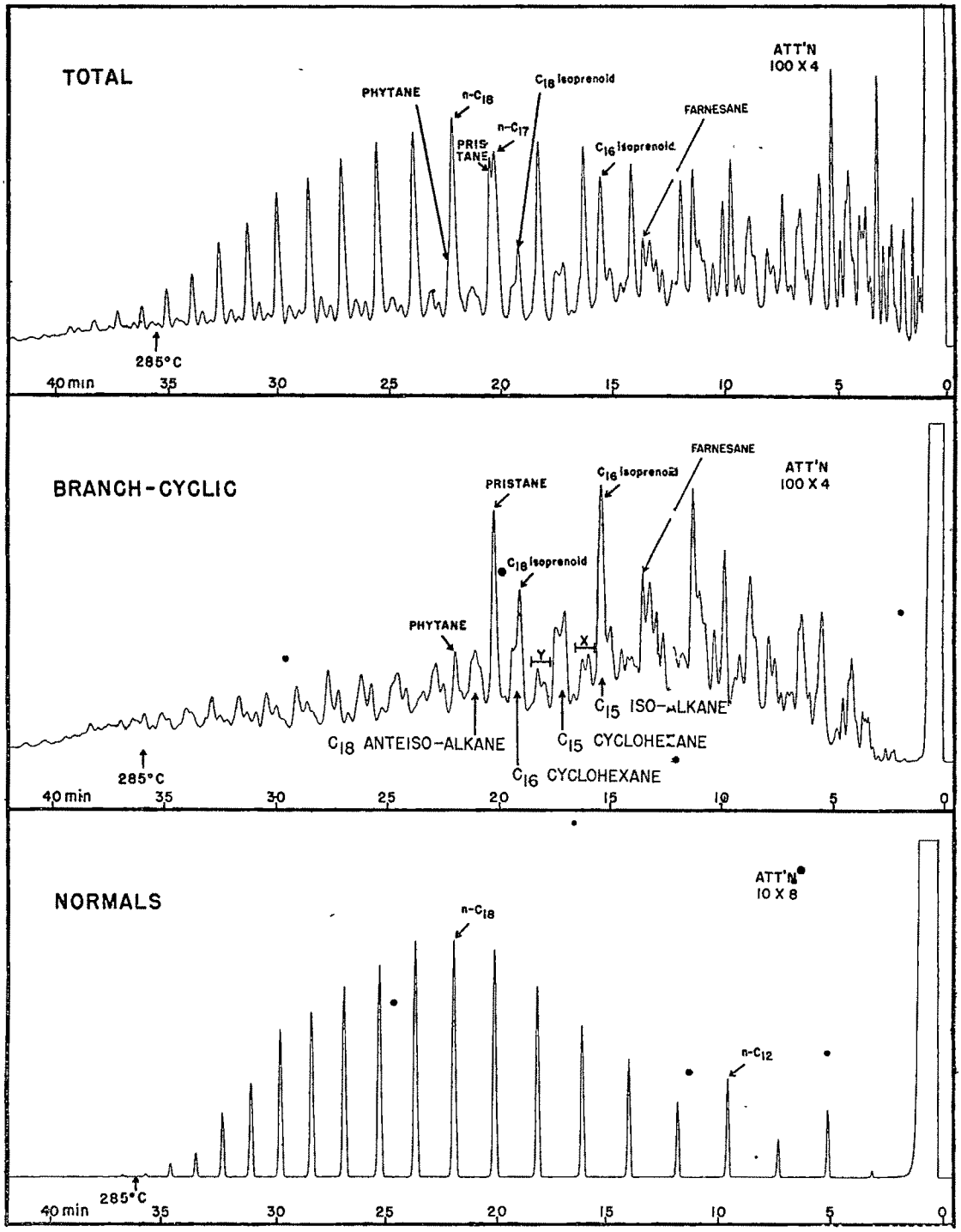
III

Similarly, a possible interpretation of compound X_2 is given by Structure III (4,9-dimethyltetradecane).

Table 2. Relative Retention Times (min)

Compound	10 ft. × ¼ in., *SE*-30 (programme rate 4°/min)	25 ft. × ¼ in., F2 5 per cent PPE (isothermal 136° C)
C_{16} Isoprenoid	22·7	10·8
X_1	23·5	11·9
X_2	24·2	12 8
Y_1	26·7	19·0
Y_2	28 2	20·8
C_{18} Isoprenoid	28·6	23·1

The formation of such compounds might be possible by cracking of squalane. Table 2 presents the relative retention times of compounds X_1, X_2, Y_1 and Y_2 on gas chro-

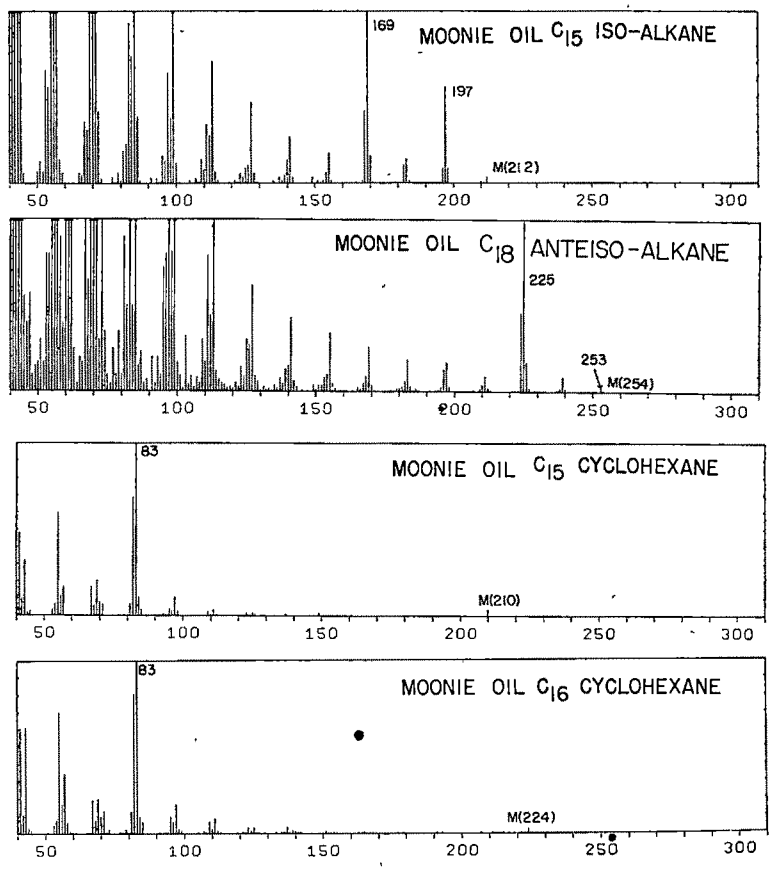


MOONIE OIL, HYDROCARBON FRACTIONS

~200 X 10^6 YEARS

Fig 9. Gas chromatograms of total, branched-cyclic and normal hydrocarbon fractions of the Moonie oil.

matographic phases. High resolution mass spectrometric studies verify that these are saturated branched hydrocarbons. Fig. 18, a gas chromatogram on a 3 per cent *SE*-30 capillary column (150 ft. × 0·010 in., programme rate 1°/min), reveals the actual complexity of these regions. A thorough mass spectrometric analysis of the C_{17} isoprenoid region (as determined by "co-injection" of the synthetic 2,6,10-trimethyltetradecane) indicates that this compound is not present in significant amounts. Mass spectrometric ambiguities in the identification of the exact structure of the C_{19} isoprenoid[4] have been eliminated (and the presence of pristane definitely established) by co-injection of pristane[6]. An investigation of the high mass region has not provided evidence of steranes or triterpanes, although high molecular weight unsaturated compounds are present.



Figs. 10–13. Mass spectra of Moonie oil C_{15} iso-alkane, C_{18} anteiso-alkane, C_{15} *n*-alkyl-*cyclo*hexane and C_{16} *n*-alkyl-*cyclo*hexane.

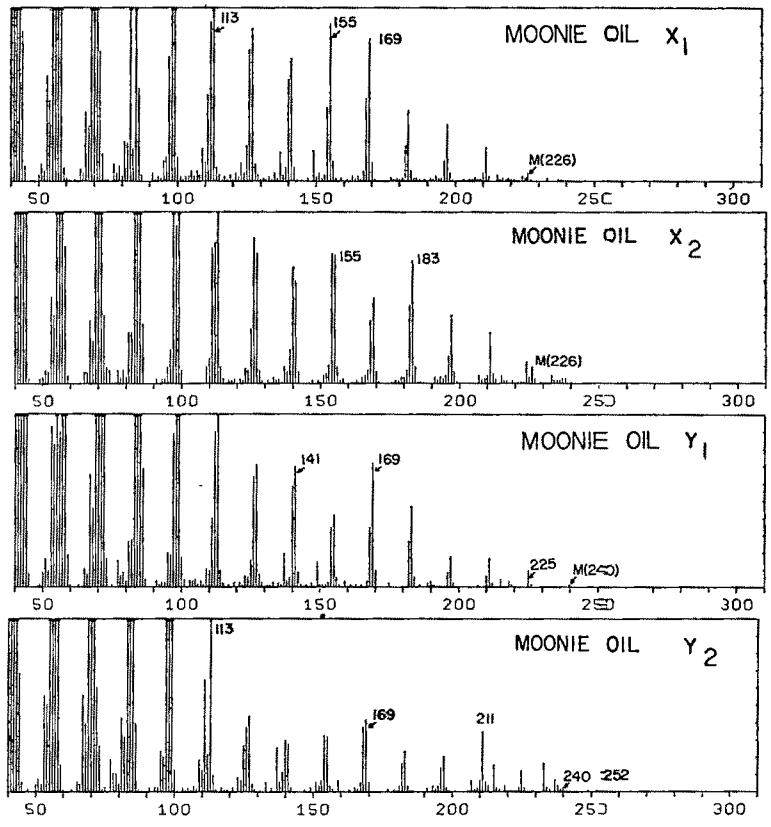
Table 3. APPROXIMATE RELATIVE ABUNDANCE OF ALKANES, DETERMINED FROM GAS CHROMATOGRAPHIC PEAK HEIGHTS

Sample	Age	Isoprenoids						
		C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}
Soudan shale	2.7×10^9		0.08		0.6	1	0.6	0.1
Nonesuch oil	1×10^9	0.4	1.2			1	0.3	0.1
Nonesuch calcite vein	1×10^9	0.2	0.8			1	0.3	
Antrim shale	265×10^6			0.2	1.3	1	0.6	
Moonie oil	200×10^6	0.3	0.9			1	0.3	< 0.05
Green river shale	50×10^6		0.4	< 0.1	0.2	1	5.5	
San Joaquin oil	30×10^6				0.9	1	0.9	
Abbott seep oil	3×10^6					1	0.3	
Abbott rock oil	3×10^6				0.4	1	0.5	

Sample	Age	Steranes			Triterpane
		C_{27}	C_{28}	C_{29}	C_{30}
Soudan shale	2.7×10^9		0.03		1.2
Green river shale	50×10^6	0.05	0.5	0.8	1·2

Sample	Normal alkane																									
	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{20}	C_{21}	C_{22}	C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}	C_{34}
Soudan shale						0.01	0.07	0·5	1	0·9	0·5	0·9														
Nonesuch oil				0.01	0.05	0·3	0·7	0·95	1	0·9	0·75	0·55	0·4	0·3	0·2	0·1	0.09	0.07	0.05	0.04	0.03	0·02	0.01	0.01	0.01	
Nonesuch calcite vein			0·2	0·1	0·3	0·4	0·6	0·8	1	1·2	1·35	1·35	1·30	1·2	1·1	1·0	0·8	0·7	0·6	0·4	0·3	0·2	0·1	0·08	0·05	0·03
Antrim shale		0·005	3·0	5·0	5·0	4·0	3·0	1·5	1	0·5	0·2	0·005														
Moonie oil	0·03	0·3	0·2	0·4	0·3	0·5	0·7	0·8	1	1·04	1·03	0·9	0·8	0·7	0·65	0·4	0·3	0·1	0·06	0·01	0·005					
Green river shale					0·01	0·02	0·05	0·04	1	0·06	0·1	0·02	0·08	0·25	0·1	0·01	0·15	0·01	0·25	0·02	1·1	0·02	1·1	0·01	0·09	
San Joaquin oil		0·2	1·3	2·0	2·4	2·2	1·8	1·4	1	0·6	0·4	0·2	0·1	0·05												
Abbott seep oil							0·1	0·7	1	0·4	0·2	0·08	0·06	0·05	0·04	0·025	0·025	0·025	0·06	0·04	0·07	0·08	0·3	0·1	0·05	0·03
Abbott rock oil					0·01	0·25	0·2	0·6	1	1·2	1·3	1·0	0·8	0·5	0·3	0·2	0·15	0·1	0·08	0·01						

Sample	Age	Iso-Alkane				Anteiso-Alkane			*n*-alkyl-*Cyclo*hexanes					X_1	X_2	Y_1	Y_2
		C_{15}	C_{16}	C_{17}	C_{18}	C_{16}	C_{17}	C_{18}	C_{15}	C_{16}	C_{17}	C_{18}	C_{19}	C_{16}	C_{16}	C_{17}	C_{17}
Nonesuch oil	1×10^9		< 0.1	0.2	0.07	< 0.1	0.2	0.05		0.1	0.1	< 0.1	0.1				
Moonie oil	200×10^6	0·1						0·1	0·2	0·3				0·1	< 0·1	0·05	0·1



Figs. 14–17. Mass spectra of Moonie oil X_1, X_2, Y_1 and Y_2.

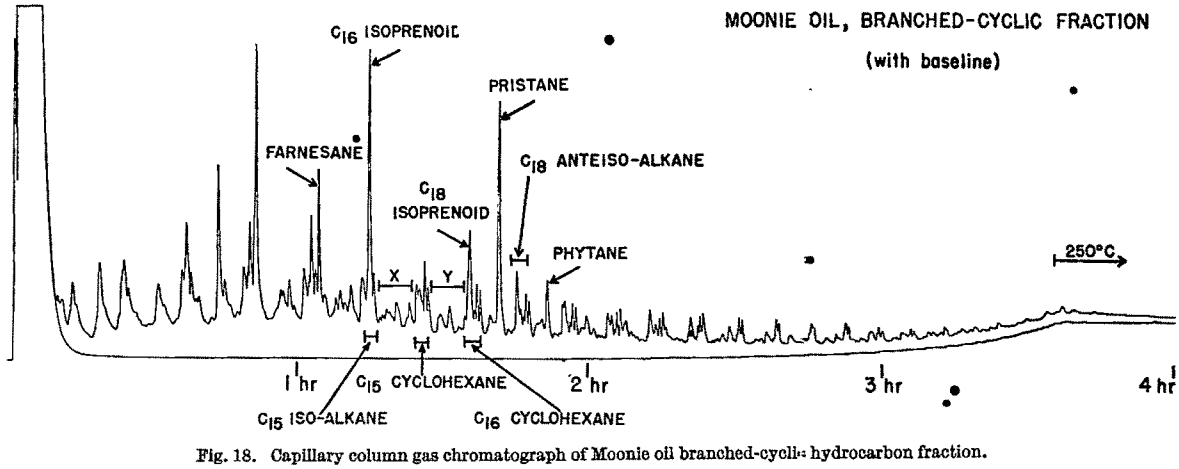


Fig. 18. Capillary column gas chromatograph of Moonie oil branched-cyclic hydrocarbon fraction.

Hydrocarbons from the Australian Moonie oil, which is two hundred million years old, therefore resemble very strongly the hydrocarbons found in other sediments varying in age from thirty million to two thousand seven hundred million years. Because we have found indications of isoprenoids, iso-alkane, anteiso-alkane, *n*-alkyl *cyclo*-hexane, and normal alkane series in the oils and sediments examined, as indicated in Table 3, it is becoming increasingly evident that among biogenic sediments, variations resulting from individual sediment history and ecology are to be found primarily in the relative amounts of these compounds present, and possibly in the hydrocarbons present only in small quantities, and not in the types of hydrocarbons present.

We thank Prof. J. B. Johns and Dr. H. K. Schnoes for many helpful discussions, A. S. Keller for samples of crude oil, D. Smith for helping in preparing the mass spectra and E. D. McCarthy for providing a sample of synthetic C_{17} isoprenoid, 2,6,10-trimethyltetradecane. This work was supported by the National Aeronautics and Space Administration and the U.S. Atomic Energy Commission.

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ISOLATION OF A GLUCOSE-BINDING COMPONENT FROM HUMAN ERYTHROCYTE MEMBRANES

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GLUCOSE is transported across the human erythrocyte membrane by a relatively specific facilitated diffusion system, the kinetics of which have been extensively studied[1-3]. Attempts to isolate this system from the cell membrane[4,5] have not hitherto been successful. We wish to describe a sensitive column chromatographic technique for studying the binding of glucose by membranes and by membrane fractions and to describe how this technique has been used to monitor the isolation from the membrane of a glucose-binding component. The properties of this glucose-binding component suggest that it is closely associated with glucose transport.

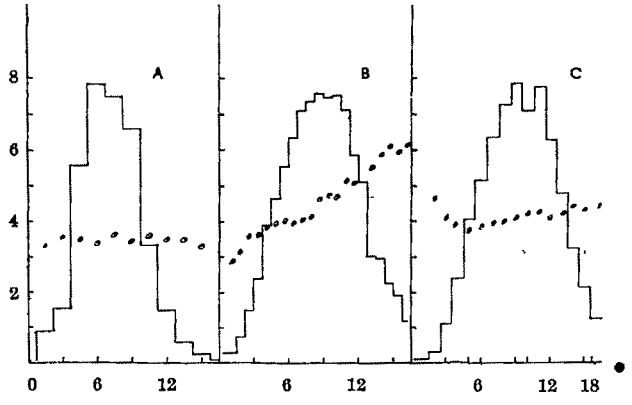


Fig. 1. Retardation chromatography on 'Celite' columns. *A*, 'Celite' alone. *B*, 'Celite' + membranes from 10 ml. of erythrocytes. *C*, as *B* but in the presence of 10^{-4} M phloretin. Solid line, radioactivity associated with glucose (abscissa scale; 1 unit = 0·1 counts/sec). Circles, ratio of tritium to carbon-14 counts (ordinate scale; 1 unit = unit ratio of tritium to carbon-14 counts), (abscissa scale: effluent volume in ml.). The sugar added was here 2 ml. of a mixture of 2 mM glucose (tritiated) + 2 mM sorbose (labelled with carbon-14) in a *tris*-succinate buffer at *p*H 7·4.

We prepare chromatographic columns in which the stationary phase is composed of membranes or of membrane fractions immobilized by adsorption on to 'Celite' or on to DEAE-cellulose. (The idea of making columns of cell membranes is due to Dr. S. Hunt and the further development of this technique has been greatly assisted by his collaboration—see the following communication.) After exhaustive washing, a procedure which removes all residual haemoglobin bound to, or occluded within, the membranes, the columns are loaded with a mixture of sorbose labelled with carbon-14 and tritiated glucose, both at the same concentration (generally 2 mM). The volume of the sugar solution is such that the concentration of sugar emerging from the bottom of the column attains a plateau value (Figs. 1–4). Fractions of the emerging solution are collected and analysed for radioactivity by liquid scintillation counting, the leading and trailing edges of the plateau being particularly carefully examined. If such a column bears a component which binds glucose in preference to sorbose, it would be expected that glucose will be retarded by the column and will appear after sorbose in the effluent. Thus the radioactivity counts in the emerging effluent will be initially low in tritium. In the plateau region the two isotopes will generally emerge together. In the trailing edge of the plateau, the counts due to glucose will be high. The amount of glucose retarded can be calculated from the difference in area between the sorbose and glucose effluent curves in the region up to the point where both curves merge in the plateau.

Although each glucose-binding component interacts with many glucose molecules during the passage of the sugar sample down the column, the total number of glucose molecules retarded will be equal to the number of glucose-binding sites. Yet this column procedure is apparently a very sensitive measure of glucose binding. Indeed, glucose binding by erythrocyte membranes has not hitherto been detected[6]. The sensitivity of the procedure depends (among other factors) on the volumes of the column and the solution; long, narrow columns will ensure that each glucose-binding site is used with maximal effectiveness.

Using this procedure with 'Celite' columns we have been able to show that (i) 'Celite' itself does not retard glucose in preference to sorbose (Fig. 1*A*), but that (ii) cell membranes adsorbed to 'Celite' do retard glucose (Fig. 1*B*), and that (iii) this retardation is largely prevented by the simultaneous presence of a low concentration

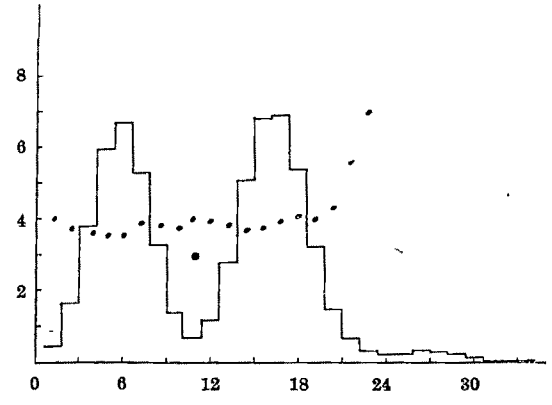


Fig. 2. Retardation chromatography on DEAE-cellulose columns, in the absence of membranes. Two successive samples (4·0 ml. and 5·6 ml.) of a mixture of 2 mM (tritiated) glucose and 2 mM sorbose (labelled with carbon-14) in 0·003 M phosphate buffer at *p*H 7·0 were added. Symbols and scale as in Fig. 1.

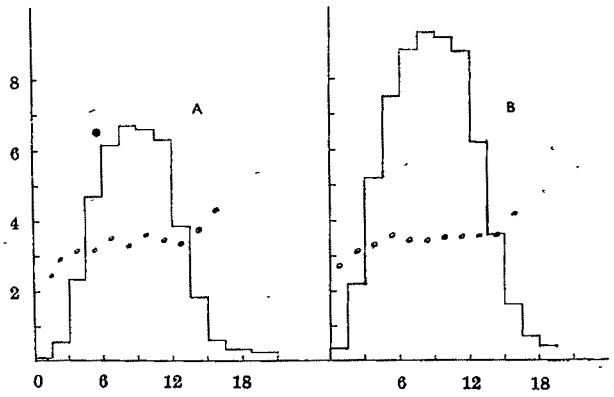


Fig. 3. Retardation chromatography on DEAE-cellulose. Column contained *A*, adsorbed membranes from 4 ml. erythrocytes; *B*, adsorbed iodide extract of membranes from 4 ml. erythrocytes. The sugar added was 6 ml. of the glucose/sorbose mixture described in Fig. 2. Symbols and scale as in Fig. 1.

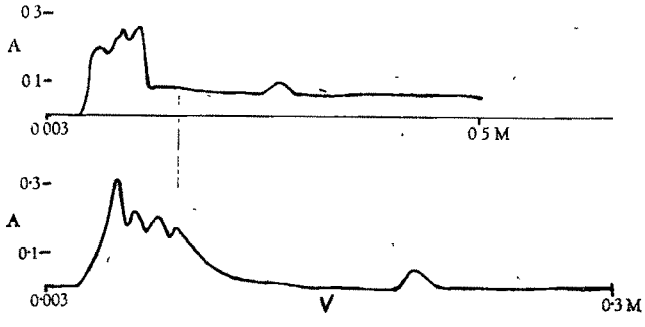


Fig. 4. DEAE-cellulose chromatography of solubilized membranes. A dialysate of the molar sodium iodide extract of the membranes from 7 ml. of erythrocytes was applied to the column, and eluted with a phosphate buffer at *p*H 7·0, a linear gradient to 0·5 M in the first experiment (upper figure) or 0·3 M in the second experiment (lower figure). The maximum specific retardation activity lies in the (compound) second peak of the upper figure and in the third peak of the lower figure (where the resolution appears to be more satisfactory). The ordinate is the absorbance of the effluent solution read at 280 mμ. The abscissa is the molarity of the effluent.

(10^{-4} M) of phloretin, an inhibitor[2] of glucose transport in human erythrocytes (Fig. 1*C*).

These 'Celite' columns have the great advantage that they do not distinguish between glucose and sorbose and do not adsorb phloretin but, in other respects, 'Celite' columns are much more difficult to handle than are the DEAE-cellulose columns used for the remainder of this work. The DEAE-cellulose columns in the absence of membranes or membrane fractions do, in our hands, distinguish between glucose and sorbose (Fig. 2), in that the trailing edge of an emergent plateau gives a clear indication of glucose retardation. Indeed a small peak of radioactivity often emerges at an effluent volume some twice that of the main sugar peak. We can show that this retardation is due to the binding by DEAE-cellulose of a particular form of glucose or of a decomposition product of glucose, in preference to the major form of the glucose, or to sorbose. Little evidence for glucose retardation is, however, found at the leading edge of such plateaux and in the following discussion we shall consider only the leading edges, where the distinction between the behaviour of DEAE-cellulose alone (Fig. 2) and of membranes bound to DEAE-cellulose (Fig. 3) is clear.

By studying the percentage retardation of glucose at different concentrations of glucose, we have been able to demonstrate that the glucose-binding capacity of such columns is saturable and we have obtained an estimate of the dissociation constant for interaction between glucose and the glucose-binding site, on the assumption that this binding is of the simple Michaelis–Menten or Langmuir form. The data from three different experiments at 23° C are collected in Table 1. (We are indebted to Mrs. M. Levine for allowing us to use her data, recorded as the second of these experiments.) The concentration of glucose at the plateau (listed in column 2 of Table 1) must not be used to calculate the effective binding constant for the sugar-site complex, as the real sugar concentration here increases from the value of zero at the leading edge of the sugar front. We have calculated an "equivalent glucose concentration" (recorded in column 3 of Table 1) from the values of the real concentration at different points on the leading edge on the assumption that binding is a saturable function of the glucose concentration. In general, the "equivalent concentration" is between one-half and one-third of the plateau concentration. The data from these three experiments taken together yield a mean value for the Michaelis constant for binding of 18·2 mM with a standard error of ±4·2 mM. The Michaelis constant for the complex formed between glucose and the glucose transport system as determined in kinetic experiments on intact erythrocytes[3] is 11–13 mM at 25° C.

Table 1. Retardation of Glucose as a Function of Glucose Concentration

Experiment	Nominal sugar concentration (mM)	Equivalent concentration (mM)	Glucose retardation (percentage of total glucose added)	Retardation as percentage of value at lowest concentration in the series
4 ml. membranes 0·9 × 27 cm column	2	1	1·9	(100)
	12	5·5	1 1	58
	72	22	0·80	42
Sonicate of 4 ml. membranes 0 8 × 28 cm column	2	1	1·36	(100)
	20	9	0·48	35
	60	20	0 70	51
Iodide extract of 7 ml. membranes 0 8 × 60 cm column	4	2	1·4	(100)
	20	9	0·70	50
	100	26	0·55	39

We have prepared columns from erythrocyte ghosts obtained from cells treated under different conditions with dinitrofluorobenzene (DNFB), an inhibitor of glucose transport[7], and have compared the amount of glucose retardation by such columns with the effectiveness of the residual sugar transport by the inhibited but otherwise intact cells. (We are indebted to Mrs. C. Caygill of this department for the preparation of these DNFB-treated cells and for the permeability assays, performed by measurements of the uptake of sorbose labelled with carbon-14 using procedures described previously)[3]. Table 2 shows that the inhibition of sugar transport and of glucose retardation closely parallel one another. A striking feature of this finding is that the inhibition of glucose retardation also displays the peculiar kinetics of DNFB-inhibition of sugar transport[7]—the rate of inhibition is higher than first order with respect to the DNFB concentration.

Table 2. Inhibition of Sugar Transport in Intact Erythrocytes and of Glucose Retardation by Columns of Erythrocyte Membranes on Treatment with Dinitrofluorobenzene

Concentration of inhibitor DNFB in mM	Time of incubation with DNFB at 25° C (min)	Sorbose permeability (percentage of control)	Glucose retardation (percentage of control)
0 (control)	0	100	100
0·4	20	130	140
4	2	48	58

The cells (control as well as inhibited) were all pre-treated with 0·4 mM DNFB at 4° C for 1 h, a procedure which we have shown to be without effect on sugar transport, but which blocks many DNFB-reacting sites on the cell membrane.

We feel that we are justified in identifying glucose retardation by columns of membranes as a reflexion of a property of the glucose transport system, on the grounds that this retardation is, in parallel with sugar transport, inhibited by phloretin and by DNFB, and saturates with glucose in the same concentration range as does glucose transport in the intact cell. We have, therefore, proceeded with the isolation of the glucose-binding material from erythrocyte membranes.

Our erythrocyte membranes are pink ghosts prepared by washing with distilled water. These ghosts sediment readily at 2,500 r.p.m., in 15 min. Treatment of such a membrane preparation with a 1 M solution of sodium iodide solubilizes the ghosts in that, after dialysis, much of the preparation no longer sediments under these conditions of centrifugation nor in the ultracentrifuge. The material solubilized in sodium iodide, when held on DEAE-cellulose columns at *p*H 7·0 in the presence of 0·003 M sodium phosphate, retains intact its ability to retard glucose in preference to sorbose (Fig. 3). Elution of such a column with a linear gradient of 0·5 M sodium phosphate at *p*H 7·0 yields five major peaks of material absorbing at 254 mμ (and at 280 mμ) (Fig. 4) and, simultaneously, the ability of the column selectively to retard glucose is lost. When the material eluted from the column is, after dialysis, put back on fresh columns of DEAE-cellulose, glucose retardation can once again be demonstrated. The major retardation activity is associated with peak II (60 per cent) while lesser amounts of retarda-

tion are found in peak I (23 per cent) and peak III (17 per cent). Peak IV shows no retardation.

Thus our peak II, a component of the human erythrocyte membrane which is (i) extracted from pink ghosts by molar sodium iodide, (ii) held on to DEAE-cellulose at *p*H 7·0 in 0·003 M phosphate and (iii) eluted at some 0·1 M phosphate, is associated with most of the glucose-binding capacity of erythrocyte membranes. This binding capacity seems to be closely associated with glucose transport. We believe that our peak II material may well be responsible for glucose transport in the intact erythrocyte.

The work described here was supported by a grant from the Nuffield Foundation to Prof. A. R. Birch. One of us (B. B.) holds a Research Fellowship maintained by these funds. We thank the Nuffield Foundation and Prof. Birch for this support and numerous colleagues (in particular, Drs. S. Hunt, S. R. Ayad and A. R. Kolber, and Mrs. M. Levine) for their helpful advice and criticism.

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SOLUBILIZATION OF A GLUCOSE-BINDING COMPONENT OF THE RED CELL MEMBRANE

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Department of Biological Chemistry, University of Manchester

HUMAN red cell membranes are known to contain a system capable of bringing about "facilitated diffusion" of glucose into the cell, presumably mediated by some binding component[1]. Investigations of this system have hitherto been confined, in the absence of techniques which would measure binding of glucose in cell wall extracts, to the intact cell membrane. This article describes the solubilization and assay, by a new technique, of a system which binds glucose in human red cell membranes.

A variety of techniques are available for extraction and disruption of erythrocyte membranes[2,3]. In order to evaluate the validity of these methods in extracting, without damage, active cell wall constituents we have taken as our standard the activity of acetylcholinesterase, an enzyme thought to be an integral component of the erythrocyte membrane itself[4]. On the basis that the acetylcholinesterase activity of the ghosts, when still present after solubilization, is a measure of maintained integrity of other membrane components, we were then able to proceed to studies of glucose binding by the method of retention or retardation analysis.

The technique of retention analysis is based on a method postulated by S. Hunt and W. D. Stein for measurement of binding in general and was subsequently developed by W. D. Stein in this department as an experimental technique. (We are indebted to Dr. Stein for providing us with the practical details for the technique used here.) The basis of this method is the concept of a column composed of binding molecules (either free or held in cell membranes) through which is passing a solution containing both molecules with affinity towards the binding molecules and also molecules with no affinity. Obviously the molecules with affinity will, providing the geometry of the column is correct, eventually lag behind those without affinity. In the present case the column consists of either red cell ghosts or solubilized ghosts supported on some medium (DEAE cellulose) through which is passed a mixture of glucose (with affinity) and sorbose (without affinity). Labelling of the sorbose with carbon-14 and the glucose with tritium permits ready assessment of the lag of glucose behind sorbose.

Retention or retardation analysis is, in essence, the same as any other chromatography in that it involves partition of the separating mixture (glucose and sorbose) between a moving phase (the solution containing the glucose and sorbose) and a stationary phase (the glucose-binding agent). It follows, therefore, that the elution volume of a substance retained by the binding agent is dependent on the equilibrium constant between the free and bound forms. The difference between the elution volume of an interacting molecule and that of a non-interacting molecule will be referred to as the retention volume.

Theoretically the shape of a peak eluted from a column is Gaussian and corresponds to the equation

$$y = \frac{1}{c\sqrt{2}} \cdot e^{-(x-\bar{x})^2/2c^2}$$

where y is the peak height at the elution volume x, $\bar{x}$ is the elution volume at y_{max} and c is a constant which is a function of the peak spread and is dependent on the diffusion rate.

The ratio $y_{retarded}/y_{unretarded}$ for two similar but displaced Gaussian curves plotted against x is an exponential. If the log of this ratio is plotted against x, a straight line results the gradient of which depends on the displacement, that is on the retardation, of one peak relative to the other.

Thus, $y_r/y_0 = e^{(x-x_0)^2-(x-x_r)^2/2c^2}$

Therefore $\log (y_r/y_0) = (x(\bar{x}_r - \bar{x}_0) + \bar{x}_0^2 - \bar{x}_r^2)/k$

and $\log (y_r/y_0) \propto x(\bar{x}_r - \bar{x}_0)/k$

where subscripts 0 and r refer to the unretarded and retarded peaks respectively and where k is a function of c.

To calculate k for a known peak shape it is only necessary to construct a second peak retarded by some predetermined elution volume $(\bar{x}_r - \bar{x}_0)$ and to obtain ratios of y_r to y_0 for a series of values of x. A plot of y_r/y_0 against x then yields a straight line from the gradient of which k may be calculated, knowing $(\bar{x}_r - \bar{x}_0)$. The value of k determined theoretically may then be applied to the determination of $(\bar{x}_r - \bar{x}_0)$ in the real system.

To attain the ideal Gaussian peak shape, in chromatography, the rate of elution must be very slow and there must be only one type of interaction with the stationary phase. At practical elution rates the lower part of the leading edge of the peak is steeper than the ideal; moreover, secondary interactions such as that between DEAE cellulose and glucose cause tailing of the peak. The upper two-thirds of the leading edge do, however, correspond reasonably to the mathematically ideal situation. Thus analysis of this part of the peak in the manner described enables an accurate estimate of retention volume to be made. The analysis can, however, only be assumed to obtain when the amount of spread of the two peaks is the same. For the sorbose–glucose system used here this is assumed to be the case.

Haemoglobin-free erythrocyte ghosts were prepared by a modification of the procedure of Maddy[2]. Washed cells were haemolysed in 0·005 M phosphate buffer at *p*H 8·0 for 1 h followed by washing, on the centrifuge, in 0·01 M phosphate buffer at *p*H 8·0. After standing in the latter buffer at 4° C overnight the ghosts were washed alternately with 0·005 M and 0·01 M phosphate buffers at *p*H 8·0 until white. The white ghosts were finally washed three times in distilled water.

Four separate methods of solubilization or extraction were attempted. The technique of extraction with butanol and water was carried out according to the procedure of Maddy[2], only the aqueous soluble fraction being examined in this instance. Extraction of ghosts with 0·5 M phosphate buffer was performed at *p*H 7·0 and the soluble fraction freed of phosphate by dialysis. Extraction by 1·0 M sodium iodide was carried out according to a procedure developed by Dr. H. Bobinski in this department and we are therefore indebted to him for permission to use this technique before publication. Iodide was removed from extracts by dialysis. Extraction with the detergent 'Triton *X*100' was carried out by mixing equal volumes of ghost suspension, and a 5 per cent v/v aqueous solution of 'Triton *X*100'; clarification of the suspension occurs almost immediately. An exact estimation of the degree of solubilization was not attempted. The following observations might, however, be noted. Whereas, in the solubilization of bovine erythrocytes by butanol, Maddy[2] reports little material remaining at the butanol–water interface, we find a very heavy flocculent white material to be formed. The aqueous extracts were clarified by centrifugation at 20,000*g*. In the case of the 'Triton' extract, centrifugation yielded negligible residue whereas the phosphate extract yielded a considerable solid residue and the iodide extract a fairly considerable residue.

Acetylcholine esterase activity was estimated in ghosts and extracts according to Michel[5]. The aqueous extract resulting from butanol extraction possessed only 3·7 per cent of the original acetylcholine esterase activity of the ghosts while iodide extraction solubilized 8·2 per cent of the activity. Treatment with phosphate failed to extract any acetylcholine esterase activity. The 'Triton' extraction, however, resulted in complete extraction of acetylcholine esterase; moreover, the activity was enhanced, the recovery in the extract being of the order of 130 per cent. On this basis 'Triton' extracts were examined for glucose-binding properties.

Retention analyses were carried out on columns of DEAE cellulose equilibrated with 0·003 M phosphate buffer at *p*H 7·0. In order to attain even distribution of ghosts or solubilized material throughout the column, samples were introduced into the DEAE cellulose before packing the column. After packing, the columns were eluted with 100 ml. of 0·003 M phosphate buffer at *p*H 7·0 followed by careful application to the top of the column of a solution containing tritiated glucose, sorbose labelled with carbon-14, "cold" glucose and "cold" sorbose to a total glucose and sorbose concentration of 2 mM each. Columns were then eluted with 0·003 M *p*H 7·0 phosphate buffer at a rate of 1 ml. per min and fractions of 6 to 10 drops collected after rejecting the first 25 ml. of eluate (hold up volume). Suitable aliquots of each fraction were taken for counting in a Packard liquid scintillation counter. Results were analysed as described. Control columns of DEAE cellulose alone showed no retention of glucose relative to sorbose.

Figs. 1–3 illustrate the results of a typical retention analysis carried out on a 'Triton' solubilized erythrocyte ghost preparation. Fig. 1 shows the eluate plot (or retentiogram) of glucose and sorbose counts versus fraction number while Fig. 2 shows the plot of the ratio of glucose counts to sorbose counts versus fraction number. In addition, Fig. 2 also shows the result of theoretically retarding the glucose effluent curve by two and by four fractions. Fig. 3 indicates the plots of log glucose–sorbose ratio versus fraction number and the plots of log ratio versus fraction number for the theoretical curves. Derivation of $1/k$ from the theoretical curves yields a retention for real glucose curve of 1·36 fractions or 0·205 ml. This value agrees fairly well with the value obtained by observing the displacement at half the peak height on the leading edges of the two curves, that is, approximately 1·25 fractions or 0·188 ml.

In a comparative experiment in which equal volumes of ghost suspension were taken from the same ghost preparation, only one portion solubilized and the whole ghosts and extract then separately subjected to retention analysis and acetylcholine esterase assay, a retention of 0·408 and 0·615 ml. for the ghost preparation and the extract respectively was detected. Acetylcholine esterase activity was enhanced in the extract, in this case by 42 per cent.

The foregoing results thus appear to suggest that treatment of erythrocyte membranes with the detergent

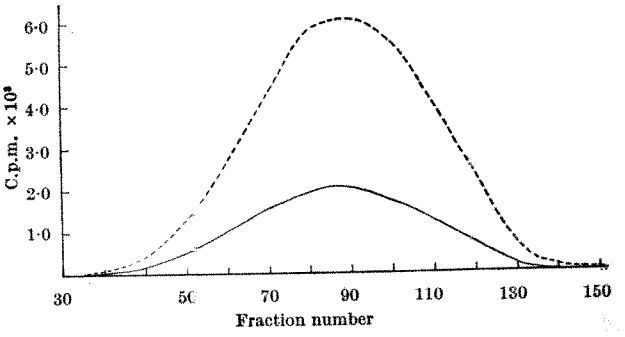


Fig. 1. Retentiogram of tritiated glucose (– – – –) and sorbose labelled with carbon-14 (———) on DEAE cellulose loaded with a 'Triton X100' extract of erythrocyte ghosts.

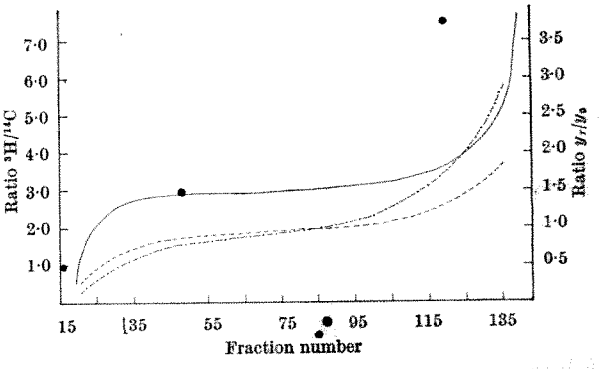


Fig. 2. Ratio y_r/y_0 versus fraction number derived from the retentiogram of Fig. 1, where y is peak height at any value x of fraction number and where r and 0 refer to the retarded and unretarded peaks respectively. Left hand scale, ratio of tritium/carbon-14 counts (———). Right hand scale, ratio $^{14}C_x/^{14}C_{x+2}$ (– – – –) and $^{14}C_x/^{14}C_{x+4}$ (—·—·—).

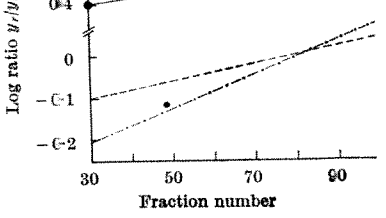


Fig. 3. Log y_r/y_0 versus fraction number. ———, Ratio of tritium/carbon-14; – – – –, $^{14}C_x/^{14}C_{x+2}$; —·—·—, $^{14}C_x/^{14}C_{x+4}$. Symbols as in Fig. 2.

'Triton X100' produces the solubilization of membrane components without loss of biological integrity, the method of retention or retardation analysis permitting identification of the presence of a glucose-binding system both in the whole membranes and the extracts. Work is at present in progress to fractionate the extracts. The system solubilized need not necessarily be the actual glucose transport system. Further investigations would be needed to confirm or deny this. The bovine erythrocyte possesses no such glucose transport system and a comparison, by the methods used here, with human erythrocytes might be illuminating.

This work is complementary to that of Dr. H. Bobinski and Dr. W. D. Stein in the preceding article.

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HYPERSENSITIVITY INDUCED IN ALBINO MICE BY MELANOCYTE-STIMULATING HORMONE

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DURING the course of experiments using melanocyte-stimulating hormone (MSH), it was found that albino mice became hyperexcitable after injections of this hormone; that is, they reacted to stimuli with greater force, rapidity and frequency. The change in behaviour induced by MSH was further investigated because such a response might shed light on the physiological significance of this ubiquitous hormone[1].

Mice injected with MSH typically moved in short, fast bursts and were difficult to catch. They struggled more vigorously than control animals during injections and would often attempt to bite. Because of their more frequent and more rapid movements, they could easily be distinguished from controls by the increased noise they made in their cage. If mice injected with MSH were observed without stimulation several hours after injection, their behaviour and movements were similar to those of control mice. If they were stimulated by loud noises, or by tapping or moving their cage, or if attempts were made to catch them, however, their movements would again become rapid and "jerky".

When mice injected with MSH were suspended by their tails, they consistently arched their bodies closer to a horizontal or upright position than did control mice (Fig. 1). At times they climbed up their own tails—a manoeuvre not attempted by control animals.

It was possible to detect hyperexcitability after the fourth to sixth injection of MSH, and the onset of this condition was earlier in younger mice and later in older. The maximum response occurred at 2–3 weeks and it gradually declined over a period of 2 months.

Hyperexcitability was quantified by measuring the increase of motor activity in reaction to stimuli recorded graphically on the motor activity recorder of Easterday and Higinbotham[2]. In this method mice are placed on a 'Styrofoam' platform in contact with four needles attached to gramophone cartridges. The impulses from the cartridges are amplified and recorded.

In the experiments reported here, Swiss albino male mice (*BNL* strain) 5–8 weeks old were randomly divided into groups of from four to seven for each hormone. βMSH, in doses of 0·1–1·0 mg (Armour, sp. act. 2×10^6 U/mg), was generally used. The hormones were mixed with 16 per cent gelatine, and the gelatine solution was used as control material. They were warmed in a water bath at 40° C just before injection, and injected subcutaneously once daily 6 days per week.

Large doses of βMSH were not toxic—the mice injected were vigorous and healthy and gained weight (two groups of mice injected with 0·2 and 1·0 mg for 7 weeks gained 18 per cent of their original weight, compared with controls which gained 25 per cent of their original weight).

After a week or two of injections when hyperexcitability became obvious, one group of mice at a time was placed on the platform of the motor activity recorder[2] for 2–3 min about 15 min after all injections were completed for the day. The process of moving the animals from their own cage to another location plus the previous parenteral injection provided enough stimulation to cause the mice injected with MSH to move about more rapidly and more frequently than the controls. If the mice were tested in the motor activity recorder 2 or 3 h after injection, a stronger stimulus than the mere process of transfer from cage to platform was often necessary to bring about hyperexcitation. Of the various stimuli tried (the alarm of a clock, electric shock, bright lights, etc.) the simplest and most effective in showing the difference between test and control animals seemed to be two light strokes of the rubber eraser of an ordinary pencil beginning from the lower abdomen cephalad to the tip of the nose. Excessive stimuli, such as electric shock, evoked the same response in both test and control animals. Further, when mice were tested individually rather than in groups, it was difficult to obtain consistent results.

The tracings from the motor activity recorder were quantified by summing up the amplitudes of each excursion. Each deflexion represents a burst of vibrations created by a movement of the mouse on the platform.

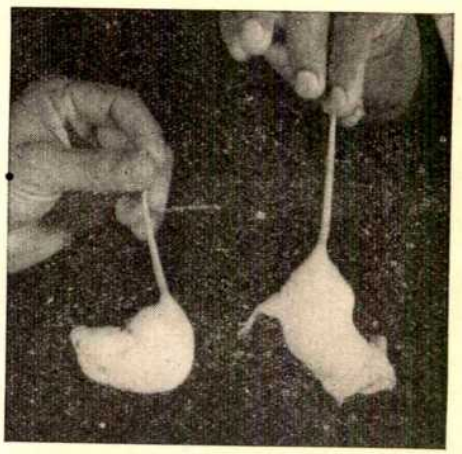


Fig. 1. Albino mice suspended by their tails, showing that the mouse injected with MSH (left) arches its body more closely to horizontal than does the control (right).

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The amplitude of the excursions increased with increase of the loudness of vibrations made by the mouse. Fig. 2 shows the amplitudes of excursions when weights were released from a fixed distance from the platform. The amplitudes are averages of four or five such "drops". The units are arbitrary as the response of the recorder can be varied by a gain control in the motor activity recorder.

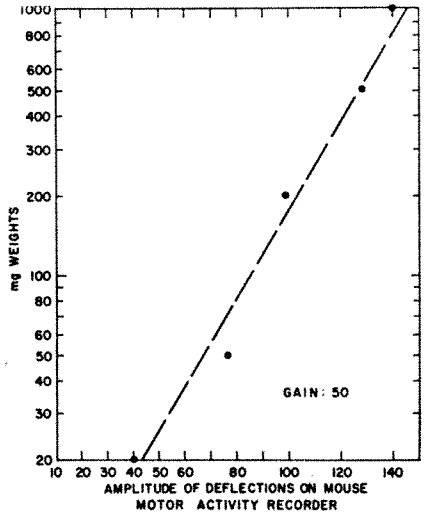


Fig. 2. The response of the motor activity recorder to varying weights dropped 10 cm from surface.

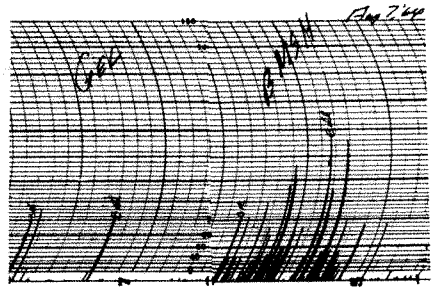


Fig. 3. Graph taken from the motor activity recorder showing increased activity of a group of mice injected with βMSH (right) compared with controls (left).

The increase of motor activity following stimulation of mice injected with βMSH is clearly shown in a tracing taken from the recorder (Fig. 3). A quantitation of such a tracing (Table 1) shows that the degree of hyperexcitability declines after the third week.

We also carried out experiments to find (*a*) whether this hyperexcitability may be due to another well known effect of MSH on the thyroid gland (its "TSH-like" effect)[3], and (*b*) whether the reaction is a result of contamination of the MSH preparation by a polypeptide from the original pituitary preparation.

Question (*a*) was studied by thyroid ablation and by injections of thyroid stimulating hormone (TSH). Two groups of mice were "thyroid ablated" with iodine-131. They were placed on a low iodine diet (whole milk) for 7 days, injected with 200 mc. of $Na^{131}I$, and maintained on the same diet for 10 days more. The "thyroid-lethal" dose was estimated from data presented by Gorbman[4]. One of these two groups was treated with 0·1 mg βMSH and the other with gelatin solution.

Another group was injected with 2·0 mU of TSH—an amount shown to produce more than twice the increase in blood iodine-131 than 0·1 mg βMSH does[3].

The second question, as to whether hyperexcitability may result from a contaminant, was studied using a highly purified αMSH (supplied through the courtesy of Dr. A. V. Schally, sp. act. $1–2 \times 10^7$ U/mg). Small amounts of adrenocorticotropic hormone (ACTH) were tested as its structure is closely related to that of MSH.

In Table 2 the motor activity after stimulation of three groups of mice (those treated with αMSH, βMSH, and βMSH after thyroid ablation) is compared with four groups of mice (those injected with ACTH, TSH, gelatine, and gelatine after thyroid ablation).

Table 1. SUMMARY OF DATA FROM MOUSE MOTOR ACTIVITY RECORDER

Groups	Material injected	Amount per day	Duration	No. of mice	Total activity	Activity per mouse
A	β MSH	0·1 mg	5 weeks	5	2,068	414
	Gelatin	0·05 ml.	5 weeks	5	110	22
B	β MSH	0·1 mg	8 weeks	4	354	88
	Gelatin	0·05 ml.	8 weeks	4	104	26
C	β MSH	0·2 mg	3 weeks	6	6,766	1,127
	β MSH	1·0 mg	3 weeks	6	4,371	725
	Gelatin	0·05 ml.	3 weeks	6	77	13

Average activity per mouse for groups *A*, *B* and *C*:
Mice injected with βMSH; 588 ($t = 2·2$).
Mice injected with gelatin; 20 (P between 0·025 and 0·05).

Table 2. SUMMARY OF DATA FROM MOUSE MOTOR ACTIVITY RECORDER

Groups	Material injected	Amount per day	Duration	No. of mice	Total activity	Activity per mouse
A	β MSH	0·1 mg	1 week	6	510	85·0
	α MSH	0·1 mg	1 week	6	820	136·7
	β MSH and ^{131}I	0·1 mg	1 week	7	640	91·4
B	Gelatin	0·05 ml.	1 week	6	190	31·7
	ACTH	2 U	1 week	6	230	38·2
	TSH	2 mU	1 week	6	200	33·3
	Gelatin and ^{131}I	0·05 ml.	1 week	7	220	31·4

Average activity per mouse:
Group *A*, 104·4 ($t = 5·1$)
Group *B*, 33·7 ($P < 0·005$)

From the results presented in Table 2, it is clear that the hyperexcitability noted was not the result of a "TSH-like" effect of MSH. Also, a highly purified αMSH obtained from a laboratory different from that which supplied the βMSH did produce a state of hyperexcitability. Furthermore, small amounts of the related hormone ACTH were devoid of such activity. It therefore appears likely that the MSH polypeptide is the hormone responsible for this behavioural change.

Recently I noticed that βMSH affects albino rats in a manner entirely different from that reported here for albino mice. It induces drowsiness, hyperpnoea, hypertension, and piloerection. Dyster-Aas and Krakau have reported similar effects in the rabbit, in which MSH produced drowsiness, hyperpnoea, hypotension, and increase of cutaneous temperature[5].

Krivoy, Lane and Kroeger have shown that βMSH "augments excitability" in (*a*) the dorsal root preparation in cats and Lloyd's preparation by increasing the response to submaximal stimulus, and in (*b*) the electric knife fish, by causing a decrease in the frequency of electrical signals emitted, in a manner similar to the response when startled[6]. Krivoy and Guillemin suggest that "one of the possible roles of βMSH in the central nervous system is to maintain and modify the central excitatory state of some neuronal systems' [1]. The increased excitability observed in albino mice could possibly be related to the same group of phenomena.

Further experiments are in progress on the effect of MSH on the behaviour and central nervous system of mammals, to find whether such phenomena are a manifestation of a biological role of this hormone.

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IS γ-AMINOBUTYRIC ACID AN INHIBITORY TRANSMITTER?

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γ-AMINOBUTYRIC acid (GABA) was first identified as a normal constituent of brain tissue in 1950[1,2]. Some years later, GABA was found to have a marked depressant effect on cortical neurones[3], and to be a constituent of Factor I—an extract of brain tissue found to have a strong inhibitory action on certain isolated neurones[4] of Crustacea. The possibility was therefore considered that it might act as a chemical transmitter at synapses, or as a controller of the excitability of neurones. The inhibition of crayfish neurones (and muscle) proved to be particularly significant as it became clear that GABA acts in every respect like the inhibitory transmitter[5–7] of crustacean neurones, and is only found in the inhibitory axons of lobster nerves[8].

The hypothesis that GABA might be the main inhibitory transmitter in the central nervous system (CNS) of mammals was soon discounted, however, because it was considered incompatible with certain experimental observations[9,10]. The most consistent and serious objections were those raised by Curtis and his associates[11–14], who claimed that the action of GABA in the spinal cord differs significantly from that of the inhibitory transmitter. Post-synaptic inhibition of the spinal cells is associated with an increase in membrane potential. But experiments with co-axial pipettes failed to detect any hyperpolarization of motoneurones during the administration of GABA. As there was no evidence that GABA had a hyperpolarizing action in other parts of the CNS, Curtis concluded that GABA could not be the main central inhibitory transmitter[13,14].

On the other hand, more detailed investigations of the effects produced by GABA on cortical cells[15,16] showed a remarkably potent inhibition—more potent, in fact, than the effects of any other substance known to be present in the brain in appreciable amounts. In spite of certain preliminary investigations from which it appeared that GABA had no strong hyperpolarizing action on cortical cells[16], it was therefore concluded that before making a thorough comparison between the effects produced in the cortex by GABA and by post-synaptic inhibition[16,17], the possibility that GABA might be the inhibitory transmitter should not be dismissed. This article reports the results of such a direct comparison.

Our experiments were performed on cats under pentobarbitone anaesthesia. Twin micropipettes were used for intracellular recording and for the extracellular release of GABA; they consisted of conventional single glass micropipettes fixed side by side with dental cement, in such a position that the respective tips were separated by a distance of 20–100μ. One pipette was filled with 3 M potassium chloride or 2 M potassium citrate. Its tip was sufficiently fine (< 1μ) to penetrate at least some cells without excessive injury. The second pipette had a somewhat larger tip (1–2μ) and it was filled with 2 M GABA acidified with hydrochloric acid to *p*H 4·5. The recording electrode was connected to a cathode follower through a saline : silver : silver chloride link and a second cathode follower made contact with muscle on the back of the animal's neck, via a comparable silver : silver chloride electrode. Before further amplification, the outputs of the two cathode followers were fed into a balanced Wheatstone bridge. This bridge allowed short pulses or steady currents to be passed through the intracellular electrode while the membrane potential was being recorded. It was therefore possible to measure changes in cell resistance, or to polarize the cell, while testing the action of GABA. The pipette filled with GABA was connected to a high-impedance polarizing circuit which provided small outward currents (10–100 n.amp) for the iontophoretic release of GABA as a cation. Electrical coupling between the two pipettes was checked regularly, *in situ*. Apart from transients at "on" and "off", artefacts resulting from coupling seldom exceeded a few millivolts.

The twin micropipettes were inserted into the sensorimotor cortex, on either side of the cruciate sulcus. Neuronal inhibition was evoked by cathodal stimulation of the exposed surface of the cortex at a rate of 4–5 sec. The shocks were 1 msec. square waves, at an intensity of 5–20 V. This kind of stimulation has a widespread and prolonged inhibitory effect on neurones throughout all layers of the cortex[18–20]. A single shock causes an inhibitory post-synaptic potential which typically lasts for more than 100 msec.

The cells observed formed two populations: those giving inhibitory or excitatory responses, or firing "spontaneously"; and others which neither fired spikes nor responded to any kind of stimulation. The first type of cells we assumed to be neurones: the second, we suggest, are likely to be glial cells. The "glial" cells yielded significantly higher and more stable membrane potentials than did neurones.

Resistance measurements showed a substantial drop in resistance of the neurones during the initial phase of the inhibitory post-synaptic potential; this can be seen as a diminished pulse immediately after the stimulus artefact in Fig. 1*A*. When GABA was released, there was a marked drop in resting membrane resistance within a few seconds (Fig. 1*B*). At the same time, all inhibitory and excitatory post-synaptic potentials and neuronal discharges (Figs. 1*B*, 2, 3*B* and *C*) were much reduced or abolished. With electrodes filled with potassium nitrate, we recorded hyperpolarizing inhibitory post-synaptic potentials, and GABA regularly had a hyperpolarizing action which occluded the inhibitory

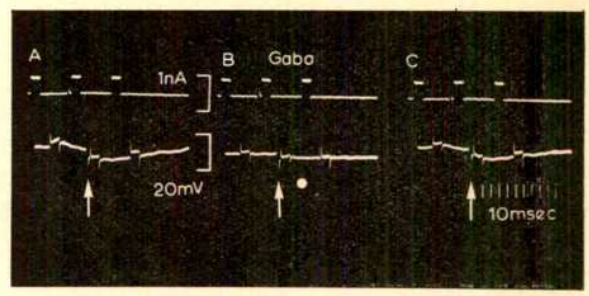


Fig. 1. Intracellular records showing the effect of GABA on the membrane properties of cortical neurones. Upper traces indicate a d.c. reference level and the intensity of outward current pulses delivered through the intracellular electrode for resistance measurements. Lower traces show changes in membrane potential and resistance before and during an inhibitory post-synaptic potential evoked by stimulating the surface of the cortex immediately above the point of recording; arrows indicate the stimulus artefact. The membrane resistance is proportional to the amplitude of the pulses recorded in the lower trace. Downward deflexions are in hyperpolarizing direction. *A*, Control record; *B*, during iontophoretic release of GABA from the extracellular pipette (60 n.amp): note hyperpolarization and fall in resting resistance; *C*, 8 sec after release of GABA was stopped.

* NATO Postdoctoral Fellow.

post-synaptic potentials (Figs. 1*B* and 2). All these effects disappeared soon after the end of the release of GABA (Figs. 1*C*, 2 and 3*D*). If the state of the cell remained satisfactory, the experiment could be repeated several times without any sign of desensitization.

When an inhibitory post-synaptic potential recorded with an electrode filled with potassium citrate was reversed by suitably polarizing the neurone, the action of GABA was also reversed. Furthermore, complete reversal of inhibitory post-synaptic potential was readily obtained when chloride ions were released into the cells (from electrodes filled with potassium chloride); under these conditions GABA also had a depolarizing action, and it even became possible to excite a cell strongly by a steady application of GABA. We did not take records for a sufficiently long time in any one case to test

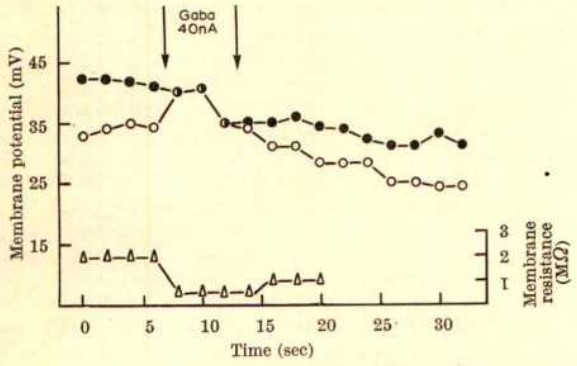


Fig. 2. Graph showing changes in membrane potential and resistance of a neurone produced by an extracellular application of GABA (40 n.amp), indicated by two arrows. ○, Resting potential; ●, potential at peak of inhibitory post-synaptic potential evoked by cortical stimulation; △, resting resistance.

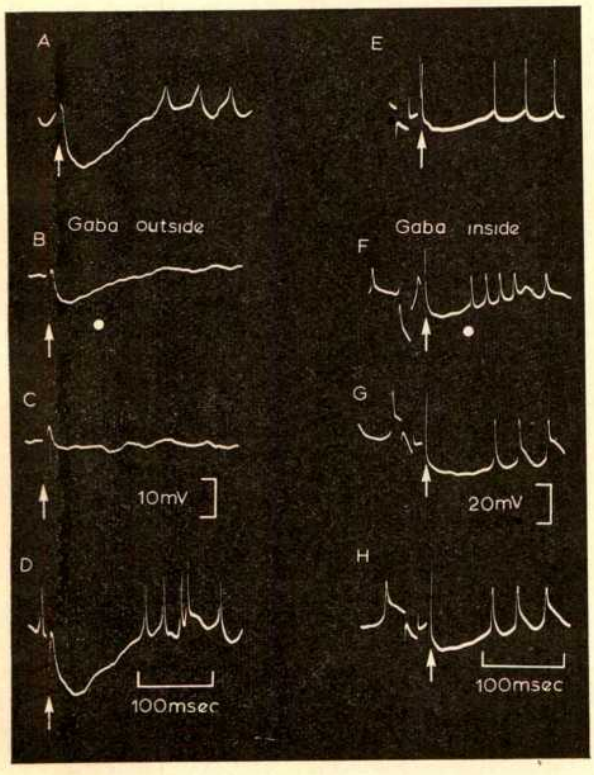


Fig. 3. Intracellular records from two cortical neurones (*A–D*) and (*E–H*), which show that GABA is much more effective when applied outside than inside cells. *A* and *E*, Control traces of injury discharges interrupted by inhibitory post-synaptic potential evoked by single shocks to surface of cortex; *B*, during extracellular release of GABA (40 n.amp); *F*, during intracellular release of GABA (1·2 n.amp); *C* and *G* immediately after iontophoresis of GABA; *D* and *H*, 3–6 sec later.

GABA systematically over a wide range of membrane potentials. The fact that in various cells its action always shifted the membrane potential in the same direction as the inhibitory post-synaptic potential, however, suggests that the two processes must have a similar equilibrium level.

We also released GABA inside cells, by using recording electrodes filled with GABA. Three of these cells were neurones which were firing spontaneously and had clear inhibitory post-synaptic potentials (Fig. 3*E*). Even repeated injections of GABA by currents of 1–3 n.amp, for as long as 45 sec, did not alter greatly their membrane potential, inhibitory post-synaptic potential or rate of discharge (Fig. 3*F* and *G*).

In contrast to the marked and predictable effects of GABA on the membrane potential and conductance of the cortical neurones, the effects produced on the cells in the second population (the glial cells as already defined) were quite different. There was no detectable change in cell conductance, and only a relatively slight, but reversible and repeatable depolarization with large doses of GABA.

Because the action of GABA on cortical neurones is so similar to that of synaptic inhibition, one must seriously consider the possibility that GABA is, in fact, the main inhibitory transmitter in the cortex. It is perhaps worth repeating that the cortex contains very substantial amounts of GABA but relatively little of the other ω-amino-acids (or the corresponding guanidino compounds) which have a comparable depressant action[21]. Weaker depressants, such as noradrenaline, dopamine and 5-hydroxytryptamine[15,22], are found there only in relatively small amounts[23] and there is no histochemical evidence of any important mono-aminergic innervation at the cortical level[24]. Moreover, cortical inhibition is not affected by adrenergic blocking agents[17]. The effects produced in the cortex by acetylcholine and its antagonists suggest that the inhibitory synapses cannot be cholinergic[17].

The rather widespread distribution of free GABA in subcellular fractions (and particularly in the supernatants) of cortical homogenates has been adduced as evidence against the possibility that GABA might be a transmitter[14,25]. The inhibitory effect of suspensions of nerve endings or synaptic vesicles, however, can be accounted for by their GABA content[26]. Like other known transmitters, GABA would probably be distributed throughout the relevant neurones; but only GABA present in the nerve endings is likely to be trapped or bound. As all the evidence suggests that the cell bodies of cortical inhibitory fibres lie mostly within the cortex[20] most of the cortical GABA would not appear in a bound form when the tissue is homogenized.

Despite its relatively high concentration in the cortex, GABA cannot be readily detected in the fluid bathing the surface of the cortex[27]; only under certain special conditions can the release of GABA be demonstrated at all regularly[28]. This observation is not inconsistent with a transmitter function. Clearly, a powerful transmitter could not be allowed to accumulate in the extracellular fluid: in fact, any substance continuously exuding from a tissue in large amounts is extremely unlikely to be a transmitter in the usual sense.

GABA must exert its principal action on the outer surface of the cortical neurones (compare crayfish muscle)[7] as intracellular injections tended to be ineffective. GABA could thus be functionally inactivated by a simple uptake into cells. The uptake of amino-acids, including GABA, by slices of brain is a well known process[29,30], which could take place either in the nerve endings (compare the sympathetic system), or in the perikaryon. Its depolarizing action on "glial" cells, without a corresponding increase in conductance, may indicate that glial cells are concerned in the active transport of GABA. It may not be easy to demonstrate the release of GABA by nerve endings until some means of specifically blocking such an uptake mechanism can be found. It would also be of

great help in the analysis of inhibitory effects in the cortex and elsewhere in the CNS if a specific antagonist for the inhibitory action of GABA were available; so far there has been no convincing evidence that in the cortex one can selectively block either the action of GABA or synaptic inhibition.

In conclusion, it is evident that GABA imitates the action of the cortical inhibitory transmitter at least qualitatively: it usually raises the membrane potential and conductance of cortical neurones, just like the normal inhibitory mechanism at synapses, and when the latter inhibitory effect is artificially reversed, the action of GABA is reversed in a similar way. Hence, in so far as the cortex is concerned, there is no evidence against the hypothesis that GABA is an inhibitory transmitter.

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FACTORS CONTROLLING THE DIFFERENTIATION OF THE HYDATID ORGANISM, *Echinococcus granulosus*, INTO CYSTIC OR STROBILAR STAGES *IN VITRO*

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IT is generally recognized that the development of *in vitro* culture techniques for parasitic helminths is a fundamental problem in parasitology. In recent years, a number of cestodes have been cultured *in vitro* from the larval stages to maturity or near maturity[1–5].

In vitro culture of the causative organism of hydatid disease, *Echinococcus granulosus*, has presented some very special problems, for not only is the period of maturation relatively long (40 days), but the larval stages (protoscoleces), which are found in a hydatid cyst, are remarkable for being capable of differentiating in two directions. Thus, when a hydatid cyst is eaten by a dog, the freed protoscoleces develop into adult strobilated tapeworms in the duodenum. On the other hand, if a hydatid cyst bursts, while still within the intermediate host (sheep, man, etc.), each of the released protoscoleces is capable of differentiating into a new hydatid cyst should it reach a suitable tissue site. Thus, a protoscolex has the potentialities of developing either into a tapeworm (in an intestinal site) or into a hydatid cyst (in a tissue site)—a degree of heterogeneous morphogenesis unusual for a metazoan organism. This suggests that there are factors present in the intestinal and tissue environments which initiate differentiation in these two directions. Attempts to determine the nature of these factors by *in vitro* experiments have been, to date, largely unsuccessful.

Yamashita *et al.*[7] and Smyth[8] found that, when cultured *in vitro* in a variety of media, protoscoleces of *Echinococcus* differentiated only in a cystic direction, and eventually developed into miniature hydatid cysts with laminated envelopes. This occurred either (*a*) by forming a "posterior bladder" which secreted a laminated envelope (Fig. 1), or (*b*) by becoming vesicular and similarly secreting a laminated envelope (Fig. 1). Webster and Cameron[9], using a succession of complex media, succeeded in obtaining several segmented forms of *E. multilocularis* and *E. granulosus*, but the precise conditions under which this result was obtained were not clarified experimentally or confirmed by further work.

In a series of experiments, extending over a period of some years, we have attempted to initiate the growth of *E. granulosus* in a strobilar direction so that the tapeworm stage could be obtained *in vitro*. On account of the high risk of infection from handling the hydatid organism in infected dogs in the laboratory, it is the *in vitro* culture of this stage which is of special interest and importance. Although a wide range of modified tissue culture techniques were used, as well as techniques which have been used successfully for other cestodes[1], cultures always resulted in growth in a cystic direction as already described. Attempts to alter this pattern of development, by varying the usual physiological conditions of culture (*Eh*, *p*H, pO_2, pCO_2), or the nutritional constituents (amino-acids, vitamins, etc.), were without success.

These consistently negative results led us to speculate that some wholly unsuspected and unusual requirement was missing from the culture conditions provided. Attention was therefore turned to the conditions under which a worm lives while attached to the intestinal mucosa. We have shown[10,11] (*a*) that, in the small intestine of the dog, the worm penetrates deeply between the villi with each sucker, drawing a plug of villus tissue into its cavity, (*b*) that the extended rostellum of the worm normally penetrates a crypt of Lieberkühn, and (*c*) that the rostellar tip contains gland cells which secrete externally. The significance of these observations was not at first clear, but

the failure of *in vitro* methods led us to re-examine them in more detail. This led to the conclusion that the contact of the scolex with the mucosal epithelium was so close that the worm could perhaps be regarded (at least at the early stage in which it consists largely of scolex) as more of a tissue parasite than an intestinal parasite. If this hypothesis were sound, the organism would not only be absorbing nutritive materials from the intestinal contents, but it would also be absorbing directly through its contact with mucosa. In other words, the scolex of *Echinococcus* could be regarded as essentially placental in nature. These speculations were reinforced by investigations of the ultrastructure of the tegument, which showed that the protoplasmic projections termed microtriches were present only on the scolex region of a freshly evaginated protoscolex (Fig. 2) and not on the region posterior to the suckers. This latter region was covered in a dense periodic acid–Schiff positive (PAS-positive) layer beneath which were found what appeared to be undeveloped microtriches. If microtriches are concerned with absorption (and they have been reported from the tegument of a number of cestodes[12,13]) it is likely that absorption can take place initially only from the newly evaginated region.

This led us to speculate that the requirement missing *in vitro* was a solid substrate which could act as a supporting surface and from which, at the same time, nutriment could be obtained directly. We had previously used such a nutritive substrate for the *in vitro* culture of an intestinal trematode[14].

Experiments have shown, in fact, that this hypothesis appears to hold, although a number of basic problems had to be overcome before it could be adequately tested. The chief of these was concerned with obtaining satisfactory evagination of the protoscoleces. Although some evagination takes place without preliminary treatment, the process is greatly accelerated by treatment with enzymes. It is known that pepsin plays some part in evagination[15], but further treatment with pancreatin and trypsin in the presence of bile serves the multiple role of accelerating evagination, stimulating activity and removing cystic debris. Pretreatment with pepsin (0·025 per cent) three times at *p*H 2·0 (20 min) followed by treatment with 0·3 per cent pancreatin (5 N.F.) and 0·1 per cent trypsin (twice) plus 5 per cent dog bile or the equivalent bile salt (for 1–3 h), all in balanced salt solutions, produce almost immediate evagination of more than 90 per cent of protoscoleces. In any one batch of cultures, the degree of evagination appears to be related to the source and freshness of the material and in most cases the percentage falls to about 30–40 after about 3 h.

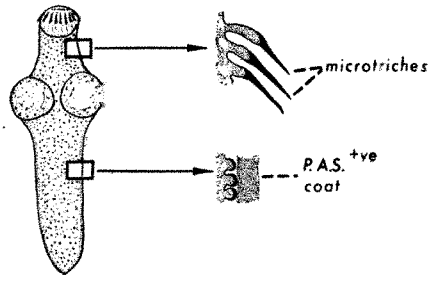


Fig. 2. Ultrastructure of the tegument with anterior and posterior regions of an evaginated protoscolex of *Echinococcus granulosus*. Microtriches only occur in the anterior region.

Once the problem of evagination was essentially overcome, attempts were made to culture the organisms using diphasic media, with liquid and solid phases. The culture system, in screw-top bottles, consisted of a solid base of bovine or canine serum coagulated by heat, into which a series of small holes had been made with a fine glass pipette; alternatively, the surface was roughened. The whole was covered by a liquid phase of 'Parker 199' and 20 per cent hydatid fluid. A gas phase of 8·8 per cent oxygen and 5 per cent carbon dioxide in nitrogen was used. In this diphasic system, a marked change came over the cultures; organisms which were evaginated remained so, became visibly elongated and eventually strobilated. Under the best conditions to date (using bovine serum base covered with 'Parker 199' and 20 per cent hydatid fluid) segmentation has been obtained in 32 days compared with 15 days *in vivo*.

That this growth has resulted from actual placental contact with a suitable substrate and is not merely due to diffusion of soluble materials from the solid base into the liquid phase was shown by the experiment illustrated in Fig. 3. Cultures were set up as follows: *A*, contained a bovine serum base coagulated by heating; *B*, contained the equivalent volume of coagulated serum retained within a cellulose tubing. As a control, culture *C* had no base, but had 10 ml. of serum added to the liquid phase; culture *D* had a non-nutrient agar base instead of serum.

In culture *A*, organisms grew and became segmented as described above. In *B*, although organisms survived for considerable periods, they gradually became abnormal, vesicular, formed posterior bladders (Fig. 1),

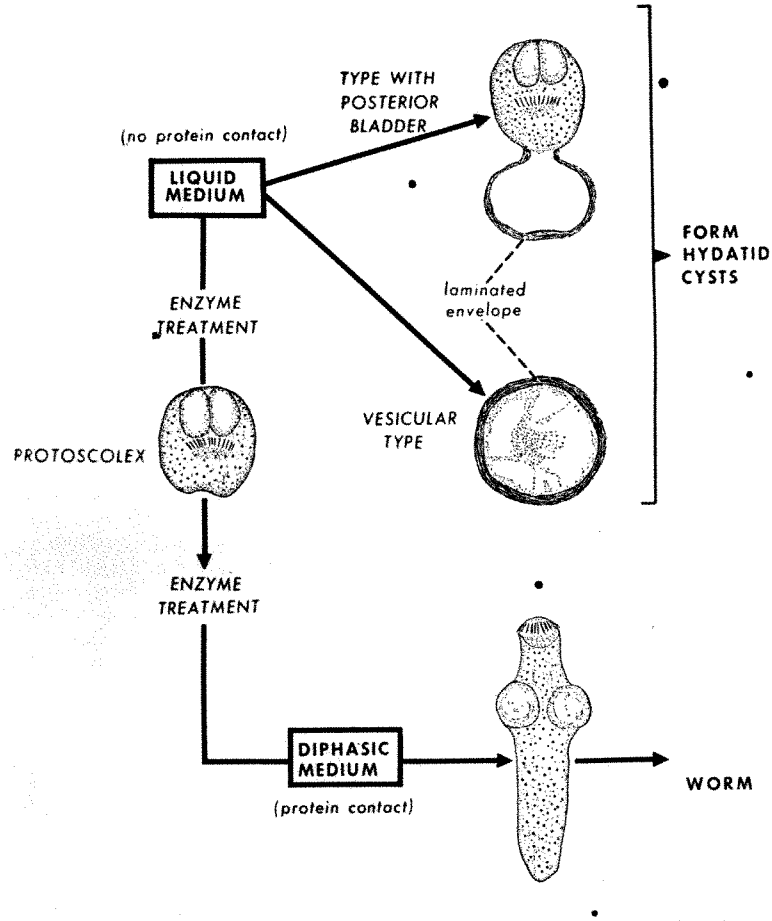


Fig. 1. Pattern of differentiation of the protoscoleces of *Echinococcus granulosus* in liquid (monophasic) and diphasic media: strobilization only occurs in diphasic medium. Unevaginated protoscoleces form miniature hydatid cysts.

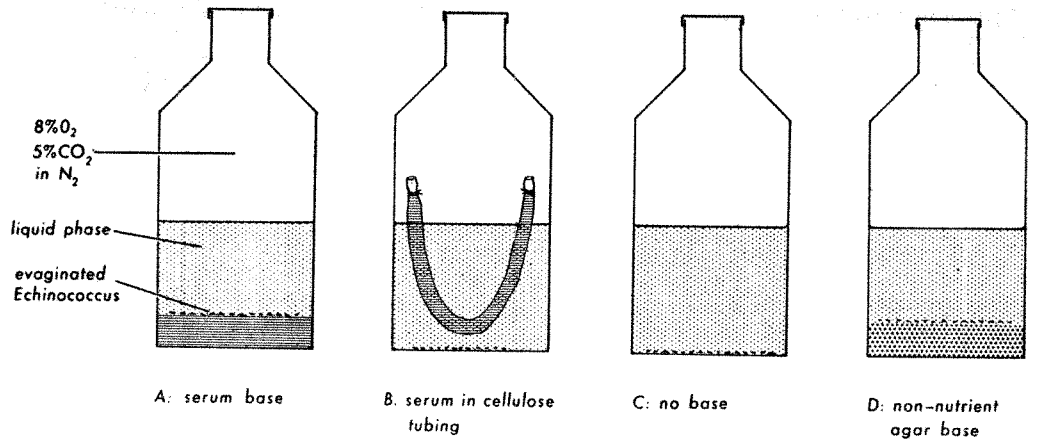


Fig. 3. Various monophasic and diphasic media used for *in vitro* culture of *E. granulosus:* strobilization only occurs in *A*.

or died, as did the organisms in the controls *C* and *D*. This and comparable experiments have been repeated a number of times and, in every case in which a nutritive substrate has not been provided, growth and strobilization have not taken place. That the role of the substrate is truly nutritive and is not merely to provide physical support is shown by the fact that the non-nutrient solid base of agar (culture *D*, Fig. 3) was not effective in supporting growth in a strobilar direction. Contact as such may, of course, be a part of the stimulus necessary for inducing growth in a strobilar direction, but if such a contact stimulus is involved it is clearly not effective in the absence of the appropriate nutriment. In this respect it may be noted that the tip of the rostellum carries a number of delicate hairs, the occurrence of which appears to have escaped previous observation. These structures undoubtedly represent sensory receptors, and their exposure by evagination of the scolex and subsequent contact with a suitable substrate may result in the production of a stimulus necessary for strobilization. This stimulus could perhaps operate through a neuro-secretory mechanism.

The importance of the protein base is further emphasized by observations of the activity of the evaginated protoscoleces during the early phases of cultivations. During this period, in all cultures (even those with bases), there is a decline of activity and the appearance of a remarkable phenomenon, namely the onset of a period of "tetanus"—characterized by the organisms becoming rigid and the suckers ceasing activity. In cultures with bases (A_1 and A_2, Fig. 4), however, organisms slowly regain activity, whereas in those without bases activity further declines until it finally ceases altogether (*B* and *C*, Fig. 4). This result suggests that after a period of contact with the substrate, organisms are able to obtain nutriment from it and utilize it for metabolic activity. We do not yet know precisely how this is done. We have been unable (to date) to detect the occurrence of pinocytosis in the rostellar region, but have some suggestive evidence that the scolex is secreting proteases, digesting the base and absorbing the products at the tegument/base interface. Undoubtedly, the microtriches present on the anterior region play a part initially in establishing close contact with the protein base. Once the organisms begin to grow, further microtriches develop in the posterior region previously covered by the PAS layer.

The failure of unevaginated protoscoleces to develop can be explained by the fact that no microtriches are exposed and intimate contact with the protein substrate cannot take place. The few strobilate worms obtained by Webster and Cameron[9] were undoubtedly obtained as a result of the use of a filter paper support in some of their cultures.

These experiments demonstrate that the conditions required for protoscoleces to differentiate in a strobilar direction *in vitro* are (*a*) evagination of the scolex and (*b*) a nutritive substrate with which intimate contact can be established. Failure to provide both these conditions results in differentiation in the cystic direction. In addition, the usual physiological requirements and conditions (*p*H, *Eh*, gas phase, etc.) must be satisfied within reasonable limits.

At the time of writing, development of *Echinococcus* to a three-proglottid worm *in vitro* has repeatedly been obtained, although the genitalia in such worms are only poorly developed and eggs have not yet been produced. Extensive experimentation may be necessary before appropriate solid and liquid phases, which permit maturation to the egg producing stage *in vitro*, are developed. It is unlikely that the conditions in our experiments are anything approaching optimal, and factors such as gas phase, *p*H, *Eh*, clearly require detailed examination as does the composition of the liquid and solid phases in terms of levels of the usual growth requirements, amino-acids, vitamins, sugars, etc. The *in vitro* system developed should, however, enable quantitative investigations of the relative importance of these factors to be carried out, and perhaps the basis for the strobilar and cystic differentiation at a biochemical or molecular level determined.

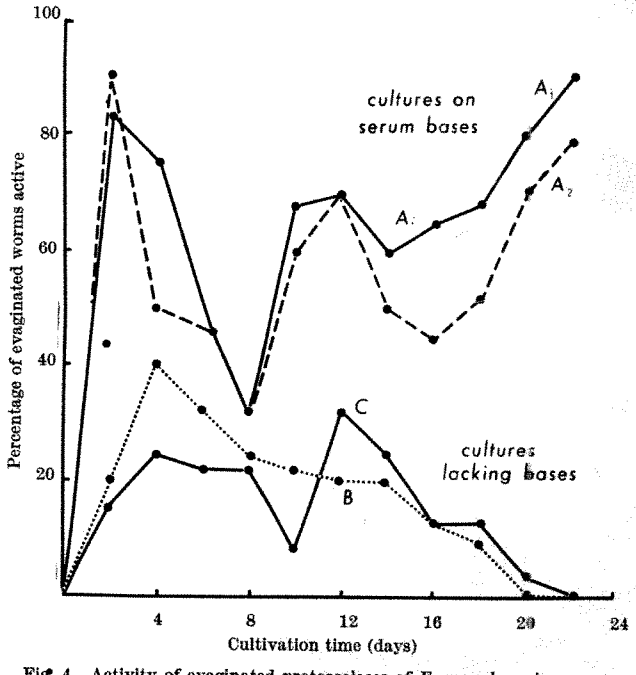


Fig. 4. Activity of evaginated protoscoleces of *E. granulosus* in monophasic and diphasic media. Activity is only maintained in the diphasic cultures A_1 and A_2 (see Fig. 3 and text).

It is interesting to note that Schiller[6] has recently shown that in the case of *Hymenolepis diminuta* a diphasic medium can replace the complex synthetic medium developed by Berntzen[1,2] for the culture of this species. Since, unlike *Echinococcus*, *H. diminuta* can develop in a liquid medium alone, its nutritional demands appear to be substantially simpler than those of *Echinococcus*.

As already mentioned, protoscoleces of *Echinococcus* which fail to evaginate develop in the cystic direction and form miniature hydatid cysts either by the growth of a small posterior bladder or by the swelling up of the protoscolex to become a vesicular form (Fig. 1). The latter appears to be a response to abnormal condition such as a low *p*H (for example 6·5) or mechanical damage to the tegument, although we have not examined this phenomenon in great detail. In both types of growth, a thick laminated envelope (Fig. 1), similar to that found round a hydatid cyst, is secreted which eventually encloses the whole organism.

In summary, then, it has been shown that protoscoleces may be induced to differentiate in either a strobilar or a cystic direction *in vitro* by provision of suitable culture conditions. This culture system is somewhat unusual for a helminth parasite, for differentiation, comparable to that occurring in either the definitive or the intermediate host, is possible.

Apart from the parasitological aspects of these results, the protoscoleces of *Echinococcus* may represent unique biological material. As all the protoscoleces within a hydatid cyst have developed from a single fertilized egg, each has an identical genotype. Moreover, as a single sheep hydatid cyst contains very large numbers of organisms it is possible to obtain perhaps as many as 10^5 organisms with the same genotype. We know of no other metazoan organism which possesses these unusual characteristics, and whose differentiation can be controlled and modified *in vitro* in this way. It would appear to represent an experimental model *par excellence* for experimental investigations in genetics, cell differentiation and morphogenesis.

These experiments have been based on *E. granulosus* but are likely to be used with more success on *Echinococcus multilocularis*, a "species"[10] which matures more rapidly than *E. granulosus*.

We thank the Australian Wool Board, the National Institutes of Health, U.S. Public Health Service and the World Health Organization for financial assistance in support of this work.

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OXIDASE ACTIVITY IN ECOTYPIC POPULATIONS OF *Typha latifolia* L.

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In 1922 Turesson suggested that plant species are differentiated into sub-specific populations which differ in their ecological characteristics according to the habitat they exploit[1]. Since then, genecologists have reported occurrence of morphological[2], phenological[3], and physiological[4] differences between populations adapted to different climates. These investigations provide strong circumstantial evidence for the occurrence of differences in enzyme activity between ecologically distinct populations[5]. This article provides additional evidence for this and indicates that biochemical activities which are dependent on temperature can be influenced by the environment.

Populations of broad leaved cattail (*Typha latifolia* L.), a species which is temperate circumboreal in distribution[6] (predominantly Arctic to 30° S.), were selected from marshes at Beaverton and Redmond, Oregon. Two populations were sampled at each site. Redmond (940 m above sea level) is located in the Deschutes River valley, just east of the Cascade Mountains. Beaverton (60 m above sea level) is in the Willamette River basin, between the Cascades and the Coast Range, and is subject to coastal influence from the Columbia River gorge. Weather reports[7] from stations near the populations sampled show that the climates of the two regions differ substantially (Table 1). The Willamette Valley vegetation is principally grain and truck crops but was originally *Pseudotsuga menziesii* Mirb. (Franco) and *Acer macrophyllum* Pursh[8]. Vegetation at Redmond is *Juniperus occidentalis* Hook. with *Artemisia tridentata* Nutt. at lower altitudes[9].

Rhizomes attached to shoots excised 30 cm above the soil were transplanted to the greenhouse in May and June and experiments were performed with this material in late June and early July and again in late August. The old shoots rarely grow after such transplantation; essentially all growth is from the attached rhizomes which, however, grow only poorly when detached from the old shoot. Mass leaf samples were collected from the natural populations in late August and were iced or refrigerated until experiments could be performed. The maximum storage time was 3 days and previous experiments indicated that no loss in activity occurred during storage for 5 days at 2° C.

Isolation of the chloroplasts was accomplished by a technique modified from standard methods[10]: 20 g of leaves were blended in 80 ml. 0·35 M sodium chloride for 1 min; the slurry was filtered through a double layer of cheesecloth and centrifuged at 1,000*g* for 1 min; the supernatant was centrifuged at 37,000*g* for 10 min; the pellet was suspended in 0·067 M phosphate buffer, *p*H 7·8, to give 0·008 g/ml., and 2·5 ml. of this suspension of chloroplast fragments added to each Warburg vessel. All operations were carried out near 0° C. The departures from standard technique were the longer blending time which was made necessary by the less succulent nature of *Typha* leaves and the higher centrifugation speeds required by the smaller size of the plastids. The preparation was undoubtedly contaminated with mitochondria. All experiments were performed in a dimly lighted room on a Gilson *GRP* 14 respirometer with potassium hydroxide in the centre well of the flasks. The vessels were allowed to equilibrate for 20 min and the rate of uptake of endogenous oxygen measured in two 5 min periods before adding the substrate (this usually approached

zero and was never more than 10 per cent of the rate in the presence of substrate). Substrates and *tris*-hydrochloric acid, buffered to *p*H 7·8, were tipped in from the sidearm to make the suspension 0·008 M. Control flasks lacking plastids were always run but fluctuation in these was negligible. Because of the occurrence of non-enzyme ascorbate oxidation in the presence of cupric ions (ref. 11), rates obtained with boiled plastid preparations (usually about 40 per cent of the enzyme rates) were subtracted from experimental ascorbate sets.

With catechol as a substrate, it was found that the consumption of oxygen at warmer temperatures depended on the place of origin; greater activity occurred in populations from regions with a continental climate (Fig. 1). Activity was stimulated more by temperature in the Redmond plants. The rate of increase of enzyme activity (Q_{10}) over the range of 17°–27° C was 1·4 for maritime and 3·7 for continental plants. It seems probable that several different enzymes, or forms of the same enzyme, are responsible for phenol oxidation and that the enzyme frequently referred to as "polyphenol oxidase" is in fact a group of enzymes rather than a distinct species[12]. Nevertheless, in the populations of *T. latifolia* studied, the ability to oxidize catechol is greater in populations growing in a continental climate, particularly at warm temperatures. This catalytic activity has been related to the possibility of providing protection from attack by micro-organisms[13,14] and also to lignin biosynthesis[15,16], but the precise function of this enzyme has not yet been determined. Because the activity measured is probably the result of that of several enzymes, however, the functions are almost certainly multiple. The data indicate, however, that the activity in the populations of *T. latifolia* investigated correlated with the climate, which therefore suggests that adaptation occurs.

With ascorbic acid as substrate, there were no differences between the natural and greenhouse populations (Fig. 2). Mapson[17] has shown that the activity of ascorbic acid oxidase increases considerably with increased light intensity, and that more of the ascorbate is converted to dehydroascorbate. Once the conditions of darkness are restored, the equilibrium lies in the opposite direction and the quantity of dehydroascorbate declines. Other investigators[18,19] have shown that an NADP–glutathione reductase system links the ascorbate oxidizing system to enzymes oxidizing respiratory substrates with nicotinamide–adenine dinucleotide phosphate. The function of this sytem is not known. The data on *Typha* indicate that activity of ascorbic acid oxidase is not of selective importance in differentiating the four populations sampled.

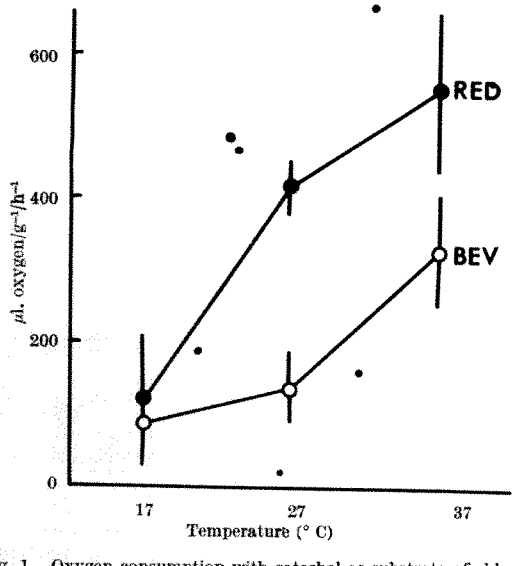


Fig. 1. Oxygen consumption with catechol as substrate of chloroplast fragments from two continental (RED) and two maritime (BEV) populations of *Typha latifolia* L. Points are means of twelve replications, six per population. Vertical bars indicate 95 per cent confidence intervals.

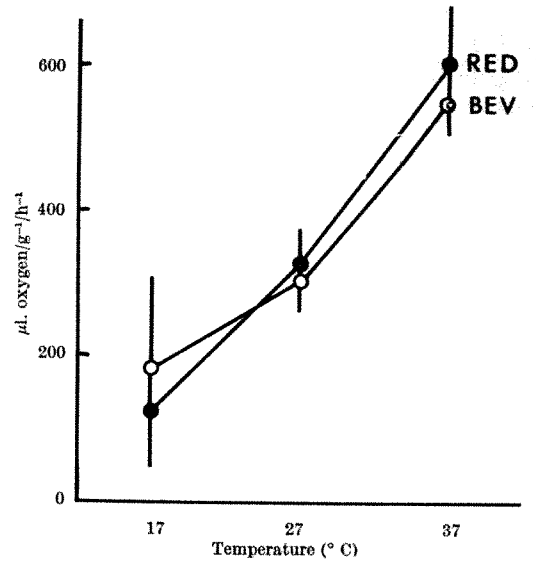


Fig. 2. Oxygen consumption of chloroplast fragments with ascorbic acid as substrate.

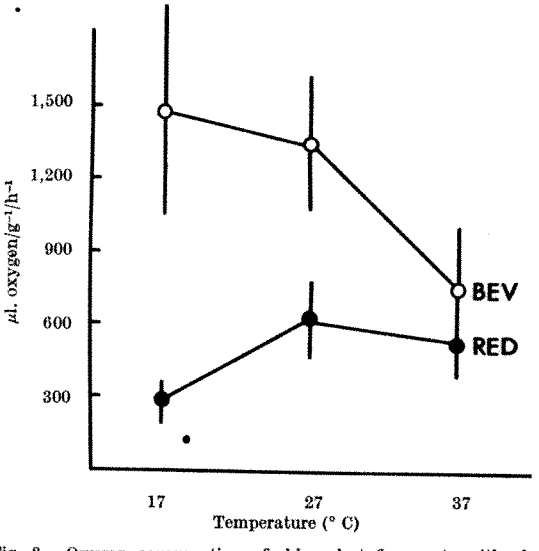


Fig. 3. Oxygen consumption of chloroplast fragments with glycolic acid as substrate.

The most conspicuous enzyme difference found between the populations of *T. latifolia* from different climates was in glycolic acid oxidase activity (Fig. 3). There were pronounced differences in the magnitude and temperature dependence of oxygen consumption with glycolate as a substrate. Populations growing in a maritime climate were much more active at 17° and 27° C. Glycolate oxidation increased with increased temperature in the continental populations while the relationship was an inverse one in the maritime plants. Zelitch[20] has shown that more than half the carbon fixed in photosynthesis may be metabolized by glycolic acid oxidase. This suggests an important adaptive function for this enzyme in assimilatory metabolic pathways. It is known that the Beaverton marsh is most productive in June, August and September. The mean maximum day temperatures during these months (1964) are 21°, 24° and 22° C, respectively. Data concerning the productivity for the Redmond marsh are not available. The frost-free period is only 90 days, however, and average maximum day temperatures during

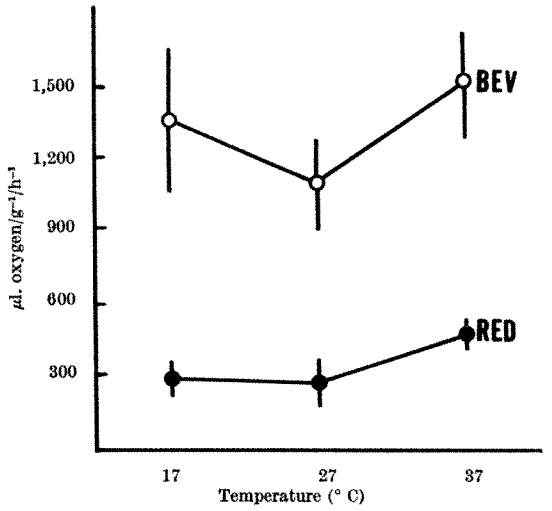


Fig. 4. Oxygen consumption of chloroplast fragments from greenhouse-grown plants with glycolic acid as substrate.

these months are 23° (June), 30° (July) and 27° (August). It seems clear from the data available that the dependence on temperature of glycolic acid oxidase activity in natural populations of *T. latifolia* is related to the daytime temperatures at the native site. If, as Zelitch's recent data indicate[21,22], glycolic acid is an early and important product of carbon fixation by photosynthesis, the ecological importance of this temperature dependence in integrating assimilatory pathways with temperature climate is considerable.

In early studies of plants from the natural populations transplanted into the greenhouse, the dependence on temperature of glycolic oxidase activity was similar to that of natural populations[5]. After 3 months in the greenhouse, however, the dependence on temperature had shifted (Fig. 4). Oxidase activity in all populations was relatively independent of temperature, but with slightly greater rates at 37° C. The maritime populations were still much more active in glycolate oxidation, but temperature dependence was similar in plants from different climates. For Beaverton plants at 37° C, a comparison of the enzyme activity of plants growing in their natural habitat and those grown in the greenhouse gave a t value of 65·3—a highly significant difference ($P \ll 0{\cdot}001$ with $d.f. = 10$). Glycolic acid oxidase activity in Redmond plants was significantly different at 27° C, depending on the growing conditions ($t = 37$ with $d.f. = 10$; $P \ll 0{\cdot}001$). In view of the similarity of the activities at 17° and 37° C, however, it is doubtful whether this is a significant difference in any practical sense. The greenhouse conditions did not resemble the temperature climate of either native site, although the maximum day temperatures (about 38° C) were closest to those at Redmond. As can be seen in Table 1, the frequency of days approaching this temperature is much greater at Redmond. There were 4·5 times as many days at 32° C, or warmer, at the continental site compared with the maritime site.

Table 1. WEATHER DATA FROM TWO OREGON SITES DURING THE PERIOD WHEN MINIMUM TEMPERATURES WERE GREATER THAN −2° C AT REDMOND

Location	Month (1964) June	July	August
Beaverton (Hillsboro)			
Avg. temp. (° C)	15	18	17
Avg. maximum	21	25	24
Avg. minimum	8	11	9
No. days max. ⩾32	0	1	3
No. days min. ⩽0	0	0	0
Redmond (Redmond Airport)			
Avg. temp.	14	18	16
Avg. maximum	23	30	27
Avg. minimum	4	6	4
No. days max. ⩾32	2	10	6
No. days min. ⩽0	5	2	3

This is probably reflected in the negligible modification of the temperature curve in the plants from Redmond compared with those from Beaverton. Recent studies of photosynthetic and respiratory rates in reciprocal transplants have shown that temperature dependence may be altered by climate[23–25]. Björkman *et al.*[26,27] have shown that photosynthetic activity can be dramatically altered by growing ecotypes under different conditions.

Enzymatically distinct races have been known since Mitchell and Houlahan's work on *Neurospora*[28]. The most intensively studied multiform enzyme is the lactic dehydrogenase system[29]. As many as fifteen electrophoretically distinguishable isoenzymes, all presumably formed by different tetrameric combination of three polypeptide chains, have been discovered[30]. Dorn[31,32] has shown that there are three mutants of *Aspergillus nidulans* which produce active phosphatases when grown at 35° C and partially or wholly inactive phosphatases when grown at 25° C. He has proposed that the various isoenzymes are produced by different combinations of distinct protein monomers. Glycolic acid oxidase is a tetramer in its most active form[33]. It is attractive to envisage a combination of various monomers producing various forms of the enzyme in the *T. latifolia* ecotypes under the control of different environmental conditions.

The data presented here indicate that populations of *T. latifolia* from climatically distinct sites are enzymatically distinct. The results also indicate that activity may be regulated by environmental conditions. There is no reason to suppose that the differences in catechol oxidation in populations from different sites would not disappear if they were grown under the same conditions. Similarly, populations might differ substantially in ascorbic acid oxidase activity when grown under the same conditions even though activity was identical in the native populations. The data are indicative, not definitive.

The investigation reported here was done while I was a faculty member of Portland State College, Portland, Oregon. It was supported by grants from Tektronix Foundation and Portland State College. The technical assistance of Mrs. Leta Burnham is gratefully acknowledged.

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NEW ASPECTS OF VISUAL PERCEPTION THRESHOLDS

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THE statistical hypothesis advanced by Rose[1,2], and independently formulated by de Vries[3] and Pirenne[4], to account for visual contrast and acuity thresholds in conventionally illuminated scenes, was extended to X-ray images by Sturm and Morgan[5], and further elaborated by Coltman[6] and by others. This extension has been tacitly assumed, though with some reason, to apply to all visual images in which random fluctuations of luminance tend to obscure the regular patterns (so called "noisy" images).

The conventional image of common experience and the noisy image differ in that only the latter exhibits marked spatial and temporal correlations between the emission of light photons; the former does not appear to be noisy, although stochastic processes in the eye must certainly result in a noisy pattern of visual stimuli from the retina. Albrecht[7] has therefore rightly remarked that the validity of a common theoretical approach to perceptibility in both types of image is by no means obvious. Although there seems to be no experimental evidence in support of this equivalence of treatment, the necessary computational facilities which man has acquired by evolutionary development for dealing with the conventional visual image appear to be equally effective for the frankly noisy image. There is no protracted learning process involved in the perception of the grainy photograph, the half-tone reproduction, the noisy television image (X-ray or conventional), the *pointilliste* painting, etc., suggesting that the same computational mechanism is used for both types of image.

The essence of the Rose theory is that the perception of image detail is a sampling process in which the fluctuations in the image at that point in the system where the event flux density is smallest are integrated over the size of the detail and over the so-called storage or integration time of the system. If then the detail contrast, against its background, is greater by a certain factor k (the threshold signal-to-noise ratio) than the root mean square fluctuation so observed, the detail will be perceived. Clearly the effect of noise on detail perceptibility on this hypothesis is less for larger details, for longer storage times and for larger event flux densities.

Despite its usefulness, many criticisms of the Rose hypothesis are possible; some of these have recently been voiced by Morgan, who has proposed[8] an alternative model of visual perception. Morgan has raised two main objections to the Rose theory.

First, at a given level of event flux density the measured perceptibility of both large and small detail falls below the value predicted from the theory, a value which holds good for medium-size detail. Rose recognized this fact and attributed it correctly, but unspecifically, to "local limitations" in the eye. Morgan has elaborated this idea in the light of later developments and has described it in terms of light diffusion for small detail and of the level-correcting feed-back mechanisms in the eye for large detail. Both effects can be represented generally by the modulation transfer function (assuming the system to be linear over small amplitude excursions).

Second, although in the noisy image Rose assumes the eye to integrate the noise over the whole area of an image detail, nevertheless finer noise within that detail is still clearly visible and might therefore be reasonably assumed to interfere with the detail perceptibility. By way of analogy, Morgan points out that the noise amplitude in the output of an electrical transmission system is a function not of the frequency spectrum of the signals passing through it but of the physical bandwidth of the system itself. He therefore supposes that in an image system the effective noise amplitude is constant for a given event flux density and independent of the size of the detail; it is to be calculated from the input noise and the overall modulation transfer function, by way of the concept of noise-equivalent bandwidth. This type of hypothesis, based on a physically realizable noise level, is conceptually more plausible and satisfying than the Rose model in which the effective noise must be assumed to change from instant to instant as the attention of the observer turns to detail of varying size. Its introduction seems, however, to leave the following important points unresolved.

First, the calculation of the noise amplitude from available data may not be simple or even possible. If the spatial bandwidth limitations are predominantly external to the eye, there is in principle no difficulty, as the diffusion characteristics of a physical system may in general be determined as accurately as desired. If the properties of the eye significantly limit the system bandwidth, however, adequate diffusion data may not be available. In the limiting case of the conventional visual image, when the diffusion limitations are solely those of the eye, to calculate noise amplitude it is necessary to know the overall modulation transfer function of the visual system, including the optical components and the retina, but dissociated from any noise limitations. So far as is known, no separation of diffusion and noise processes in the eye has ever been made, and it is my belief that no valid information about the overall modulation transfer function of the eye exists. The reciprocal perceptibility thresholds for sinusoidal details commonly referred to as "sine-wave response", etc. (for example, Schade[9]), must necessarily include a visual noise component and therefore may not properly be used in the calculation of noise-equivalent bandwidths.

Second, Morgan asserts that the perceptibility differences between details of different size arise solely from diffusion and similar processes and not at all from spatial noise integration. No experimental evidence is offered in support of this proposition. I believe, however, that such experimental data as are available appear to be more consistent with the suggestion that the effect of noise on detail perceptibility is area-dependent. As only one example, Fig. 1 shows the results of subjective measurements of disk perceptibility in an X-ray image intensifying system, carried out by Hay, Clarke and Marquerinck and reported by Marquerinck and Recourt[10]. The viewing conditions were such that the diffusion limitations were effectively those of the physical system and were approximately known: the noise limitations were predominantly of X-ray quantum origin, as interpreted by the visual mechanisms of the observer.

The relative noise contribution to perceptibility, calculated from all the experimental data and normalized to a single value of event flux density, is shown in Fig. 1 as a function of detail size. Shown also are the theoretical noise contributions calculated from both the Rose and the Morgan models. (The upper limit to the "Rose" noise is set by the spatial bandwidth of the intensifier system: clearly the noise cannot possibly exceed this value, however small the image detail.) Similar evidence is reported

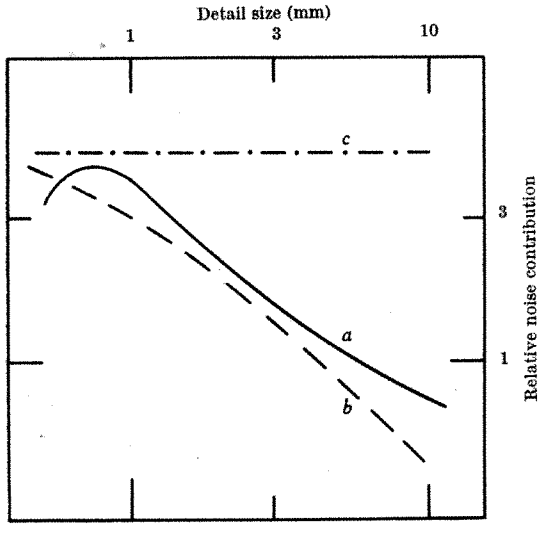


Fig. 1. Relative noise contributions to the perceptibility of circular detail. (*a*) Calculated from experimental data using an X-ray image intensifier television system; (*b*) calculated from the Rose theory; (*c*) calculated from the Morgan theory.

elsewhere, for example, for sinusoidal image detail in measurements by Coltman and Anderson[11]. It must be emphasized, however, that because of the complexity of the processes involved, quite different interpretations are possible for the data so far available. Clearly there is a great need for a new series of experimental studies performed with careful attention to all the relevant disturbing factors.

Third, the temporal characteristics of the visual system are expressed by Rose in the form of an integration time having the often quoted but poorly substantiated value of 0·2 sec; this in calculation yields plausible values for k of 2–5 depending on the nature of the image. Morgan, on the other hand, makes use of Kelly's measurements[12] of the temporal frequency response of the eye, corresponding to much smaller integration times from 0·02 to 0·07 sec; he calculates far less plausible values for k of 0·1 to 0·2. If k has indeed this order of magnitude then it necessarily loses its significance as a threshold signal-to-noise ratio, that is, as a ratio based on a statistical confidence level: it could be suggested that the wrong signal is being compared with the wrong noise. The conclusion seems inescapable that the visual system is at the same time capable of high-speed response as well as effectively long integration time.

The evidence outlined here reveals not only a remarkable symmetry between the spatial and temporal aspects of visual system behaviour but also a hitherto unformulated duality in frequency response, whether spatial or temporal. It suggests to me the possibility of a third type of hypothesis to account for visual perceptive thresholds. Three broad conclusions can be drawn from the evidence, of which two at least have not, so far as I am aware, been previously enunciated.

First, the spatio-temporal symmetry suggests that one might reasonably look for stochastic limitations to temporal response. Further analysis of Kelly's results[12], as well as of similar measurements at lower values of retinal illumination, is clearly desirable.

Second, the combination of good spatial and temporal high frequency response with low frequency "integration" is most satisfactorily accounted for by the assumption of a receptor of good spatial and temporal resolution, together with a computer which does not integrate and therefore destroy the fine detail from the receptor, but examines and analyses its pattern according to the needs of the moment. For example, in the temporal domain it becomes possible to reconcile Kelly's results[12] with the traditional 0·2 sec or thereabouts of the Rose theory by supposing that Kelly's data refer to the receptor (as is indeed most likely) and that the computer is normally effective over longer periods of the order of a second. This latter period has been referred to by previous authors (for example, Albrecht[7]) as a "short term memory"; it may well be identical, in mechanism as well as in magnitude, with that called by the psychologists "the observed present", which from quite different types of experiment is found to have a value between 0·2 and 1·0 sec. I find it difficult to imagine how any of these computational processes can occur in the retina, because as a result of rapid saccadic movements the co-ordinates of the input image relative to the retina must change many times in the longer computational period. It seems essential to postulate that these processes occur within a stable framework of reference relative to the mental presentation and this can surely be only central.

Third, we must look for a model to represent the type of analysis performed by the computer on the basic high frequency data from the receptor; this model must preserve the fine detail and yet permit its assessment in larger domains. I believe that in the spatial domain at least the meagre experimental evidence so far available, as well as objections of the kind raised by Morgan, can be accounted for by the adoption of a hypothesis of visual perception thresholds based on the Wiener or power spectrum of the noise in relation to the Fourier transform of the relevant image detail—the noise amplitude being determined in principle on the basis of Morgan's model.

There is virtually no experimental evidence from which such a hypothesis can be elaborated, but if it were assumed, in very general terms, that the perceptibility of image detail of a given linear dimension were disturbed only by noise of spatial frequency corresponding to a wavelength greater than that dimension, an effective noise dependence on detail size similar to that predicted by the Rose theory (with Morgan's modifications to account for diffusion processes) would be obtained. The important difference is that the idea of integration, with its implication of the destruction of fine-grain information, is avoided. Analogous behaviour has been observed in the aural system by Wegel and Lane[13], who have found that a tone of given frequency is well obscured by masking tones of frequency lower than its own but far less effectively by higher frequencies. Whether or not such a mechanism is operative visually in the temporal domain can at present be only a matter of conjecture.

The truth of this new hypothesis cannot be verified from existing experimental data because in almost every case the noise has been of the kind known as "white"; its Wiener spectrum has been uniform from zero to the upper frequency limit of the system. However, one isolated piece of supporting evidence is reported by Coltman and Anderson[11], who found that a reduction in noise bandwidth from 5 Mc/s to 600 kc/s (a noise amplitude decrease of 2·8) caused an improvement in the perceptibility of a 170 kc/s sinusoidal detail of only 25 per cent. Moreover, this small change could well have been due to the disappearance of low frequency noise intermodulation products resulting from the non-linearity of the display monitor.

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LETTERS TO THE EDITOR

ASTRONOMY

Observations on Stellar Extinction obtained in the *Gemini* 9 Space Flight

LINK[1] has suggested that there is a high absorbing layer in the Earth's atmosphere. This layer, presumably dust, was suggested in order to explain the anomaly in the size of the Earth's shadow during eclipses. Some support for the suggestion came from measurements of stellar extinction from Mont Blanc[2]. The height of the layer has been estimated as 100–150 km above the surface and it is presumed to have a vertical optical depth of 0·025–0·05. The purpose of this communication is to show that on the basis of stellar extinction measurements made in the *Gemini* 9 flight, no layer at an altitude above 80 km exists with a vertical optical depth of more than 0·0015. Either Link's layer does not exist at all or it is at a lower altitude than 80 km.

Table 1

1 Star	2 Magnitude $\frac{B+V}{2}$	3 Log_{10} brightness Frame 3	4 Log_{10} brightness Frame 2 normalized to Frame 3	5 Average of columns 3 and 4
α C Ma	−1·4	4·36	4·29	4·32
α C Mi	+0·56	3·47	3·58	3·53
ε C Ma	1·39	3·14	3·21	3·17
β C Ma	1·84	3·04	3·00	
δ C Ma	2·17	3·00	2·93	2·96
η C Ma	2·58	2·88	2·78	2·83
0^2 *C Ma*	2·98	2·65	2·58	2·61
ω C Ma	3·4	2·40	2·50	2·45

The extinction experiment was performed with two negatives of the airglow viewed in profile from above. These negatives were taken with a special camera designed for dim light photography in manned space flights. The 30 sec exposures were taken by astronauts Cernan and Stafford on June 4 during the *Gemini* 9 flight. In the first photographs (Frame 2) all the bright stars of Canis Majoris are above the airglow layer. In the second photograph (Frame 3), the star β *C Ma* is in the airglow layer. The experiment consists of comparing the brightness of β *C Ma* when it is above and when it is in the airglow layer. The height of the tangent ray to β *C Ma* in Frame 3 is about 80 km. The extinction produced in a thin layer viewed in profile is seventy times the vertical extinction. (The ratio of the tangent path to the vertical path is $\sqrt{(8R)}/y$, where R is the radius of the Earth, and y is the layer thickness or the scale height. For $y = 10$ km, the ratio is 70.) The brightness of the airglow layer viewed in profile from above is, of course, increased by approximately the same factor.



Fig. 1. Wide angle photograph of the sky, the airglow layer viewed in profile and the moonlit Earth obtained in *Gemini* 9 on June 4, 1966. The star β *C Ma* is in the airglow layer. *α C Mi* is left of centre in the picture. The obscuration in the lower left is due to the spacecraft window.

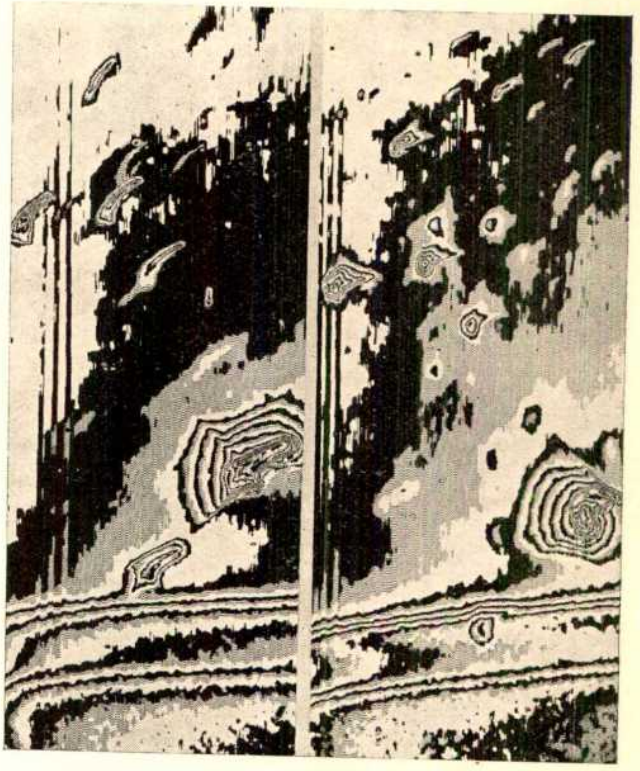


Fig. 2. Isodensity tracings of the portions of Frame 2 and Frame 3 which contain the constellation Canis Majoris. In Frame 3 right hand tracing β *C Ma* is in the airglow layer.

Fig. 1 shows the complete field of view of Frame 3. The field is 130° by 50° and the constellation Canis Majoris is at the left with the star β *C Ma* in the airglow layer. The star Procyon is just left of the centre of the picture.

Fig. 2 shows an isodensitrace of the portions of Frame 2 and Frame 3 which include Canis Majoris. Seven stars including β *C Ma* in Canis Majoris and *α C Mi* were accurately measured with a densitometer in Frame 2 and Frame 3. The calibration of the film was obtained from a step wedge exposure on the same film. The magnitude scale was determined by using all the stars except β *C Ma* in both frames. Table 1 shows the pertinent data, including the known magnitudes of the stars involved. Because of the wide spectral range of the film ('Tri *X*') the magnitudes used are the average of *B* and *V* magnitudes.

In Fig. 3 the experimentally determined values in column 5 of Table 1 are plotted against the magnitudes in column 2. The two values for β *C Ma* are shown with respect to this calibration. No extinction is detectable. Also shown in Fig. 3 is the position expected for β *C Ma* in Frame 3 if the vertical optical depth of the layer were 0·025, as previously proposed. This is clearly ruled

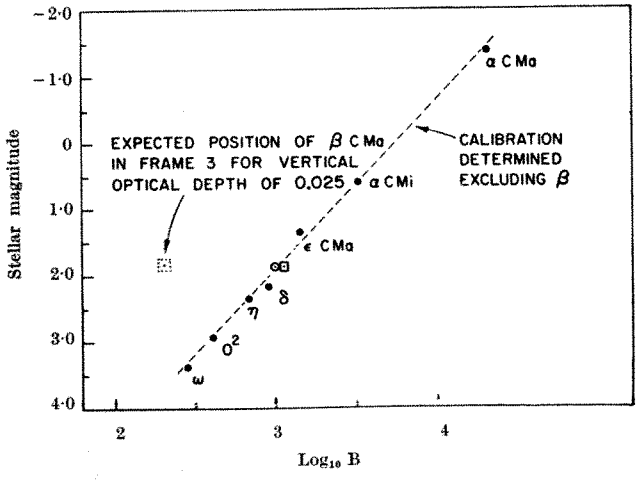


Fig. 3. Plot of the measured brightness of the stars in Canis Majoris and *α C Mi* establishing the film calibration. The values obtained for *β C Ma* in Frame 2 and Frame 3 are also shown and there is no measurable extinction in either case. The predicted value of the measurement in Frame 3 is shown for the vertical optical depth of 0·025. □, *β C Ma* Frame 3; ○, *β C Ma* Frame 2.

out by the experiment. For a pure Rayleigh scattering atmosphere the vertical optical depth above 80 km is of the order of only 10^{-5} and our experiment, although ruling out the dust layer, sets an upper limit of $1{\cdot}5 \times 10^{-3}$, corresponding to our measurement of extinction in Frame 3 of magnitude $0 \pm 0{\cdot}1$.

We acknowledge the excellent work of astronauts Cernan and Stafford in carrying out the experiment. The backup crew astronauts Lovell and Aldren were very helpful. We also thank Dr. Jocelyn Gill, John Lintott, W. F. Huch and Fred Gillett.

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PHYSICS

Proposed Search for Antimatter among Hydrogen-like Atoms in the Ambiplasma

H. Alfvén and O. Klein have developed[1,2] an appealing idea in which antimatter plays an important part in cosmology. N. A. Vlasov[3] has suggested an optical method for observing hydrogen-like atoms in an ambiplasma consisting of protons, anti-protons, electrons and positrons.

We have made some calculations relating to this question in which we include the positive and negative muon in the ambiplasma together with the four particles in Alfvén's model[2]. Radiation from positronium (e–$\bar{e}$), protonium (p–$\bar{p}$) or muonium (μ^+–e, or μ^-–$\bar{e}$), if observed in the centre of our galaxy, or in one of the quasi-stellar radio sources, would provide significant support for the Alfvén–Klein cosmology. Vlasov has suggested that our own galaxy might consist of an arm and an anti-arm and that the centre of the galaxy would be a logical place to search for an ambiplasma. The immense amount of radiant energy which apparently comes from quasars makes it plausible to assume that a significant mechanism is matter–antimatter annihilation. (The possibility that pair annihilation is the source of energy in quasars has recently been discussed by A. G. Ekspong, N. K. Yamdagni and B. Bonnevier[4].) This is the reason why we believe that the quasars may be ambiplasmas, that is contain significant admixtures of matter and antimatter.

Table 1. Wavelengths in Ångströms for Several Hydrogen-like Atoms

		Positronium	Protonium	Muonium	Hydrogen
Lyman	α	2430·9	1·3234	1220·9	1215·7
	β	2050·3	1·1167	1030·1	1025·7
	γ	1944·0	1·0588	976·71	972·55
	δ	1898·5	1·0340	953·83	949·75
Series limit		1822·5	0·99261	915·67	911·76
Balmer	α	13122	7·1468	6592·9	6564·7
	β	9720·2	5·2939	4883·6	4862·7
	γ	8678·7	4·7267	4360·4	4341·7
	δ	8201·4	4·4667	4120·5	4102·9
Series limit		7290·1	3·9705	3662·7	3647·1
Paschen	α	37492	20·419	18837	18756
	β	25629	13·959	12877	12822
	γ	21870	11·911	10988	10941
	δ	20093	10·943	10095	10052
Series limit		16402	8·9335	8241·1	8206
Brackett	α	81001	44·116	40697	40523
	β	52489	28·587	26371	26259
	γ	43299	23·582	21754	21661
	δ	38881	21·175	19534	19451
Series limit		29161	15·882	14650	14588
Pfund	α	149120	81·214	74919	74599
	β	93025	50·665	46737	46538
	γ	74771	40·723	37566	37406
	δ	65904	35·894	33111	32970
Series limit		45563	24·815	22892	22794

In Table 1 we list some wavelengths from the "Lyman", "Balmer", "Paschen", "Brackett", and "Pfund" series of positronium, protonium, muonium and hydrogen. These are calculated from the Bohr theory formula

$$E_n = -\frac{\mu c^2 \alpha^2}{2n^2} \qquad (1)$$

with accurate values[5] used for the reduced mass μ in each case.

The wavelengths for positronium are almost exactly double those for hydrogen and span the ultra-violet, visible and near infra-red portions of the spectrum. The wavelengths for protonium are in the X-ray region. It is necessary to consider much higher values of the principal quantum number n to reach wavelengths for this atom in the ultra-violet. The $n_i = 14$ to $n_f = 13$ transition in protonium has a wavelength $\lambda = 1217{\cdot}8$ Å which is close to the Lyman-α line in hydrogen. The wavelengths for muonium differ from the corresponding values for hydrogen by just a few Ångströms.

If only hydrogen-like spectra from a quasar were to be observed it would be possible to confuse positronium with hydrogen. It could be said, for example, that the $1{\cdot}13122\mu$ line came from positronium in a quasar with $Z = \Delta\lambda/\lambda_0$ equal to two (no red-shift) or from hydrogen in the same quasar but with a Z number equal to 3. The ambiguity can be resolved by positive identification of spectral lines from other atoms, so that there is no appreciable uncertainty in the Z number. Or one could look, with radio telescopes, for the wavelengths associated with the hyperfine splitting of the ground state of these various atoms. These are well known[6] and well understood[7] for positronium, muonium and hydrogen and are listed in Table 2.

Table 2. Ground State Hyperfine Split Frequencies and Wavelengths for Several Hydrogen-like Atoms

Positronium	Muonium	Hydrogen
$\nu = 2{\cdot}0337 \times 10^5$ Mc/s	$\nu = 4{,}463$ Mc/s	$\nu = 1{,}420$ Mc/s
$\lambda = 0{\cdot}147$ cm	$\lambda = 6{\cdot}72$ cm	$\lambda = 21$ cm

It is natural to enquire whether or not a matter–antimatter hydrogen-like atom will suffer annihilation or

decay before radiation occurs. We have found that protonium in the $n = 2$ state will annihilate before the Lyman-α radiation takes place, using the approach of Bethe and Hamilton[8]. Consequently, we do not believe that the 1·32 Å line of protonium can be observed. In all other cases the annihilation or decay lifetime is long compared with the radiative lifetime.

N. A. Vlasov has estimated that there is a very small flux—0·0054 photons/cm²/sec/steradian at the top of our atmosphere—due to hydrogen-like atoms like protonium. This means the detection problem can be solved only by using large apertures and long exposure times. Vlasov's estimate may well be pessimistic in view of the observations of Giacconi *et al.*[9], who report a soft X-ray flux of 5 photons/cm²/sec¹/steradian from a source near the galactic centre. We believe this search is well worthwhile. Positive identification of an ambiplasma source of positronium, protonium, or muonium radiation would clearly open up new and exciting fields of spectroscopy and enrichen observational astronomy.

We are grateful to Dr. Frederick R. West for his encouragement and comments.

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PLANETARY SCIENCE

Implications of the *Mariner* IV Photography of Mars

LEIGHTON *et al.*[1] and Loomis[2] have recently provided brief assessments of various aspects of the *Mariner* IV photographs of Mars. Their conclusions imply an almost universal acceptance at the present time of the impact theory of lunar cratering. Loomis states "little disagreement remains that the Moon's craters were formed by hypervelocity impact rather than volcanism". I would suggest, however, that the facts do not justify this generalization. There is, on the contrary, a wide body of international opinion favouring endogenous—volcanic or quasi-volcanic—causes for the development of the large lunar craters, and I myself hold such views[3,4].

The principal objection to the volcanic theory is posed by the question "Where have all the meteorites gone?" But this objection is no more insurmountable than those which have been ranged against impact theory[3,4]. The truth is that there are valid objections to both theories, and the controversy is far from being resolved—contrary to Loomis's statement. Gold[5] refers to the volcanic theory being abandoned "because a large amount of the detail could not be accommodated into it". His arguments involve the assumption that the cratering is explosive, and that volcanism involves immense lava outpourings. Neither is necessary. I have demonstrated that there is a good case for the alternate theory in the case of the Moon[3,4], and that there is much detail irreconcilable with impact theory. I shall endeavour to show that the same applies to the martian cratering.

Certain modifications of the impact theory have become popular, particularly since the successful *Ranger* project. Some modifications of the "all-out" impact theory were needed, and two have been suggested. The first involves a clear-cut separation of the large lunar craters into impact explosion craters (for example, Tycho and Copernicus) and caldera craters of volcanic origin (for example, Alphonsus and Ptolemeus). The second assumes considerable secondary volcanic modification of initial impact explosion craters. Neither modification of the impact theory seems to have been taken beyond a vague statement, and it is doubtful if either would stand up to a rigorous analysis of the detailed relationships between craters and linear (tectonic) structures, and of the integral structure of the craters. To emphasize this point, in the case of the first modification I would refer the reader to a photograph published by Moore[6], showing the craters Azarchel, Alphonsus and Ptolemeus. Can it be doubted that these three craters are broadly congenetic? Yet a comparison between Azarchel and Copernicus will reveal remarkable similarity. Both show high, terraced walls; depressed floor; a little disrupted outline; median sector graben structure corresponding to a ridging of the central peak. There is a complete gradation between lunar crater types, and no clear-cut separation into contrasting genetic types can be substantiated. The second modification of the impact theory cannot seriously be maintained because the rays clearly overprint everything else around the large rayed craters and this is fatal to any theory of secondary volcanic modification. Another answer will surely have to be found to explain the anomaly of caldera features in rayed craters such as Copernicus.

Leighton *et al.*[1] conclude that the martian craters, like those of the Moon, resemble impact craters on Earth, but this conclusion would be difficult to justify by detailed analysis. I believe that lunar craters do not closely resemble terrestrial impact explosion craters, but do closely resemble terrestrial volcano-tectonic craters[3]—calderas and surface cauldrons. Which particular terrestrial impact explosion craters are the lunar craters supposed to resemble? Leighton *et al.* have produced good statistical evidence for the congenesis of lunar and martian craters, but by no accepted standard of scientific evaluation could this be considered to be evidence for anything more. The unresolved controversy concerning the formation of craters has simply been extended to Mars by the *Mariner* IV probe. I do not doubt that the barren, cratered surface revealed by both these small planetary bodies reflects a shared fundamental cause: but I believe that what we are seeing is the surface reflexion of a primitive phase of quasi-volcanic activity (such as has been suggested by Fish, Goles and Anders in another context[7]), and that these surfaces have never progressed beyond this primitive state, lacking the more complicated internal and surface processes which have affected the Earth.

The eleventh frame of the *Mariner* photographs (Figs. 1 and 2) shows a focal bright spot in the crater in the upper right-hand corner. Without doubt this is a central peak structure, such as has been recognized in other frames, and suggests that the phenomenon responsible for crater formation was endogenous rather than impact explosion. Such a structure has never been satisfactorily explained in terms of impact in the case of lunar craters: chance "bull's eye" strikes by other meteorites, rebound and isostatic adjustment are all untenable[3]. Of considerable significance are the perfectly formed scalloped facets in the walls of the crater shown near the top of the frame, left of centre. Previously, I had recognized a restricted development of such scalloped facets in the walls of lunar craters (Clavius and Schiller, ref. 3), but I treated this

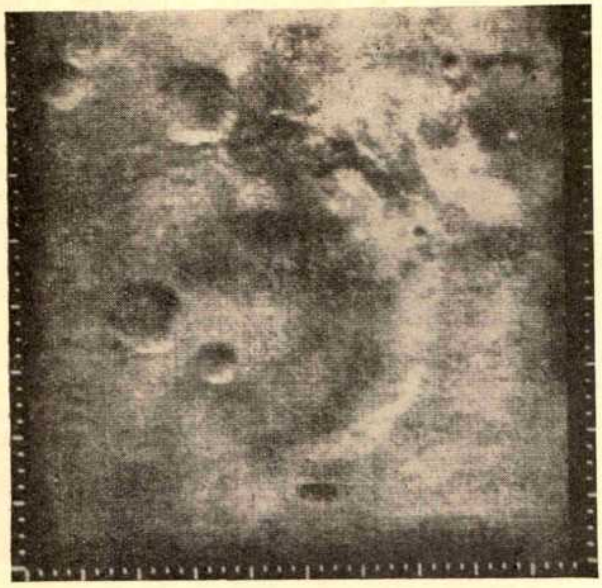


Fig. 1. *Mariner* IV Frame 11 (by permission of the National Aeronautics and Space Administration). East—West 270 miles; North—South 240 miles.

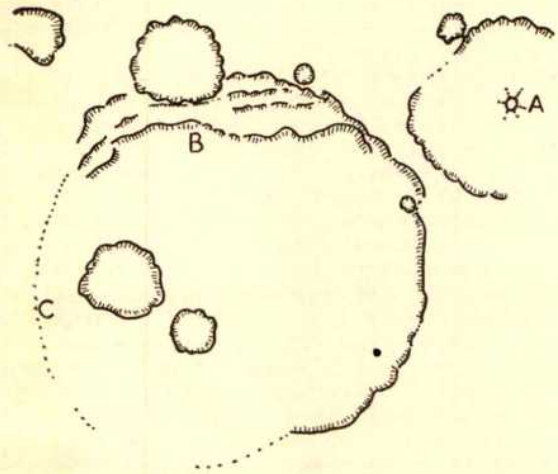


Fig. 2. Tracing of Fig. 1, in which *A* represents the bright spot at the crater focus, *B*, the large scale step-faulted zone of the largest crater, and *C*, the section of wall of the largest crater which is either obscured or not developed.

evidence with some reserve. The martian scalloping, however, is so perfect that it must be regarded as important evidence.

Many terrestrial subsidence calderas of volcano–tectonic origin show scalloped facets in their walls[8], and among the better known examples are Krakatau, Aso, Crater Lake, Santorin and Menengai[8,9]. While the exact origin of this phenomenon is debatable, such a structure has not been found developed to any significant degree in any crater produced by eviscerating explosion. My inference[3] of a subsidence origin for the Trou au Natron crater, Tibesti, was based entirely on this feature—contrary to an opposing interpretation. (Recently, in a personal communication, Prof. H. Tazieff has confirmed this view.) I therefore take pronounced scalloping of crater walls to be a valid indication of volcano–tectonic subsidence.

Scalloping is also evident in the walls of the largest of all the known martian craters, and in the two smaller craters sited within it (Figs. 1 and 2). (Since this communication was first prepared, Fielder[10] has commented on this scalloping.) Not only is simple scalloping evident in the walls of the largest crater, but also giant scalloped steps, which can only reflect step-faulting on an immense scale. Scalloping is also evident in the walls of the crater showing the bright focal spot already mentioned.

Scalloping seems to be emphasized in the case of the martian craters, and not very conspicuous in the case of the lunar craters. The walls of martian craters tend to be less complicated by linear fractures and thus exhibit more circular outlines. Such divergences in cratering style from the surface of one planetary body to that of another are to be expected with endogenous cratering—eruptive processes are very flexible. It may be questioned, however, whether such marked disparity is likely to develop between craters on two separate planetary surfaces by the agency of the much less flexible process of impact explosion. But then it is difficult to see how scalloped facets could be developed in the walls of impact explosion craters at all!

Loomis[2] refers to the obscuration of a section of the walls of the large crater shown in Fig. 1. Obscuration by superimposed volcanics is also possible—this has actually occurred in the case of the outer caldera of Suswa volcano, Kenya[11]. I prefer, however, yet another possible alternative explanation—that the ring fracture was simply incompletely developed in the first place. Terrestrial caldera walls, and annular fractures in ring-complexes are by no means always entire, and most calderas show unequal displacement downwards of the floor. This large crater is, I believe, a surface cauldron of subsidence.

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Theory of some Quiet Magnetospheric Phenomena related to the Geomagnetic Tail

THIS is an outline of a theory which correlates four quiet aeronomic phenomena—(1) the ionized plasma in the geomagnetic field, particularly the tail; (2) the maintenance of the polar F region in winter; (3) type A red polar cap aurora and (4) some aspects of the scattering of solar Lyman alpha radiation (L_α) into the night sky.

Briefly, the theory is that a very small fraction (generally < 1 per cent) of the solar wind plasma impinging on to the geomagnetic field diffuses into it at times of magnetic quiet. The plasma helps to form the geomagnetic tail. In the outer geomagnetic field (B) the plasma cools and heat is conducted away along B to the polar ionosphere. The cooled plasma contributes to the gravitational diffusive equilibrium distribution along B and thus to the polar F region ionosphere. Sufficient heat is given to the F region electrons of the polar ionosphere to generate red auroral glow over the polar caps by thermal excitation of atomic oxygen. In a steady state, the capture of proton–electron pairs from the solar wind is balanced by the ejection of hydrogen atoms from the outer atmosphere of the Earth. This is achieved by charge exchange between protons moving faster than escape velocity and hydrogen atoms. The escaping hydrogen atoms provide interplanetary centres for scattering of solar L_α into the night sky.

The physical quantities used in what follows to illustrate the theory mostly apply to conditions at sunspot minimum and to magnetically quiet times.

The plasma in the outer geomagnetic field consists of a thermal part comprising electrons and protons of energy 5–7 eV and density ~ 5–20 cm^{-3}, and at least two non-thermal parts comprising electrons at 22 eV, and protons at 350 eV, respectively, both at a density of ~ 1–2 cm^{-3}. (The actual numbers are taken from the work of Sagalyn and Smiddy[1].) It is supposed that at least the non-thermal parts diffuse into the geomagnetic field from the solar wind. This assumption is necessary for the part of the theory concerned with the maintenance of the F region, but is not essential for the theory of the red arcs as such or of the aspects of L_a. Non-thermal plasma has also been observed in the outer geomagnetic field by Gringauz *et al.*[2] and Serbu[3]. The energy density of the plasma is in the range 6–14×10^{-10} ergs cm^{-3}. This is the same as in a magnetic field of strength 12–19×10^{-5} gauss. It is therefore of the correct magnitude to contribute significantly to the formation of the observed geomagnetic tail[4].

The polar region is considered to be connected to the geomagnetic tail[4]. In the cooling of the plasma in this tail, the cooled particles contribute to the thermal electron content of the tube of force and fall into the polar atmosphere where they contribute ionization to the F region. The rate at which the tail provides this ionization is

$$\mathcal{J}_{(\max)} = \sum_c n'_c\,(t'_{sc})^{-1}\,B_I\,B_T^{-1}\,L \text{ cm}^{-2}\text{ sec}^{-1} \qquad (1)$$

where n'_c represents the density of a component (c) of non-thermal plasma, t'_{sc} the slowing down time of this component in the thermal plasma, B_I the geomagnetic field at polar cap, B_T the magnetic field in tail, and L the characteristic length of geomagnetic tail.

Consider the maximum contribution to $\mathcal{J}$ by non-thermal electrons. Put $n'_c = 1$–2 cm^{-3}, $t'_{sc} \approx 5 \times 10^5\ n_e^{-1}$ sec, where n_e, the thermal electron density, is 5–20 cm^{-3}, $B_I \approx 0{\cdot}6$ gauss, B_T is 10–30×10^{-5} gauss, $L \approx 30$ Earth radii (R_E) (ref. 4). It is found that

$$7{\cdot}5 \times 10^7 \lesssim \mathcal{J} \lesssim 1{\cdot}5 \times 10^9 \text{ cm}^{-2}\text{ sec}^{-1} \qquad (2)$$

These fluxes are large and it can be shown theoretically[5] that a substantial polar F region can be maintained by them. In particular, assuming a recombination coefficient of about 5×10^{-8} sec^{-1} at 300 km, a maximum electron density of $0{\cdot}25$–5×10^5 cm^{-3} can be sustained at altitudes of 300–400 km. The upper limit of the flux ($\mathcal{J}$) may be lowered somewhat by consideration of the next section.

The high red polar cap aurora can be attributed partly to dissociative recombination of F region electrons and partly to thermal excitation of atomic oxygen by F region electrons. The latter is the same hypothesis as was used earlier for the stable auroral red arc which is reported in middle latitudes during geomagnetic storms[6]. If the red glow at wavelengths of 6300 and 6364 Å resulted entirely from thermal excitation by F region electrons, the ratio of the intensities $I(6300)/I(5577)$ would be very large and $\gtrsim 50$. If the red resulted entirely from recombination, then $I(6300)/I(5577) \approx 4$. Although the ratio is frequently very large[7,8], sometimes it is small[8]. When the index of local magnetic disturbance (K) is zero, $I(5577)$ is not increased[7] above its value at middle latitudes where it is predominantly influenced by some photochemical process at the height of the lowest E region. Photometric measurements show that on one occasion in a polar cap arc $I(6300) \approx 1{,}000\ R$ (where $R = 10^6$ photons cm^{-2} vertical column sec^{-1}) while $I(5577) \lesssim 100\ R$ (ref. 8). On the basis of recombination, the latter intensity puts an upper limit of about 5×10^8 cm^{-2} sec^{-1} on $\mathcal{J}$. This limit would be decreased if allowance were made for generation of some light at a wave-length of 5577 Å—in this case in the E region by some photochemical process. The considerations applied to existing observations suggest that 10^8 cm^{-2} sec^{-1} is a reasonable order of magnitude estimate of the average value of $\mathcal{J}$ but that it may extend over the values given in relation (2).

We shall now assume that the red polar cap glow is due dominantly to thermal excitation by F region electrons. It should be noted, however, that the arcs near the auroral zone are considered to be caused predominantly by corpuscular bombardment by electrons of energy 1–10 keV and only partly by the thermal excitation. This discussion is intended to account for the $K = 0$ type of regime found by Sanford[7] (see his Fig. 8) in which green arcs at the auroral zone apparently do not exist.

The height integrated cooling rate (Φ) of the electrons in the red polar aurora[7,8] can be found by the theory given elsewhere[6]. Knowing the intensity of the aurora, the electron temperature can be found and thus also Φ. I have found that Φ is matched by the integrated cooling rate of non-thermal particles in the tail which is

$$F = \sum_c n'_c\,E'_c\,(t'_{sc})^{-1}\,B_I\,B_T^{-1}\,L \text{ ergs cm}^{-2}\text{ sec}^{-1} \qquad (3)$$

where E'_c is a representative energy of a non-thermal component (c). Again non-thermal electrons contribute most to F and we find, using the parameters following equation (1),

$$1 \times 10^{-2} \lesssim F \lesssim 3 \times 10^{-1} \text{ ergs cm}^{-2}\text{ sec}^{-1}$$

These values of F span those of Φ. Heat conduction is invoked to transport the heat from the geomagnetic tail to the polar ionosphere.

Although the accumulation of hydrogen at rates given by relation (2) usually represents far less than 1 per cent of the solar wind flux impacting on the geomagnetic field, it represents a significant rate of accumulation for the Earth. Integrated over both polar caps the fluxes are from $2{\cdot}3 \times 10^{25}$ to $4{\cdot}6 \times 10^{26}$ sec^{-1}. Averaged over the Earth, these fluxes become 5×10^6—1×10^8 cm^{-2} sec^{-1}. On their own these fluxes would change the amount of hydrogen in the atmosphere above 100 km by a factor 2 in 10^5–10^6 sec. This is unacceptable. Given that the hydrogen does not combine with oxygen and descend into the oceans, an equal amount of it must escape from the Earth. This can be achieved by charge exchange of magnetospheric plasma with hydrogen atoms in the outer atmosphere. The protons in the outer atmosphere are relatively energetic, and have energies of from 1 eV to 5 eV (refs. 1 and 9). If these protons could only be made neutral by charge exchange they would have sufficient energy to escape. The rate of charge exchange is given by

$$\mathcal{J}(\text{H}^+)\,n(\text{H})\,V\sigma(\text{H}^+ \text{ in H}) \qquad (4)$$

where $\mathcal{J}(\text{H}^+)$ is the flux of H^+, $\sigma(\text{H}^+ \text{ in H})$ the charge exchange cross-section of H^+ in H, $n(\text{H})$ the density of hydrogen atoms, V the volume of space in which charge exchange takes place. Between 2 R_E and 6 R_E altitude, the flux of protons is 10^9–10^{10} cm^{-2} sec^{-1} (ref. 1), $\sigma = 5 \times 10^{-15}$ cm^{-2} (ref. 10), $V \approx 2 \times 10^{29}$ cm^3. A flux of 5×10^{26} sec^{-1} hydrogen atoms out of the gravitational field would require $n(\text{H}) \approx 50$–500 cm^{-3}. Johnson[11] considers that this is the concentration of hydrogen atoms which does exist at sunspot minimum between 2 R_E and 6 R_E, although his argument does not take into account the charge exchange process considered here. The hydrogen escaping from the Earth consists of an amount approximately equal to the capture rate of proton–electron pairs from the solar wind together with the rate of thermal escape. Outside the shadow of the Earth these hydrogen atoms can scatter solar L_a into the night sky. The flux required to account for a significant background of intensity of night L_a is, for example, on one model[12] of the order of 10^8 cm^{-2} sec^{-1}. Charge exchange appears capable of accounting for fluxes of this order of magnitude. Moreover, about 15 per cent of the L_a of the night sky requires interplanetary hydrogen atoms with

energy in excess of about 1 eV (ref. 13) for its explanation. Clearly, these hydrogen atoms may come from the geomagnetic source suggested here, although an alternative has been suggested[14].

The theory is compatible with and constrained by sunspot minimum observations of geomagnetic plasma, red aurora, and electron densities in the polar cap F region, and night L_a. Although the four phenomena are consistent on an order of magnitude basis, the theory calls for better measurements of the parameters involved. There will be further tests of it when measurements appropriate to sunspot maximum conditions and geomagnetic quiet are available.

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Temperature Conditions of Salt Dome Intrusions

IN discussing the effect of temperature on salt dome intrusions, Dr. W. C. Gussow[1] quoted the example of salt domes in Iran, and implied not only that raised temperatures are necessary for inception of salt (halite) intrusion at depth, but that they must also be involved in the movement of salt glaciers at the surface.

Temperatures of the order required are well attested at depth by the kind of data quoted, and also by alteration of exotic blocks of igneous and other rocks in the salt in Iran, and by metasomatism and pneumatolytic action elsewhere, as in Arctic Canada[2,3]. O'Brien[4] believes movement to be begun by igneous intrusion, but this hypothesis has not been accepted as generally applicable[5,6]. Once large salt bodies have started moving, however, they remain mobile at shallow depths where temperatures are demonstrably far lower than the 200° C which Gussow regards as the limit for flow, and their movement can be traced by stratigraphical anomalies in the rocks through which they are forced. There is ample evidence of shallow movement of plugs during sedimentation, and of their occasional temporary emergence at the surface. Recent work has emphasized that the upper part of a plug is not intruded as an inert piston, but that it changes its shape in response to varying stresses while still moving through relatively shallow overburden[7].

The active surface salt plugs and salt glaciers of Iran, however, demonstrate most clearly that salt flow occurs at nearly normal temperatures. The climate of southern Iran is arid but nevertheless has a measurable rainfall, and there are numerous examples to show that if intrusion ceases the dome begins to dissolve, and eventually becomes a thickly detritus-cloaked mountain, or even an empty crater[6,8,9]. There has never been any doubt that salt mountains such as Kuh-i-Namak, near Bushire, owe their elevation (in that case 4,000 ft. above the adjacent plain level) to salt extrusion continuing to the present day. There is no thick mantle of detritus; the salt flanks are precipitous and salt avalanches are recorded. But this salt is not hot, nor is that of the salt glaciers, contrary to Gussow's implication. The glaciers are related to the modern topography and may show surface flow lines similar to those of ice glaciers. They demonstrably waste by solution so that like melting ice tongues) they attenuate distally and terminate as thin salt layers sandwiched between "moraines" of insoluble matter. Solution, soil creep and rainwash as well as glacier movement are continually active in moving the salt and its clastic content downhill; it is only by the continuing feed from the active dome above that their precipitous unstable slopes are maintained. All this takes place at temperatures which in summer may reach 46° C, but which are unlikely to be significantly higher.

Whether solid flow or the development of shear planes is the dominant element in salt movement has not been established, but it is abundantly clear that movement does take place at the temperatures occurring both at shallow depths and at the surface in tropical climates.

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Possible Relationships between Continental and Oceanic Basalt and Kimberlite

BASALTIC rocks conform to two standard compositions—alkali-basalt or tholeiitic basalt. Both types of magma occur in continental as well as in oceanic areas[1], but tholeiitic basalts are much more abundant than alkali-basalts. For example, the volcanoes of Hawaii were shown to consist largely of tholeiitic basalts with only a very thin cap of alkali-basaltic rocks[1]. The same holds for the volcanic rocks of the Mid-Atlantic Ridge[2]. In continental areas the amounts of alkali-basaltic rocks are likewise very small in comparison with tholeiitic rocks.

Most authors agree that the origin of basaltic magmas must be attributed to some process of differential melting of peridotitic rocks in the upper mantle. To reach the required temperature, convection currents in the mantle are invoked by several authors[3]. The origin of alkalic and tholeiitic basalts is controversial. Yoder and Tilley's experiments[4] indicate that at pressures of more than 19 kbars, corresponding to a depth of more than 60 km, crystallization of basaltic magma produces a "high-pressure" mineralogy, that is pyrope and omphacite instead of plagioclase and augite. Settling of garnet, observed in some of their experiments, would yield a residual liquid enriched in alkalis. At depths greater than 60 km, gravitational separation of pyrope crystals from a tholeiitic basalt magma might thus produce residual magmas of alkali-basaltic compositions. Similar ideas have been expressed by Evans[5] and Holmes and Harwood[6].

As a source of basaltic magmas, Yoder and Tilley suggest widespread occurrence of garnet peridotite in the upper mantle. A more likely hypothesis, in my opinion, has recently been put forward by De Roever[7], based on

ideas of Bernal[8], and experimental work by Ringwood[9–13]. According to Ringwood, it is plausible that at very high pressures, equivalent to depths exceeding 1,000 km, forsteritic olivine will no longer be stable and will transform into a magnesium and silicon-rich spinel of higher density. This cubic "olivine" would be capable of dissolving considerable quantities of foreign elements in solid solution in its crystal lattice, for example, chromium, aluminium, calcium, sodium and volatiles. De Roever suggested that basaltic magma might originate as the fluid by-product of the decay of this spinel into forsteritic olivine, enstatite, chrome-diopside and chrome-spinel in the rising parts of convection cells in the mantle. Squeezing out and coalescence of the interstitial magma droplets formed in this way would produce magma pockets in the mantle that would, under favourable conditions, give rise to basaltic volcanism. It is possible that this magma might be of tholeiitic composition.

There is a similarity, in the type of basaltic volcanism observed in continental shield areas such as those of northern Siberia and southern Africa, and in the oceanic areas of Hawaii and the Mid-Atlantic Ridge. This re-resemblance may well indicate a similar origin of basalt volcanics in both environments. In continental shield areas as well as in oceanic areas, the volcanism of alkali-basaltic type is preceded by a long period of volcanism of tholeiitic basalts. The tholeiitic type of volcanism is characterized by a quiet and fairly continuous outpouring or intrusion of very large quantities of uniform tholeiitic basalts and, to a lesser extent, of tholeiitic differentiates. In the volcanic area of Hawaii, the initial tholeiitic basalt volcanism is gradually decreasing, and changing into a volcanism of alkali-basaltic type. This is of explosive, discontinuous and much less uniform character, comprising true alkali-basalts, melilite basalts, nepheline basalts, etc. The alkali-basaltic rocks are also characterized by peridotitic and occasionally eclogitic xenoliths. Many authors have argued that these xenoliths represent parts of the upper mantle[14–23]. So far as I am aware, peridotitic or eclogitic upper mantle xenoliths are absent in tholeiitic basalts. This may be attributed to the non-explosive character of the tholeiitic basalt volcanism, making transport of xenoliths out of the mantle impossible.

In the continental shield areas of southern Africa and northern Siberia, there is a similar early period of tholeiitic volcanism which is devoid of peridotitic and eclogitic xenoliths, and which is followed by alkali-basalt volcanism. Here, however, there is a very long period between the end of the tholeiitic volcanism and the beginning of a peculiar type of alkali-basalt volcanism. For example, in southern Africa the intrusion of the tholeiitic Karroo dolerites ceases in Lower Jurassic time, whereas it is not before Upper Cretaceous time that new volcanism starts: the highly explosive kimberlite volcanism. Similar relations are found in the shield area of northern Siberia. In this area, there is a gap from Upper Trias to Lower Cretaceous time between the end of the outpouring of the tholeiitic trapbasalts and the beginning of kimberlite volcanism.

Explosive kimberlite volcanism is an initial, extremely gas-rich phase of alkali-basalt volcanism, comparable with Maar-type volcanism[24,25], in which the alkali-basaltic magma is obviously obscured by large quantities of upper-mantle xenoliths and fragments of the crust[26,27]. Both types of xenoliths are often disintegrated into loose crystals. Most xenoliths are of peridotitic composition. As in the oceanic alkali-basalts, eclogitic xenoliths are much less abundant. According to several authors[26,27], melilite basalt is the magmatic component.

The possible genetic connexion between kimberlite volcanism and an older period of tholeiitic basalt volcanism had been put forward as early as 1920 by Du Toit[28], and seems to be accepted by many Russian authors[29,30]. As was stated by Du Toit: "It may even be suggested that this younger eruptive phase has been derived from the ultrabasic differentiate from the dolerite magma still remaining in the subcrustal reservoir . . . ."

After the short kimberlite phase, the alkali-basalt volcanism seems to have acquired a less gas-rich and less explosive character. The amounts of xenocrysts and xenoliths decrease and the nature of the magmatic component becomes more conspicuous: melilite basalts, nepheline basalts, etc. These rocks also form pipes and dykes, possibly by the expulsion of older kimberlite fillings[31]. Apparently, after the explosive opening of escape ways for the magma from the upper mantle to the surface, a more quiet outpouring of magma can take place. In many Maar-type volcanoes of the kimberlite phase, however, volcanism ceases, except for post-volcanic action causing calcification, serpentinization, etc.

The origin of tholeiitic basalt is thought to be connected with the rising convection currents in the mantle. In the areas where rising currents approach the Earth's surface, strong tensile stresses and heat-flow will affect the crust and upper mantle, giving favourable conditions for the magma to reach the surface.

If a genetic connexion between alkali-basalt volcanism and the older tholeiitic basalt volcanism is accepted (in shield areas tholeiitic basalt volcanism being very much older), it is plausible that, at the termination of a convective mantle current, pockets of tholeiitic magma remain in the upper mantle. These magma pockets cool slowly and this is especially so under continental shield areas because of the higher production of radiogenic heat in the overlying crustal material. At depths greater than 60 km they consolidate to form high-pressure minerals. Variations in temperature, pressure, form and volume of the magma reservoirs may cause alkali restmelts of widely varying compositions to develop and also possibly carbonatitic magmas. Excessive vapour pressures may build up in the reservoirs of consolidating magma. Under continental shield areas, this may give rise under favourable conditions—for instance, renewed movements in the mantle yielding deep fault zones from the upper mantle through the crust—to explosive ejection of the rest magmas, that is kimberlite volcanism. These explosions cause fragments of crust and upper mantle to be ejected with the magma. It seems feasible that the most forceful explosions and very rapid transport of mantle material through the crust cause diamonds to be brought to the surface, "frozen" in the high-pressure carbon modification.

Oceanic regions will require a much shorter period than continental shield areas for the upper mantle to cool after the termination of a convection current. Consequently, differentiation of tholeiitic magma portions in the upper mantle into residual melts of alkali-basaltic compositions should be a much more rapid process. Escape of alkali-basaltic magma through the relatively thin crust is also much easier. Explosions of the kimberlitic type apparently do not occur under these conditions, and transport of upper-mantle xenoliths by the outpouring magmas is also relatively rare. Diamonds evidently do not survive a relatively slow ascent to the surface in a hot magma.

If the conditions favourable for escape of the magma to the surface do not occur, the magma pockets in the upper mantle will finally consolidate to form eclogitic rocks or, at a depth less than about 60 km, gabbroic rocks. Only relatively small masses of eclogitic rock should therefore be present in a mainly peridotitic upper mantle. This is in accordance with the relative rarity in alkali-basalts of eclogitic xenoliths, compared with peridotitic xenoliths. Similarly, only relatively thin lenses and bands of eclogitic compositions are present in alpine-type peridotite masses, which may be considered to represent the best samples available from the Earth's upper mantle.

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Low Spin Ferrous Iron in the Iron Silicate Deerite

AGRELL[1] recently found a group of new minerals occurring in ferruginous cherts metamorphosed under conditions of the glaucophane–lawsonite schist facies. This metamorphic environment is characterized by a combination of pressures near 10 kbar and temperatures in the range 200°–300° C. The partial pressure of water must be high. High-pressure phases such as jadeite, lawsonite, omphacite and aragonite are found in related rocks. Deerite, one of the new minerals, is essentially a hydrated ferrous–ferric silicate. The mineral is almost opaque to visible radiation and this property suggested that the magnetic behaviour might be of interest.

Deerite was separated from a sample from the type locality, Laytonville, California. The chemical analysis is listed below in Table 1. The formula is appproximately $Fe^{2}_{13}Fe^{3}_{7}Si_{13}O_{44}(OH)_{11}$ with minor substitution of titanium and aluminium for ferric iron and manganese for ferrous iron.

The inverse magnetic susceptibility is shown as a function of temperature in the range 80°–300° K in Fig. 1. The susceptibility is independent of field strength between 2,500 and 6,500 G over the temperature range studied. But the marked deviation from Curie–Weiss law behaviour suggests that ferromagnetism might be observed at lower temperatures. It is hard to suggest an alternative explanation for the temperature dependence of the susceptibility but some field dependence would be expected at lower temperatures if the system is ferromagnetic.

The observed susceptibility per formula unit (that is, 20 iron atoms), corrected for diamagnetic components, is $147{,}700 \times 10^{-6}$ c.g.s. units at 295° K. Expected susceptibilities for various configurations are summarized in Table 2 and no high spin arrangement will account for the observed susceptibility.

Table 1

Chemical analysis		Formula based on 55 oxygen atoms	
SiO_2	32·95	Si	13·02
TiO_2	0·19	Ti	0·06
Al_2O_3	0·19	Al	0·09
Fe_2O_3	22·81	Fe^3	6·78
FeO	35·24	Fe^2	11·64
MnO	4·00	Mn	1·34
MgO	0·08	Mg	0·05
CaO	0·15	Ca	0·06
Na_2O	—	OH	10·87
K_2O	—	O	44·13
H_2O^+	4·12		
H_2O^-	0·10		
Total	99·79		

Table 2

Configuration	$10^6 \times$ Susceptibility at 295° K		
	Fe^3	Fe^2	Formula unit
High spin, octahedral	14,700	10,200	235,500
Low spin, octahedral	2,250	0	15,750
High spin, tetrahedral	14,700	11,000	245,900

As there is no evidence for antiferromagnetic behaviour, it may be concluded that some of the ions present are in a low spin configuration. It has been suggested[2] that ferrous ions will assume a low spin configuration at lower ligand field strength than ferric ions. This assumption would suggest that of the thirteen ferrous ions about four are in the high spin configuration, perhaps due to a distribution between octahedral and tetrahedral sites. This must be considered an approximation and further refinement would require knowledge of the crystal structure. It seems certain that a range of sites for the M^2 cations will be found in the structure.

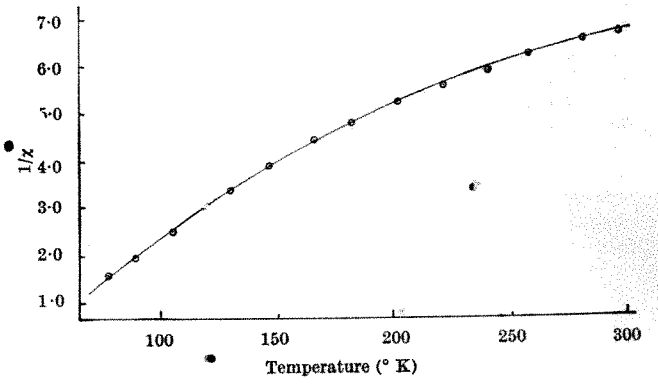


Fig. 1. Temperature dependence of inverse susceptibility of deerite. Units shown correspond to observed pull on the sample.

Deerite is a moderately dense substance in the sense that reactions between silicates and oxides leading to deerite indicate a large negative volume change. This would be expected on the basis of a small low spin ferrous ion. It is also possible that π interaction is enhanced with shorter bond distances and may account partly for the anomalous optical properties. The magnetic properties show that low spin ferrous iron in oxygen co-ordination does not require extreme pressures and leaves little doubt that olivine or spinel phases in the mantle of the Earth will contain iron in this diamagnetic state as previously suggested[3].

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ENGINEERING

Effect of Polymer Additives on Boundary Layer Separation and Drag of Submerged Bodies

THIS communication describes a simple experiment which demonstrates that in some circumstances the use of polymer additives can increase drag owing to a change in the position of boundary layer separation which results in a change of wake size and thus form drag.

Recently, there has been considerable interest in the turbulence damping properties of certain long chain polymers when dissolved in water. It has been found that very small traces in solution can reduce turbulent friction by a very significant amount and drag reductions of more than 50 per cent are possible by the addition of just a few parts per million by weight of the polymer[1,2].

The large drag reduction produced by these visco-elastic polymer solutions is being considered for practical application in many fields. The effect has already been made use of in certain aspects of oil field technology and experiments are at present being carried out with the view of using it in marine technology[3] in the testing of ship models[4].

If we consider flow around a submerged surface, flow separation is likely in a region of adverse pressure gradient and the point of separation will depend on whether the boundary layer is laminar or turbulent—the separation point being nearer to the leading edge with a laminar boundary layer.

For this experiment a concrete sphere 7·75 in. in diameter was dropped into a 'Perspex' sided tank which contained either water or dilute poly (ethylene oxide) solution ('Polyox' *W.S.R.* 301), and the wake pattern was recorded by high speed ciné photography. A comparison of the drag coefficients has also been obtained by analysis of the film record.

The sphere was first made hydrodynamically smooth by careful rubbing down and painting and was then dropped into water from a suitable height so that the average Reynolds number was just below the critical value ($\rho Vd/\mu < 2 \times 10^5$). The wake is shown in Fig. 1, which indicates a laminar boundary layer with characteristic early separation.

The front of the sphere was then roughened by glueing on a patch of sand, and the sphere was again dropped into the water from the same height. Fig. 2 shows the wake pattern from this test which exhibits a much later separation point caused by the turbulent boundary layer. The drag coefficient was about half the value found from the previous test. These results are well known and are described in any standard fluid dynamics text[5].

For the third test the roughened sphere was dropped into a solution of 'Polyox' (60 parts per million by weight). Fig. 3 shows that the polymer additive has suppressed turbulence in the boundary layer and caused the separation point to move forward again with a resulting increase in form drag. The drag coefficient was about the same as for the first test.

It is therefore seen from this rather basic experiment that although polymer addition can significantly reduce skin friction, the effect can in certain circumstances be more than offset by an increase in form drag caused by a change in the separation point and wake size.

The effect was anticipated by Gadd[6] and could lead to difficulties with tests of ship models in polymer solution.

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Particle Migration in Cone-plate Viscometry of Suspensions

VISCOMETERS with a cone and plate configuration are often used for viscosity measurements on non-Newtonian fluids, and for suspensions provided that a particle cone truncated near its open end is used. In the latter case it has been assumed that radial motion of particles occurs too slowly to affect the accuracy of the measurements involved.

Recently, however, while investigating the behaviour of suspensions of spherical particles in certain non-Newtonian fluids using a cone and plate viscometer, rapid radial migration of the suspended solids was observed at low cone speeds (up to 40 r.p.m. corresponding to a shear rate of 184 sec^{-1}). Significant migration was observed after shearing for only 5 min whereas centrifugal

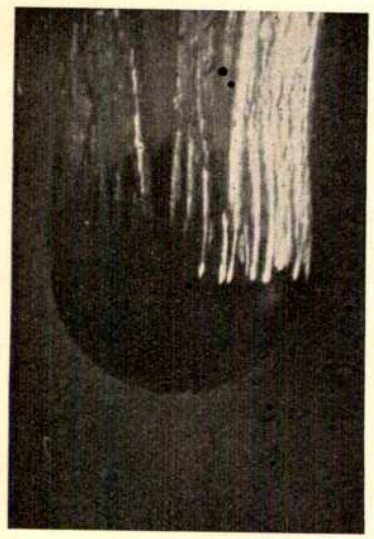


Fig. 1.



Fig. 2.

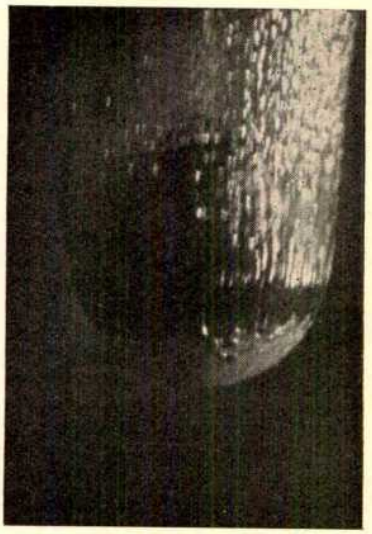


Fig. 3.

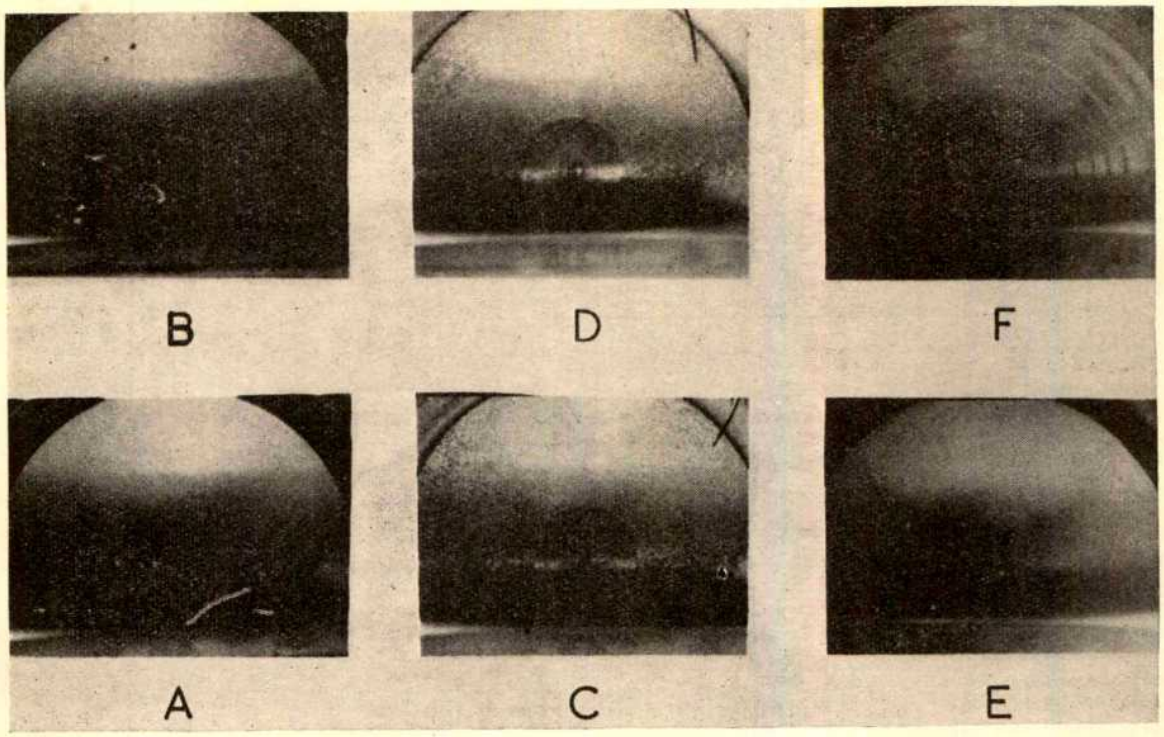


Fig. 1. Suspensions of 'Kallodoc' in: *A* and *B*, a Newtonian fluid, 'Rheoplex 200', before and after shearing for 30 min. *C* and *D*, in a non-Newtonian fluid with only small normal stress effects, 3 per cent sodium-carboxy-methyl cellulose in water, before and after shearing for 30 min. *E* and *F*, in a non-Newtonian fluid producing large normal stress effects, a 10 per cent solution of poly-iso-butylene in 'Tetralin', before and after shearing for only 5 min.

forces would require many hours to produce comparable effects.

The apparatus used was a Ferranti-Shirley cone and plate viscometer with a particle cone 2 cm in radius and of 0·0228 radians gap angle. The instrument was modified to accept a glass prism in place of the lower instrument plate so that observations of the distribution of particles were possible during shearing.

Fig. 1 shows the comparative effects of shearing suspensions of 'Kallodoc' spheres, about 100μ diameter, in three media at 138 sec^{-1}. The extended periods of shearing produced only minimal effects on the distribution of particles present in the control experiments, while in the poly-iso-butylene a marked clearance of the cone area is apparent.

Numerous tests[2] on the poly-iso-butylene solution indicated certain general features of the phenomenon. First the particles, initially at a uniform concentration of 9 per cent by volume, fell into distinct circular rings of high particle density (~25 per cent by volume) within seconds of the start of the shearing motion. Second, the whole particle population (whether free or in the rings) migrated towards the outer edge of the cone at a rate which increased with the rate of shear resulting in complete clearance of the cone area within 20 min at 30 r.p.m. (134 sec^{-1}). Finally, in control experiments using 'Rheoplex' and sodium-carboxy-methyl-cellulose no appreciable clearance resulted during long periods of shearing (up to 60 min), thus directly eliminating centrifugal effects, or non-linearity of the flow curve, as a primary cause of this phenomenon.

A theory for the overall radial migration of the particles has been developed in terms of the effect on a single particle of the normal stress gradient $\left(\frac{\partial p_{33}}{\partial T}\right)$ present in a cone and plate system during the shearing of a normal stress producing material such as poly-iso-butylene solution[3]. This approach led to a successful prediction of the overall clearance time and its dependence on the rate of shear; however, the mode of formation of the rings remains obscure, and is not accounted for by the present theory.

The effect of the migration of particles was to produce a fall in the measured value of the apparent viscosity of the suspension by up to 10 per cent of its initial value, in the first moments of shearing at a constant rate of shear. The pure solutions used as the liquid phases in this work did not exhibit any such change of apparent viscosity with time of shearing and the entire effect resulted from particle motion.

Many materials with complex rheological properties show a similar drop in apparent viscosity (at a constant rate of shear) during the first moments of shearing. For this reason, in work which involves suspensions and cone and plate measuring systems it is essential to distinguish between changes in apparent viscosity resulting from transient effects in the base fluid and a re-distribution of the suspended solids.

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THE SOLID STATE

Direct Solution of Organic Crystal Structures from Molecular Packing Considerations

A LARGE body of information on bond lengths and angles, and on intra- and inter-molecular contact distances has been amassed. Because of this, and because high speed computers are now available, the solution of crystal structures without diffraction data (except for the

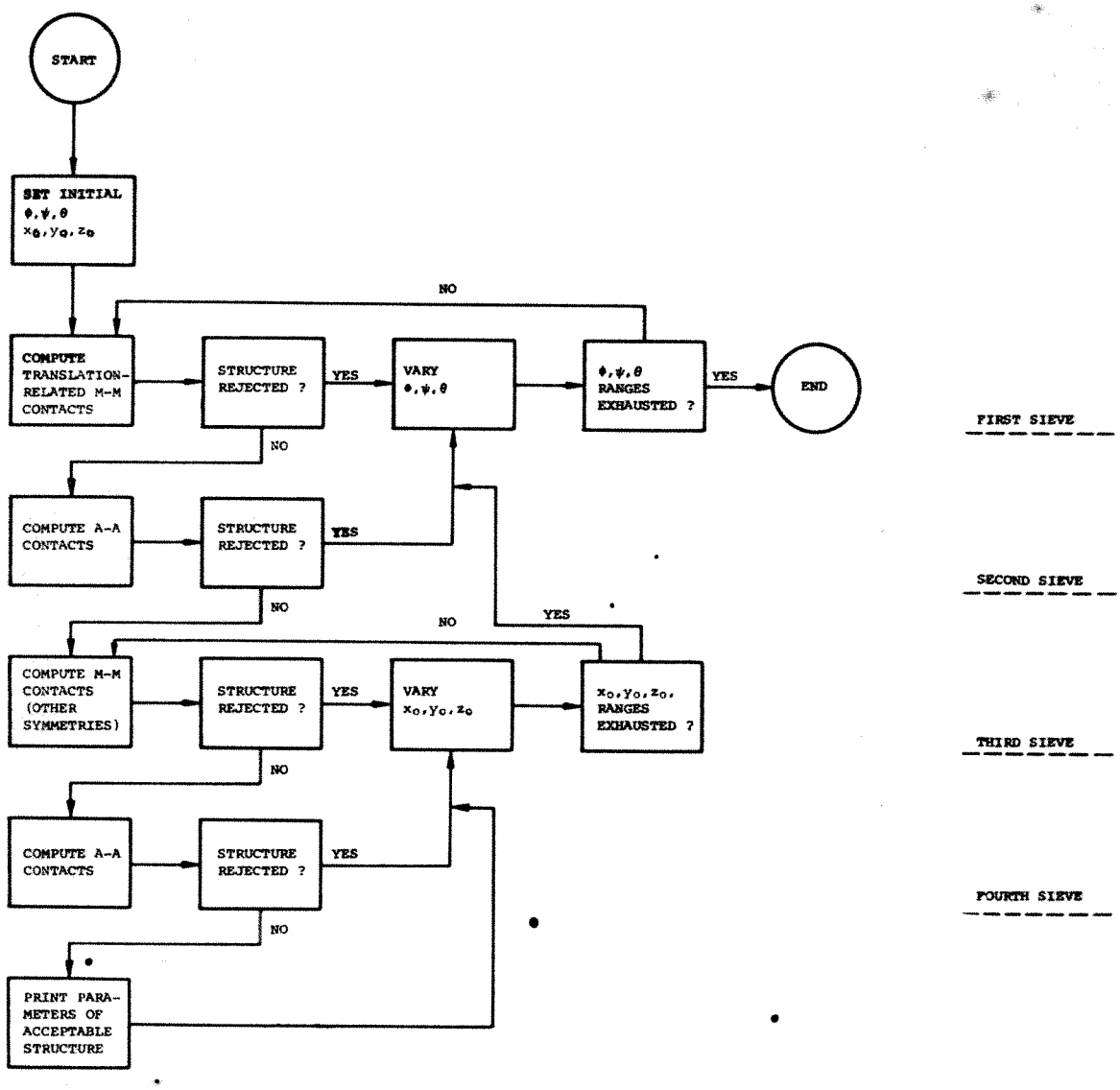


Fig. 1.

crystallographic constants of the unit cell) is an interesting challenge[1]. We wish to report some experience with a FORTRAN computer programme (SEARCH) currently being developed[2,3] which has successfully tackled this task in several instances.

We define the model of the generating unit (which as, for example, in centrosymmetrically paired carboxylic acids need not be identical with the asymmetric unit of the unit cell) by specifying its atomic co-ordinates in an orthogonal molecular system, M_i, preferably the system defined by the three principal axes of inertia of equally weighted atoms. The crystal structure is now defined by (*a*) the orientation of M_i with respect to the unit cell edges, (*b*) the position in fractional co-ordinates (x_0,y_0,z_0) of the origin of M_i in the unit cell, and (*c*) the space group symmetry elements operating on the generating unit. The orientation of the M_i system in the unit cell may be conveniently defined by the use of three Eulerian angles (φ,ψ,θ) specifying the rotation of M_i with respect to another, auxiliary, orthogonal system L_i fixed in the unit cell.

By stepwise variation of the six parameters $(x_0,y_0,z_0, \varphi,\psi,\theta)$ and by applying the symmetry operations on the generating unit we derive a set of trial structures. An acceptable solution is defined as a structure that involves no inter-atomic contact shorter than a preset minimum.

In order to reduce computations to reasonable dimensions, a sieving procedure was adopted which handles the entire molecule, rather than the atoms, as the fundamental unit for contact calculations; the molecules are treated as rectangular parallelopipeds or ellipsoids. For an initial orientation, the molecule-to-molecule contacts are computed between the generating unit and its translation-related neighbours in order of successive proximity to the former up to a preset radial distance determined by the dimensions of the unit. These distances depend solely on the orientation of the unit and not on the position (x_0,y_0,z_0) of its origin. An orientation is rejected if the interpenetration of the units for that orientation is deeper than a preset minimum. Rejection bypasses the scan of the (x_0,y_0,z_0) parameters; if the orientation has passed this first coarse sieve, actual interatomic contacts are computed for atoms of translation-related units. An interatomic contact shorter than a preset value leads to rejection and to stepped changes of the angular settings. If the orientation has passed this second sieve, molecular contacts across other elements of symmetry are computed. Because these contacts are dependent on (x_0,y_0,z_0) a scan with stepped changes in these co-ordinates is executed. (Obvious restrictions by symmetry elements on space occupancy reduce the volume of the unit cell to be scanned.) If no position of

Table 1

Compound	a (Å)	b (Å)	c (Å)	β (°)	Space group	n	No of atoms in generating unit	Symmetry of generating unit	No. of search parameters	Agreement factor R§	
Dibenzoyldiimide	7·35	3 95	19 80	96 0	$P2_1/c$	2	28	$2/m$	3	$(hOl+hll)$:	0·080
β-Methyl-*cis*-cinnamic acid	16·49	7·46	7·51	98·8	$P2_1/n$	4	38*	$\bar{1}$	3	(hkl):	0·065
o-Methoxy-*cis*-cinnamic acid	11·97	10·71	7·29	93·5	$P2_1/a$	4	46	$\bar{1}$	3	$(hkO+hk1)$:	0·065
β-(2-furyl)-*trans*-Acrylamide	9·87	23 76	5·84	—	$Pbca$	8	34	$2/m$	3	$(hkO+hk1)$·	0 107
cis-trans-Muconic acid	15 65	7·03	6 09	—	$Pna2_1$	4	16	1	5†	$(hkO+hOl)$.	0·158
o-Phthalaldehyde	7·49	13 21	6·78	—	$Pna2_1$	4	16	1	5‡	(hkO).	0 107
N-Phenylsydnone	22·06	3 75	9 58	103 2	$P2_1/a$	4	17	1	6	(hOl)·	0·174
9-Methyl-anthracene	8·91	14 63	8 06	96 6	$P2_1/c$	4	28*	mm	6	$(hkO+hk1+Okl+1kl)$·	0·065

* Hydrogen atoms of the methyl group omitted.
† Limited precession around the normal to the very strongly reflecting (001) plane.
‡ Limited precession around the normal to the very strongly reflecting (201) plane.
§ After least-squares refinement.

the molecular centre survives this sieve the orientation of the generation unit is changed, and the programme repeats the cycle described. Alternatively, all interatomic contacts are computed. The parameters of a structure model, resulting from this final sieve, with contacts not less than the preset minima, are printed.

The flowchart of the programme is shown in Fig. 1. The input consists of: (1) unit-cell information (cell constants, symmetry operations; definition of the auxiliary co-ordinate system L_t); (2) information on the generating unit (co-ordinates of all atoms, including hydrogens, in the inertial system; dimensions of the molecular envelope; (3) contact information (maximum molecular penetration; minimum interatomic distances); (4) parameter information (intervals and ranges to be varied, the latter determined by the symmetries of the generating unit and of the unit cell[3]). Minimum interatomic distances are given for pairs of atoms rather than computed as sums of individual atomic radii so as to allow for contacts not necessarily of the van der Waals type, such as polar (hydrogen) or ionic bonding. The values to be used as preset contact minima are correlated with the degree of uncertainty in the assumed molecular model and with the size of intervals in the search parameters. Restrictions on molecular orientation other than those mentioned so far have been used in compounds crystallizing in unit cells having short axes (4 Å) or showing outstandingly heavy reflexions.

We have found experimentally that 80 per cent van der Waals radii, and steps of 9° in φ,ψ,θ, and of 0·4 Å in (x_0,y_0,z_0), yield a limited number of solutions, including the correct crystal structure, within reasonable time on the *GOLEM* computer of the Department of Applied Mathematics of this Institute.

Table 1 lists the structures that have been solved *ab initio* by this technique. We have not given the number of spurious solutions nor the computing times required for each of these structures as these change with the procedure adopted and with the continued improvements now being made; of the two cinnamic-acid structures the β-methyl derivative gave one correct and one spurious solution, and the *o*-methoxy acid only the correct solution, both in a matter of 60 min. The individual structure analyses will be reported elsewhere.

We have developed this programme for several fields of applications: first, in our studies[4] of the topochemical control of organic solid-state reactions we required information on the molecular packing arrangement and the contact geometry of functional groups. Second, in order to be able to vary and thus to control this contact geometry we are analysing the crystal structure types of organic molecules having common functional groups (for example, $-CO_2H$, $-CONH_2$, $-CN$, $-NO_2$). Third, we are investigating in a related study the problem of limited polymorphism within chemically related molecular types[5], for example, $Ar(CH:CH)_n.CO_2H$ (where Ar = phenyl substituted by halogen, alkyl, alkoxyl, nitro, groups; n = 0,1). These problems can be attacked by structure analyses accurate enough to supply packing data to within 0·02 Å; because we consider that limited diffraction data are adequate for this purpose particularly when, as in our situation, collection of (photographic) data is the decisively limiting factor, a programme based on molecular rather than diffraction data seemed to us a desirable addendum to existing crystallographic techniques.

In a further extension of the procedure outlined we intend to discard fixed contact distances and to introduce interatomic potential energy functions[6,7].

We thank Drs. Bart, Coppens, Hirshfeld, Leiserowitz, and Shmueh for intensity data of some of the structures listed, and for suggestions concerning the molecular configuration of the input models. We are grateful to Prof. C. L. Pekeris and members of the Computing Laboratory of the Applied Mathematics Department of this Institute for time on the *CDC* 1604 *A* and the *GOLEM* computers.

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Synthesis of an Interstratified Mineral from Micas

Sericite, otherwise known as hydromuscovite, is a product of hydrothermal alteration of granodiorite and occurs as aggregates of fine grains with a silky lustre. It has the chemical composition shown in Table 1 (1).

Table 1. Chemical Compositions of the Starting Material and of the Synthetic 25 Å Mineral

	(1) (per cent)	(2) (per cent)
SiO_2	47·65	17 59
TiO_2	0·10	
Al_2O_3	37 03	35·78
Fe_2O_3	0 01	0 65
FeO	tr.	
MnO	tr.	
MgO	0 04	1 40
CaO	tr.	0·65
K_2O	9·02	6 36
Na_2O	0 76	0 83
SO_3		24·11
P_2O_5	0 02	
$H_2O(+)$	4·97	11·68
$H_2O(-)$	0 73	0·84
Total	100·33 (Analyst: H. Kodama)	99 89 (Analyst: S. Ueda)

A sample was pulverized to a fine powder in an agate mortar and amounts consisting of 0·2 g of this were mixed with powdered aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) and magnesium carbonate (both of reagent grade) in a range of proportions (0–0·1 g aluminium sulphate and 0–0·2 g magnesium carbonate). The resulting mixtures were packed into small silver crucibles, set over a water-level in a Morey autoclave with an inner volume of 500 ml., and maintained at 250°–300° C and at a pressure

Table 2. X-RAY POWDER DIFFRACTION DATA FOR THE SYNTHETIC 25 Å MINERAL (1) AND FOR ALLEVARDITE (2)

	(1)		(2)	
	d(Å)	F	d(Å)	F
001	25·61	47·0	24·21	45·4
002	12·81	45·8	12·28	−41·4
003	8 51	10·4	8·27	−3·7
004	–	–	6·16	−4·7
005	4 98	33·4	4·941	−36·0
006	–	–	4·086	tr.
007	–	–	3 520	19 7
008	3 12	60·4	3 089	−65 4
009	2·840	8·4	2·722	6·4
00·10	2·478	8·4	–	0·0
00·11	2·284	31·8	2·240	−14·1
00·12	–	–	2·053	23·6
00·13	1 910	30 4	1·899	38 8
00·14	–	–	1·768	9·0
00·15	1·653	6·0	–	0 0
00·16	1·568	16·0	1·542	16 2
00 17	1·497	4·6	1 446	−5·4
00·18	1·393	25 0	1·370	−18·0
00·19	1·337	2 0	1·301	−9 6
00 20	–	–	1·234	11·3

F values were derived from observed intensities. The signs of the values of allevardite were assigned from the probable structure (ref. 1).

of 60–120 atm. for 2–3 h. The mean heating rate was about 2° C per min. The autoclave was then allowed to cool in air at a mean cooling rate of 0·5° C per min. When the material in the crucibles had cooled to room temperature it was immediately washed with distilled water in order to remove any water-soluble materials. The residue was examined by X-ray crystallography and other mineralogical techniques.

As a result of these examinations, it was evident that various kinds of complex clay minerals had been formed together with alunite. These included minerals with interplanar spacings of 30, 25, 15, 9 and 7 Å. This communication is concerned with the 25 Å mineral.

Usually, the 25 Å mineral was formed together with the 15 Å mineral and alunite, and was the main component when aluminium sulphate and magnesium carbonate were used in the proportions 0·5 : 0·08.

The following properties are noteworthy: (*a*) The long spacing was particularly sensitive to the surrounding humidity. In cold dry weather there is a 23–25 Å reflexion which is relatively weak compared with the second order reflexion. In wet warm weather a strong 25–27 Å reflexion is apparent. When the spacing was 25 Å, a complete series of orders of this reflexion could also be observed as shown in Table 2. (*b*) Treatment of this sample with ethylene glycol resulted in the 25 Å and 12·5 Å reflexions being replaced by weak reflexions at 27 Å and 13 Å, and an extremely strong 17 Å reflexion became apparent. (*c*) Treatment with 1 N potassium chloride led to the 25 Å and its second order reflexions being almost entirely replaced by a 10 Å reflexion. (*d*) The chemical composition of this sample is as shown in Table 1 (2). Subtracting the chemical composition of alunite the following formula for the 25 Å mineral was obtained:

$$(Mg_{0\cdot57}Ca_{0\cdot25}(Na,K)_{0\cdot24})(Al_{3\cdot65}Fe_{0\cdot18}Mg_{0\cdot17})(Si_{6\cdot28}Al_{1\cdot72})O_{20}(OH)_4$$

(*e*) The electron micrograph of this sample showed that the original shape and size of the parent mica flakes were still well preserved and gave a hexagonal pattern by electron diffraction. Patches of alunite were also present.

These data indicate that although the 25 Å mineral is similar geometrically it is quite different dynamically from the regular mixed-layer mineral of the 15 Å and 10 Å layers because both of these are expandable.

Table 2 shows *F*-values which were derived from intensities by the factor $(1 + \cos^2 2\theta)/\sin 2\theta$, which is a valid procedure because the specimen was formed on a slide glass at the optimum orientation. These *F*-values are close to those of allevadite[1], which has been shown to be identical to rectorite[2].

When potassium alum ($KAl(SO_4),12H_2O$) (0·025 g–0·1 g) was added to the sample together with aluminium sulphate and magnesium carbonate, the X-ray behaviour of the 25 Å mineral approached that of the natural regular mixed-layer mineral, mica-montmorillonite. This suggests that potassium ions inhibit the expansion of the 10 Å layers.

Similar results were obtained when flakes of muscovite or phlogopite (1 *M* type) were used as the starting materials. It would seem that magnesium carbonate plays an important part in providing favourable conditions for the formation of 15 Å, 25 Å and 30 Å minerals from the dioctahedral micas. These minerals were successfully formed, however, from phlogopite even without magnesium carbonate. The use of magnesium oxide or magnesium sulphate instead of magnesium carbonate did not provide conditions favourable for the formation of the 25 Å mineral from the dioctahedral micas.

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CHEMISTRY

Structural Changes in Poly-β-benzyl-L-aspartate

DIFFERENTIAL thermal analysis has been applied over a wide field in the study of thermally induced structural changes in polymers[1]. This communication reports the application of this technique to an investigation of such changes in poly-β-benzyl-L-aspartate. It is generally recognized that polypeptides can form crystalline, helical structures in the solid state, in particular the α-helix of Pauling, Corey and Branson[2]. The stability of such a helix depends on a number of factors, in particular the nature of the side-chain[3], and the degree of polymerization of the molecule[4]. Of particular interest is poly-β-benzyl-L-aspartate, which adopts a relatively unstable left-handed α-helical conformation in the solid state, at room temperature. It has been suggested that the preferential formation of the helix with this anomalous sense results either from steric interference between the side-chain and the main-chain atoms[5,6], or from the competitive hydrogen bonding between the ester carbonyl groups of the side-chain and the amide groups of the main-chain[7], which precludes the formation of the more common right-handed helix. In this investigation, changes in the heat-energy of the polymer when heated at a constant rate were followed by differential thermal analysis and were correlated with structural changes, characterized by X-ray diffraction and infra-red spectroscopic methods.

The sample used was obtained from Yeda Research and Development Co., Rehovoth, Israel, and had a molecular weight of about 5,000 (lot *AS*10). The thermogram obtained using a DuPont 900 differential thermal analyser is shown in Fig 1. The double exothermic peak at about 100° C, and the endotherm at about 180° C divide the thermogram into three regions, *A*, *B* and *C*. Neither peak is observed on cooling from *B* or *C*, or on reheating the polymer from room temperature.

The X-ray diffraction powder photograph of the unheated polymer can be identified from its three clearly defined rings; but after heating to the temperature of the exotherm the pattern changes and many more reflexions become obvious. This diagram is further replaced by one with two sharp rings as soon as the temperature of the endotherm is exceeded. The many spacings exhibited by the sample after it had been heated to temperatures between those of the two peaks could be indexed on a tetragonal cell, with $a = b = 13{\cdot}8$ Å, $c = 5{\cdot}42$ Å, and we

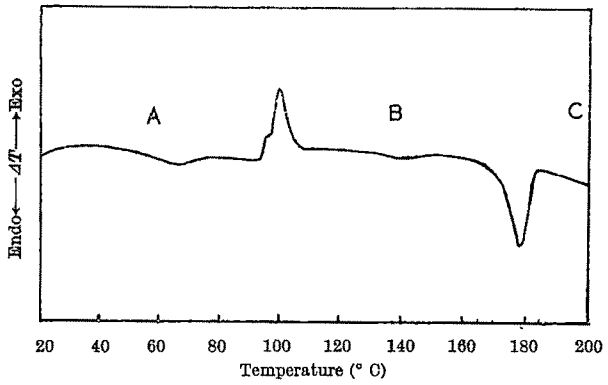


Fig. 1. Thermogram of poly-β-benzyl-L-aspartate. Heating rate 10° C/min. Sample *in vacuo*.

associate this pattern with the ω-helical conformation of the polymer. This value for the axial repeat is greater than that found by Bradbury *et al.*[6]; it is, however, not so high as that observed in the ω-form of poly-S-benzyl-thio-L-cysteine[8].

Corroborative evidence for the existence of the intermediate ω-helical state was obtained from the infra-red spectra (using the potassium bromide disk technique) of samples which had been heated to temperatures in the regions *A*, *B* and *C*. The specimens gave strong absorption bands at the frequencies indicated in Table 1. From these results it seems that the exotherm results from the transition of the polymer from the α- to the ω-helical conformation, with subsequent crystallization on a tetragonal lattice. The appearance of a sharp band at 1,639 cm^{-1} in samples heated above the temperature of the endotherm suggests that this is associated with the conversion to the cross-β conformation[4], with the formation of inter-chain hydrogen bonds.

Table 1. INFRA-RED ABSORPTION FREQUENCIES (cm^{-1})

Region	Ester C=O	Amide I	Amide II	Aromatic C—C
A	1,739	1,664	1,549	1,501
B	1,733	1,669 1,677	1,539	1,502
C	1,726	1,639 1,703 (weak)	1,528	1,501

Differential thermal analysis would thus seem to be a promising technique for the investigation of the relative stabilities and heats of transition of polypeptide structures in the solid state. Results so far seem to indicate that the stability is highly dependent on the crystallinity and the molecular weight of the sample.

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Detection of Thorium-234/Protactinium-234*m* in Sediments from the Ribble Estuary, Lancashire, England

SAMPLES of silt and sand are collected regularly from the estuary of the River Ribble as part of the programme of environmental monitoring conducted by the Ministry of Agriculture, Fisheries and Food to measure the distribution of radioactivity discharged into the Irish Sea. This discharge occurs from the United Kingdom Atomic Energy Authority's factories at Windscale (in Cumberland), and Springfields (in Lancashire).

Gamma spectrometric analyses of the dried samples collected in October 1965 showed γ-photons at 1·47 MeV from naturally occurring potassium-40, and at 0·51, 0·62, and 0·75 MeV from ruthenium/rhodium-106 and zirconium/niobium-95 from the Windscale factory discharges. A further γ-photon at 1·00 MeV was detected in some of these samples, notably in the sample collected near the mouth of the Springfields factory effluent pipeline, as shown in Fig. 1. Although ruthenium-106 emits a γ-photon at 1·06 MeV, the abundance of the emissions at 1·00 MeV exceeded those expected from ruthenium-106 alone, and the presence of protactinium-234*m*, which emits γ-photons with energies of 0·76 and 1·00 MeV, was suspected; this isotope is part of the uranium $(4n+2)$ series

$$^{238}U \xrightarrow[4.5\times10^{9}\text{ years}]{\alpha} {}^{234}Th \xrightarrow[24\cdot1\text{ days}]{\beta} {}^{234m}Pa \xrightarrow[1\cdot18\text{ min}]{\beta} {}^{234}U \xrightarrow[2\cdot5\times10^{5}\text{ years}]{\alpha} \text{etc.}$$

(Branch to ^{234}Pa)

Confirmation of the presence of protactinium-234*m* was obtained in two ways: first by comparison of the γ-spectrum obtained from the sample with that of a standard of pure uranium (in equilibrium with thorium-234 and protactinium-234*m*) distributed on sodium phosphate; and secondly by a chemical separation of thorium from the sample. This separation involved leaching the ignited silt with 5 M nitric acid and passing the acid extract through 'Dowex 1' anion exchange column in the nitrate form. After thorough washing with 5 M nitric acid to remove uranium and other metals, the thorium was eluted with 0·2 M nitric acid[1,3]. The γ-spectrum of this eluate contained the two peaks corresponding to γ-photons at 0·76 and 1·00 MeV from protactinium-234*m* and was identical with a γ-spectrum of thorium-234 separated from pure uranium (Fig. 2); the decay of this eluate was followed and the half life was found to be 25·3 days,

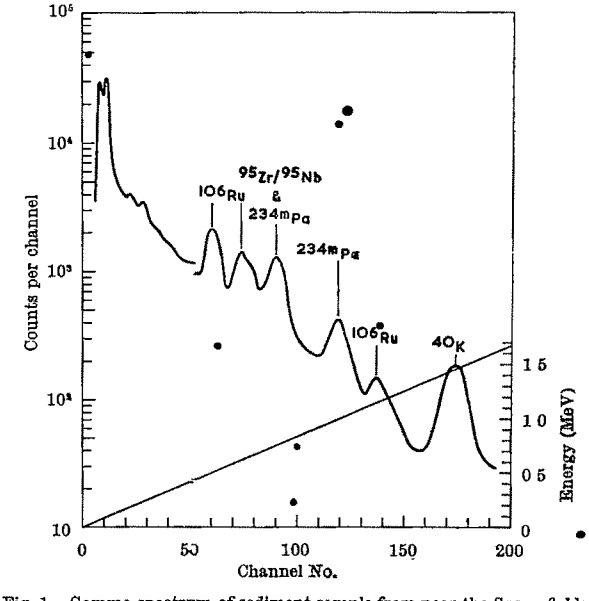


Fig. 1. Gamma spectrum of sediment sample from near the Springfields effluent pipeline.

Table 1

	Yards upstream of effluent pipeline					Yards downstream of effluent pipeline			
	12,500 (Samlesbury)	3,000	1,000	550	Effluent pipeline	100	1,000	5,500	12,000
Thorium-234 pCi/g dry	>300	700	1,280	680	1,450	>300	>300	>300	>300
Ruthenium-106 pCi/g dry	>1	39	57	28	20	12	32	52	41

confirming the presence of thorium-234 (half life 24·1 days).

The decay of the 1·00 MeV γ-photon in the original sample was also followed and the measured half life was about 28 days. The protactinium-234*m* in the sediments is therefore associated with thorium-234 and not with uranium-238.

Sediments which contain naturally occurring uranium normally emit γ-photons characteristic of the whole of the $(4n+2)$ series and additionally uranium-235. The γ-spectrum of such a sample, from the River Ribble above the tidal limit at Samlesbury, is shown in Fig. 3. In addition to photopeaks from fall-out nuclide γ-emission (cerium/praseodymium-144, antimony-125 and ruthenium/rhodium-106) and potassium-40, peaks corresponding to γ-photons with energies of 0·185 MeV from uranium-235, 0·242, 0·295 and 0·342 MeV from lead-214, and 0·610 MeV and other higher energies from bismuth-214 can be seen. These γ-photons are only just discernible in the Ribble estuary sediments where protactinium-234*m* occurs, so

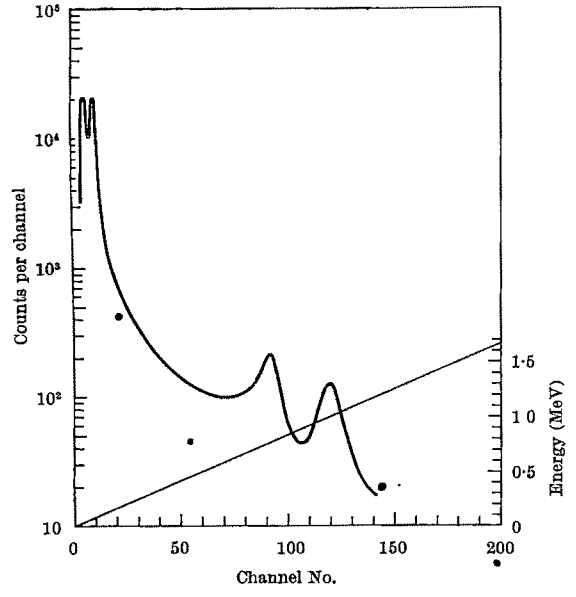


Fig. 2. Gamma spectrum of thorium extract.

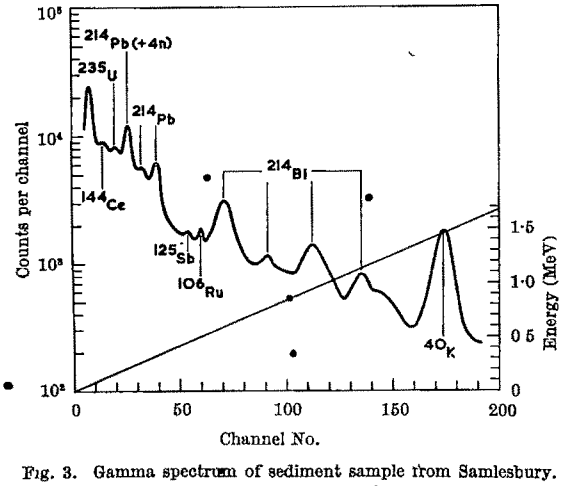


Fig. 3. Gamma spectrum of sediment sample from Samlesbury.

the thorium-234 in these samples is not associated with radium-226 and its daughters.

The probable source of the thorium-234 is the Springfields factory, where it arises as a result of uranium purification by a solvent extraction process and is subsequently discharged in the effluent in accordance with authorizations issued by this Ministry and the Ministry of Housing and Local Government.

Comparison of the count rates obtained from the samples with those from standards indicated the levels of thorium-234 and ruthenium-106 in the silts at the time of collection (Table 1).

Although ruthenium-106 occurs throughout the estuary, in this survey thorium-234 has been detected only between the Springfields factory pipeline and the upper tidal limit. This distribution may be due to the heavy dredging of the estuary; levels of radioactivity are of course affected by the dredging history of an area, and a later survey (carried out in March 1966) indicated the presence of thorium-234 downstream of the pipeline mouth. All these activity concentrations are considerably less than those which could result in a significant radiation dose to people or to marine organisms.

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Effect of Excess Energy on the Reactivity of Methylene

ALTHOUGH earlier work had suggested that the species methylene, CH_2, could participate in "insertion" reactions into C—H and C—Cl bonds[1,2], it has recently been shown that in the gas phase reaction of methylene with methyl chloride the course of reaction is best described by a radical abstraction–recombination mechanism[3,4]. It was shown that both ethane and dichloroethane appeared at rates at least as great as the rate at which ethyl chloride (the true "insertion" product) was formed in the reaction. The electronic state of the methylene may, however, determine the reaction path, and there is some evidence that it is excited singlet methylene which undergoes the insertion reaction[5,6]. Methylene is most conveniently prepared by the photolysis of ketene[7] or diazomethane[8], and the proportion of triplet (ground state) and singlet methylene formed depends on the precursor and the wave-length of the radiation used for photolysis[9,10], more singlet being formed at shorter wavelengths. We wished to investigate the effect of change of excess energy in methylene on the course of reaction, and we present here preliminary results.

Ketene was used as the source of methylene, and was prepared as previously described[3]. Ultra-violet radiation was derived from a 125 W *MB/D* high pressure mercury vapour lamp, and was incident on a quartz reaction cell maintained at 25° C. The wavelength region 2500–3400 Å was isolated by means of a chance *OX*7–nickel sulphate solution filter, while the region 3000–3650 Å was obtained by use of a glass filter (the optical absorption of ketene[11]

Table 1. RELATIVE RATES OF FORMATION OF ETHANE AND ETHYL CHLORIDE AT VARIOUS TOTAL PRESSURES

Total pressure (cm of mercury)	$\lambda = 2500$–3400 Å $d[C_2H_6]/d[C_2H_5Cl]$	$\lambda = 3000$–3800 Å $d[C_2H_6]/d[C_2H_5Cl]$
2·6	5 0	2·4
7·8	3·0	1·7
10 4	2 8	1·8
13·0	2 0	1·1
26·0	1·1	0·9
52 0	1 1	0·8

defined the longer wavelength limit). A reaction mixture of 5 parts ketene to 8 parts methyl chloride at various total pressures was photolysed in each wavelength region. The products formed were analysed by vapour phase chromatography.

Since ethane can be formed only by a radical mechanism, while ethyl chloride could be formed by insertion (although we believe it is not), the relative rates of formation of these two products are of significance. Table 1 shows the function $d[C_2H_6]/d[C_2H_5Cl]$ at a number of different total pressures in the two wavelength regions. Ethylene, dichloroethane and vinyl chloride, together with smaller amounts of methane and dichloromethane, were detected in addition to ethane and ethyl chloride. Experiments were carried out with the light intensity reduced by a factor of nine: the relative rates of ethane and ethyl chloride formation were unaffected. Thus the wavelength used for the photolysis does alter the product distribution, and the effect cannot be ascribed to differing absorbed intensities in the two spectral regions. It is seen that the rate of formation of ethyl chloride never exceeds significantly that of ethane, and that the ratio $d[C_2H_6]/d[C_2H_5Cl]$ is in general higher in the shorter wavelength region. Such a result is in direct contrast with that expected on the basis of the insertion theory, since singlet methylene—the species believed to insert[5,6]—is formed preferentially at shorter wavelengths[9,10].

A satisfactory interpretation of the results may be made in terms of the radical abstraction theory, if the exothermic (about 90 kcal/mole) recombination of methyl and CH_2Cl radicals produces a "hot" molecule of ethyl chloride which may either be quenched, or undergo a unimolecular decomposition to ethylene and hydrochloric acid (a process which requires about 55 kcal/mole). The only decomposition for ethane is the direct dissociation to two methyl radicals, a process not thought to be important under our experimental conditions. Setser gives a review of these processes[4]. Thus, for the formation of ethylene, ethane and ethyl chloride, our simplified reaction scheme (omitting abstraction reactions of the radicals) is

$$CH_2CO + h\nu \rightarrow CH_2 + CO \quad (1)$$

$$CH_2 + CH_2CO \rightarrow C_2H_4 + CO \quad (2)$$

$$CH_2 + CH_3Cl \rightarrow CH_2Cl + CH_3 \quad (3)$$

$$2CH_3 \rightarrow C_2H_6 \quad (4)$$

$$CH_3 + CH_2Cl \rightarrow C_2H_5Cl^* \quad (5)$$

$$C_2H_5Cl^* + M \rightarrow C_2H_5Cl \quad (6)$$

$$C_2H_5Cl^* \rightarrow C_2H_4 + HCl \quad (7)$$

The recombination of two CH_2Cl radicals gives rise similarly to "hot" dichloroethane which may be quenched, or decompose giving hydrochloric acid and vinyl chloride (observed product).

It is apparent that the rate of decomposition of $C_2H_5Cl^*$ in (7) depends on the wavelength used to photolyse the ketene, and this suggests that the excess energy carried by the methylene is transferred to the methyl or chloromethyl radicals. At least at the lowest pressures used by us, the radicals cannot become "thermalized" before recombination, as otherwise the wavelength of photolysis would not affect the $d[C_2H_6]/d[C_2H_5Cl]$ ratio; however, at the higher pressures the radicals may reach thermal equilibrium before they recombine. The excess energy of the methylene which is carried over to $C_2H_5Cl^*$ may be in the form of the electronic excitation known to occur, but it may equally well be that vibrational or translational excitation is important in the present connexion. The experimental data do not permit us to reach a conclusion about the mode of excitation. The effect of total pressure on the ratio is also easily understood: increase in pressure leads to greater stabilization of $C_2H_5Cl^*$ in (6). The ratio should, therefore, tend to a limiting value dependent on the rate constants, k_4, k_5 of (4) and (5) (our limiting ratio of ~ 1 suggests that if $[CH_3] \sim [CH_2Cl]$, then $k_4 = 2k_5$).

The hypothesis that $C_2H_5Cl^*$ needs to be quenched may be tested by adding an "inert" gas under conditions where $C_2H_5Cl^*$ would otherwise decompose to a considerable extent. We made up to 1 atm. with nitrogen or carbon dioxide a reaction mixture at a total pressure of 2·6 cm of mercury, and photolysed it by light of the shorter wave-length region. The ratio $d[C_2H_6]/d[C_2H_5Cl]$ dropped from 5·0 without the gas to 1·9 with nitrogen and 1·4 with carbon dioxide, which is entirely consistent with the mechanism proposed.

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Thermochemical Data Storage for Inorganic Compounds

THE method proposed by Alcock[1] as a means of correcting an approximate free-energy change calculated from the simple two-term equation

$$(\Delta G^\circ_T)_{\text{approx.}} = \Delta H^\circ_{298} - T\Delta S^\circ_{298}$$

leads to a system of thermochemical data storage which possesses many advantages over the free-energy function and polynomial methods. This "free-energy deviation" does not, however, allow for transformations or changes in state of reactants and products, whereas in many processes the standard free-energy change would be required for examples such as

$$M_{(l)} + O_{2(g)} = MO_{2(l)} \quad (1)$$

$$M_{(c)} + 2Cl_{2(g)} = MCl_{4(g)} \quad (2)$$

to provide the basis for calculating equilibrium activities in solution and equilibrium partial pressures in a vapour phase. It is immediately apparent that the heat and entropy changes associated with such transformations must be taken into account and for reaction (1), where it is assumed that the solid state represents the normal equilibrium form of the metal and its oxide at 298° K, an approximate free-energy change is given by

$$(\Delta G^\circ_T)_{\text{approx.}} = \Delta H^\circ_{298} - \Delta H_{f,M} + \Delta H_{f,MO_2} - T(\Delta S^\circ_{298} - \Delta S_{f,M} + \Delta S_{f,MO_2})$$

For many purposes this approximate free-energy change is sufficient, but in the terms in which it is written it

cannot be combined with one or more free-energy deviation functions to give a true value for the change in free-energy.

To demonstrate this, consider the reaction

$$M_{(l)} + O_{2(g)} = MO_{2(c)} \quad (3)$$

at a temperature T °K > T_1° K, the melting point of the metal M. The true change in free-energy is given by

$$\Delta G^\circ_T = \Delta H^\circ_{298} - \Delta H_{f,M} + \int_{298}^{T_1} \Delta Cp_1 dT + \int_{T_1}^{T} \Delta Cp_2 dT$$

$$- T\left[\Delta S^\circ_{298} - \Delta S_{f,M} + \int_{298}^{T_1} \frac{\Delta Cp_1}{T} dT + \int_{T_1}^{T} \frac{\Delta Cp_2}{T} dT\right]$$

where ΔCp_1 and ΔCp_2 are the heat capacity equations appropriate to the temperature ranges 298 – T_1° K and T_1 – T° K.

Writing

$$\int_{T_1}^{T} X dT \quad \text{as} \quad \int_{298}^{T} X dT - \int_{298}^{T_1} X dT$$

we have

$$\Delta G^\circ_T = \Delta H^\circ_{298} - T\Delta S^\circ_{298} - \Delta H_{f,M} + T\Delta S_{f,M}$$

$$+ \left\{\int_{298}^{T} \Delta Cp_2 dT - T\int_{298}^{T} \frac{\Delta Cp_2}{T} dT\right\}$$

$$- \left\{\int_{298}^{T_1} (\Delta Cp_2 - \Delta Cp_1)\, dT - T_1 \int_{298}^{T_1} \frac{(\Delta Cp_2 - \Delta Cp_1)}{T} dT\right\}$$

$$+ (T - T_1) \int_{298}^{T_1} \frac{(\Delta Cp_2 - \Delta Cp_1)}{T} dT$$

The terms in braces are Alcock's free-energy deviations and

$$\Delta G^\circ_T = \Delta H^\circ_{298} - \Delta H_{f,M} - T(\Delta S^\circ_{298} - \Delta S_{f,M}) + \Delta(G.D.F.)_T$$

$$- \delta(G.D.F.)_T + (T - T_1) \int_{298}^{T_1} \frac{\delta Cp}{T} dT$$

where $(G.D.F.)_T$ stands for the free energy deviation function and is to be calculated for reaction (3) at T° K, and $\delta(G.D.F.)_{T_1}$ stands for the similar function for the melting of the metal, with

$$\delta Cp = (\Delta Cp_2 - \Delta Cp_1) = Cp_{M(l)} - Cp_{M(c)}$$

It can be shown that for each transformation or change of state between 298° K and T° K in the reactants and products the additional terms

$$\left(\pm \Delta H_t \pm T\Delta S_t \pm \delta(G.D.F.)_{T_t} \pm (T - T_t) \int_{298}^{T_t} \frac{\delta Cp}{T} dT\right)$$

are required. In general, for i transformations or changes in state

$$\Delta G^\circ_T = \Delta H^\circ_{298} - T\Delta S^\circ_{298} + \Delta(G.D.F.)_T$$

$$+ \sum_i \left(\pm \Delta H_t \pm T\Delta S_t \pm \delta(G.D.F.)_{T_t} \pm (T - T_t) \int_{298}^{T_t} \frac{\delta Cp}{T} dT\right)$$

where the immediately accessible approximate free-energy change is given by

$$(\Delta G^\circ_T)_{\text{approx.}} = \Delta H^\circ_{298} - T\Delta S^\circ_{298} + \sum_i (\pm \Delta H_t \pm T\Delta S_t)$$

As a result of this extended treatment, the particular value of the free-energy deviation as the difference between a true and an approximate free-energy change is lost. By referring the transformation data to 298° K, that is

$$\Delta H_{t,298} = \Delta H_{t,T_t} - \int_{298}^{T_t} \delta Cp\, dT;$$

$$\Delta S_{t,298} = \Delta S_{t,T_t} - \int_{298}^{T_t} \frac{\delta Cp}{T} dT$$

a second approximate free-energy change is obtained as

$$(\Delta G^\circ_T)_{\text{approx 2}} = \Delta H^\circ_{298} - T\Delta S^\circ_{298} + \sum_i (\pm \Delta H_{t,298} \pm T\Delta S_{t,298})$$

which differs from the true free-energy change only by

$$\int_{298}^{T} \Delta Cp\, dT - T \int_{298}^{T} \frac{\Delta Cp}{T} dT$$

where ΔCp is the difference in heat capacities for the states of the reactants and products appropriate to the temperature T °K. This is the required free-energy deviation and thus

$$\Delta G^\circ_T = (\Delta G^\circ_T)_{\text{approx.2}} + \Delta(G.D.F.)_T$$

In this expression the change in free energy is now in the form proposed by Alcock, and is rigorously applicable to all processes irrespective of the states of the reactants and products. It is obvious that for many purposes the first approximate value for the free-energy change suffices, while the correction of this by means of the free-energy deviation function will satisfy most of the remaining requirements. The calculation of true changes in free energy requires the tabulation of heats and entropies of transformation and changes in state for the temperature 298° K. It is noted that heats of evaporation and sublimation at 298° K are already tabulated in a compilation[2] of thermochemical data which enjoys a large following, and it is suggested that an extension of the tables to include 298° K values for all such processes would facilitate and encourage the use of Alcock's free-energy deviation function.

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Reactions of the Hydroxyl Radical in the Radiolysis of Aqueous Solutions of Di-ethyl Acetal

IT has recently been suggested[1] that the decomposition of aqueous solutions of di-ethyl acetal induced by radiation is due to hydrolysis, catalysed by hydronium ions formed in the spurs in the primary radiolytic process.

$$H_2O \rightarrow H_2O^+ + e^- \quad (1)$$

$$e^- + nH_2O \rightarrow e^-_{aq} \quad (2)$$

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH \quad (3)$$

As the result of our experiments on the reaction between hydroxyl radicals produced by the photolysis of hydrogen peroxide, and acetal, we have come to the conclusion that this reaction may account for at least a part of the decomposition observed in the radiolysis.

$$OH + CH_3CH(OC_2H_5)_2 \xrightarrow{k_4} CH_3CHO + C_2H_5OH + \text{other products} \quad (4)$$

The experiments were carried out as follows: solutions of acetal (10^{-2} M) and hydrogen peroxide (10^{-1} M) in neutral water (phosphate buffer) were irradiated with a low pressure mercury lamp, virtually monochromatic with respect to the 2537 Å component. The intensity of the 2537 Å light was determined by the uranyl oxalate actinometer as modified by Pitts *et al.*[2] and was $1{\cdot}1 \times 10^{19}$ quanta l./sec.

At the concentrations used virtually all the light is absorbed by the hydrogen peroxide for which an extinction coefficient of 20 l./mole/cm was found, in agreement with other determinations[3]. The extinction coefficient

Table 1. YIELDS IN THE PHOTOLYSIS OF AQUEOUS HYDROGEN PEROXIDE, ACETAL SOLUTIONS

Conditions*	Concentration in moles/l. Acetal	Ethanol	Acetaldehyde
10^{-2} M Acetal unirradiated. After 20 min	$9{\cdot}75 \times 10^{-3}$	$0{\cdot}4 \times 10^{-3}$	$0{\cdot}24 \times 10^{-3}$
10^{-2} M Acetal pH = 2 unirradiated. After 20 min	$0{\cdot}8 \times 10^{-3}$	$1\ 93 \times 10^{-2}$	1×10^{-6}
10^{-2} M Acetal irradiated 20 min	$9{\cdot}65 \times 10^{-3}$	$0\ 4 \times 10^{-3}$	$0\ 24 \times 10^{-3}$
10^{-2} M Acetal, 10^{-1} M H_2O_2 unirradiated. After 20 min	$9\ 40 \times 10^{-3}$	$1{\cdot}5 \times 10^{-3}$	$1{\cdot}0 \times 10^{-3}$
10^{-2} M Acetal, 10^{-1} M H_2O_2 irradiated 15 sec	$8\ 95 \times 10^{-3}$	$2\ 1 \times 10^{-3}$	$1{\cdot}45 \times 10^{-3}$
10^{-2} M Acetal, 10^{-1} M H_2O_2 irradiated 30 sec	$8\ 55 \times 10^{-3}$	$2\ 65 \times 10^{-3}$	$1{\cdot}9 \times 10^{-3}$
10^{-2} M Acetal, 10^{-1} M H_2O_2 irradiated 45 sec	$8{\cdot}15 \times 10^{-3}$	$3{\cdot}2 \times 10^{-3}$	$2{\cdot}4 \times 10^{-3}$
10^{-2} M Acetal, 10^{-1} M H_2O_2 irradiated 60 sec	$7{\cdot}8 \times 10^{-3}$	$3{\cdot}65 \times 10^{-3}$	$2{\cdot}8 \times 10^{-3}$

* All at pH 7 except where otherwise stated.

for acetal at 2537 Å was found to be less than 0·6 l./mole/cm.

After irradiation the products were determined by gas chromatography on a 1 m 'Carbowax 400' column operated at 138° C. The main products of the photolysis were found to be ethanol and acetaldehyde together with a small amount of what has tentatively been identified as di-ethyl ether. For short irradiations the concentration of these products increases linearly with irradiation time while that of acetal decreases linearly with approximately 1 mole each of ethanol and acetaldehyde formed for each mole of acetal destroyed.

Other reactions leading to the same products were also observed. First, even at pH 7, a small amount of hydrolysis took place and, second, reaction between acetal and hydrogen peroxide was found. Direct photolytic decomposition of acetal at this wavelength was not observed in peroxide free solutions. The results of these and the other experiments are summarized in Table 1.

As the irradiation time is increased beyond 1 min the yields of the products tend to fall off, indicating competition with acetal for hydroxyl radicals. The ethanol concentration reaches a maximum of $4{\cdot}5 \times 10^{-3}$ moles/l. after 6 min of irradiation while for acetaldehyde this maximum is $6{\cdot}2 \times 10^{-3}$ moles/l. after 15 min. This is qualitatively in accord with the reaction rate constants of hydroxyl with ethanol and acetaldehyde in aqueous solution which have been determined[4] as $1{\cdot}2 \times 10^{9}$ l./mole/sec and 7×10^{8} l./mole/sec respectively.

The fact that ethanol competes with acetal for hydroxyl radicals introduces a complication as the product of this reaction in the presence of oxygen is also acetaldehyde[5]. The mechanism is probably the following:

$$OH + C_2H_5OH \rightarrow C_2H_4OH + H_2O \quad (5)$$

$$C_2H_4OH + O_2 \rightarrow C_2H_4(OH)O_2\cdot \quad (6)$$

$$2C_2H_4(OH)O_2\cdot \rightarrow O_2 + H_2O_2 + 2CH_3CHO \quad (7)$$

In confirmation of this we have photolysed aqueous solutions of ethanol and hydrogen peroxide and found that one mole of acetaldehyde is formed for each mole of ethanol destroyed. That the reaction is due to hydroxyl radicals and not to direct decomposition was shown by irradiating a peroxide free solution, which did not decompose.

The problem which now arises is whether acetaldehyde is a primary product of the reaction of hydroxyl and acetal or is formed by the secondary attack of hydroxyl on ethanol. We believe that at low doses it is a primary product. First, its dose-yield curve when corrected for thermal contributions is linear and passes through the origin with no indication of an induction period. Second, it has been found that the addition of chloride ion (10^{-1} M) has no effect on the rate of formation of acetaldehyde. Chloride ions react with hydroxyl radicals in competition with ethanol to yield chlorine atoms which appear to be unreactive toward ethanol[6]. Thus if attack on ethanol were responsible for acetaldehyde formation, the addition of chloride would be expected to suppress the rate of this reaction. The fact that no competition between chloride and acetal is apparent can be explained if it is assumed that chlorine atoms react with acetal to produce the same radical, a precursor of ethanol and acetaldehyde, as that produced by the hydroxyl radical

$$CH_3CH(OC_2H_5) + Cl \text{ or } OH \rightarrow R\cdot \leftarrow \text{Products} \quad (8)$$

So far, we have only considered the reaction of hydroxyl radicals produced by the primary decomposition of hydrogen peroxide

$$H_2O_2 \rightarrow 2\ OH \quad (9)$$

These radicals will, however, react with the peroxide in competition with (4), and the possibility thus exists that the effects we have observed may be due to reactions of the peroxy radical

$$OH + H_2O_2 \rightarrow H_2O + HO_2 \ (k_{10} = 4{\cdot}5 \times 10^{7} \text{ l./mole/sec}) \quad (10)$$

$$HO_2 + \text{Acetal} \rightarrow \text{Products} \quad (11)$$

Without a determination of k_4 it is not possible to assess the importance of (10), but if (10) and (11) are responsible for acetaldehyde production in these experiments then it is likely that reaction (11) is the source of this product in the radiolysis experiments. The latter were carried out in the presence of air so that the reducing radicals will react according to (12) and (13) to form the peroxy radical or its basic counterpart

$$H + O_2 \rightarrow HO_2 \ (k_{12} = 2{\cdot}6 \times 10^{10} \text{ l./mole/sec}) \quad (12)$$

$$e^-_{aq} + O_2 \rightarrow O_2^- \ (k_{13} = 2{\cdot}2 \times 10^{10} \text{ l./mole/sec}) \quad (13)$$

In conclusion we can state that the present work strongly indicates that in the radiolysis of aqueous acetal the formation of acetaldehyde is partly due to reaction between acetal and either the hydroxyl or the peroxy radical. Because of the effects of competition on the part of the products the present work is not extensive enough to ascertain whether this is the only effect or whether hydrolysis induced by radiation and catalysed by acid contributes to the formation of the products. A detailed investigation of the problem is being made with the emphasis on photolysis at low doses, on the effect of hydroxyl scavengers on the radiolysis and on methods for the determination of the rate constant of reaction (4). These results will be communicated at a later date.

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Rapid and Specific Effects of Bromine on the System Keratin/Formic Acid

FORMIC acid interacts strongly with many fibrous proteins, polypeptides and polyamides; it produces either complete dissolution or considerable swelling together with a marked contraction of the fibre resulting from melting of the crystalline material[1]. Fibres of α-keratin, however, show considerable radial swelling (about 50–60 per cent) in concentrated formic acid, but virtually no change of length. The swelling will tend to lengthen the fibre while the melting of α-helices will tend to contract it, but the major cause for the constant length must be

sought in the stability of a residual proportion of the crystallites which have been shown by X-ray measurements to remain in the α form in 98 per cent formic acid[2]. This residual stability has been shown to persist even in dilute solutions of the helix-forming protein extracted from wool[3].

In the absence of mechanical stress, the melting-point of the crystallites in a normal α-keratin (for example, wool or hair) in water[4,5] is above 100° C. Partial melting at least can, however, be induced below 100° C, either by stretching combined with heat treatment[5,6] or by immersion in a suitable medium such as lithium bromide solution[7,8]. At room temperature, the contraction obtainable in 8 M lithium bromide is only about 10 per cent, but at 100° C contractions up to about 50 per cent can be achieved in times of the order of an hour. Very small amounts of bromine can strongly inhibit the contraction produced in lithium bromide and this has been attributed[7] to the action of the tribromide ion $(Br_3)^-$ on keratin.

The influence of small amounts of bromine on the interaction of wool with formic acid has now been investigated, with surprising results. In contrast to the behaviour in formic acid alone, rapid elongation is produced. This shows that the constraint of the residual α-helical keratin has been overcome. This could be the result of a direct attack of the bromine on the tyrosine residues, a conclusion indicated by model experiments[9] and supported by the amino-acid analyses below. As the tyrosine residues occur at regularly repeating distances along the helix[10], there will be a tendency for relatively short segments of crystalline order to survive the treatment, which would account for the phenomena described here.

In a typical experiment, a Corriedale wool fibre was immersed in 98 per cent formic acid. A slight initial contraction was observed, after about 10 sec, followed by a lengthwise swelling. Equilibrium was reached with the fibre about 1 per cent longer than its original length. A force-extension measurement revealed a substantially linear relationship up to about 15 per cent extension, with a Young's modulus of about 7×10^8 dynes/cm^2. After washing out the formic acid with water, the original length and Young's modulus (about $1{\cdot}8 \times 10^{10}$ dynes/cm^2) were largely restored.

The same procedure was then carried out with 98 per cent formic acid containing about 0·1 per cent w/v bromine. There was a small initial contraction (as in the absence of bromine) but then a rapid lengthening of the fibre, reaching an apparent equilibrium at a length about 40 per cent greater than the original. This apparent equilibrium was reached in about 20 sec and no further change was observed in the following 5 min. In this swollen condition the fibre could be extended to a length 90 per cent greater than the original without breaking: the modulus in the early stages of the extension was very low, certainly less than 10^6 dynes/cm^2.

When the fibre was transferred from the bromine/formic acid mixture to water at 20° C, it contracted spontaneously from 40 per cent above to 30 per cent below the original length. In this process it became white and opaque. The contracted length was not altered by immersing the fibre in water at 20° C for 16 h or by heating it in water at 52° C for 1 h. Further contraction did, however, take place, in boiling water, reaching a value of 38 per cent after 1 h. It would appear that some remaining crystalline segments melted in water between 52° and 100° C.

The contracted fibre, in water, was still soft (Young's modulus approximately 4×10^8 dynes/cm^2) and highly extensible with a breaking extension of about 120 per cent of the contracted length or 80 per cent referred to the initial length (extension rate approximately 15 per cent/min). Under these conditions, such high extensibility is not shown by fibres contracted in other media such as lithium bromide[7].

Table 1. CHANGES IN THE AMINO-ACID COMPOSITION OF WOOL INDUCED BY IMMERSION IN FORMIC ACID/BROMINE SOLUTION

Residue	Concentrations in μmole/g Before treatment	After treatment	Change
Lysine	205	252	+47
1/2-Cystine	901	800	−31
Cysteic acid	16	86	
Tyrosine	275	12	−263
3-Bromo tyrosine	—	178	+178
Phenylalanine	214	168	−46

Further physico-chemical investigations of these phenomena are in progress, but amino-acid analysis of the treated wool has already indicated that a very specific mechanism is involved. Most residues showed only an insignificant change (about 5 per cent) from the control, but marked alteration was shown by the residues listed in Table 1. Lysine increased and the groups containing sulphur decreased, although if half-cystine and cysteic acid are taken together, the total loss of these is only about 3 per cent. In contrast the tyrosine has been almost entirely eliminated, and this is presumably the main point of attack by the bromine (which is present here in the molecular and not the ionized form (Br_3^-) as in lithium bromide). About two-thirds of the tyrosine has been isolated as the mono-brominated derivative, and a separate experiment showed that 3 : 5 di-bromotyrosine appeared at the location of the lysine peak on the amino-acid analyser, thus accounting for the apparently anomalous increase in lysine. Altogether, then, about 80 per cent of the original tyrosine has been isolated as bromo-derivatives, 15 per cent has apparently been destroyed or otherwise modified, and 5 per cent is left intact.

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Spontaneous Bubbling at Porous Hydrophobic Membranes

IT has been observed that when a hydrophobic membrane permeable to gases is present at the interface between water and a dry gas, the gas passes through the membrane and is released in the liquid as bubbles. The interesting feature of this process is that it proceeds without the need to raise the pressure of the gas and will even operate against a positive hydrostatic head of liquid. Indeed, substantial static pressures are developed in the water chamber when escape of the gas is prevented on the liquid side of the membrane. The membrane used in these experiments was a 7-mil thick, extruded tetrafluoroethylene (TFE) film which, in the course of preparation, was never subjected to temperatures above 300° C. The morphology and pore size of the film have not as yet been determined.

The tests were performed with disks of the TFE membranes 1 in. in diameter mounted in various orientations. With the membrane oriented vertically or horizontally (with the liquid phase uppermost), buoyancy forces remove

the gas. If, however, the membrane is orientated horizontally with the gas phase uppermost, bubbles collect beneath the membrane and displace the water. This does not arrest further transport of the gas, which indicates that physical contact between the membrane and the liquid phase is not necessary for the process.

When the water temperature was 70° C, the hydrostatic head 1 in., and the gas delivered at 1 in. water pressure at 25° C, the approximate gas flow rates for hydrogen, nitrogen, oxygen and air were found to be 8·0, 1·6, 1·4 and 1·6 ml. cm^{-2} min^{-1}, respectively. These flow rates increased to 26·0, 5·2, 5·0, and 5·2 ml. cm^{-2} min^{-1}, respectively, when the water temperature was raised to 90° C.

A total head of water of 2·0 lb./in.² gauge was necessary to arrest the transport of hydrogen across the membrane at 70° C. Similarly, to arrest the passage of air, minimum water heads of 0·6, 1·6, 2·3 and 3·0 lb./in.² gauge were required at water temperatures of 70°, 80°, 85° and 90° C, respectively.

It has been shown that this phenomenon results from interdiffusion of water vapour and gas through the porous membrane, with the partial pressure gradients of the respective gases through the pores providing the driving force for the process. The translation of this familiar process into the unusual effects described is possible because of the impermeability of the hydrophobic membrane to liquid water either by direct permeation or by condensation. The liquid water thus acts as a displaceable barrier to gas transport and, at the same time, is the source of water vapour. So far as we know, the TFE membranes are unique in providing a separator with the combination of physical and chemical inertness, porosity, and high hydrophobicity necessary to give rise to the observed effects.

Separate measurements have been made of the interdiffusion of water vapour and gas across the membrane in the absence of the liquid phase. These experiments were carried out at 70° C under conditions of zero differential total pressure and with the partial pressure gradients of the two components across the membrane essentially constant. Anhydrous calcium sulphate was used as a drying agent in the gas chamber and was placed close to the membrane. In the water vapour chamber, also close to the membrane, there was a glass wool wick partly immersed in a pool of water. The increase in weight of the drying agent was taken as a measure of the quantity of water vapour transported across the membrane while the gas transported in the opposite direction was collected over water in a gas burette. Tests were performed with air, carbon dioxide, carbon monoxide, helium, hydrogen, oxygen and methane. The following relationship was found to hold within experimental error:

$$\frac{\text{flux water}}{\text{flux gas}} = \sqrt{\frac{\text{Mol. wt. gas}}{\text{Mol. wt. water}}}$$

This result agrees with the findings of other investigators who have used a variety of membranes and various sets of interdiffusing (non-condensable) gases. The relationship holds both for Knudsen effusion and for diffusion, and has been given a theoretical interpretation by Evans *et al.*[1]. As well as the flux ratio relationship we found that the flux of water and gas is an inverse function of the molecular weight of the gas.

Less satisfactory agreement with the theoretical relationship was obtained in the experiments with liquid water. The possibility that this may be related to the kinetics of bubble release is now being investigated.

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BIOCHEMISTRY

Periodic Cross-banding in Amyloid Filaments

THE presence of a fibrous component in amyloid first detected in 1959 by the electron microscopic studies of Cohen and Calkins[1] has been confirmed by all subsequent observers. The fibrillar structure of amyloid, its physicochemical properties and its exclusive occurrence in the extra-cellular portion of the connective tissue suggested that amyloid is a variant of one of the normal fibrous proteins[2,3]. We believe that the following observations are pertinent to this hypothesis.

Tissues obtained by biopsy or at necropsy from cases of familial Mediterranean fever, multiple myeloma, idiopathic primary amyloidosis and experimental leishmaniasis-induced amyloidosis in hamsters were fixed in glutaraldehyde–osmium tetroxide, embedded in 'Vestopal', contrasted with uranyl acetate–lead and examined under a Siemens Elmiskop I electron microscope.

Examination of native amyloid from these sources showed that it is made up of filamentous structures which are present only in the connective tissue ground substance. Filaments as long as 1μ and ranging in thickness from 40 Å to 140 Å were measured. Organization beyond the filament stage was never noted. At high resolutions it sometimes seemed as if the filament were composed of two strands twisted around one another[2,4].

In primary enlargements of ×30,000 or more, a clear cross-striation of alternating 30 Å dark and 25 Å bright bands was sometimes observed with a 55 Å periodicity. The grouping of bands such as occurs in ripe collagen was not apparent (Fig. 1).



Fig. 1. Cross-banding in amyloid deposits in familial Mediterranean fever. Arrow indicates filaments which appear to be composed of two strands (×120,000).

The periodic cross-banding is of considerable significance to an understanding of the ultrastructure of amyloid. Indications of this have been recorded in the past as beading; Gueft and Ghidoni reported "an irregular granular periodicity . . . of approximately 40 Å"; Shirahama and Cohen found "a regular beading which measured approximately 100 Å" in isolated amyloid filaments[4]. Recently, these indications were strengthened by the observations of Hashimoto *et al.* in a study of lichen amyloidosis[5]. In areas where amyloid and collagen were intimately intermingled, these authors found thin amyloid filaments which showed irregular 150–250 Å beading and thicker fibrils with regularly spaced cross-banding of about 150–250 Å. Boere *et al.* reported 40 Å cross-striation in isolated and negatively stained primary and secondary amyloid[6].

The properties of amyloid cited here, together with its intermingling with collagen and reticulin within a common matrix[7], and the occurrence of secreting fibroblasts and

fibrocytes as the only active elements in amyloid-laden tissues[5,8] seem to suggest that amyloid may be related to the collagen family. It is significant that collagen does not always possess 640 Å periodicity and under certain conditions a variety of periodicities occur[9,10]—for example, embryonal and young collagen show predominantly shorter periodicities, ranging from 70 Å to 210 Å (ref. 11). Proline and hydroxyproline are apparently absent from amyloid, and for this reason similarities in structure which seem to link it with collagen require careful examination before they are accepted as an indication of a family relationship.

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Purification and Characterization of Ribonuclease from Unfertilized Eggs of the Sea Urchin, *Psammechinus miliaris*

KNOWLEDGE about the various types of RNA involved in the biosynthesis of proteins has increased greatly over the past few years. Little is known, however, about the part played by the various ribonucleases (RNases) known to be present in most cells, in the metabolism of the RNA. There has been much speculation on this topic, but few attempts have been made to investigate the physiological action of these substances. This is largely because few of the many RNases identified have been isolated and purified sufficiently to allow them to be characterized. The purification of RNase has great interest at present because of its possible use in the structural analysis of RNA molecules.

Sea urchin eggs have long been intensively used in investigations of maturation, fertilization and early cell differentiation processes which have a marked effect on the metabolism of ribosomal RNA[1–3]. Investigations of the RNase content of eggs of the sea urchin, *Psammechinus miliaris*, were therefore undertaken in this laboratory. Although minute traces of RNase activity in sea urchin eggs have been reported[4] no attempt has so far been made to isolate and characterize the enzymes. This communication reports the isolation and purification of an RNase from mature, unfertilized eggs of *Psammechinus miliaris*.

A homogenate of lyophilized sea urchin eggs was extracted with chloroform to remove lipid material. After extraction, the aqueous phase was lyophilized and redissolved in water. The active part of the fraction was precipitated at *p*H 3·5 by fractionation with ammonium sulphate. 90 per cent of the activity was obtained in the fraction between 45 and 75 per cent saturation. Gel filtration on a column of 'Sephadex *G*-100' increased the specific activity (activity/$A_{280\ m\mu}$) of the preparation to about 1,000 times that of the homogenate. The enzyme solution was concentrated using an '*SE*-Sephadex *C*-50' column, and the final purification was accomplished by chromatography on a column of '*CM*-Sephadex *C*-50', which was eluted with a sodium chloride gradient in 0·1 M ammonium acetate buffer, *p*H 4·8 (Fig. 1). This step of the purification resulted in an enzyme preparation with a specific activity about 10,000 times higher than that of the homogenate. Purified yeast RNA was used as a substrate in the assays[5] of the phosphodiesterase (DEase) activity, and synthetical nucleoside 2′,3′-phosphates were used as substrates in the assays[6] of the cyclic phosphatase (CPase) activity. The activity was related to standard curves prepared using crystalline bovine ribonuclease (Armour). Because of the small amount of activity present in the egg material (75 μg RNase/g lyophilized eggs) special care had to be taken to ensure high yields in each step of the purification. The overall yield in the purification was about 50 per cent.

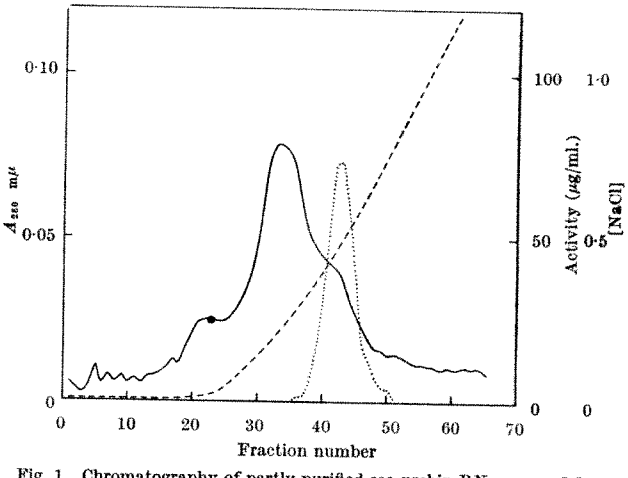


Fig. 1. Chromatography of partly purified sea urchin RNase on a 0·5 × 60 cm column of '*CM*-Sephadex *C*-50'. The load used was 220 μg of RNase in 1·2 ml. solution obtained from the '*SE*-Sephadex' step. Initial buffer: 0·1 M sodium acetate, *p*H 4·8 (– – –) sodium chloride. Flow rate 3·6 ml./h. 0·6 ml. per fraction. (——) A_{280}. (. . .) Activity.

Investigations of the specificity of this purified preparation showed that the sea urchin RNase hydrolysed RNA to both purine and pyrimidine cyclic riboside 2′,3′-phosphates. The purine cyclic riboside 2′,3′-phosphates were hydrolysed at a considerably slower rate to their corresponding dihydrogen phosphates of the 3′ isomer form. The enzyme lacked CPase activity against the pyrimidine cyclic riboside 2′,3′-phosphates, and in this respect it was similar in its specificity to tobacco leaf RNase[7] and pea leaf RNase[8]. The curve of *p*H against activity for the RNase showed an optimal *p*H of 5·4. Investigations of the heat stability at different values of *p*H revealed that the activity had higher stability in the acid region of the *p*H range, but the stability in general was found to be rather low. On the other hand, when the purified enzyme was kept at −20° C during a period of over a year, little or no loss of activity was observed. Determinations of the molecular weight of this RNase using a column of 'Sephadex *G*-100' and reference substances with known molecular weights gave a molecular weight of 32,000–35,000 for the enzyme.

During the investigation, two other separate enzymes were isolated from the egg homogenate. One showed an acid *p*H optimum while the other had its optimum *p*H at about 7. Both the enzymes showed high CPase activity but completely lacked DEase activity, and thus probably a complementary function to the RNase.

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Change in Ribonuclease Concentrations in L-Asparaginase-treated Lymphosarcomata

BROOME[1,2] suggested that L-asparaginase is responsible for the antilymphoma activity of guinea-pig serum[3]. We confirmed the hypothesis[4] and furthermore presented[5] evidence that extracts of *Escherichia coli B* contain an asparaginase which also has high antilymphoma activity. This communication presents evidence that the regression of lymphosarcoma 6*C3HED* induced by L-asparaginase is preceded by an increase in the alkaline ribonuclease of the post-mitochondrial cell fraction of the tumour, while the acid ribonuclease of that fraction is increased later during the regression.

Asparaginase assays were performed as previously described[5]. Purification of asparaginase is shown in Table 1. The enzyme was extracted from lyophilized *E. coli B* cells by sonication of the suspended bacteria (10 per cent w/v) for 30 min. The sonicate was centrifuged at 30,000*g* for 30 min. After precipitation of the nucleic acids by 1·0 M manganese chloride (0·05 × volume), the supernatant was subjected to fractionation using ammonium sulphate. The 55–100 per cent saturated fraction which contained the asparaginase was dialysed until no ammonia could be detected in the dialysate with Nessler's reagent, and applied to a hydroxylapatite column (2·0 × 23 cm). The column was washed with disodium hydrogen phosphate and the enzyme was eluted with dipotassium hydrogen phosphate, both buffers being 0·1 M with a *p*H of 8·6. Tubes containing most of the enzyme activity were pooled and purified either by additional hydroxylapatite chromatography or by column electrophoresis. Better purification was usually achieved by electrophoresis.

The effect of the enzyme on the growth of the tumours is shown in Fig. 1. The *BW* 5147 and the resistant strain of 6*C3HED* continued to grow while the sensitive strain of 6*C3HED* progressively decreased in size. The resistant tumours of animals which were treated with the buffer only grew at the same rate as those of the animals which were treated with asparaginase.

Animals bearing a tumour were injected intraperitoneally with 10–11 units of asparaginase (specific activity = 1,000–4,500 u/mg protein), while the controls were injected with an equal volume of the buffer. After either 2 or 18 h, the animals were killed and ribonuclease (RNase) activity determined by the method of Shortman[6]. The tumours were removed and immediately placed in ice-cold 0·15 M sodium chloride solution. They were then trimmed to remove connective tissue and blood vessels, weighed and homogenized in 0·44 M sucrose to give a 10 per cent homogenate. The homogenate was centrifuged at 17,000*g* for 60 min and the supernatant was assayed for RNase activity. The assay mixture contained 0·1 ml. of 1 per cent ribonucleic acid (RNA) in 10^{-2} M ethylenediamine tetraacetic acid, 0·1 ml. of water or of 10^{-2} M *para*-chloromercuribenzoate (*p*CMB), 0·2 ml. of buffer (acetate, 0·03 M, *p*H 5·8, or veronal, 0·03 M, *p*H 7·8) and 0·1 ml. of the supernatant. After incubation at 37° C for 30 min, the tubes were cooled in ice and 0·6 ml. of cold acid–alcohol (1 N hydrochloric acid in 76 per cent ethyl alcohol) was added. Cold acid–alcohol was added to controls which had not been incubated, immediately after the addition of the supernatant. The resulting

Table 1. PURIFICATION OF ASPARAGINASE

	Units (u)	Protein (mg)	Sp. act. (u/mg)	Recovery (per cent)	Purification (x)
Crude extract	25,000	4,000	6·4		
Ammonium sulphate 55–100 per cent	15,700	1,100	14·3	61	2·2
Hydroxylapatite column I	12,400	72	172	48	27
Hydroxylapatite column II	2,200	2·2	1,000	9	156

Table 2. EFFECT OF ASPARAGINASE ON THE CONCENTRATIONS OF RIBONUCLEASE IN LYMPHOSARCOMATA

Tumour	RNase (u/g tumour tissue) Acid (*p*H 5·8) Control	Treated	Alkaline (*p*H 7·8) Control	Treated	Inhibited Alkaline Control	Treated
BW 5147						
2 h	172 ± 32	163 ± 16	206 ± 72	231 ± 49	141 ± 21	131 ± 17
6*C3HED*/resistant						
2 h	64 ± 9 (10% decrease)	58 ± 18	115 ± 18 (13% decrease)	100 ± 24	48 ± 29	54 ± 23
18 h	76 ± 26 (25% increase)	95 ± 22	124 ± 37	123 ± 32		
6*C3HED*/sensitive						
2 h	76 ± 12 (18% increase)	85 ± 10	43 ± 11 (80% increase)	78 ± 7	96 ± 13	87 ± 10
18 h	71 ± 5 (43% increase)	109 ± 15	112 ± 6 (66% increase)	186 ± 25		

One unit is that amount of enzyme which will produce an optical density change of 1·0 in 30 min.

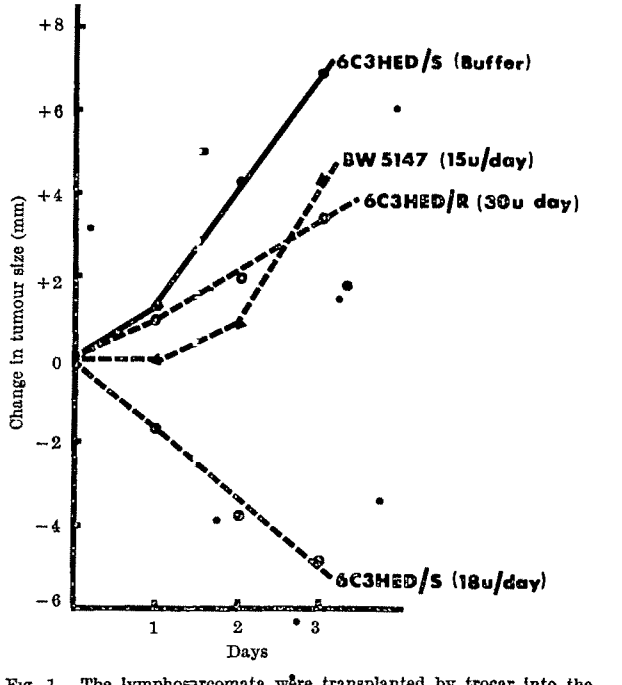


Fig. 1. The lymphosarcomata were transplanted by trocar into the right flank of the female host animals (*C3H/HeJ* and *AKR/J*) obtained from Jackson Memorial Laboratories, Bar Harbor, Maine, 4 days before testing began. The tumour-bearing mice were injected intraperitoneally twice daily, at least 6 h apart for 3 days with the asparaginase solution or an equal volume of the buffer. The tumour sizes reported represent the average diameter obtained by measuring with calipers in three directions before the first injection each day.

precipitate was removed by centrifugation, the supernatant diluted six times with distilled water, and the optical density at 260 mμ was determined. The difference between the optical density of the incubated sample and that of the unincubated sample was taken as a measure of RNase activity.

Table 2 shows that although there is little or no change in the levels of any of the RNases in either of the resistant tumours, there is an 80 per cent increase in the alkaline RNase of the post-mitochondrial fraction of the sensitive tumour within 2 h after treatment with asparaginase. The level of the acid RNase is not significantly changed in the sensitive tumour during the first 2 h, but there is a 43 per cent increase over the control 18 h after treatment. The raised level of the alkaline RNase (inhibited RNase) persists for at least 18 h. The increase in alkaline RNase seen in the presence of *p*CMB is not significantly different in tumours from treated and control animals.

Because asparaginase gives rise to increased RNase activity only in tumours which regress following treatment, and has no effect on the levels of RNase in tumours which are resistant to the antilymphoma action of asparaginase, it is possible that the mechanism of regression caused by asparaginase might involve activation of RNase. MacLeod, King and Hollander[7] have shown that *in vivo* treatment with 9-α-fluoro-prednisolone produces an increase in acid RNase of lymphosarcoma *P*1798 before any visible reduction in size of the tumour occurs. The similarity of the asparaginase effect to that of the 9-α-fluoro-prednisolone on the corticoid-sensitive lymphosarcoma suggests a generalized mechanism of lymphocytolysis involving RNase. This is supported by examples correlating regression of tumours with increased acid RNase recently reported by Ambellan and Hollander[8]. As RNase is a possible regulator of protein synthesis, its increased activity could lead to cell destruction. Furthermore, messenger RNA has been reported to be particularly sensitive to pancreatic (alkaline) RNase[9], thus causing disaggregation of polysomes[10]. This observation is relevant because the rise in RNase reported here is in the soluble portion of the cell.

The cytotoxic effect of asparaginase on *6C3HED* cells *in vitro* reported by Borella *et al.*[11] was accompanied by a reduction in the amount of tritiated cytidine taken up by the cells. Such a decrease could be caused by an increased rate of RNA destruction by RNase which has been activated by asparaginase as well as by a decrease in RNA biosynthesis.

Recently, El Asmar and Greenberg[12] reported that *in vivo* treatments of *6C3HED* lymphosarcoma with a glutaminase-asparaginase preparation from a Pseudomonad inhibited both the growth of the tumour and the incorporation of labelled glycine and adenine. The effect was ascribed to the impairment of purine synthesis by the depletion of glutamine. The same result could be obtained by the activation of RNase by means of the asparaginase activity in the preparation. The nucleotides released from RNA by the action of the activated RNase would dilute the labelled pool of purines and thereby reduce the incorporation of the labelled compounds.

Ledoux[13] has demonstrated that direct injections of pancreatic RNase are effective in causing regression of tumours, but rather large amounts of material were necessary. In the investigation reported here, a maximum of 10 μg of protein was given and no RNase activity could be detected in the samples administered. This difference suggests that internal stimulation of the endogenous enzyme is more effective than administration of the same activity from another source.

There are several possible mechanisms by which asparaginase may stimulate RNase: (1) by removing an inhibitor other than that which is destroyed by *p*CMB; (2) by releasing the enzyme from some inactive, bound form; (3) by binding to the enzyme to produce a synergistic effect. The removal of an inhibitor is the most tempting mechanism. An analogy to this mechanism has recently been reported by Wiernick and MacLeod[14], who found that the removal of inhibitors of alkaline RNase by acid treatment[15] activated the enzyme so that 9-α-fluoro-prednisolone no longer induced an increase in thymus RNase.

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The "Pink Spot" and Schizophrenia

THE idea that the "pink spot" is uniquely associated with schizophrenia[1–3] is now in doubt because of its reported presence in normal individuals[4,5] and in patients suffering from Parkinson's disease[6]. Additionally, some doubt must be cast on its identification as β-3,4-dimethoxyphenylethylamine (DMPE)[1,7]. The evidence supporting the finding that the "pink spot" is DMPE rests on its almost exact correspondence with DMPE in certain paper chromatographic[1,4,7] and gas chromatographic[8,9] systems, the chromatographic similarity of prepared derivatives[1,7] and the similarity of the melting point range of the hydrochlorides[22]. This indirect identification of the "pink spot" as DMPE is of great interest because of the hypothesis[10,11] that in schizophrenia abnormal methylation of catecholamines might occur. This hypothesis is strengthened by the chemical similarity between DMPE and the hallucinogenic substance mescaline and also by the observation that the feeding of substances capable of donating a methyl group can cause an exacerbation of schizophrenic symptoms[12–14].

In this laboratory urine samples containing about 300 mg of creatinine (usually taken from a urine specimen collected over a 24 h period) were extracted with chloroform according to the procedure described by Friedhoff and van Winkle[1]. The final dried extract in chloroform was applied in the proportions 150, 75 and 75 mg of creatinine, respectively, to three 5 cm strips of Whatman number 2 quality paper. Authentic DMPE (25 μg) was overspotted on one of the 75 mg strips. Descending chromatography in butanol : acetic acid : water, 60 : 15 : 25 (v/v), was allowed to proceed overnight (15 h). This length of run (40–43 cm) was sufficient to allow a good separation of the pink spot zone from other constituents of the urine extract,

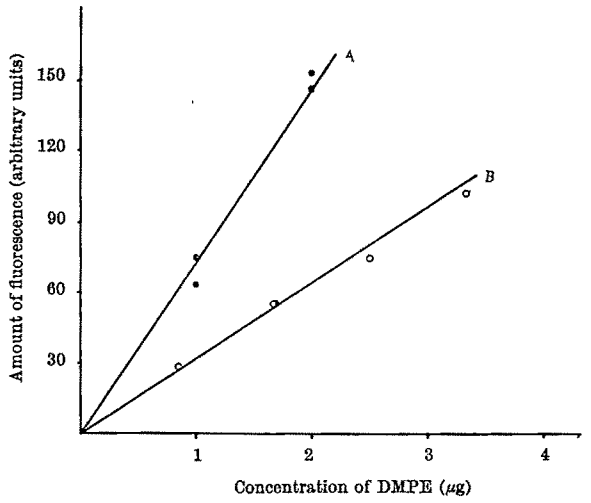


Fig. 1. Recovery of authentic DMPE added to urine. According to the procedure described by Friedhoff and van Winkle[1] 5, 10, 15 and 20 μg respectively of DMPE were added to four 100 ml. samples of "normal" urine and extracted in chloroform (3 × 50 ml.). Equal portions of each of the alkaline chloroform extracts were spotted, separated and rendered fluorescent by the glycine–formalin treatment (see text). The amount of fluorescence was measured on a paper strip scanning device, arranged for fluorescence measurements, using primary filter Chance *OX*1 (365 mμ) and secondary filter Chance *OY*8 (cut off below 450 mμ). Standards (curve *A*), run at the same time, show that over the range 0–20 μg the recovery (43 per cent) was constant.

including on occasions various drug metabolites. The 150 mg and the 75 mg+25 μg DMPE strips were sprayed with ninhydrin-pyridine reagent, heated for 2 min at 100° C, and, after noting the positions and colours of the separated zones, dipped through a modified Ehrlich reagent[15]. In order to be sure whether or not a particular sample was positive it was essential that the zone on the 150 mg strip corresponded almost exactly with the less concentrated strip containing the authentic DMPE, and that the correct sequence of colour changes occurs. This is important because several "pink spots" can be found in the urines of those patients excreting drug metabolites (see also refs. 4, 5 and 16). In a study of thirty-one urines, obtained so far as possible from patients selected according to the diagnostic criteria suggested by Bourdillon *et al.*[3], only one was found to exhibit a clearly defined "pink spot", although a ninhydrin positive substance was seen in the appropriate position in two others. Nothing was seen at this position in extracts of the urine from five "normal" subjects. Because it is necessary to have at least 5 μg of DMPE on the paper strip for the formation of a definitive "pink spot" it may be that more positives would have been found if larger amounts had been separated.

In an attempt to increase the sensitivity of the identification of the "pink spot" the fluorimetric procedures of Seiler and Weichmann[17] and Bell and Somerville[18] were investigated using urine to which authentic DMPE had been added. The acid glycine–formalin treatment of Bell and Somerville was finally selected on the basis of its greater sensitivity. It is possible to obtain quantitative values from these strips by using a paper strip scanning device[19] arranged for fluorescence measurements. Fig. 1 shows that a constant proportion (43 per cent) of added DMPE within the range 0–20 μg is recovered from urine. Other experiments have shown that, while on a particular occasion the recovery of added DMPE is reproducible, it is not necessarily the same proportion as on a different occasion.

It was surprising to find that the 75 mg strip from the positive urine did not fluoresce after the glycine–formalin treatment. In order to decide whether this lack of fluorescence was caused by inhibition of the formation of the fluorophore by a contaminating substance present in the alkaline chloroform extract and possessing a similar R_F value, 10 μg of DMPE was added to two qualitatively positive, but quantitatively different, samples of the single positive "pink spot" urine obtained on different days. After chromatography and development of the fluorophore, scanning revealed that the same proportion of the added DMPE (55 per cent) was recovered from both urines. This clearly indicates that in this particular urine the "pink spot" is not DMPE. Recent work[20,21] along different lines also concludes that the "pink spot" is not DMPE. At this stage it is difficult to assess how much importance to attach to the finding of the "pink spot" in schizophrenic patients, at least until more careful studies with respect to the influence of gut bacteria, diet, drug therapy, etc., have been performed. It is essential, however, that an unambiguous chemical identification be obtained as soon as possible.

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Identity of the "Pink Spot"

A NUMBER of authors have now reported the occurrence of "pink spot" in the urine of schizophrenics[1]. Its identification as β-3,4-dimethoxyphenylethylamine (DMPE) rests on paper chromatographic evidence and a melting point provided by Friedhoff and Van Winkle[2] together with the gas chromatographic evidence of Sen and McGeer[8]. The pink spot produced from DMPE by successive treatments with ninhydrin and Ehrlich's reagent is evanescent and some 5–10 μg are required on the paper after chromatography in order to be certain of its occurrence. Drug metabolites can seriously interfere with its determination by this technique. We find that DMPE is readily detected by its reaction with formaldehyde on paper[4], and that it can be separated by high voltage electrophoresis from urinary metabolites of 'Largactil' (2,000 mg/day), 'Stelazine' (540 mg/day), 'Nardil', 'Disipal' (300 mg/day), barbiturates and haloperidol (2·25 mg/day). We have examined urines from

Table 1. PARTITION COEFFICIENT OF DMPE BETWEEN ETHYLENE DICHLORIDE OR ISOAMYL ALCOHOL AND GLYCINE SODIUM HYDROXIDE BUFFERS (0·05 M) OF VARYING *p*H

	*p*H					
Solvent	8·68	9·15	9·65	10·08	10·4	11·6*
Ethylene dichloride	0·59	1·60	3·51	5·68	7·51	5·69
Isoamyl alcohol	0·45	1·53	3·12	5·26	7·34	6·65

* 0·1 N sodium carbonate.

patients during treatment with these drugs (at the doses quoted), and find that their metabolites give no fluorescence with formaldehyde and do not interfere with the formaldehyde fluorescence of added DMPE. ('Tofranil' metabolites, however, had a native fluorescence which may interfere at high doses.) Even when overloading of the paper has caused tailing of the DMPE and overlap with metabolite spots, the fluorescence still develops normally. The fluorophore can be eluted with 0·4 N sodium hydroxide in methanol, and after acidification with hydrochloric acid it can be measured quantitatively in a fluorimeter at 360 mμ activation/460 mμ fluorescence. In case of tailing on the electrophoresis paper, the eluted fluorophore can be re-run on thin layer plates of silica gel using *n*-butanol : water : acetic acid (60 : 20 : 20), or isopropanol : methyl acetate : water : concentrated ammonia (35 : 45 : 15 : 5), in order to separate it from traces of drug metabolites or other urinary constituents.

For the examination of schizophrenic urines we use 24 h collections and extract either 500 ml. or the whole sample (whichever is the less) with 3 × 1/3 volume of re-distilled ethylene dichloride. Since DMPE is a water-soluble base of *p*K 9·86 (determined by Mr. P. J. Taylor using potentiometric titration at an ionic strength $\mu = 0{\cdot}01$) the extraction at *p*H 9·0 used by previous workers is not favourable to complete recovery. Table 1 shows the partition coefficient of DMPE between organic solvents and aqueous buffers of varying *p*H. This was measured by shaking the DMPE in buffer (100 μg/ml.) with a third of its volume of organic solvent and reading the optical density of each phase at 279 mμ. We have adopted *p*H 10·5 as standard for the extractions and at this *p*H solutions of DMPE are stable for at least several days at 4° C. After drying the extract with sodium sulphate and evaporating it to dryness under vacuum at 40°–50° C, the residue is triturated with 50–100 μl. 0·01 N hydrochloric acid, and three equal portions of the solution are applied to Whatman No. 1 paper soaked in 0·1 M phosphate buffer *p*H 6·2. 10 μg DMPE is spotted over the middle sample and the electrophoresis is run at 4 kV for 1·5 h. After oven drying at 80° C, half the paper is stained by ninhydrin/Ehrlich and the other by glycine/formaldehyde. Markers of DMPE alone are also run on each half. Using this technique, 25 μg DMPE added to 500 ml. of normal urine can be detected by the pink spot reaction and the recovery as measured by elution of the formaldehyde fluorophore is 50–60 per cent. Two authentic "pink spot" urines supplied by Dr. A. Pauline Ridges were examined by this method and gave pink spots at the position corresponding to DMPE on both paper chromatography and electrophoresis, but no formaldehyde fluorescence. We have now examined another thirty-eight urine samples from twenty-seven schizophrenic patients selected according to the "Schneider positive, non-paranoid" criteria of Bourdillon *et al.*[5] which correlate with a high incidence of "pink spot". We have found only four positives, two of which were from one patient (N. P.), and none of these gave formaldehyde fluorescence. N. P. is a chronic patient and the first sample was obtained during treatment with 'Largactil'. The second and stronger positive was obtained when all medication had been stopped for 26 days. Intermediate samples were negative. These positive samples have all been re-examined with the same result, using fresh portions of the same urines. Standards of DMPE added to urine, extracted and run at the same time gave good fluorescence. Although the "pink spots" from schizophrenic urines had the same colour and fading characteristics as those from DMPE standards, their appearance on the electrophoresis paper was different because they tended to form streaks running ahead of the standard, rather than compact spots.

For patients who were specially selected, the incidence of "pink spots" is disappointingly low, though this could result partly from the fact that some of the samples were of small volume and in these cases there may not have been enough material on the paper for detection. From the evidence of the four positives which we have obtained, as well as those supplied by Dr. Ridges, it seems to us that these particular "pink spots" at least could not have been β-3,4-dimethoxyphenylethylamine, although at present we have no other information as to their identity.

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Effect of Reduced Glutathione on Haem Synthesis in Rat Liver during the Postnatal Period

GROWTH is subordinate to the development of function in the liver of the rat during the first 2 weeks after birth[1]. In this period the activities of some respiratory enzymes in the liver increase to adult levels. Dawkins found that the rates at which some substrates are oxidized increase greatly in the first few days after birth[2]. He also demonstrated a marked increase over this period in the concentration of co-factors of the respiratory enzymes. The rate-limiting factor controlling these processes is not at present known.

The oxidative function of these respiratory enzymes is connected with their prosthetic group, which in many cases is haem[3]. The increase in the activity of haemoproteins after birth may be related to the rate of synthesis of haem. A low level of haem synthesis would be expected to limit the development of aerobic metabolism in the liver. It has been shown[4] that haem synthesis in adult rats is increased in the presence of sulphydryl reagents such as reduced glutathione (GSH) or ascorbic acid.

The present experiments were designed to investigate the effect of GSH on haem synthetase which catalyses the insertion of iron into protoporphyrin. The activity of this enzyme was determined in extracts of isolated rat liver mitochondria from rats aged 1–21 days. The method used was a modification of that of Labbe[5]. Mitochondrial extracts were prepared in 0·25 M sucrose solution containing 1 per cent oleic acid. The incubation mixtures contained 0·08 μmoles ferrous sulphate, 0·08 μmoles protoporphyrin IX and, when indicated, 20 μmoles GSH per ml.

The protoporphyrin was dissolved in 0·1 per cent oleic acid at *p*H 8·5. Haem synthetase activity was expressed as mμmoles protoporphyrin consumed/30 min/g fresh liver. Preliminary experiments showed that proto-

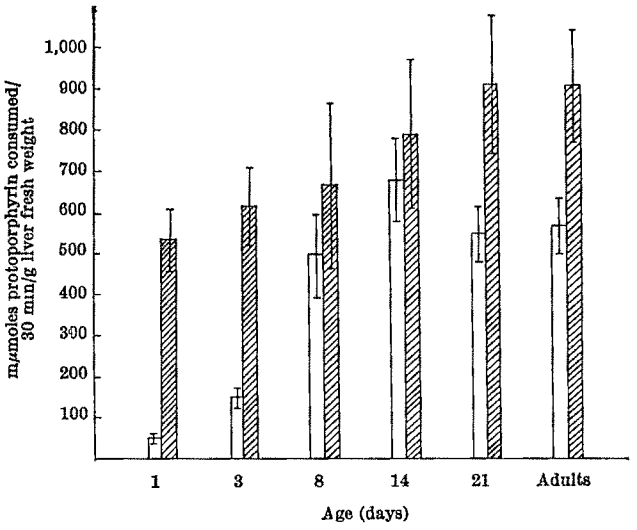


Fig. 1. The effect of GSH on haem synthetase activity in extracts of isolated rat liver mitochondria during the postnatal period. The columns represent the means of the results of between four and seven enzyme preparations. In the first 14 days the livers of between three and five animals were used for one enzyme preparation. The ranges show the standard deviations. White columns, without GSH; hatched columns, with GSH.

porphyrin consumption was stoichiometric to haem formation. Haem labelled with iron-59 was isolated and determined as haemin by the method of Labbe and Nishida[6]. The conditions used in the experiments with iron-59 were identical to those with non-labelled iron. GSH was determined by the method of Grunert and Phillips[7].

Fig. 1 shows that the protoporphyrin consumption with GSH increases within 21 days from 540 to 900 mμmoles/30 min/g liver fresh weight, whereas the activity without GSH increases from 49 to nearly 600 mμmoles/30 min/g liver fresh weight. This implies that the GSH-activation of haem synthetase is increased by a factor of eleven at the first day and only by a factor of 1·5 in adults. Two possible explanations of this change in the effect of GSH during the postnatal period can be advanced: (1) the concentration of GSH in the extracts of newborn liver might be lower, or (2) the breakdown of the added GSH might be increased. Preliminary experiments indicate that the first assumption is more likely, because the GSH-content of the mitochondrial extracts is only 25 per cent of that of adults.

Regulation of enzyme activity during development by the concentration of ascorbic acid was demonstrated for the enzyme *p*-hydroxyphenylpyruvate oxidase by Kretchmer[8]. Haem synthetase provides a further example of the concentration of a sulphydryl reagent regulating enzyme activity in the postnatal period.

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PHYSIOLOGY

Receptive Field Properties of the Superior Colliculus of the Rabbit

ALTHOUGH richly endowed with both sensory and motor information[1], the superior colliculus has in recent years been considered to play a very small part—literally that of a vestigial body—in the vision among higher mammals. In view of its intimate relationships with many central nervous centres[1] and its homologous significance (as in the frog), a fuller reappraisal of its role in vision seems timely. Analysis of the receptive fields of these collicular cells was found a particularly effective approach here since levels both more peripheral (the retinal ganglion cells[2]) and parallel (the lateral geniculate body[3]) in the main visual pathway of the rabbit have been similarly investigated. There have also been related studies at several synaptic levels in the cat[4–6], frog[7] and monkey[8].

The direction of this preliminary investigation was to determine qualitatively the scope of visual information represented in this centre—in particular its more important "trigger features"[2].

The twelve adult hybrids the fields of which are described here were investigated under moderate photopic conditions (background illumination of 1 ft.-L.). They were maintained under light urethane anaesthesia (5·5 ml./kg body weight of a 20 per cent solution in saline). This dosage was found from earlier work at the retinal ganglion and lateral geniculate levels to be no more detrimental to cell responsiveness than decerebrate and *encéphale isolé* techniques. Recordings were made only when spontaneous activity was prevalent in the midbrain and withdrawal reflexes of the forelimbs were easily obtained.

The animal was supported during the experiment in a stereotoxic frame designed not to impair the visual field. The lids of the tested (right) eye were sutured well back and the pupil dilated with two drops of phenylephrine hydrochloride (10 per cent, viscous). Eye movements were seldom encountered, making paralysis of the extraocular muscles unnecessary.

The eye was then refracted and fitted with a contact lens to protect the cornea from drying and to bring the retina into conjugacy with the testing plane. 57 cm from the eye. For plotting purposes, horizontal and vertical zero were taken from a line projecting from the area centralis (20° below the lower margin of the optic disk[9]) through the centre of the pupil into visual space.

An aperture in the skull 4 mm long and 3 mm wide over the left superior colliculus allowed the passage of a stainless steel microelectrode (average resistance, 40 mΩ) to any region of this body. Sealing the hole with a 4 per cent agar gel minimized pulsation so that cells could be maintained for from several minutes up to two hours time. One recording site, usually the deepest, was marked by a deposit of metallic iron on each penetration. These locations were readily identified in frozen sections later made of each brain, appearing as Prussian blue reactions (50–100μ in diameter) to the potassium ferrocyanide included in the formal–saline perfusion solution.

Decline of response to repeated stimulation[10] is a common property of these cells, and localization had to be based on spontaneous discharge as the electrode was lowered rather than on provocative searching techniques. Once localized, the receptive field of the cell was first mapped with a $\frac{3}{4}^{c}$ diameter light spot (6 ft.-L.) found most effective for defining the whole-field boundaries as well as the discrete response zones within. Further testing with diffuse illumination and a variety of specific targets (such as straight edges, lines, contours, etc.) both of light and shadow, moving and still, followed.

In categorizing the sixty receptive fields described here (Table 1), three major classes were evident: (1) those giving the same mode of response throughout their domain; twenty-three of these have been found and they are referred to here as homogeneous fields; (2) those of

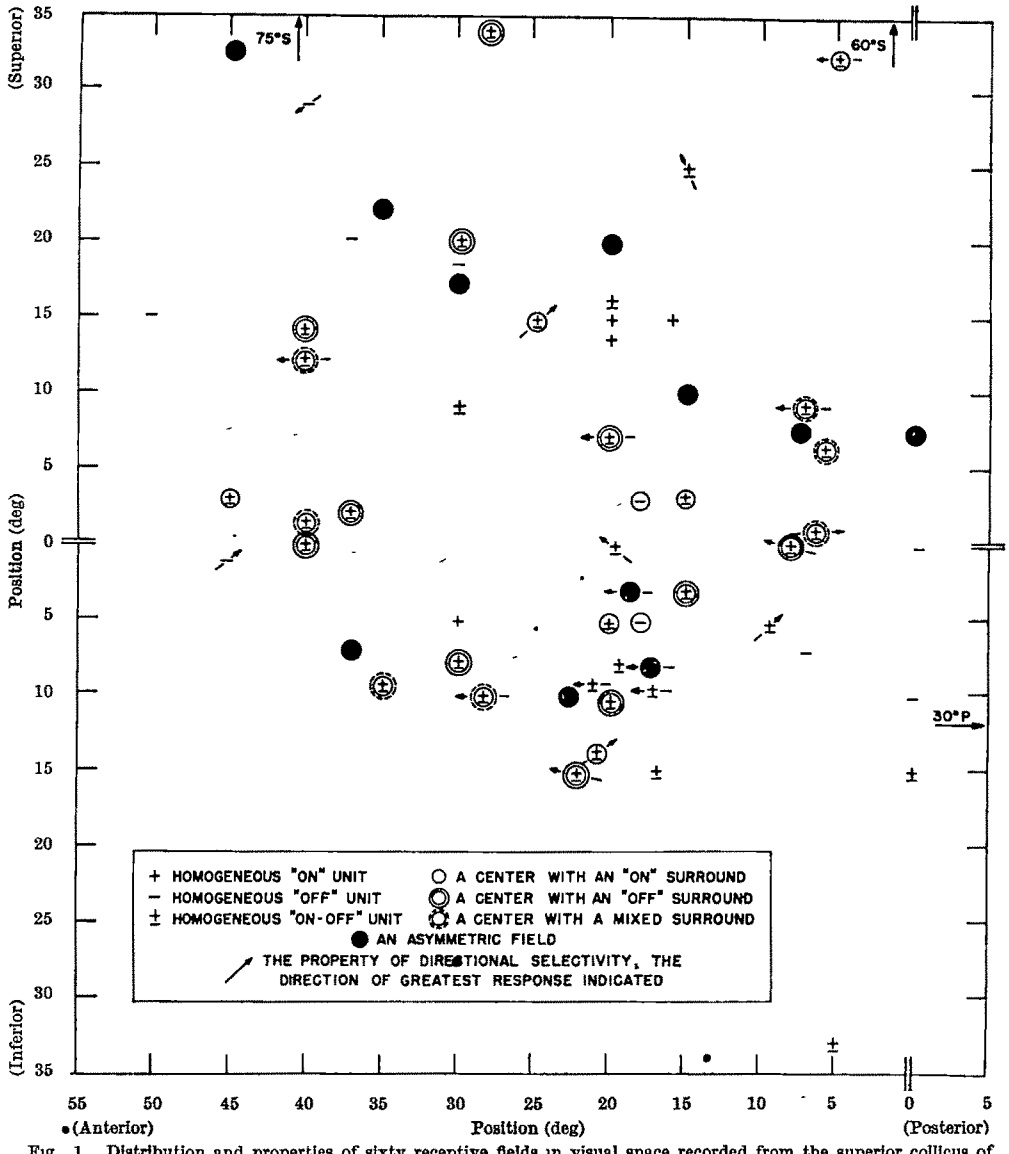


Fig. 1. Distribution and properties of sixty receptive fields in visual space recorded from the superior colliculus of rabbit.

classic concentric geometry with centre and surround producing opposing modes of response; twenty-six of these were found; and finally (3) a group of eleven fields generally asymmetric in outline and always asymmetric in internal geometry, often incorporating several response zones of distinctive character. Fig. 1 shows the distribution of these sixty fields in visual space together with some of their sub-distinctions.

Those fields "homogeneous" in response were of three descriptions: four responded only at "on" of the stimulus; eight responded only at "off"; and eleven responded at both "on" and "off". Although this uniformity was not found to change over a wide photopic range, diffuse light stimulation, generally resulting in a greatly reduced response from the field or its complete elimination, suggested the presence of lateral influence.

Most distinctive of these three forms were the "on" responders, with the smallest average diameter, 8·2° ($\sigma = \pm 3{\cdot}5°$). While reacting briskly to movement, either of light or shadow, none were directionally selective nor were they sensitive to particular ranges of velocity.

In contrast, the "off" and "on–off" responders were more varied and often considerably larger, with average diameters of 27° and 21° respectively. They were, in addition, located throughout a greater range of eccentricity in the visual field, their centres being found up to 50°

Table 1

Field geometry	*N*	Percentage	Diam. $\bar{x}°$	Diam. $\sigma°$	Diam. range°	Eccent. $\bar{x}°$	Eccent. $\sigma°$	Eccent. range	*DS* *N*	*DS* Percentage of class
(1) Homogeneous "on"	4	7	8·2	±3·5	5 to 14	25·5	±3·1	22 to 30	0	0
Homogeneous "off"	8	14	26·8	±21·3	5 to 77	32·8	±17·9	0 to 52	2	25
Homogeneous "on–off"	11	18	21·0	±23·2*	2 to 50	22·7	±7·0	10 to 36	5	45
(2) "Off" centre conc. "on" surround	2	3	14·8		14·2 and 15·5	18·6		18·2 and 18·8	0	0
"On" centre conc. "off" surround	0	0								
"On–off" centre conc. "on" surround	6	10	15·5	±6·3	4 to 23	34·2	±16·9	15 to 60	3	50
"On–off" centre conc. "off" surround	11	18	21·6	±20·5	4 to 83	29·4	±12·4	8 to 50	3	27
"On–off" centre conc. mixed surround	7	12	14·8	±6·9	5 to 24	25·0	±15·9	7 to 42	4	57
(3) Asymmetric	11	18	27·5	±25·2	7 to 103	29·6	±20 9	7 to 87	2	18
Total	60	100							19	

* Badly skewed distribution, many more large fields than small being represented. *DS*, directional selectivity.

from the optic axis. These cells were often difficult to map with a flashing spot, responding sluggishly and seeming to decline readily in response to repeated stimulation. Movements, especially of edges or detailed targets, were found to be more effective stimuli and seven of these cells showed a well-developed directional selectivity. In addition, a few were more responsive to fast targets (5°/sec or more), while one was sensitive only to very slow movement of low amplitude. Scanning for fine (or distant) movement seemed the forte of these cells.

Slightly more common were cells with fields arranged concentrically. Least frequent among those studied was the off-centre, on-surround form (on-centre, off-surround forms were not found). Since only two were mapped, little can be said about their properties except that their surrounds were sharply defined and well developed in each case. Both were extremely sensitive to small movements, but neither was directionally selective. Their overall diameters were 15° in each case.

While the zones of the fields already described were well defined, those of the balance of the concentric fields were not. All had a large central area, responding both at the onset and at the cessation of the stimulus, and were encircled by a marginally developed "off" surround (eleven cases), "on" surround (six cases), or a surround giving either "off" or "on" depending on the portion of the rim being tested (seven cases). Each group contained a large range of diameters, some as small as 4°, others reaching 80°. The eccentricity of their centres varied from 7° to 50°. Their functional characteristics included a sluggishness in responding to repeated static stimuli, a high sensitivity to direction of movement (ten out of twenty-four cells) and occasionally a predilection for limited ranges of velocity.

Apart from the two major classes just described, the homogeneous and concentric forms, one other population stood out. The common characteristic of these eleven fields was their asymmetry, as mentioned earlier. Most had a distinctive "off" responding band dividing their field irregularly, the remainder of it being either "on" or "on–off" in character. Three contained local regions which were directionally selective. While static stimuli were not particularly effective, moving targets of some detail were quite effective. Their frequent large diameters (up to 103°) and range of eccentricities (up to 85°) make them, like the homogeneous fields, well suited for scanning.

Of comparative interest is the geometrical likeness of the first two classes to the "simple" fields of the lateral geniculate nucleus of this animal described by Arden[3]—particularly the poorly developed surrounds common to both sites. Similarly, there are both functional and geometrical resemblances between the "complex" fields of the lateral geniculate and the asymmetric variety found here. There seem to be primary differences in the range of field sizes and the lower frequency of the complex forms in the superior colliculus. Of course, differences in testing conditions and selectivity of electrodes cannot be ruled out as selective factors.

In contrast to the unlayered lateral geniculate body of the rabbit, however, the stratified superior colliculus lends itself to the possible ordering of groups of cells with overlapping fields into functional columns, catering to specific orientations of stimuli and balances of ocular dominance as described in the cat cortex by Hubel and Wiesel. While the cells whose fields have been described here have been localized in successive layers (for example, stratum opticum, stratum griseum superficiale and the stratum griseum intermediate) only the first hints of such ordering are evident so far, but a clearer relationship between specific layers and receptive field geometry is promising.

This extensive interchange of sensory and motor information in the superior colliculus, representing a rich repertoire of properties—among them, movement, velocity and directional sensitivities—makes this centre an appealing one for renewed examination, not only relative to its own analytical structure, but also with regard to its functional influence on eye movements, pupillary control, and other visually dependent behaviour.

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Convergence of Inputs on Deiters Neurones

ON the basis of anatomical studies, Deiters nucleus has been divided into parts projecting to different levels of the spinal cord, and it has been suggested that these parts are not functionally equivalent[1]. We have recently shown that those Deiters cells which project to the lumbosacral cord can be strongly facilitated by electrical stimulation of peripheral nerves; the polysynaptic pathway through which facilitation is produced does not traverse the cerebellum, and can be activated by stimulation of all types of afferents other than Group I fibres from muscle[2]. In the present experiments the effects of peripheral and labyrinthine inputs on Deiters cells that send axons to the lumbosacral cord have been compared with the effects of those inputs on Deiters cells that send axons to more rostral spinal segments. The results of this single unit physiological analysis have in many respects confirmed the interpretation of the organization of Deiters nucleus proposed by Brodal and his collaborators[1].

All experiments have been performed on acutely decerebellated cats, usually anaesthetized with chloralose–urethane, paralysed with 'Flaxedil' and artificially respired. The stimulating and recording equipment, and much of the dissection and preparation of the animal, were as previously described[2]. The vestibulospinal tract was stimulated antidromically with two sets of electrodes, each consisting of a pair of narrow strips of platinum foil (thickness 0·002 in., interelectrode distance 7 mm) attached to a thin piece of plastic. One electrode pair was inserted under the spinal cord at *C3–C4*, the other at *L3–L4*. We have designated as *C* cells those Deiters cells activated antidromically only by cervical cord stimulation. This group of cells includes cells projecting to the cervical, thoracic and upper lumbar cord. Cells also activated antidromically as a result of lumbar stimulation have been designated *L* cells. These units belong to the "hindlimb" part of Deiters nucleus[3], and project to the lumbosacral cord. Glass micropipettes, filled with 2 M sodium chloride saturated with fast green FCF and selected for initial resistances of 3–8 MΩ, were used to record the activity of single units extracellularly, and, when desired, to mark the position of the electrode tip[2,4]. During an experiment, several fast green marks were made with the recording electrode and these marks were afterwards used to estimate the position of units as being in the rostral, middle or caudal part of Deiters nucleus. Cells were found by searching the appropriate region of the medulla while stimulating the spinal cord at a rate of 5 stimuli/sec. Once a cell was encountered and identified attempts were made to modify its activity by stimulation of various forelimb and hindlimb nerves with 40 μsec rectangular pulses, delivered through bright platinum electrodes at a repetition rate of 1 a sec. The effect of impulses in the vestibular nerve on Deiters cells was also

investigated in some experiments. Stimuli to the membranous labyrinth were delivered through a fine platinum ball electrode, inserted as deeply as possible through the ipsilateral fenestra vestibuli, and held in place with dental cement.

The results to be described were obtained from twenty-four cats, in which 127 *L* cells and eighty-eight *C* cells were found in electrode tracks located in all parts of the nucleus. As might be expected from previous anatomical[3] and physiological[2,5] investigations, *L* cells were relatively more numerous in the caudal part of Deiters nucleus. In caudal tracks *L* cells outnumbered *C* cells fifty to twenty-six (*L*/*C* ratio 1·9), while in rostral tracks forty-seven *L* cells and forty-five *C* cells were encountered (*L*/*C* ratio 1·05). The remaining thirty *L* and sixteen *C* cells were approximately in the middle of the nucleus.

We have previously reported that many *L* cells discharge spontaneously in the type of preparation used in our experiments[2]; and many *C* cells that fired spontaneously were found in the present investigation. Systematic tracking in different regions of the nucleus, however, revealed an interesting difference between these regions: spontaneously firing cells were much more prevalent in the caudal part of Deiters nucleus than in the rostral part. Thus of 69 cells that were studied satisfactorily in the caudal half, fifty-one (74 per cent) exhibited ongoing activity. In comparison, only thirty-six of eighty cells in the rostral half, or 45 per cent, discharged spontaneously. Rostral *C* cells showed the least spontaneous activity (15 of 39, 38 per cent) while caudal *L* cells showed the most (39 of 46, 85 per cent). Study of seventeen tracks in which three or more cells were encountered (and in which there were non-spontaneous cells) revealed that the non-spontaneous cells were most likely to be cells in the middle or at the bottom of the track.

In a previous paper[2] it was shown that stimulation of mixed or cutaneous nerves at 1/sec facilitated many *L* cells, with a latency of about 20 msec and a duration of 100–200 msec. The facilitation was usually revealed as an increase (often of 100 per cent or more) of ongoing activity, and was rarely strong enough to cause previously silent cells to discharge. With stimulation of muscle nerves single shocks were not very effective, and triple shocks (at 300/sec) were required to bring about any significant facilitation. In the present experiments similar results were obtained in a number of *C* cells: fifteen cells that were carefully studied were facilitated in the same way as *L* cells described previously and as *L* cells seen in this series of experiments. Facilitation of *C* and *L* cells was produced by stimulation of the same peripheral nerves and was of approximately the same magnitude. Of the ten *C* cells studied that had known locations, however, eight were in the caudal or middle parts of the nucleus, and therefore can be assumed to have been mainly cells projecting to the thoracic and upper lumbar (above *L*3) cord[3]. While these cells received a similar input from the periphery as did *L* cells, less is known about rostral *C* cells; many of these *C* cells presumably project to the cervical cord[3]. Only fifteen spontaneous rostral *C* cells were encountered, and it was possible to test the effect of limb nerve stimulation on eight of these; two showed typical effects and six some facilitation. Similar results were obtained with nineteen spontaneous rostral *L* cells. It is reasonable to suggest that *L* and *C* cells located in the rostral part of Deiters nucleus received the same facilitatory inputs as did cells located more caudally, and that the relative lack of information on facilitation of rostral cells resulted from the comparatively large number that were non-spontaneous and therefore could not reveal the excitability change produced by peripheral stimulation. Apparently the distribution of facilitatory inputs from the periphery to Deiters cells in decerebellated cats is more widespread than would have been predicted from anatomical data such as the distribution of spinovestibular fibres[1].

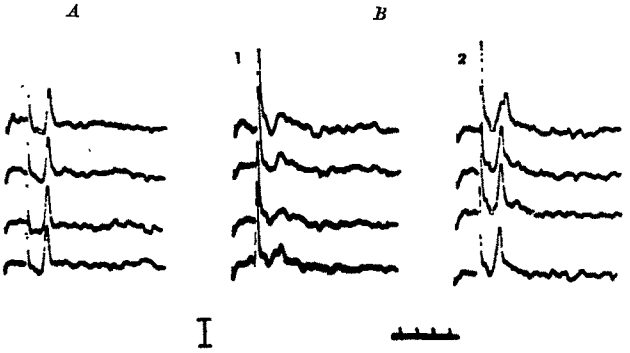


Fig. 1. Effect of stimulation of the labyrinth on a non-spontaneous *C* cell located in the very rostral part of Deiters nucleus. *A*, Antidromic response to stimulation of the cervical cord at 1/sec; *B*, response to stimulation of labyrinth; *B*1, with a stimulus of 1·8 V a small *P* wave is seen superimposed on the end of the stimulus artefact, and an *N*1 wave of about 300 μV starts at 0·9 msec; *B*2, when the stimulus is raised to 2·0 V a spike occurs in every sweep, at 1·0–1·5 msec. Extracellular recording with fast green electrode. Time marks 1 msec, voltage calibration 500 μV.

Stimulation of the labyrinth at 1/sec with an electrode inserted into the fenestra vestibuli produced, in many parts of the nucleus, field potentials similar to those recently described[6]. The delay in the arrival of afferent impulses, as measured from the stimulus artefact to the summit of the *P* wave[6], was usually 0·4 msec. A large negative (*N*1) deflexion, presumably consisting of post-synaptic potentials and spikes, usually had a latency of 0·8–0·9 msec. Of ninety-one cells studied for vestibular effects, thirty-three fired on top of this *N*1 potential at a latency of 0·9–2·0 msec after the stimulus artefact (latency with strong shocks 0·9–1·4 msec), as shown in Fig. 1. The latency of this firing was such that the responses can safely be considered monosynaptic. Almost certainly, other cells also received a monosynaptic input from the labyrinth; in the absence of firing, however, such an input cannot be detected with extracellular recording. Some polysynaptic firing was also observed; this will be described in a subsequent publication.

While stimulation of the labyrinth fired both *C* and *L* cells monosynaptically, such firing was present in a considerably greater fraction of the former. Thus, monosynaptic responses were seen in only twelve of fifty-five *L* cells (22 per cent), but in twenty-one of thirty-six *C* cells (58 per cent). While stimulation of peripheral nerves usually caused detectable effects only in spontaneous cells, stimulation of the labyrinth produced monosynaptic firing mainly in non-spontaneous cells: monosynaptic firing was seen in twenty-six of forty-four non-spontaneous cells, in only seven of forty-two spontaneous cells. The biggest single group of cells monosynaptically activated by the labyrinth consisted of twelve non-spontaneous rostral *C* cells; in contrast, only one spontaneous caudal *L* cell responded monosynaptically. Parenthetically, the monosynaptic response of non-spontaneous cells demonstrates the power of the input from the labyrinth: at 1/sec most cells responded to every shock.

As stated above, non-spontaneous cells were usually found in the middle or at the bottom of electrode tracks. Analysis of twenty-four tracks, including the seventeen already described, showed that monosynaptic activation by the labyrinth was also usually found in the middle or at the bottom of electrode tracks. This result correlates well with the finding that primary vestibular fibres terminate only on cells of the rostroventral region of Deiters nucleus[7].

Our findings are in agreement with many predictions based mainly on anatomical studies[1]. In particular our results provide further evidence for a functional subdivision of Deiters nucleus. The rostral part of the nucleus contains many cells projecting to the rostral levels of the spinal cord, and it is among these cells that monosynaptic

input from the labyrinth is found most often. The rostral and caudal parts of the nucleus are also differentiated by the level of spontaneous activity. Such activity is found especially frequently in the more dorsal, caudal cells, that include many cells projecting to the lumbosacral cord. It is interesting to note that a direct input from the labyrinth, and non-spontaneity, are largely found in the same population of cells. This suggests a need for further studies to test the responsiveness of Deiters cells, identified by antidromic invasion and location, in animals whose position in space can be modified in the course of the experiment.

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Determination of Regional Blood Flow by means of Small Semiconductor Detectors and Red Cells tagged with Phosphorus-32

THE determination of regional blood flow is of great interest both from a clinical and a physiological point of view. Several methods for doing this are available. Most of them, however, have the limitation that they give the blood flow in comparatively large masses of tissue, which may include parenchyma with different functions and different blood flows. The thermocouple[1], the hydrogen gas electrode[2] and the noble gas method (local-injection technique) offer the possibility of making measurements within small and anatomically well-defined regions. These methods must, however, be calibrated and can only be used in certain types of tissues, such as muscles and skin, because they will determine not only the blood flow but also the lymph flow, and in the kidneys the urine flow[2].

To supplement the available methods, we evaluated an indicator-dilution technique to measure local blood flow using small semiconductor detectors primarily sensitive to beta-rays and using phosphorus-32-tagged red cells as the indicator. Needle detectors were put into the parenchyma, and end detectors on the surface of the organ. We have applied the method to the kidneys.

The needle detector itself consists of a tube of stainless steel or aluminium with a wall thickness of about 50μ at the tip of the tube, where a lithium-drifted *p-i-n* silicon crystal is placed. The smallest detector used has an outer diameter of 1·1 mm. The length of the sensitive part of the semiconductor is 2·5 mm and the diameter 0·9 mm. The largest one has an outer diameter of 2·5 mm. The corresponding sensitive part is 4·5 mm long and 1·9 mm in diameter. The detectors were made by *AB* Atomenergi, Sweden, and were operated with a bias voltage of 20–50 V. The pulses were amplified by a charge-sensitive preamplifier and a linear amplifier and were recorded on a scaler with an automatic print-out (2·5 times per sec)[3].

The counting-rate efficiency for the smallest detector was 1,500 c.p.m. and for the largest 6,000 c.p.m., when the detectors were placed in a solution of phosphorus-32 of concentration 1 μc ml.

The end detector used was a common phosphor-diffused semiconductor detector. This detector is enclosed in a stainless steel capsule with a 15μ aluminium window. The analysing system was essentially the same as for the needle detector, except for the print-out, where a decimal pip method was used[4].

Twenty-five experiments were performed on six dogs which had been anaesthetized with chloralose. The left kidney and the renal artery were exposed by an incision in the flank. A lumbar artery leaving the aorta at the same level as the renal artery was dissected free and a catheter was inserted through this into the renal artery. The needle detector was inserted into the medulla and the end detector was placed on the surface of the kidney. After determination of the background activity, 0·2–0·5 ml. of plasma containing a suspension on the phosphorus-32-tagged red cells (0·2–2 mc./ml.) was injected into the catheter. The pulses from the needle and the end detector were recorded simultaneously. The indicator-dilution curves from typical experiments are shown in Figs. 1 and 2.

Five minutes later, when the tagged red cells were equilibrated in the total blood volume of the body, the intrarenal activity was determined by the detectors. Then 5–10 ml. of blood was taken and the activity determined using the same detectors. The blood volume (V) in the region investigated was calculated by dividing the

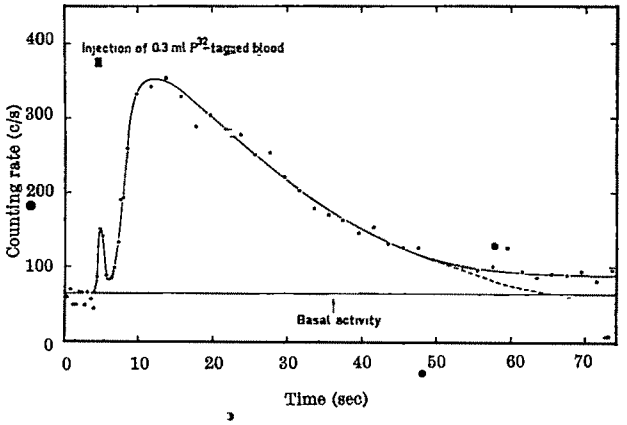


Fig. 1. Recording from a needle detector in the inner zone of the renal medulla. The dots represent the experimental values. The dashed line is extrapolated from the exponential slope of the curve. The basal activity depends on earlier injections of radioactive blood.

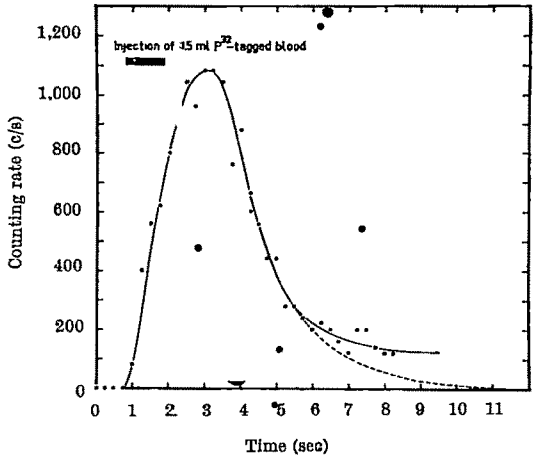


Fig. 2. Recording from an end detector on the renal cortex. The experimental values are represented by the dots and the dashed line is extrapolated from the exponential slope of the curve.

intrarenal activity by the blood activity. To determine blood flow we used the following equations[5]:

$$\bar{t} = \left\{ 1/c(t)_{max} \right\} \int_0^\infty c(t)\, dt \qquad (1)$$

where $\bar{t}$ is the mean transit time, $c(t)$ the recorded activity and $c(t)_{max}$ the peak value of $c(t)$, and

$$\varphi = V/\bar{t}, \qquad (2)$$

where φ is the blood flow and V the calculated blood volume in the region investigated.

Fig. 1 shows a recording from a needle detector placed in the inner zone of the renal medulla. The first peak arises from the activity passing through the interlobar arteries. The counting rate then rises very quickly to a peak value ($c(t)_{max}$) and then decreases exponentially. The dashed line is an extrapolation of the exponential decrease of the activity.

The mean transit time of the actual experiment was calculated to be 24 sec, corresponding to a blood flow of 0·5 ml./min. ml. tissue. Fig. 2 shows a recording from an end detector placed on the renal cortex. The mean transit time and the blood flow were calculated as 3·2 sec and 3·3 ml./min. ml. tissue, respectively.

The semiconductor fulfils excellently the requirements of a detector for local blood-flow measurements. The method is dependent primarily on the fact that the semiconductor records beta-particles. The energy of these particles determines the volume measured which in the case of phosphorus-32 is about 0·2–0·5 cm³, depending on the size of the detector used. Gamma detectors, even greatly collimated ones, do not afford the possibility of such local application. Other advantages of the semiconductor detector are its great efficiency relative to its small size, its extreme stability and long life and also its ability to record high activity[6]. In these latter respects the semiconductor detector is superior to Geiger–Müller tubes.

A drawback of the needle detector is that it damages the surrounding tissues when inserted into the organ; this in turn gives rise to a non-perfused zone around the detector. The estimated blood volume will therefore be too small. This error, however, has no effect on the determination of blood-flow described earlier, because the calculated mean transit time refers to the calculated blood volume and not to the true blood volume in the investigated region.

The results, though few, show a very low rate of flow in the medulla compared with the high flow in the cortex. These results agree with those obtained by Kramer *et al.*[7]. The calculated cortical blood flow may have been underestimated, owing to the comparatively long injection time.

We consider that the method described is valuable for blood-flow determinations not only in the kidneys but also in other organs, such as brain, liver, heart and bone marrow. To evaluate this method, it seems necessary to study the accumulation and washing-out rates after continuous injections of tagged red cells. The method also makes it possible to use krypton-85 for the blood-flow measurements, as this isotope has a suitable beta-energy.

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Central Mechanism of Hyperaesthesia

IT is common experience that inflamed tissues are hypersensitive to pain. Direct experiments have shown that this hypersensitivity may result from the increased sensitivity of the receptors in the injured tissues[1]. On the other hand, MacKenzie[2], as far back as 1893, suggested that hypersensitivity could be the result of increased sensitivity of structures in the central nervous system. According to this investigator, sensory impulses arising in the injured tissues create an "irritable focus" in the segment of the spinal cord at which they enter. As a result a stimulus which would not evoke a sensation of pain in undamaged tissue begins to do so, if the afferent impulses set up by the stimulus reach the "irritable focus". Participation of central mechanisms in the development of hyperaesthesia was then confirmed by a number of observations[3–7]. It has not yet been made clear, however, just where this "irritable focus" is created—that is, at which synaptic relay the facilitation elicited by impulses arising in the injured tissues occurs. A number

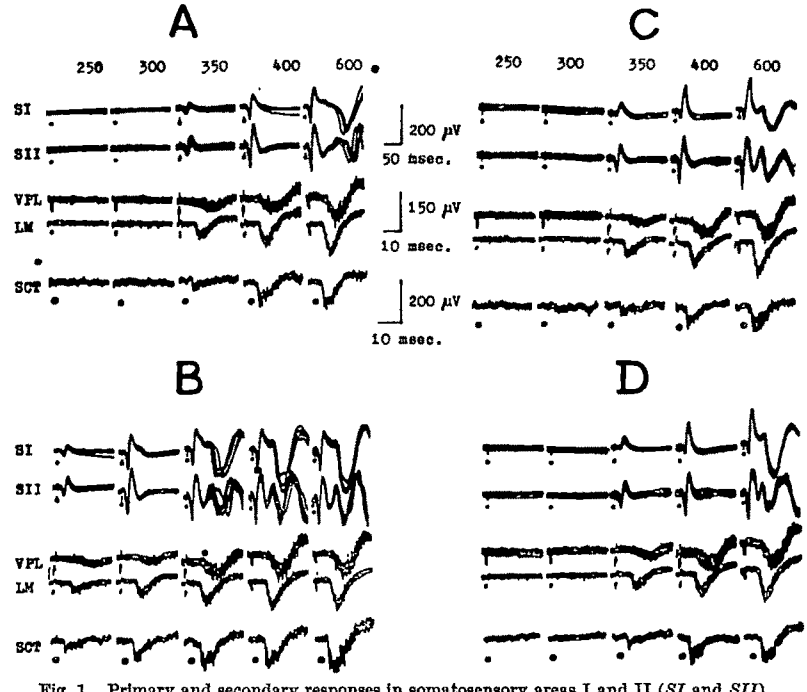


Fig. 1. Primary and secondary responses in somatosensory areas I and II (*SI* and *SII*), evoked potentials in the ventralis postero-lateralis nucleus of thalamus (*VPL*), in the medial lemniscus (*LM*), and in the spinocervical tract (*SCT*) set up by stimulation of the superficial peroneal nerves of right (*A*, *B*) and left (*C*, *D*) hind limbs before (*A*, *C*) and after (*B*, *D*) the development of the inflammation in the right hind paw. A marked facilitation of all responses elicited by stimulation of the nerve of the damaged hind limb is seen (*B*). The numbers indicate the strength of stimulus in mV. Each record has been formed by superposition of five traces. The black points beneath the individual records mark the moment of stimulation. Upward deflexions signal negativity in all records.

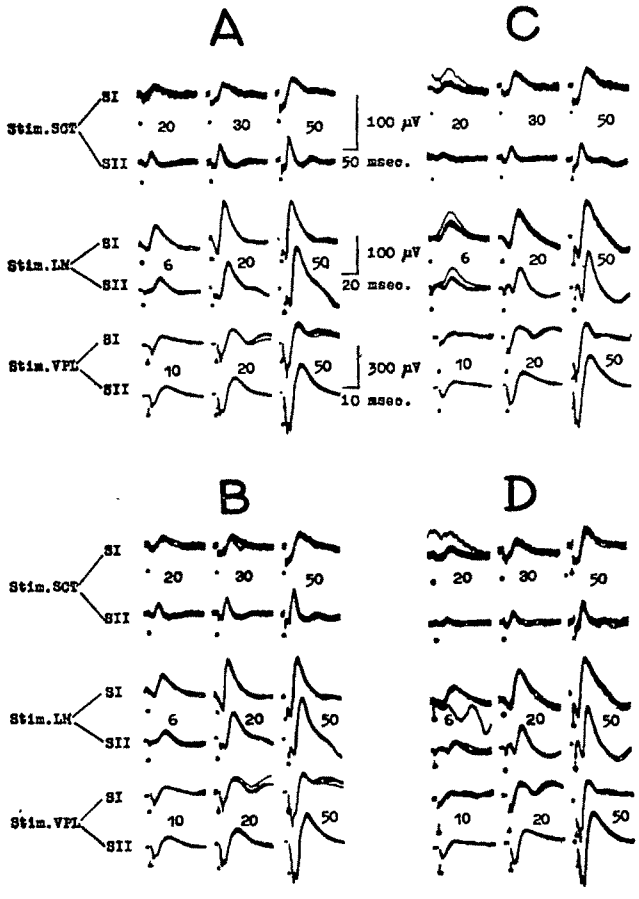


Fig. 2. Primary responses in somatosensory areas I and II (*SI* and *SII*) of the left (*A*, *B*) and right (*C*, *D*) hemispheres elicited by stimulation of the contralateral spinocervical tract (*Stim. SCT*), of the contralateral medial lemniscus (*Stim. LM*), and of the ipsilateral ventralis postero-lateralis nucleus of the thalamus (*Stim. VPL*) before (*A*, *C*) and after (*B*, *D*) the development of the inflammation in the right hind paw. It can be seen that responses did not change after the damage of the right hind paw. Each record has been formed by superposition of five traces. Remaining notation as in Fig. 1.

of suggestions have been made previously[5,8,9], but none is satisfactory.

This investigation was undertaken to elucidate the problem of finding a central mechanism. For this purpose we studied the action of the impulses arising in the inflamed area on the hind limb of experimental animals on the excitability in all synaptic relays of the specific projection pathway along which the information from the injured hind limb reaches the cerebral cortex.

The experiments were performed on eighty-five cats anaesthetized with 'Nembutal'. The inflammation was evoked in one of the hind paws of the animal by a subcutaneous injection of turpentine (0·5–1·0 c.c.). The excitability of the synaptic relays in the specific projection pathway along which the information from the injured hind limb reaches the cerebral cortex was tested by means of the evoked potentials recorded from somatosensory areas I and II, from the ventralis postero-lateralis nucleus of the thalamus, from the medial lemniscus, and from the spinocervical tract, after the first synaptic relay in the dorsal horn of the lumbosacral segments of the spinal cord[10]. Responses were elicited in these structures either by electrical stimulation of the superficial peroneal nerve along which impulses from the inflamed area on the hind limb were propagated, or by stimulation of the spinocervical tract, medial lemniscus or ventralis postero-lateralis nucleus. As a control, similar responses were recorded in the specific projection pathway of the undamaged hind limb.

As a result of our investigations it was first of all established that impulses from the inflamed area on the hind limb facilitated the primary and secondary cortical responses evoked by the stimulation of the nerve from the injured hind limb. As shown in Fig. 1 *A*, the weakest stimulus which would elicit the primary responses in somatosensory areas I and II before the damage of the hind limb was 350 mV. The secondary discharges were evoked by a stronger stimulation—600 mV. Two hours after the injection of turpentine the right paw was very swollen and by this time the threshold for the initiation of cortical responses to stimulation of the damaged hind limb nerve was markedly lowered. The primary and secondary responses in somatosensory areas I and II now began to appear on stimulation of this nerve by current pulses produced by voltages of 250 mV and 350 mV respectively (Fig. 1 *B*). Similar facilitation was then established for the responses elicited by stimulation of the nerve from the damaged hind limb in all the remaining relays of the same specific projection pathway—in the ventralis postero-lateralis nucleus, medial lemniscus and spinocervical tract (Fig. 1 *B*). By contrast, it was found that the threshold for the initiation of the same responses to stimulation of the undamaged hind-limb nerve did not usually change under very deep anaesthesia (Fig. 1 *C*, *D*), neither did the responses elicited by the stimulation of the central structures (ventralis postero-lateralis nucleus, medial lemniscus or spinocervical tract) (Fig. 2).

Thus it seems that the impulses from the inflamed area in the hind paw facilitate the first synaptic relay of the spinocervical tract and do not elicit any detectable changes in the excitability of higher relays of the same specific somatosensory projection pathway. As a result of this, the weak afferent volley from the periphery which normally does not reach the cerebral cortex will begin to do so after the injury to the hind limb. Thus, the facilitation of the first synaptic relay of the spinocervical tract may be considered as one cause of the hypersensitivity of inflamed tissue.

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Nitrogen Balance Experiments: some Theoretical Considerations

Miller and Payne[1] have raised anew the problem of how to explain net protein utilizations (NPU) greater than 100 per cent. They offer a conventional answer, that the metabolic or endogenous loss of nitrogen can be reduced in certain cases and that this results in calculated efficiencies greater than 100 per cent. The examples cited of such high utilizations are of two types

$$\mathrm{NPU} = 100\,(\mathrm{Bal}+M)/I \qquad (1)$$

$$\mathrm{Bal} = aI+bI^2+c \qquad (2)$$

$$\mathrm{NPU} = 100\,\mathrm{dBal}/\mathrm{d}I \qquad (2a)$$

where I, Bal, and M are nitrogen intake, nitrogen balance, and endogenous urinary and faecal nitrogen excretion, respectively, and a, b and c are constants.

The high utilization values referred to by Miller and Payne were taken from Allison *et al.*[2] and Anderson and Nasset[3], who obtained them by means of equation (1); other values were taken from Forbes *et al.*[4] by way of equations (2) and (2*a*). The high results by equation (1) are illogical and require explanation, while the finding that the instantaneous slope of the curvilinear regression line may be greater than unity merely indicates that the utilization of an intake increment may be greater than the increment itself. This is quite possible provided the slope is less than unity below and above the intake range where it was found to exceed this value. There are indications[5–7] that growth, nitrogen balance and nitrogen retention may vary with the intake of protein, amino acids, or vitamins by sigmoid response curves. The alleged demonstration, however, of slope values greater than unity in curves describing the relationship between nitrogen balance and nitrogen intake[1,4] refers to the intake range where no observations were recorded, and for this reason they may, therefore, be artefacts.

It remains to be explained how values greater than 100 per cent can be obtained for net protein utilization by equation (1). It has been assumed that the feeding of some special proteins or amino-acids reduces the endogenous excretion; it may, however, be that the protein-free feeding prescribed for its determination[8] results in nitrogen being excreted in excess of the inevitable nitrogen loss which it is intended to account for in the experimental animal given protein in its diet. It will be noted that when Miller and Payne[1] constructed their theoretical curves they used a higher endogenous excretion (about 250 mg N/day/kg body weight$^{0.73}$) than the mean extrapolated value corresponding to the experimentally obtained curves[4] (about 175 mg N/day/kg body-weight$^{0.75}$). This tallies with my previous objection[9] that Miller and Payne's[10] estimate of the maintenance item is higher than that usually accepted. It also emphasizes that such estimates tend to be imprecise.

Ashworth was one of the first to report biological values greater than 100 per cent[11]. He pointed out that when complete proteins are given at low levels, the endogenous excretion represents almost the total excretion and that "therefore any variations in the endogenous nitrogen excretion reflect as variations in the biological values. Hence the importance for the evaluation of biological values of protein of a broad knowledge of the factors influencing endogenous nitrogen excretion".

Acceptance of net protein utilizations and biological values greater than 100 per cent will invalidate these concepts as quantities estimating the fractions of ingested and absorbed protein utilized by the experimental animal. Reduction of the endogenous excretion would only be suspected in the few cases when the calculated values of net protein utilization were greater than 100 per cent, but it is unlikely that the hypothetical reduction would be restricted to these cases. Thus the meaning of the calculated measures will become obscure. Before these generally accepted terms are deprived of their original meaning, serious consideration should be given to the problem of whether findings at variance with it can be explained so that its significance can be retained.

It seems to be rational first to question whether the methods used for the estimation of endogenous nitrogen excretion are adequate for conclusions to be drawn regarding the possibility of reducing it by dietary means[12].

In the same way as net protein utilization greater than 100 per cent invalidates this concept, protein scores greater than 100 per cent deprive this quantity of a logical meaning. In this case the obvious answer is either that the reference values chosen are too low or that the protein sources may contain amino-acids in excess of requirements[13]. When the relationship between net protein utilization and protein score is considered[1] it should be based on the premise that both qualities should vary between zero and 100 per cent until it can be proved that this is not so.

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Driving the Sodium Pump backwards to form Adenosine Triphosphate

IF the ionic pump in the red cell membrane expels three sodium ions and takes up about two potassium ions for each molecule of adenosine triphosphate (ATP) hydrolysed[1–5], under physiological conditions the free energy available to drive the reaction forwards must be quite small—about 3,000 cal. By arranging that the concentration gradients for sodium and potassium are even more adverse, it should be thermodynamically possible to run the pump backwards and synthesize ATP, though, of course, theory cannot predict that the reaction would occur at a measurable rate. Investigators who have looked for ATP–inorganic phosphate (P_i) exchange associated with "transport ATPase" activity[6,7] have never found it, but they have always looked in broken cell preparations where ionic concentrations must be the same on both sides of the membrane and where, in consequence, the free energy change for the overall reaction is likely to be heavily in favour of ATP breakdown. An ingenious attempt to reverse the sodium pump in perfused squid axons failed, but it failed in such a way as to leave the possibility of reversal still an open question[8]. More encouraging was the finding that red cells with intact membranes show peculiar behaviour when placed in solutions lacking potassium, and the effect of internal phosphate concentration on this behaviour suggests that the final stage of ATP hydrolysis—P_i release—may be reversible[9].

About a year ago, we attempted to reverse the sodium pump by preparing resealed red cell ghosts containing potassium in high concentration, ADP, AMP and $^{32}P_i$, and incubating them in media rich in sodium and containing no potassium. After 15 min, heavy labelling of both ATP and ADP could be demonstrated, but this labelling was not prevented by ouabain and occurred equally well when the outside solution contained potassium instead of sodium. The labelling was therefore presumably the result of residual glycolysis rather than reversal of the pump. Schrier and Doak[10] have shown that triose phosphate dehydrogenase and, to a lesser extent, phosphoglycerate kinase are partly bound to the membrane in red cells, and glycolytic ATP–P_i exchange in red cell membranes has recently been reported by Ronquist and Ågren[11].

Since triose phosphate dehydrogenase is very sensitive to iodoacetate and iodoacetamide, whereas the transport ATPase is rather insensitive[12,13], it seemed worthwhile to repeat the original experiment in the presence of fairly high concentrations of these inhibitors. A further modification was to omit AMP and include ATP. The inclusion

Table 1. INCORPORATION OF $^{32}P_i$ INTO ATP IN FOUR SEPARATE EXPERIMENTS

Composition of ghosts before sealing (mM)	Fraction of total counts incorporated into "energy-rich" phosphate* in 15 min (per cent) "Na medium"†	"Na medium" + ouabain (5×10^{-5} g/ml.)	"K medium"
ATP 1; P_i 5; Na 2; K 140; Mg 7; IAc 2	0·663	0·412	0·391
ATP 1; P_i 5; ADP 2; Na 4·8; K 134; Mg 9; IAc 2	0·697	0·571	0·538
ATP 1; P_i 5; Na 2; K 147; Mg 7; IAc 2; Iodoacetamide 5; NADH 1	0 344	0 261	0 228
ATP 1; P_i 5; Na 0; K 147; Mg 7; IAc 2; Iodoacetamide 5	0·302	0·235	0·206

* The ratio ATP counts/ADP counts was always about 3 : 1, but as ADP is presumably labelled from ATP by adenylate kinase activity it is convenient to consider merely total "energy-rich" phosphate.

† The "Na medium" contained (mM). Na, 140; Mg, 7; *tris* (*p*H 7·4), 5; IAc, 2. The "K medium" was similar but with potassium replacing 95 per cent of the sodium.

Table 2. INCORPORATION OF $^{32}P_i$ INTO ATP AND ADP

	Relative specific activities of nucleotides (Counts per mole nucleotide × 100) "Na medium"*	Counts per mole P_i "Na medium" + oligomycin (10 μg/ml.)	"K medium"
ATP	1 89	1·57	1·37
ADP	0·89	0·83	0·72

* The "Na medium" was the same as in Table 1. The "K medium" was similar to the "Na medium" but with potassium replacing 90 per cent of the sodium. The composition of the ghosts before sealing was (mM): ATP 1; P_i 5; Na 0·2; K 150; Mg 7; IAc 2.

of AMP in the original experiment was intended to hold ATP at a low concentration through residual adenylate kinase activity[2]. It is possible, however, that ATP may be needed to "prime" the system[8], so in most of the later experiments the cells were lysed in solutions containing ATP as the only nucleotide. With 5 mM P_i and 1 mM ATP in the lysing solution, about one-third of the ATP trapped in the cells is split during the pre-incubation necessary for resealing; for the main part of the experiment the ratios P_i : ADP : ATP are therefore something like 15 : 1 : 2.

The results of four experiments are shown in Table 1. In these experiments cells were prepared with the compositions shown in column 1 and, after sealing[14] and washing, were incubated in sodium media with and without ouabain and in potassium media. After 15 min the cells were treated with trichloroacetic acid, unlabelled P_i, ATP and ADP were added to act as carriers, and the nucleotides were separated on 'Dowex' 1 columns. The amount of radioactivity found in each fraction was expressed as a percentage of the total radioactivity originally in the inorganic phosphate.

It is clear that with a high concentration of external sodium and zero external potassium there appears to be an extra incorporation of P_i into ATP, and this extra incorporation is greatly diminished in the presence of ouabain. A separate experiment showed that ouabain did not affect the rate of incorporation of P_i when cells were suspended in a potassium medium.

A weakness of this kind of experiment is that because unlabelled nucleotides are added it is not possible to estimate the specific activities of the nucleotides in the cells. In the presence of external potassium, extra splitting of ATP through activation of the transport ATPase could lead to a lower concentration of ATP and therefore to fewer ATP counts even if the rate of formation of ATP were the same. With the very low concentration of internal sodium and the short period of incubation, the effect is likely to be small, and in any event it would not explain the ouabain result. Nevertheless, the difficulty can be overcome by carrying out the experiment on a scale sufficiently large to avoid the need for carriers, and estimating specific activities by direct comparison of counts and 259 mμ absorption. The results of such an experiment are summarized in Table 2 and show that $^{32}P_i$ incorporation really is greater in the sodium medium.

In this experiment oligomycin was used instead of a cardiac glycoside. At a concentration of 10 μg/ml. (60 μg/mg protein) it partly inhibited the extra P_i incorporation associated with external sodium, and this result is in agreement with the partial inhibition of ATPase activity and of sodium movements caused by similar concentrations of oligomycin[12,15–18].

Reversal of the sodium pump leading to the formation of ATP is interesting because of its possible relevance to oxidative phosphorylation in mitochondria as well as for the light it may throw on the mechanism of the pump. The rate of the reversed reaction observed in these experiments was, very roughly, 40 μmoles/l. sealed ghosts/h, that is, about 46 per cent of the rate of the forward reaction in ghosts containing similar amounts of ATP, ADP and P_i but with a normal distribution of cations, and about 2 per cent of the rate of the forward reaction in fresh red cells under physiological conditions.

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PHARMACOLOGY

Determination of Nicotine and its Metabolite, Cotinine, in Urine by Gas Chromatography

A VARIETY of methods have been described for the identification and determination of nicotine in biological fluids[1–4]; however, they lack sensitivity, specificity and are tedious for routine analysis.

Gas chromatography has been used both qualitatively and quantitatively to detect nicotine and related compounds in tobacco and in cigarette smoke[5]. In investigations of the excretion of amphetamine, nicotine was found to be eliminated in the urine of smokers[6,7] and a gas chromatographic method for the determination of nicotine and its metabolite cotinine in biological fluids was described by McNiven *et al.*[8].

We have been using a simple quantitative gas chromatographic method for the determination of nicotine and cotinine in urine for about a year. In this method, nicotine and cotinine showed single well resolved symmetrical peaks, and no interfering substances near these peaks were found in the hundreds of extracts of urine analysed from many subjects. Absolute recoveries of these compounds and their standards from urine were as follows: nicotine (90–95 per cent), chlorphentermine hydrochloride (90–95 per cent), cotinine (85–90 per cent), and lignocaine hydrochloride (85–90 per cent). A relative recovery of 95–100 per cent for nicotine and cotinine was obtained with respect to their internal standards which are added to the urine at the start of the assay procedure.

The sensitivity of this method makes it possible to use small samples and, in all, the analysis takes about 6 h for sixteen samples.

The procedure for nicotine was to add 5 ml. of urine and 0·1 ml. of 5 N hydrochloric acid to 1 ml. internal marker solution (chlorphentermine hydrochloride, 5 μg base/ml. in distilled water) in a glass stoppered centrifuge tube. The solution was extracted with 3 × 2·5 ml. of freshly distilled 'AnalaR' diethyl ether, centrifuging to break any emulsion. The ethereal extract was discarded and the urine made alkaline with 5 N sodium hydroxide (0·5 ml.). A further 3 × 2·5 ml. diethyl ether extracts were combined in a 'Quickfit' test-tube, finely tapered at the base, and concentrated to about 50 μl. at 42° C; 3–5 μl. of this solution was injected on to the column using a 10 μl. Hamilton syringe.

A similar procedure was used for cotinine except that there was: (*a*) no acid solvent extraction; (*b*) N ammonium hydroxide in place of the 5 N sodium hydroxide; (*c*) freshly distilled methylene dichloride as the extracting solvent; (*d*) lignocaine hydrochloride, 5 μg base/ml. in distilled water, as the internal standard.

A Perkin Elmer *F*11 chromatograph equipped with a flame ionization detector and a 0–5 mV Leeds and Northrup Speedomax *G* recorder, model *S*, were used. The chromatographic column was stainless steel tubing 1/8 in. outer diameter, 1 m in length and packed with 80–100 mesh 'Diatoport *S*' coated with 5 per cent w/w potassium hydroxide and 2 per cent w/w 'Carbowax 20 *M*'. The operating conditions used were: hydrogen pressure, 15 lb./in.²; air pressure, 25 lb./in.²; nitrogen flow rate, 33 ml./min.; stream split ratio 1 : 3; injection block temperature about 250° C; oven temperature 135° C (nicotine), and 209° C (cotinine). The column was silanized *in situ* with 2 × 5 μl. of hexamethyldisilizane after equilibrating for 24 h at the operating conditions.

The concentrations of nicotine and cotinine were obtained by calculating the ratio of peak height of the compound to that of the internal marker and relating this to a previously constructed calibration curve of the compound in urine.

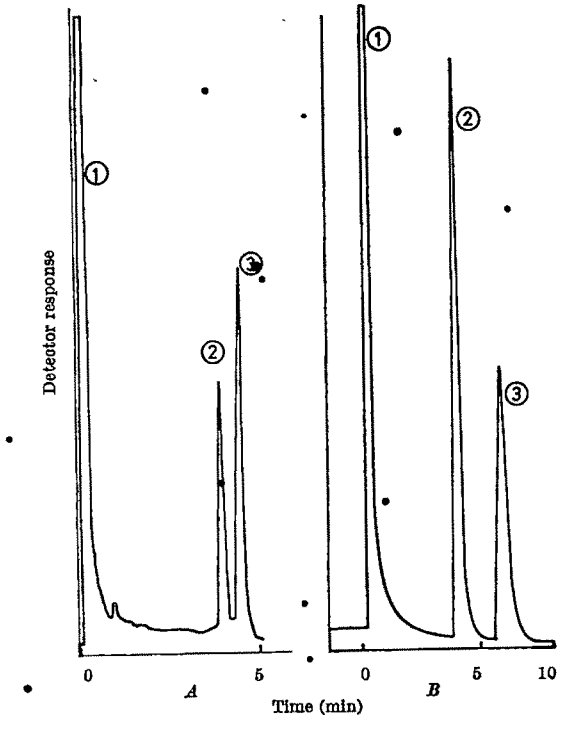


Fig. 1. *A*, Chromatogram of nicotine from urine: (1) ether (solvent); (2) nicotine; (3) chlorphentermine. *B*, Chromatogram of cotinine from urine; (1) methylene dichloride; (2) lignocaine; (3) cotinine.

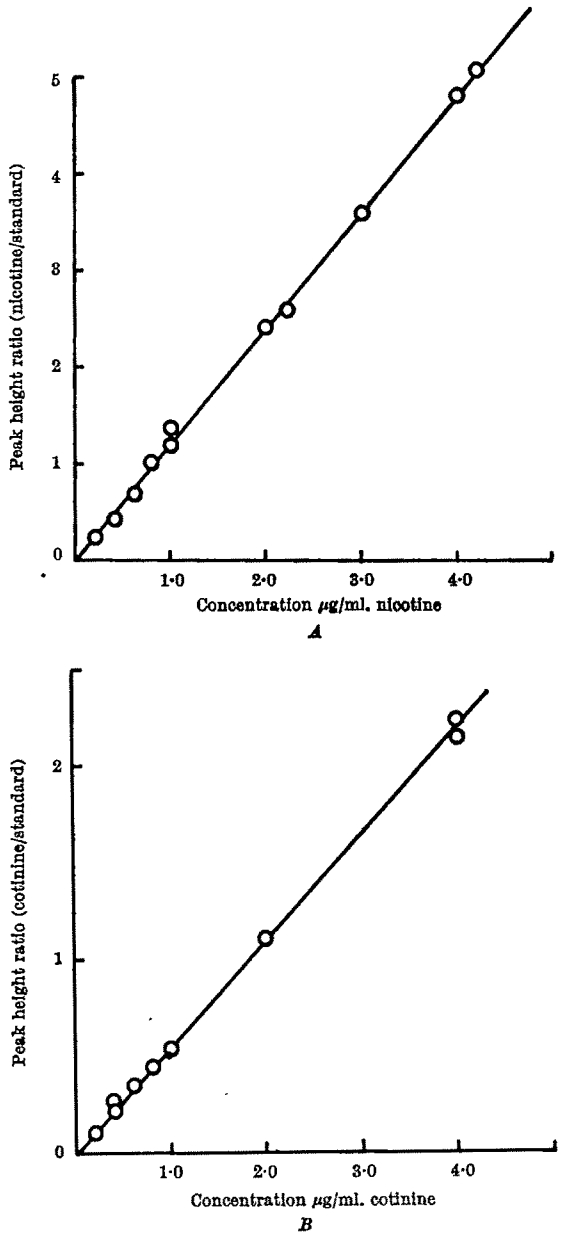


Fig. 2. *A*, Calibration curve for nicotine. *B*, Calibration curve for cotinine.

Figs. 1*A* and 1*B* show chromatograms of nicotine, and cotinine with their internal standards. No deterioration of the compounds in urine occurred when they were stored for 2 weeks at 4° C, and the extracts and concentrates were stable for 24 h at 4° C.

Figs. 2*A* and 2*B* show calibration curves for nicotine and cotinine. A consistent linear detector response was obtained for both compounds over the range 0·2–10·0 μg base/ml. urine. Sixteen analyses of a urine sample containing 1·0 μg of nicotine base/ml. gave a standard deviation of 0·04 μg/ml.; similarly for cotinine (2 μg base/ml. sample), a standard deviation of 0·05 μg/ml. was obtained.

Using the procedure described for the determination of nicotine, it was found that the rate of nicotine excretion in urine was influenced by the *p*H of the urine (see also refs. 7, 9), and by the rate of flow of the urine; the greater the rate of flow, the greater the excretion of nicotine. This was also found to apply to the urinary excretion of cotinine. Subjects who simultaneously smoked two cigarettes after abstaining from smoking for a period of 24 h excreted 0·25–0·60 mg cotinine (in

24 h) and 0·10–0·50 mg nicotine (in 24 h) under normal conditions, 0·30–0·90 mg cotinine (in 22 h) and 0·50–0·80 mg nicotine (in 8 h) when the urine was made acidic after the oral administration of ammonium chloride, and 0·30–0·40 mg cotinine (in 36 h), and less than 0·06 mg nicotine (in 36 h) when the urine was made alkaline after the oral administration of sodium bicarbonate.

Absorption of nicotine from the lungs and excretion in the urine were found to be extremely rapid, as the greatest rate of excretion was in the period 0–15 min after smoking. The rate of nicotine excretion under acidic conditions fell very rapidly to less than 0·1 μg/min 8 h after smoking the two cigarettes. The elimination of nicotine did not appear to follow first order kinetics, and this is being further investigated. The peak level of cotinine excretion was found to be at about 2 h after smoking.

The subjects were heavy smokers (twenty cigarettes a day), and intermittent smokers (between two and five cigarettes a week), and for the purpose of the experiments inhaled the smoke from the non-filter cigarettes used.

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HAEMATOLOGY

Rapid Induction of *in vivo* Fibrinolysis with D-Gluconyl-L-tyrosine

RECENTLY I reported[1] lysis of thrombi in the veins and arteries of animals with sodium gluconate and a dialysable constituent of fibrin extract or whole blood. The ultraviolet absorption spectrum of fibrin extract displayed a maximum at 275 mμ and a minimum at 247 mμ. These values happen to coincide closely with those recorded for tyrosine. This finding suggested that L-tyrosine might be successfully substituted for fibrin extract and, if this was so, that a peptide formed from the amino-acid and gluconic acid would also be fibrinolytic.

Clots were induced in the jugular veins of dogs as previously described[1], and infusions administered locally without the admission of blood. A mixture of 100 mg of L-tyrosine and 1 g of sodium gluconate lysed such thrombi, while L-alanine with gluconate or L-tyrosine with sodium glucuronate were inactive. D-Gluconyl-L-tyrosine was prepared by the method of Iacobellis[2]. One hundred milligrams in 1 ml. of distilled water injected into a leg vein was sufficient to restore within 15 min the flow of blood in occluded jugular veins of dogs weighing 12–13 kg.

The compound did not lyse fibrin clots *in vitro*. It may be visualized as the prosthetic group of a larger molecule, presumably a protein.

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IMMUNOLOGY

Complement-fixing Antibodies in Monkeys bearing Tumours induced by Rous Sarcoma Virus

ROUS sarcoma virus (RSV) produces tumours in rodents, monkeys, sheep and dogs[1–7]. RSV tumours in hamsters, guinea-pigs and rats contain complement-fixing (CF) antigens which elicit antibodies to homologous virus-free RSV-induced hamster tumour antigens, virus-containing RSV-induced chicken tumours, and antigens induced by the growth of several strains of avian leukosis viruses in chicken embryo fibroblast (CEF) tissue cultures[7,8].

Recently, using methods previously described[8–10], we observed the production of antibodies specific for avian leukosis in the serum of a rhesus monkey (kindly supplied by F. J. Rauscher) which developed tumours after inoculation at birth with the Schmidt–Ruppin (*SR*) strain of RSV. This monkey, the offspring of a healthy young mother, was bled for a serum sample when less than 24 h old and then inoculated subcutaneously over both the right and left lower back with 0·5 ml. of *SR*-RSV prepared from chicken wing web tumours[8].

A small subcutaneous tumour was first noted at the site of injection on the right side 21 days after inoculation of the virus. Several subcutaneous tumours afterwards developed and grew progressively at both inoculation sites. Tumours which developed on the left side were excised 7 days after they were first noted. An attempt to establish cells from the excised tumours in tissue culture was unsuccessful. Tumours recurred rapidly at the site of the operation and were readily detectable 7 days after excision. In a second attempt to establish tumour cells in tissue culture, sub-total excisions of the tumours on both sides were performed 85 days after their onset. Cells from this biopsy were successfully grown in tissue culture and were carried through sixteen subpassages (cultures established and carried by C. H. Calisher). Postoperatively, we noticed at the excision sites the formation of firm subcutaneous masses which were indistinguishable from tumour tissue by palpation, but these regressed rapidly and left no gross evidence of tumours 6 weeks later.

Five to six weeks after the onset of tumours, CF antibodies appeared in the monkey serum which reacted with *SR* strain hamster tumour antigens and the antigens found in chicken tumours induced by both *SR* and Bryan strains of RSV but not with the virus-induced hamster tumour antigens of *SV*40 virus, polyoma virus or adenovirus types 7 and 12. The monkey sera with the highest antibody titres against RSV hamster and chicken tumour antigens also reacted specifically with antigens present in CEF cultures of both resistance-inducing factor (RIF) and avian erythroblastosis virus (AEV), but all sera were negative when tested against cell pack preparations of uninoculated CEF cultures used at the same dilutions as the avian leukosis antigens. The progress of specific CF antibody formation is illustrated in Table 1 which shows the reactions of representative serial sera tested against 4–8 units of the specific antigens listed. Antibody titres generally increased during the period of tumour residence but fell to undetectable levels within 3 weeks of subtotal excision and subsequent regression of the tumours. Three of the sera with high CF antibody titres were negative at 1 : 10 dilution when tested for neutralizing antibodies against both *SR* and Bryan strains of RSV. Ten months after excision, tumours did not recur, and serum taken at that time did not react in CF tests with RSV antigens.

Despite the development of CF antibodies, we were unable to demonstrate CF antigens in a 20 per cent extract of tumour tissue excised from this monkey when it was tested at 1 : 5 dilution versus three of the highest titred homologous monkey sera or against standard

Table 1. COMPLEMENT-FIXING ANTIBODIES IN A RHESUS MONKEY CARRYING RSV TUMOURS

Days post-inoculation	Tumour size (mm)† Right	Left	CF titre of serum versus antigen* indicated RSV tumours *SR* Hamster	*SR* Chick	Bryan Chick	CEF inoculated with virus RSV *SR*	RSV Bryan	RIF	AEV
0 (*a*)			0‡	0	0	0	0	0	0
70 (*b*)	19	15 (*c*)	0	0	0	0	0	0	0
84	19	19	0	0	10	0	0	0	0
94	17	16	20§	10	10	10	0	0	0
198	*NM*	*NM*	20	10	10	20	20	20	10
104	19	17	40	10	20	20	20	20	20
111 (*d*)	(*e*)		40	20	20	20	10	20	10
118	(*f*)		40	10	10	20	20	20	20
125	0		20	10	10	0	0	0	0
129	0	(*g*)	0	0	0	0	0	0	0
433	0	0	0	0	0	0	0	0	0

* Antigens used at 4–8 units based on titration against RSV-tumoured hamster sera.

† Longest diameter of tumours.

‡ No reaction at 1 : 10.

§ Reciprocal of titre.

NM, not measured.

RIF, resistance-inducing factor.

AEV, avian erythroblastosis virus.

a, Pre-inoculation serum.

b, Tumours first noted on 21st day post-inoculation.

c, Tumours recurred following excision from left side on 58th day post-inoculation.

d, Tumours subtotally excised from both sides on 106th day post-inoculation.

e, Rapid formation of firm subcutaneous masses at excision sites after operation.

f, Regression of mass on right side.

g, Regression of mass on left side.

sera of high titre from hamsters bearing transplanted *SR* strain tumours[8]. Cells grown in tissue culture from the second tumour biopsy of this monkey were prepared into cell pack antigens[11]. Such preparations remained negative for RSV CF antigens after sixteen subpassages in tissue culture.

In another experiment, a 20 per cent extract of a tumour induced by *SR*-RSV from a second rhesus monkey (kindly provided by Dr. Sarah Stewart of the National Cancer Institute) was found to react at 1 : 2 dilution with a single serum from the host monkey and with a positive serum from the monkey reported above. This extract of monkey tumour, however, failed to react with 4–8 units of standard antiserum pools from hamsters bearing tumours induced by RSV. On the other hand, the reactive serum from the second monkey contained CF antibodies to titres of 1 : 40 with RSV hamster tumour antigen and 1 : 20 with RSV chicken tumour antigen. This serum and a CF-negative serum drawn 2 weeks later during a period of spontaneous rapid tumour regression did not contain neutralizing antibodies against either *SR* or Bryan strains of RSV.

Cells from the second monkey's tumour were established in tissue culture but could not be propagated beyond the first subpassage. Concentrated cell pack antigens[11] prepared from these cultured cells did not fix complement with sera from monkeys or hamsters bearing RSV-induced tumours. Cells from the primary culture and first subculture were disrupted with ultrasonic vibrations at various intervals after they were planted in tissue culture. Clarified tissue culture fluids from the sonicated tumour cells were negative for infectious virus when tested in CEF roller tubes for the formation of RSV foci[12] and in COFAL tests[13] for the induction of specific antigens.

In summary, the data indicated that tumours induced by RSV in rhesus monkeys elicited CF antibodies to both hamster and chicken RSV tumour antigens and to the antigens of several avian leukosis viruses grown in CEF; although no CF antigens could be detected in these tumours with standard sera from hamsters carrying RSV-induced tumours. While it is possible that the avian sarcoma CF antibodies, demonstrated in the sera of tumorous monkeys, were elicited by replication of infectious RSV in the host, the absence of neutralizing antibodies in these sera and the inability to recover infectious virus from tumour tissue seem to make this event unlikely. The antibodies appeared quite late after virus inoculation, reached highest titres at the peak of tumour growth and fell to undetectable levels with regression of the tumours. Similar dependence of serum antibodies on the presence of intact progressively growing tumours was noted by Huebner *et al.*[8] in hamsters and guinea-pigs which developed tumours after inoculation with RSV.

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Origin and Specificity of Mononuclear Cells in Delayed Hypersensitivity Reactions

UNDER the microscope, infiltrations of mononuclear cells are characteristic of delayed hypersensitivity reactions[1–3]. Several investigations have indicated that the reactivity is bound to these cells[4–7], and the majority of the infiltrating cells have been shown to be haematogenous[8].

Two groups of the same inbred Sprague–Dawley strain rats were sensitized simultaneously; the donor group was injected with heat killed tubercle bacilli in mineral oil and the recipients with bovine serum albumin (BSA) in complete Freund's adjuvant[9] (all injections were made into the left hind foot pad). Three times a day on days 10–12 after the sensitization, 1 μc./g of tritiated thymidine was injected intraperitoneally into the donors. On the fourteenth day the donors were killed and separate cell suspensions were made from the draining popliteal lymph nodes, axillary lymph nodes, spleen and thymus. Various recipients, which had been skin tested 4 h earlier with tuberculin, BSA and turpentine, were injected intravenously with 44×10^6 cells of each suspension. The recipients were biopsied 20 h after the transfer of the cells. The number of labelled cells was determined from autoradiographs exposed for 6 weeks. The results are given in Table 1, where the data are calculated to correspond to 4×10^6 labelled donor cells, which was the actual number of injected labelled cells from the draining lymph node. In this suspension, the proportion of labelled cells was 9 per cent, in the axillary lymph node 5 per cent, in the spleen 14 per cent and in the thymus 36 per cent.

Cells taken from the draining lymph node were found in the delayed skin reactions in larger numbers than cells taken from the non-draining lymph nodes and the spleen. Only an insignificant number of thymus cells were found in the skin sites. Cells from the draining node also seemed

to have some specificity, because the donors were only sensitized to tuberculin and in this reaction there were more labelled cells than in the BSA reaction. Scarcely any labelled cells could be found in the non-specific and non-immunologic turpentine reaction. The spleen of all the recipients contained labelled cells in the same order of magnitude at the time of biopsy. Only occasionally could single cells be found in the recipient thymuses among the 10,000 cells counted.

Table 1. NUMBER OF LABELLED CELLS FOUND IN RECIPIENTS (Per 100 63 power fields)

Donor cells taken from	Tuberculin (OT 1 : 25)	Bovine serum albumin (30 μg)	Turpentine (1 : 10)	Spleen*
Draining lymph node	28	13	1	4
Non-draining lymph node	9	7	0	3
Spleen	4	3	2	2
Thymus	0	0·5	0	5

* Per 10,000 cells.

In an analogous experiment in which the cells were labelled *in vitro* with tritiated leucine (100 μc./ml.), the findings confirmed the previous results except in specificity. Equal numbers of labelled cells were found in both tuberculin and BSA reactions, and again only very few cells in the turpentine reaction. Among the injected cells as well as in the recipients, the number of labelled cells was proportionally higher (90–95 per cent of the injected cells were labelled) than among the cells labelled with thymidine[10].

Thus the thymus cells from a sensitized animal do not have the same capacity to remain in delayed skin reactions as do cells of the peripheral lymphoid organs and spleen. The draining node contains more "informed" cells than the other lymphoid organs studied, and by using thymidine a cell population could be labelled that showed some degree of specificity, whereas with leucine no immunological specificity was observed. This would indicate that synthesis of deoxyribonucleic acid and cell division are involved in the formation of cells reacting specifically in the delayed hypersensitivity reactions. This work has been supported by the U.S. Public Health Service and by the Sigrid Jusélius Foundation, Helsinki.

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Adjuvant Principle of Pertussis Vaccine in the Mouse

IT is well known that the vaccines of many Gram-negative bacteria greatly enhance the antibody response to unrelated antigens administered to animals at about the same time, or as combined immunizing agents. The most familiar example is the increased potency of diphtheria and tetanus toxoids when combined with pertussis vaccine. Many investigators have attributed this heightened response to the heat-stable lipopolysaccharide endotoxins of this class of bacteria. Farthing[1] tested the effect of heating pertussis vaccine for 1 h at 100° C on its ability to enhance antibody response to diphtheria toxoid in guinea-pigs. He found no effect which he could ascribe to heat, and went on to show that extracted endotoxin was responsible for the increase in antibody titre.

Although *Bordetella pertussis* shares with other bacteria this ability to enhance antibody formation to unrelated antigens, it appears unique in respect to the variety and intensity of its biological effects. Pertussis vaccine and fractions thereof have been found capable of sensitizing certain strains of mice and rats to a wide spectrum of physical and chemical stresses and stressor agents, including histamine, endotoxin, anaphylactic shock and experimental allergic encephalomyelitis[2]. Several of these effects have been shown to be markedly depressed by heat[3]. This led us to question whether the antigenic adjuvant property of this vaccine for the mouse might not also be heat labile.

Groups of 36 *CFW* female mice were injected subcutaneously with four graded (two-fold) injections of plain tetanus toxoid, from 0·8 to 6·4 flocculatory units (Lf). Each dosage group was divided into three subgroups of twelve animals; one of these received at the same time an intraperitoneal injection of 1 ml. of pertussis vaccine (19×10^9 cells), a second received a similar injection except that the vaccine had been heated 100° C for 40 min, while the third was not given the associated injection. A group of twelve additional mice served as untreated controls. Fourteen days after these injections all mice were challenged by intramuscular injection of fifteen times the minimum lethal dose of tetanus toxin. The time taken for the animals to die and the associated symptoms were scored according to the method devised by Ipsen[4], as follows

Score	Day of death
0	1, 2
2	3, 4
3	5, 6, 7
4	>7 with tetanus
6	>7 no tetanus

All the untreated controls died in the first 2 days. Fig. 1 shows the mean response for each group of twelve mice and the least squares regression lines drawn to the average slope. At all dose levels, toxoid associated with the unheated vaccine injection resulted in greater protection than toxoid alone. Conversely, toxoid associated with heated vaccine was actually less protective than the injection of toxoid only. These results confirmed an earlier experiment in which there had been a similar rank order correlation among the three treatments at all dose levels of toxoid used.

The unexpected depression of protection resulting from the heated vaccine may possibly be explained as antigenic competition, for the "foreign" solids in the vaccine exceeded those of the toxoid in weight by a factor of more than 500. (The high dose of toxoid contained 0·022 mg dry weight whereas the vaccine solids weighed 12 mg.) If this were the correct explanation, it would emphasize the extraordinary potency of this heat-labile adjuvant which first had to overcome the competition for immunocompetent cells and then effect a further marked increase in apparent toxoid antigenicity.

Neither the identity of this thermo-labile adjuvant nor its mechanism of action can be determined from this experiment. A growing body of evidence leads us to suspect, however, that the protective antigen is the immunological adjuvant for mice in pertussis vaccine. The destructive effect of heat on this antigen is well known. Furthermore, we recently presented evidence that the protective and histamine sensitizing properties of pertussis vaccine can be dual manifestations of a single bacterial component[5]. It has been found[6] that the histamine-sensitizing factor (HSF), purified by various methods, is capable of enhancing the sensitivity of mice to active anaphylaxis; presumably this is mediated by way of

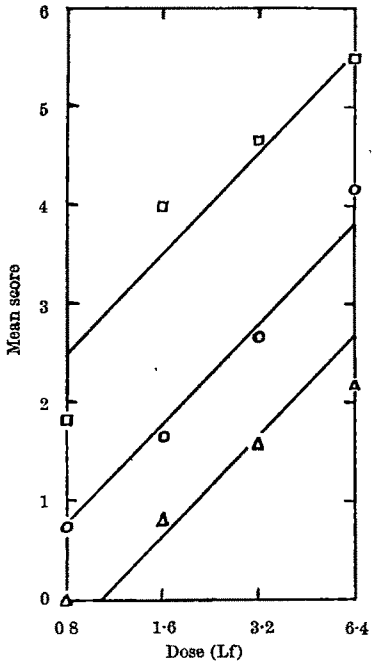


Fig. 1. Effect of the intraperitoneal injections of heated and unheated pertussis vaccine in mice on the protective potency of tetanus toxoid injected subcutaneously at the same time. Upper line: pertussis vaccine, intraperitoneally, toxoid subcutaneously. Middle line: toxoid only, subcutaneously. Lower line: heated vaccine intraperitoneally, toxoid, subcutaneously. Each point is the mean response of twelve mice challenged after 2 weeks with fifteen times the minimum lethal dose of tetanus toxin.

stimulation of the mechanism responsible for antibody formation[7]. Thus the presence of an adjuvant in the mouse may be linked with the specific protective antigen of pertussis vaccine.

In addition to altering the reactivity of certain experimental animals to a wide variety of stresses, administration of pertussis vaccine, as well as overt whooping cough infection, result in a marked and characteristic lymphocytosis. Although the mechanism by which *B. pertussis* exerts this leucocytotic response has not been delineated, stimulation of the production of immunocompetent cells could possibly explain the adjuvant effect associated with *B. pertussis*. Morse has recently demonstrated that the ability of pertussis vaccine to promote leucocytosis closely resembles its ability to make organisms sensitive to histamine. Both these phenomena are labile to heat, and take the same time to achieve maximum response (about 4 days). Moreover, intravenous administration of *B. pertussis* markedly augments both sensitivity to histamine and the degree of leucocytosis, as compared with other routes of inoculation[8].

Recently, Fishel presented evidence that hypersensitivity to histamine induced by pertussis vaccine is mediated primarily through a functional neurological imbalance—specifically, blockade of the beta-receptors of the adrenergic division of the autonomic nervous system[9]. His results have been confirmed by others[6]. Although certain of the characteristic effects of endotoxins are also believed to be induced by way of autonomic nervous pathways, Rosenblatt and Johnson[10] presented evidence that the adrenergic system is not involved in the adjuvant effect of endotoxins in rabbits. It is quite possible, however, that such neurological mechanisms are basically responsible for the heightened antibody response in mice vaccinated with pertussis vaccine. Guinea-pigs and rabbits are reported to be incapable of exhibiting enhanced sensitivity to histamine as a consequence of pertussis vaccination[11], whereas injection of either HSF or beta-adrenergic blocking agents greatly increases the sensitivity of mice to histamine. This species difference in reactivity to histamine following pertussis vaccination could be related to our finding that a heat labile *B. pertussis* component, quite possibly the immunogen, is the effective adjuvant in mice, whereas the heat stable endotoxin performs this function in guinea-pigs[1] and rabbits[12]. Purified protective and endotoxin fractions of *B. pertussis*, as well as adrenergic blocking agents, are at present being evaluated in these three species in order to ascertain their effects on haemodynamic responses and leucocytic mobilization in relation to enhanced antibody production.

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Modification of Poor Growth of Parental Marrow Cells in F_1 Hybrid Mice

ACCORDING to the present genetic theory, parental (P) strain cells transplanted to an F_1 hybrid introduce no foreign antigen to the host, incite no homograft response, and survive indefinitely. This has been shown experimentally with $P \rightarrow F_1$ skin grafts. Some P-strain tumour cells, however, have been shown to grow poorly in F_1 hybrids[1]; and transplants of antibody-forming and haemopoietic cells from certain P strains fail to grow in F_1 hybrids at a rate comparable to that seen in isologous controls[2,3]. One of the puzzling features of this phenomenon is the fact that morphologically intact $P \rightarrow F_1$ skin grafts survive even in strain combinations where grafts of dissociated cells fail.

Recently, morphologically intact grafts of lymphoid tissue have also been shown to survive in $P \rightarrow F_1$ strain combinations. After early changes indicative of a graft-versus-host response, the histological appearance of such lymphoid slice grafts reverts to that of the normal host lymphoid tissues. Indefinite survival of at least some donor elements is shown by successful secondary transplantation of these grafts back to P-strain mice after more than two years of residence in an F_1 host[4].

The present experiments were undertaken to determine whether the presence of such morphologically intact P-strain lymphoid grafts in an F_1 host may potentiate the survival of a suspension of P-strain bone marrow cells. It seemed that stroma of the parental type might prove a more suitable "home" for transplanted parental cells than the F_1 stroma. It also seemed possible that the

Table 1. ERYTHROPOIESIS IN IRRADIATED $B6D2F_1$ MICE WITH SPLEEN-SLICE GRAFTS GIVEN 10^6 *P*-STRAIN BONE MARROW CELLS

Exp.	Recipient strain	Group	Donor of spleen slice graft‡	No. of mice	Iron-59 uptake* by Erythrocytes	Spleens
I	$B6D2F_1$	1	*B6*	10	17·19 ± 2·84	10 03 ± 1·24
		2	$B6D2F_1$	10	0·57 ± 0·08	0·36 ± 0 03
		3†	None	10	2·13 ± 0·36	1·21 ± 0·16
II	$B6D2F_1$	1	*B6*	7	22·82 ± 2·73	10·34 ± 1 20
		2	$B6D2F_1$	7	1·18 ± 0·18	0·39 ± 0 05
	B6	3	None	10	20·90 ± 2 80	12·33 ± 1·13

* Means ± standard errors. 0·5 μc. iron-59 as citrate given intravenously 7 days after treatment with bone marrow. 24 h uptake by washed red cells (corrected to total blood volume) and spleens expressed as percentages of injected dose of iron-59.

† These mice came from a different stock pool from those of groups 1 and 2: mice of group 3 were only 8 weeks old, more than 2·5 weeks younger than others represented.

‡ Grafts were in place 19 days before irradiation in Exp. I, 21 days in Exp. II.

Injection of *B6* spleen cell suspensions before irradiation was also able to reproduce the augmented growth of *B6* marrow in $B6D2F_1$ hosts (Table 2, group 1). Furthermore, a spleen slice graft in place for 12 days, but removed before irradiation of the host (Table 2, group 5), was as effective as an intact spleen slice graft (Table 2, group 3) or an injection of dissociated cells (Table 2, group 1).

Injections of parental lymph node cells before irradiation were just as effective as spleen cell injections in potential of parental marrow growth, whereas injections of parental thymus or bone marrow resulted in little or no increase in the growth of subsequent marrow transplants (Table 2, groups 6–9). This suggests that the effect depends on the immunological capability of the cells injected. Cells derived from lymph node and spleen

Table 2. ERYTHROPOIESIS IN IRRADIATED, PRETREATED $B6D2F_1$ MICE GIVEN 10^6 *P*-STRAIN BONE MARROW CELLS

Recipient strain	Group	Preirradiation treatment	Days between treatment and X-rays	No. of mice	Iron-59 uptake* by Erythrocytes	Spleens
$B6D2F_1$	1	2×10^7 *B6* spleen cells intraperitoneally	14	10	13 24 ± 1·66	11 84 ± 1·03
	2	2×10^7 $B6D2F_1$ spleen cells intraperitoneally	14	10	0 65 ± 0·26	0 38 ± 0·06
	3	*B6* spleen slice graft	11	10	9·68 ± 1·35	7·90 ± 1·00
	4	$B6D2F_1$ spleen slice graft	12	10	1·41 ± 0·14	0 60 ± 0·08
	5	*B6* spleen slice graft, removed after 12 days	1	10	12 00 ± 2·57	11·58 ± 1·56
	6	*B6* spleen 3×10^7† cells intraperitoneally	21	25	13·40 ± 1·95	7·63 ± 0·81
	7	*B6* thymus 3×10^7† cells intraperitoneally	21	25	1·21 ± 0·11	0·67 ± 0·08
	8	*B6* bone marrow 3×10^7† cells intraperitoneally	21	22	0·47 ± 0 06	0·30 ± 0·02
	9	*B6* lymph node 3×10^7† cells intraperitoneally	21	24	15 38 ± 1·37	9·83 ± 0·66

* Means ± standard errors. 0·5 μc. iron-59 as citrate given intravenously 7 days after treatment with bone marrow. 24 h uptake by washed red cells (corrected to total blood volume) and spleens expressed as a percentage of the injected dose of iron-59.

† Doses used in groups 6–9 were calculated on basis of cells excluding eosin; those in groups 1 and 2 represent total numbers.

parental tissue graft might provide necessary elements found in intact tissues, but not in cell suspensions.

Spleen slices from *P*-strain mice (*C57 BL/6*, hereafter called *B6*) were therefore grafted on to the kidney surface of F_1 hybrids (*C57BL/6* female × *DBA/2* male, hereafter called $B6D2F_1$) according to the technique described by Wheeler *et al.*[5]. There is good evidence of poor growth after $P \rightarrow F_1$ bone marrow transplantation in this combination of strains. The host animals were 10–14 weeks old and were subjected to 900 r total body irradiation about 3 weeks after spleen slice grafting. In all cases, mice received 10^6 *B6* marrow cells intravenously 18–22 h after irradiation. Proliferation of donor erythropoietic cells was assessed a week later by measuring the uptake of iron-59 into red cells and spleens of these chimeras (details of method given by Goodman and Mathews[6]). Spleen weights were recorded in some experiments, and gross and microscopic observations were made of the spleen slice graft and the host spleen. The results were compared with groups of non-operated animals, sham operations, and grafts of F_1 spleen. Other experimental groups were injected with *P* or F_1 spleen cell suspensions before irradiation. In a final group, *B6* spleen grafts which had resided 12 days in a $B6D2F_1$ host were excised the day before irradiation of the host.

Table 1 presents the results of two experiments which show clearly that the growth of *B6* marrow cells in irradiated $B6D2F_1$ hosts was dramatically improved by the presence of a previous *B6* spleen slice graft on the kidney of the host. In addition to showing marked potentiation of the uptake of iron-59, those mice carrying *B6* slice grafts had large nodular spleens weighing 97 mg (average of seven mice), whereas control animals with $B6D2F_1$ grafts had small dark spleens without nodules and with an average weight of 21 mg (average of seven mice). Thus, the experimental data strikingly confirmed the original concept that the presence of a *P*-strain spleen graft in an F_1 hybrid may facilitate the subsequent growth of *P*-strain marrow cells injected after total body irradiation.

One explanation which comes quickly to mind is that the transplanted *P*-strain marrow cells "home" to the *P*-strain spleen slice graft and proliferate there preferentially. Further experiments leave little doubt, however, that the continued presence of the *P*-strain tissue stroma is not essential to modify the phenomenon of poor growth. are known to be capable of initiating a much more marked graft-versus-host response than those derived from thymus or bone marrow. Presumably by the time of irradiation, cells grafted from spleen or lymph node have gone through many cell divisions and have widely colonized the host. These cells may also have produced antibodies directed against the foreign F_1 antigens.

Moller has reported that antigenically different cells were mutually cytotoxic when brought into close contact *in vitro*[7]. Extracts of foreign antigens have also been found to inhibit the growth of tumour cells *in vitro*[8]. The inhibition of *P*-strain marrow cell growth seen in the present experiments may likewise be due to contact with foreign F_1 antigens. If so, the augmentation of marrow growth by pre-treatment with lymphoid grafts could be explained by masking these inhibiting foreign antigens or by the presence of syngeneic sites where transplanted *P*-strain stem cells can grow free of contact with foreign antigens.

In summary, these experiments indicate that the growth of *P*-strain marrow grafts in irradiated F_1 hosts can be dramatically augmented by various techniques which have in common the introduction of immunologically competent *P*-strain lymphoid cells into the F_1 host before irradiation.

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PATHOLOGY

Skin Tumours and Sarcomata induced in Rats by *N*-Hydroxy-2-acetylaminofluorene

IT has been recently found that when 2-acetylaminofluorene (AAF) and several other carcinogenic amines are administered to rats, *N*-hydroxylation of the compound occurs *in vivo*[1,2]. Using AAF, or several other amines, it has been shown that the *N*-hydroxy metabolite (N—OH—AAF) was a more active carcinogen than the parent compound[3].

It was therefore proposed that N—OH—AAF may be the proximate carcinogen when AAF is administered to rats[4]. In the course of some preliminary experiments we have obtained additional evidence for this proposition.

Ten 8-week-old male MRC rats (210 g), were injected once subcutaneously on the back with 5 mg of N—OH—AAF dissolved in 0·5 ml. of warm olive oil. A week later a shaved area of interscapular skin was painted with a 2 per cent solution of N—OH—AAF in acetone, using a fine hair brush. The rats were painted at first three times, and later five times a week until the fortieth week, when they had been treated 125 times. The animals were given powdered laboratory chow and as much water as they wanted to drink. Once a week they were weighed and carefully inspected. At the twentieth week signs of skin irritation and exudation appeared, and by the thirty-third week ulcers were present on most of the animals. At the thirty-fifth week a crop of skin tumours suddenly arose, and their progress was recorded weekly on graph paper maps. One rat died of pneumonia at 25 weeks without any other lesions, and the remaining nine rats were killed when their tumours caused a decline in health.

A total of ten subcutaneous tumours occurred near the site of the initial injection in seven of the nine surviving rats. Eight of these were invasive fibrosarcomata, and two were well differentiated, relatively benign, fibrous tumours. Most of the subcutaneous tumours were a little distant from the original site of injection, no doubt as a result of migration of the oily solution. The latent period for the subcutaneous tumours varied from 20 to 44 weeks, with a mean latency of 29·6 weeks.

The earliest skin tumour in the painted area was found in the twenty-first week, and by the thirty-seventh week all the surviving rats had tumours in the painted areas. Six out of nine rats survived longer than 35 weeks, and had a total of sixty skin tumours. The number of skin tumours on each rat increased steadily from the twenty-fifth to the fifty-second week, when the final animal was killed. Only four of the original sixty tumours regressed. Of the fifty-six tumours which persisted, twenty (36 per cent) showed a steady increase in size, and were found on histological examination to be invasive carcinomata. The majority of the carcinomata were of the squamous type, but a few were basal-cell carcinomata. The morphology of the benign tumours was similar to that described for mouse skin tumours[5]. Six rats had eight primary tumours which arose at a distance from the painted areas, namely in the Zymbal glands, breast, and lungs. One of the Zymbal tumours and one of the breast tumours metastasized widely.

The induction of malignant skin tumours in rats by painting with N—OH—AAF was striking in view of the usual refractoriness of rat skin to chemical carcinogens. In rats painted with the parent compounds 2-AAF and 2-AF, skin tumours are very rare, and nearly always occur at a distance from the painted area. The present results, therefore, provide strong evidence for the local action of N—OH—AAF as a direct carcinogen for rat skin and subcutaneous tissue, without metabolic modification. It is considered that the single subcutaneous injection at the beginning of the experiment could not have much influenced the response of the skin. The experiments are being repeated and skin tumours have already been obtained by painting alone. The occurrence of sarcomata at or near the site of injection is also of interest in that they appear to have been caused by a single injection, whereas in work reported previously, multiple injections were given[6]. The fact that combined treatment was given complicates this interpretation, however, because the occurrence of distant neoplasms indicates that there must have been significant absorption either of N—OH—AAF or of its parent amines. Furthermore, it is impossible, at present, to state what influence the painting may have had in the appearance of the subcutaneous tumours.

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Extraction of a Cancer Factor from Ehrlich Ascites Carcinoma

INVESTIGATIONS at present in progress in our laboratory on the inhibitory action of several nucleases of cobra venom on the growth *in vitro* and *in vivo* of the Ehrlich ascites carcinoma[1] have prompted us to study the RNA metabolism in that tumour cell.

Besides several studies[2–6] on the behaviour of the Ehrlich cell in its natural environment, some workers[4,7,8] have examined its behaviour in an artificial environment. They conclude that at low temperatures, and after washings repeated over relatively long periods, the carbohydrate metabolism of these cells remains almost unaffected, whereas their phosphorus metabolism is modified.

These investigations show that when the Ehrlich cell is maintained *in vitro* in an isotonic solution, it survives but undergoes at the same time a gradual decrease in vitality which corresponds to a decrease in virulence; it appears, moreover, that this decrease is associated with the modification of various metabolic pathways, particularly those of the intracellular ribonucleic acids.

It is important to notice that all these workers studied the behaviour of metabolites inside the tumour cell. Our experiments, however, were designed to observe what happens outside the tumour cell and to examine whether or not the decrease in virulence is simply related to the escape of essential metabolites.

What is the fate of the nucleic acids responsible for protein synthesis? We considered the possibility that they escape from the cell by diffusion and have tried to verify this experimentally.

When centrifuged from the cells and examined in ultraviolet light, freshly withdrawn ascitic fluid shows an absorption curve with a peak of 278 mμ, which is characteristic of proteins. In agreement with Klein *et al.*[5], we did not find that the ascitic fluid contained nucleic acid or oligonucleotides.

To follow the course of the diffusion of materials out of the cells, we studied their absorption spectra. If the cells, separated from the supernatant fluid, are placed again in suspension in a saline isotonic solution for 10

to 30 min and centrifuged once more, the maximum absorption of the supernatant fluid occurs at 265 mμ. In the course of the subsequent washings, the maximum is soon stabilized at 260 mμ, which is the characteristic wavelength of nucleic acids.

During the successive washings, the optical density at 260 mμ increases, then decreases, and is finally stabilized, indicating that nucleic acids escape out of the cell by diffusion, until some equilibrium is reached.

The cells were examined under the microscope in the course of several experiments consisting of six to twelve washings. Their morphological structure seems to be unaffected.

Are those Ehrlich cells, which have thus been deprived of their extractable nucleic acids and have remained apparently unchanged, still alive and virulent?

To answer this question, mice were given 2,000,000 cells treated in the way described earlier, by intraperitoneal injection. After 26 days, only one animal out of ten developed an ascites tumour, whereas the ten reference animals that had been injected with fresh unwashed cells all developed a cancer on the ninth day and died about the twenty-third day.

As noted by the authors mentioned, the loss of intracellular RNA leads to a decrease in virulence. Our experiment has shown that an almost total loss of virulence can be obtained by diffusion of nucleic acids outside the cell.

Is it possible that the materials that have escaped out of the cell by diffusion would restore the original virulence?

In order to investigate this point, we carried out some preliminary experiments, the exact conditions of which remain to be further defined.

One batch of ascites cells was washed 8 times. Ten Swiss mice were given 2,000,000 cells intraperitoneally (at a count of 100 million cells per ml.). By the twenty-third day, only one animal had developed ascites.

Meanwhile, ten other mice received 2 million cells from the same batch, but mixed with the extracted factor, passed through a 'Millipore' filter (1·2μ). From the tenth day onwards, six animals developed ascites. This experiment seems to show that the cells have recovered their vitality and virulence.

Is the separated factor by itself able to induce a cancer in a healthy animal?

So far, we have not been able to observe any tumour formation in a mouse or in its offspring by injecting the extractable factor directly by subcutaneous or intraperitoneal routes. This is not surprising because, in such an experiment, the extractable factor does not come into direct contact with an adequate cellular sub-stratum. We think it would be rewarding to inject the product directly into mammary glands containing the type of cells from which the Ehrlich carcinoma originated. The few observations that we had the opportunity of making lead us to think that, under definite conditions, the healthy cells of mammary glands of mice in lactation are liable to develop an ascites or solid tumour by induction.

Those experiments, which still require closer verification, seem to point to the existence of a cancer factor escaping from the Ehrlich cell, when this cell is kept in an isotonic but non-nutritive environment, in this case a saline isotonic solution.

This factor would tend to restore their virulence to cells which have formerly been deprived of it and might even induce a cancer in cells that are healthy but embryologically receptive.

We then tried to determine the chemical nature of the separated factor. For that purpose, the supernatant fluids were mixed, centrifuged and subjected to dialysis in order to eliminate sodium chloride. The obtained solution was then lyophilized. Our measurements show that, on average, the total substance lost by diffusion amounts to 80×10^{-12} g per cell. The separated substance consists mainly of a ribonucleoprotein mixture or combination. The presence of proteins was revealed by the Folin–Ciocalteau reaction; that of ribonucleic acids by the following techniques: (*a*) pyronin reaction; (*b*) ultraviolet spectrometry: maximum at 260 mμ with *p*H effects; (*c*) orcinol reaction: shows the presence of ribose; (*d*) phosphomolybdic reaction: reveals the presence of phosphorus; (*e*) diphenylamine test: shows that the DNA is absent; (*f*) ultracentrifugation: main peak corresponding to a sedimentation coefficient, $S = 2$.

We have separated materials from ascites cells which may be ribonucleic acid, with or without protein in association, and which seem to interfere with the propagation of the Ehrlich carcinoma in the peritoneal fluid of the Swiss mouse.

Further experiments are necessary to characterize the material concerned (S) and a wider range of experimental conditions will make it possible to assess the biological significance of this separated ribonucleic factor.

These experiments were carried out in the Laboratory of Biochemistry of the I.I.F.–I.M.C., CERIA, Brussels.

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CYTOLOGY

Synapses on Neurosecretory Cells of the Supra-optic Nucleus in White Mice

Few observations have been reported on the ultrastructure of synapses between nerve fibres and the perikarya of neurosecretory cells. Electron microscope observations on synapses on perikarya and on branches of neurosecretory cells of the pre-optic nucleus in teleosts —perch and trout—have been published[1,2], and recently short preliminary reports on the ultrastructure of synapses on cells of the supra-optic nucleus of white mice[3] and rats[4] have appeared. Further details of synapses on the neurosecretory cells of the supra-optic nucleus of white mice are reported here.

Adult male white mice weighing 20–24 g served as material for our investigations. Pieces of brain were fixed by immersion in 1 per cent osmium tetroxide solution after Caulfield and Sjöstrand. Sections were studied with a *JEM-5g* electron microscope.

A considerable number of synapses was observed within the supra-optic nucleus on the perikarya as well as on the branches of the neurosecretory cells. Sometimes several axosomatic synapses were seen on the surface of the perikaryon of one neurosecretory cell. These were of typical ultrastructural form. The synaptic plates varied in length from 0·9μ to 1·4μ, and showed a clearly defined presynaptic membrane. Some concentration of osmiophilic material was found in the axoplasm close to the presynaptic membrane, together with optically empty synaptic vesicles 250–550 Å in diameter. In some plates, many of these vesicles were concentrated in groups near the presynaptic membrane in the region of the trans-

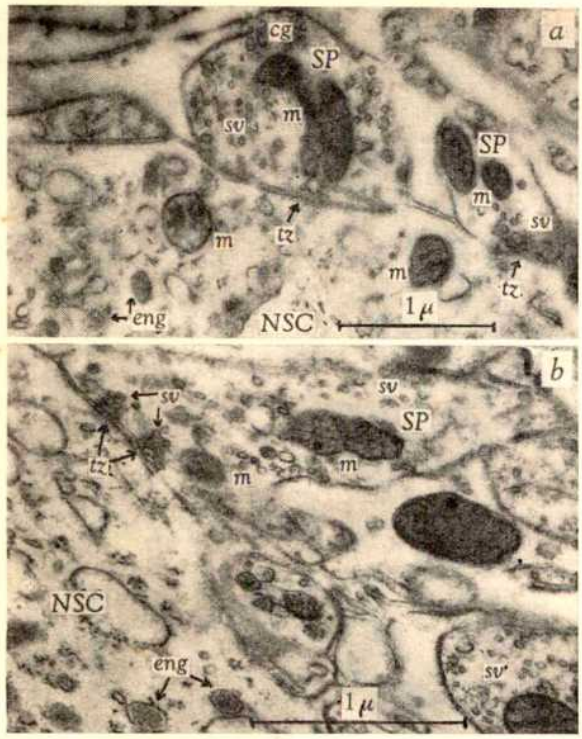


Fig. 1. Synaptic plates (*SP*) containing synaptic vesicles (*sv*) and "catecholamine" granules (*cg*) on a perikaryon of neurosecretory cells (*NSC*) in the cytoplasma of which elementary neurosecretory granules (*eng*) and mitochondria (*m*) can be seen. *tz*, Transmission zone. *a*, × *c.* 20,900; *b*, × *c.* 27,870.

mission zone; in others they were distributed more evenly through the axoplasm.

The postsynaptic membranes were sharply outlined, and a concentration of osmiophilic substance in the form of a narrow strip in some places near the postsynaptic membrane was sometimes observed. The synaptic cleft was enlarged, in some places reaching 640 Å, and contained a narrow strip of a substance of low osmiophility.

Vesicles with electron dense content were observed in the perikarya of the neurosecretory cells and in many synaptic plates (Fig. 1). The vesicles of 1600–3000 Å in diameter in the neurosecretory cells and in some branches are typical elementary neurosecretory granules which have been described in the posterior pituitary gland in our earlier publications[5]. Vesicles in synaptic plates of 900–1000 Å in diameter having electron dense content resembled in their size and appearance catecholamine granules in adrenergic synapses[6]. Besides these granules and synaptic vesicles, mitochondria were usually found in the synaptic plates; in these mitochondria the cristae were few, and usually longitudinally arranged.

Thus our observations indicate that neurosecretory cells of the supra-optic nucleus in white mice are innervated by the neural fibres which seem to belong to adrenergic neurones. Further investigations, however, are required to determine the nature of these fibres.

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BIOLOGY

Organochlorine Insecticide Residues in Soil Invertebrates

THE uptake and transmission of organochlorine insecticide residues in food chains has been described for an aquatic ecosystem[1]. There is, however, little information relating to the terrestrial sphere in respect of insecticide concentrations occurring in invertebrates which form the food of many common species of birds[2,3]. During 1965, an attempt was made to remedy this situation by collecting soil and invertebrates from ten widely separated arable fields and from two apple orchards around Huntingdon which were known to have received recent applications of a number of organochlorine insecticides. The soils varied from sandy loam to clay loam and peaty loam.

The availability of soil invertebrates to birds was determined by the use of pitfall traps which contained a solution of 0·1 per cent phenylmercury acetate and 0·8 per cent formaldehyde to prevent predation and decay. Beetles, predominantly Carabidae, small numbers of other arthropods and earthworms of up to eight species were caught at all twelve sites. Worm samples from the orchards were also collected by the application of dilute formaldehyde[4] and slugs were collected at some sites by searching the vegetation and soil surface. Analyses were performed whenever the sample collected was large enough to be representative. The beetle samples analysed consisted of separate 4–10 g quantities of *Harpalus rufipes* and *Agonum dorsale* (Carabidae) from most of the sites, two samples of mixed large carabids, two of Staphylinidae and one of Elateridae. Twenty soil cores 15 cm deep were taken in each field, bulked and sub-sampled. In the orchards ten cores were taken underneath trees one to two metres from the trunks and ten cores from the lanes between trees.

The samples were ground with anhydrous sodium sulphate and extracted with mixtures of acetone–hexane by means of a Soxhlet apparatus. The extracts were cleaned on a partially de-activated alumina column[5] and then examined by gas–liquid chromatography using silicone and 'Apiezon' columns and electron capture detectors. Considerable interference with the working of the electron capture detectors was noticed when examining those samples which had come into contact with the mercury compound in the traps. This interference was satisfactorily reduced by subjecting the sample extracts to a dimethylformamide–hexane partition[6] before the alumina column treatment. The interfering substance was identified by infra-red and X-ray fluorescence spectroscopy as diphenylmercury, which, being covalent in character, was extracted by the hexane. It was at first thought that diphenylmercury might be a metabolic product formed by the invertebrates, but further investigation revealed its presence as an impurity in the phenylmercury acetate.

The results for the arable sites are summarized in Table 1. The soil residues are probably typical of many arable soils[7]. Samples of beetles from all ten sites contained both dieldrin and *pp'*-DDE in amounts up to 0·2 and 2·2 p.p.m. respectively and there was no obvious difference between groups of beetles. Residues of dieldrin in worm samples were between two and ten times those in soils from corresponding sites and both dieldrin and *pp'*-DDT were generally rather higher than in corresponding beetle samples. The presence of aldrin in three of the worm samples was confirmed by thin layer chromatography. It was found, however, that, when hexane containing dieldrin and phenylmercury acetate was injected on to the gas chromatographic columns, a new peak corresponding to aldrin appeared on the chromatogram. It is therefore possible that the peaks for aldrin from the worm samples derive partly from reduction of dieldrin on the chromatographic columns and partly from

Table 1. RESIDUES FROM TEN ARABLE SITES

Range of values found (p.p.m.) with the number of samples containing measurable quantities in parentheses

Sample	No. of samples analysed	Aldrin	Dieldrin	γ-BHC	*pp'*-DDT	*op'*-DDT	*pp'*-TDE	*pp'*-DDE
Soil	10	0·03–0·7 (4)	0·03–0·7 (9)	0·01 (1)	0·04–0·7 (7)	0·04–0·08 (2)	0·03–0·05 (3)	0·02–0·2 (6)
Beetles	20	—	0·01–0·2 (20)	—	0·09–0·1 (2)	—	0·03 (1)	0·02–2·2 (20)
Worms	4	0·02–0·4 (3)	0·06–0·8 (4)	0·05 (1)	2·1 (1)	0·2 (1)	0·2 (1)	0·9 (1)
Slugs	1	—	0·3 (1)	—	—	—	—	—

Table 2. RESIDUES FROM TWO APPLE ORCHARDS

Sample	No. of samples analysed	γ-BHC	*pp'*-DDT	*op'*-DDT	*pp'*-TDE	*pp'*-DDE	*pp'*-DME
Soil	2	0·08 (1)	1·5–13·3 (2)	0·2–1·2 (2)	0·3–0·6 (2)	0·3–2·1 (2)	—
Beetles	2	—	0·5 (2)	—	0·09–0·1 (2)	2·6–4·6 (2)	—
Worms	2	0·3 (1)	11·7–30·4 (2)	0·9–1·6 (2)	1·5–2·9 (2)	2·1–6·5 (2)	—
Slugs	3	—	5·3–23·8 (3)	0·4–2·6 (3)	1·2–9·9 (3)	0·4–3·4 (3)	0·3–0·4 (2)

the aldrin present in the soil in the food tract of the worms. Dieldrin residues may be expected to decline in the future as a result of the restrictions on the use of aldrinated fertilizers and of dieldrin treatment for carrot fly.

All samples from the two orchard sites (Table 2) contained *pp'*-DDT, *pp'*-TDE and *pp'*-DDE, usually in large concentrations compared with those from the arable sites. The orchard soils also differ from the arable soils in that the organochlorine compounds are very largely concentrated at the top of the soil profile. The rate of application of DDT in one orchard was considerably higher than that recorded by Stringer and Pickard[3] and the residues in worms and slugs from this site are among the highest recorded for invertebrates.

All the animals were active and apparently healthy at the time of capture and there is no evidence to suggest that the concentration of residues found in them are unusual. None of the samples was collected within 2 months of the final application of organochlorine insecticide; higher residue levels than those reported here might therefore be expected in samples of invertebrates collected, or eaten by birds, shortly after spray applications.

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Variation associated with an *Aegilops umbellulata* Chromosome Segment incorporated in Wheat

THE polyacrylamide gel electrophoresis technique for the analysis of soluble plant proteins has amply demonstrated its usefulness and capacity to be repeated[1,2]. Using the method of disk electrophoresis[3,4] it is possible to resolve the proteins into a number of fractions, based on their electrophoretic mobility and the molecular sieving action of gels, with high reproducibility. The technique is being used in this laboratory to investigate variation in the spectrum of soluble plant proteins associated with known genetic loci, chromosome segments and individual chromosomes. The observations reported here show variation in protein pattern associated with a chromosome segment from *Aegilops umbellulata* Zhuk. transferred to *Triticum aestivum* L. ssp. *vulgare* (Vill., Host) Mac Key, variety 'Chinese Spring' by Sears[5]. This is of interest as a well analysed example of introgression or translocation of genetic material between allied genera which incorporated resistance to leaf rust (*Puccinia triticina* Eriks.) in bread wheat. Recent reports indicate that the *umbellulata* segment is substituted for a terminal piece of the wheat chromosome 6*B* (ref. 6), is inherited as a single dominant[7], and controls seedling and field resistance to common races of leaf rust.

Seeds of single plants of wheat variety 'Chinese Spring' and of 'Chinese Spring' with the introgressed *umbellulata* segment (*CI* 13296) obtained from Dr. E. R. Sears as well as bulk seeds provided by Dr. J. C. Craddock, were used in this investigation. Ten grams of dry seeds (about 12 per cent moisture) were ground in a hand operated coffee grinder. The flour obtained was stored overnight in a cold room at 5° C ± 2; all further work was done at this temperature. The flour was homogenized in 100 ml. of 0·01 M sodium pyrophosphate buffer containing 10^{-4} M EDTA and 0·7 ml. of mercaptoethanol. The slurry was filtered through cheese cloth and the filtrate centrifuged at 16,000 r.p.m. in a 'Lourdes' centrifuge for 1 h. The supernatant was gel filtered through 'Sephadex *G*-50' coarse beads (Pharmacia, Uppsala, Sweden) and eluted by 0·01 M phosphate buffer *p*H 7·5, containing 2 ml./l. mercaptoethanol. The protein fraction eluted was concentrated in cellulose dialysing tubing under partial vacuum for 12 h.

For extracting leaf proteins, seedlings were raised in stainless steel trays lined with cellulose wadding hydrated with Hoagland's nutrient solution. Seeds were germinated in the dark at 24° C for 48 h and later grown under continuous illumination of about 800 ft.-candles in a controlled environment. Leaves were harvested 12 days after planting and immediately frozen at −20° C. Fifty grams of frozen leaf tissue was used for each extraction. Chlorophyll was removed by repeated homogenization of the leaves in cold acetone (−5 to −10° C) until the residue was free from green pigment. The residue was dried in the cold to remove traces of acetone and then dissolved in 50 ml. of sodium pyrophosphate buffer of *p*H 8·2. The remainder of the procedure was the same as that followed for seed proteins except that with the leaf proteins an unresolved fraction was obtained as a thick band at the upper part of the gel, which prevents the separation of other proteins into distinct bands. To reduce this unresolved protein fraction, leaf extracts were gel filtered through a 7 per cent agar column and eluted by phosphate buffer of *p*H 7·5. The concentrated extracts were made to 0·25 M sucrose to increase the density, before placing on the gels for electrophoresis. Protein estimations in the final extracts were in the range 0·4–0·5 per cent, as shown using Folin-Ciocalteau reagent.

Electrophoresis was carried out in an apparatus similar to that described by Davis[4], mainly using standard 7·5 per cent acrylamide gels. In some cases, 15 per cent acrylamide gels were also used as the supporting medium, but most of the protein bands were found to resolve better on 7·5 per cent gels. The gels were prepared by mixing the stock solutions *A* and *C* of Davis[4] with boiled distilled water in the proportions 1 : 2 : 1, respectively, and an equal volume of 0·1 per cent ammonium persulphate in boiled distilled water was added to the mixture. The solution was degassed and placed in 'Pyrex' glass

tubes (6 mm × 10 cm). Gels were allowed to polymerize at room temperature. Gels obtained this way stack at *p*H 8·3 and run at *p*H 9·5.

Tris-glycine buffer of *p*H 8·3 (*tris* hydroxymethyl aminomethane 6 g and glycine 28·8 g made to 1 l. and diluted ten times) or *tris*-boric buffer (*tris* 431 g, disodium ethylenediaminetetracetate 37 g and boric acid 220 g made to 1 l. and diluted twenty times) of the same *p*H were used in the electrode vessels. *Tris*-boric buffer was found to give better resolution in certain regions. Seed proteins were run for 1 h at a potential difference of 480 V from a power supply of constant output, resulting in a current of 20 m.amp in *tris*-glycine buffer. In *tris*-boric buffer, a potential difference of 300 V gave a current of 30 m.amp. During the 60 min period, the front formed by the tracking dye (bromophenol blue) moved a distance of about 8 cm. Leaf proteins were better resolved when electrophoresis was continued for 45 min, and the front migrated 5–6 cm. After the run, gels were removed from the glass tubes and stained with 1 per cent Amido-Schwarz 10 *B* (Schmid and Co., Stuttgart, Germany) in 7 per cent acetic acid for 1 h. Gels were washed with 7 per cent acetic acid and destained electrophoretically until they were clear in an apparatus similar to that used for making the run. Destained gels were stored in 7 per cent acetic acid for observation.

Representative electropherograms of seed and leaf proteins of 'Chinese Spring' wheat and of the same variety with the introgressed *umbellulata* segment are shown in Figs. 1 and 2 along with diagrammatic representations. Fig. 1 shows the presence of two bands (marked with arrows) in the seed proteins of the introgressive type (*CI* 13296) that are not found in the parental variety, and one band in the latter is not present in the electropherogram of *CI* 13296. Similarly, in the electropherogram of leaf proteins (Fig. 2), one additional band is present

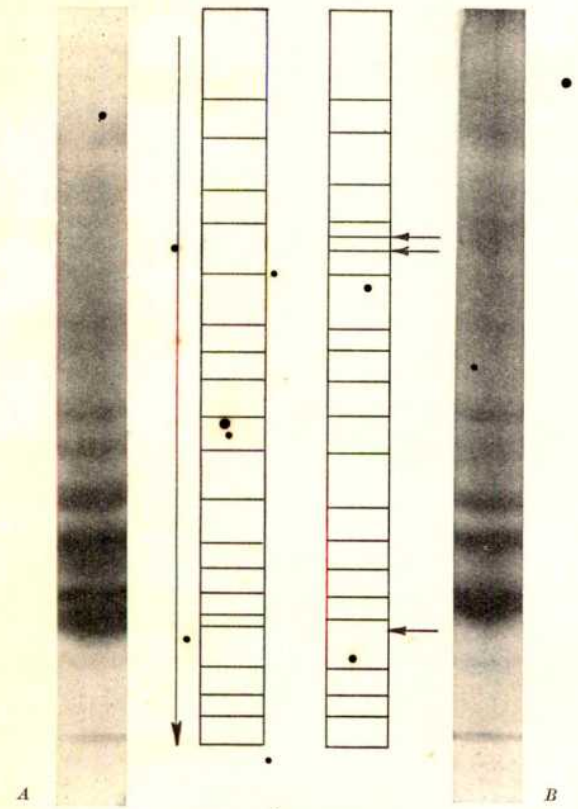


Fig. 1. Electropherograms for soluble seed proteins of wheat extracted in pyrophosphate buffer along with a diagrammatic representation; *A*, 'Chinese Spring'; *B*, 'Chinese Spring' with substituted *umbellulata* chromosome segment. Origin is at the top and the vertical arrow indicates migration of proteins toward anode. Variation in the two patterns is marked with arrows.

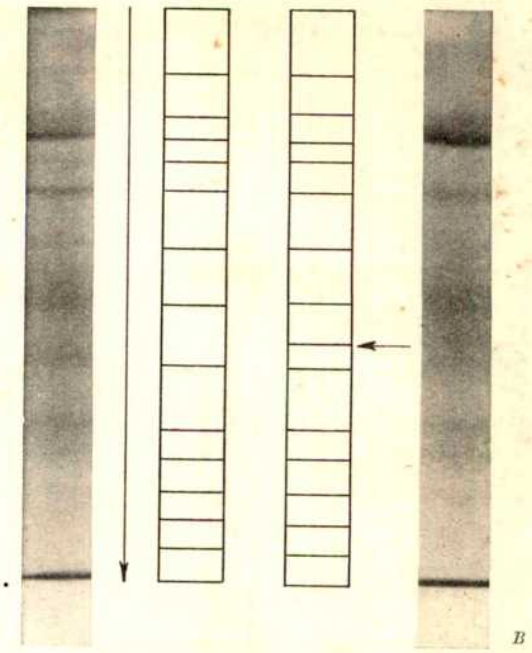


Fig. 2. Electropherograms for soluble leaf proteins of wheat extracted in pyrophosphate buffer along with a diagrammatic representation; *A*, 'Chinese Spring', *B*, 'Chinese Spring' with substituted *umbellulata* chromosome segment.

in *CI* 13296. These additional bands have been found in all four seed samples from single plants grown at Columbia, Missouri and in the bulk seeds obtained from Beltsville, Maryland. It is known that qualitatively similar patterns are obtained under uniform conditions of extraction and electrophoresis; and varieties of similar genotype have similar protein components[8,9]. In this material, therefore, the additional protein bands can confidently be interpreted as causally related to the genetic information carried by the introgressed *umbellulata* segment. The missing band in seed proteins may be due either to loss of the terminal region of wheat chromosome 6*B* or to suppression of synthesis of this protein by the introgressed *umbellulata* segment. It is planned to isolate and identify the missing and additional proteins and determine their possible relationship, if any, to the mechanism of rust resistance incorporated with the addition of the *umbellulata* segment. These results also indicate possibilities for analysing effects of introgressed genetic material in higher plants in terms of the proteins produced.

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Spiral Growth of Nacre

INTERESTING spiral patterns (Fig. 1) were found on the growing inner surface of nacre when the fresh inner surface of pelecypod shells was observed under a microscope using a vertical illuminator. The pattern was explained by invoking the theory of Liesegang's rings[1,2]. This communication deals with the phenomenon as an aspect of spiral growth.

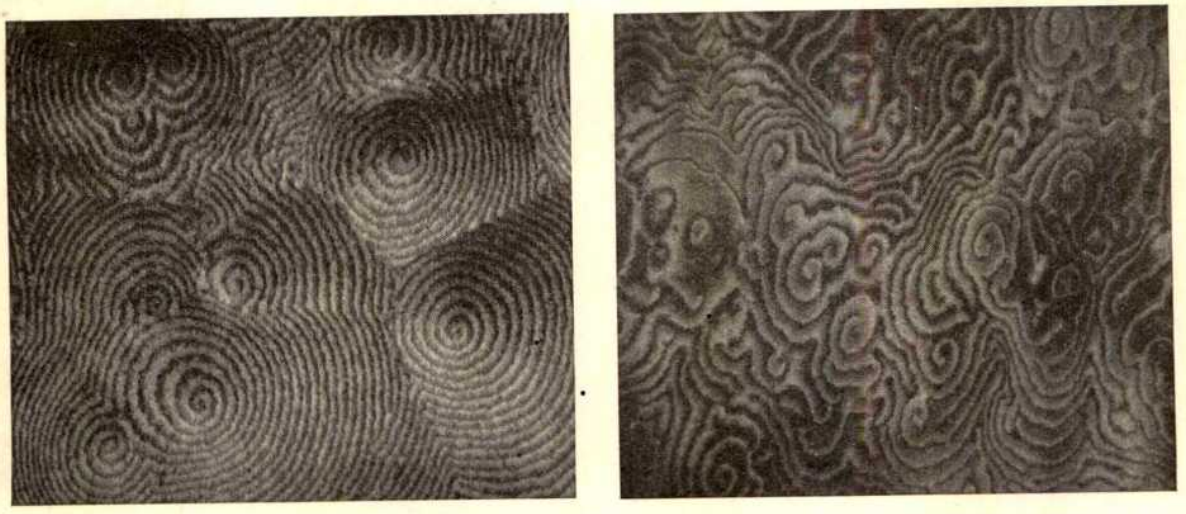


Fig. 1. Typical spiral growth of nacre in *Pinctada martensii* (left, ×85) and in *Hyriopsis schlegeli* (right, ×50).

It has been considered that the shells of molluscs crystallize in complicated ways from a specific solution ("extrapallial fluid") which is below the critical supersaturation required for homogeneous nucleation. They are built up from an organic substance "conchiolin" combined with microcrystals of calcium minerals. Investigations using Laue photographs and polarizing and electron microscopes revealed that the nacre of pelecypod shells was not a single crystal but consisted of mosaic aggregates of aragonite microcrystals having *b* and *c* axes nearly parallel to the horizontal and vertical directions of growth and with the (001) plane of aragonite nearly parallel to the growing inner surface of the nacre. Spiral patterns in the genus *Pinctada* are more often found in the anterio-ventral regions and the inside regions along the pallial impressions, where the vectors[3] indicating the direction of growth are usually deflected, than in other flat ventral regions.

The spiral pattern is observed at the edges of mineral lamellae which emerge on the growing surface of the nacre. Generally speaking, the highest point is the top of the spiral from which a ledge descends step by step outwards. The height of each step of the spirals is in the range 1000–5000 Å, which is equal to the thickness of one lamella, except near the central parts of the spirals, where the step height is less than 1000 Å. The spirals are usually nearly circular; their precise shape, however, varies with the species and physiological condition of the animal, and from region to region in a single shell. It was further shown[3] that the density of spirals per unit area, the distance between two steps, and the number of steps in one spiral depend on the precipitation rate of calcium carbonate.

Nacre grows by the advance of growth fronts of mineral terraces which begin from deformed, misfitting or boundary portions on the nacreous surface. Spiral growth hills are concentrated in particular portions of the surface of the nacre. For example, in the genus *Pinctada*, growth of nacre starts in the narrow parts along the pallial impression where curvature of nacre occurs during the morphogenesis of the shell and where misfit occurs between two groups of microcrystals of different orientations at small angles. In the nacre of *Pinctada martensii*, new microcrystals tend to grow on the boundary between two underlying microcrystals, though this does not always occur in the nacre of *Penna attenuata*. It is thought that a small amount of conchiolin condenses along these boundary regions.

It is interesting to note that the ratios of right-handed to left-handed spirals in anterior and posterior regions of a shell tend to bear an inverse relationship to each other where the vector of the shell growth in the anterior region is opposite in direction to that in the posterior region. When two adjacent spirals have the same sign they tend to develop into a superior spiral, whereas if they have opposite signs a closed loop is developed.

These observations show that tilted or twisted boundaries at small angles tend to occur between two microcrystals or between any two blocks with slightly different vectors of shell growth which interfere with each other during the morphogenesis of a shell. Screw dislocations then develop, giving rise to spiral growth hills on the nacreous surface. It should be emphasized that the spiral pattern of nacre can be reasonably explained by Frank's screw dislocation theory[4] of the spiral growth of single crystals.

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RADIOBIOLOGY

Effects of X-rays on Metamorphosis and Adult Life Span of Flour Beetles

IT has been shown that the adult life span is reduced when young mice are irradiated with X-rays[1] and when the parasitoid wasp, *Habrobracon*, is irradiated during the immature stages of its life cycle[2–4]. Lamb and Maynard Smith[5] recently reviewed the reduction of insect life spans by radiation and postulated that radiations shorten life by inducing mutations in somatic cells. When the young pupa is irradiated, it might be expected that any lethal effect of alteration of genetic material due to radiation would be eliminated during the subsequent developmental crises (stages) instead of occurring after the adult stage had successfully developed.

This investigation was undertaken to determine whether metamorphosis and length of life could be altered when flour beetle pupae were treated with X-rays.

T. confusum Duval ('Chicago Standard' stock) pupae, age 25–26 days, were sexed and irradiated. The X-ray unit operated at 250 kV, 0·86 mm copper half value layer, 0·25 mm copper and 1·0 mm aluminium filtration. The anode was 2·5 in. from the subject and the apparatus delivered approximately 1 kr./min. Doses were 0, 1, 2·5, 5, 7·5, 10, 20, 30, 40, 50, 60 and 70 kr. Each of five replicates at each dose contained ten pupae of each sex in 10 g food. Deaths were noted twice weekly. Culture conditions were $29 \pm 1^{\circ}$ C and 60–75 per cent relative humidity.

Not all dose curves are presented (Fig. 1); those omitted fall in the position expected. Lethality data for males and females were combined because, as with *Habrobracon*[6], sex makes no significant difference. Rockstein *et al.*[7] reported, however, that the emergence of adult female house flies from pupae treated with X-rays was more sensitive than that of males. Age and physiological condition are other factors which might also cause organisms to show differential sensitivities to radiations, but in this experiment these variables were controlled.

The emergence of adults from pupae given 1 or 2·5 kr. was comparable to that of controls (Fig. 1, day 0). The numbers of individuals unable to complete metamorphosis increased progressively for doses from 5 to 70 kr., but metamorphosis was not delayed. Failure of metamorphosis reached about 35 per cent for 7·5–50 kr. and about 55 per cent at 70 kr. Some individuals given 5 kr. and greater doses died when partially metamorphosed, that is, when the thorax and head were adult but the elytra, although pigmented, was pupal in size and position and the abdomen retained pupal characteristics. In addition to these abnormalities, some individuals dying after 30 kr. or more were unable to cast off their meconium, making the gut a cul-de-sac. Death probably resulted from intestinal obstruction rather than from starvation, because heavily irradiated insects are known to feed after exposure to radiation[8–11].

The reduction of the length of life of adults irradiated as pupae was also a sensitive indicator of X-ray damage (Fig. 1). Mortality increased progressively with increasing exposures. The precipitous curves of lethality from 7·5 kr. and higher exposures indicated an early and frequent type of lethality which approached 100 per cent after 9 days. The lethality curve for 2·5 kr. rose gradually, reaching a plateau by day 9 at 10–15 per cent. The 5 kr. curve had a gradual slope which levelled off by day 16 at about 60 per cent lethality.

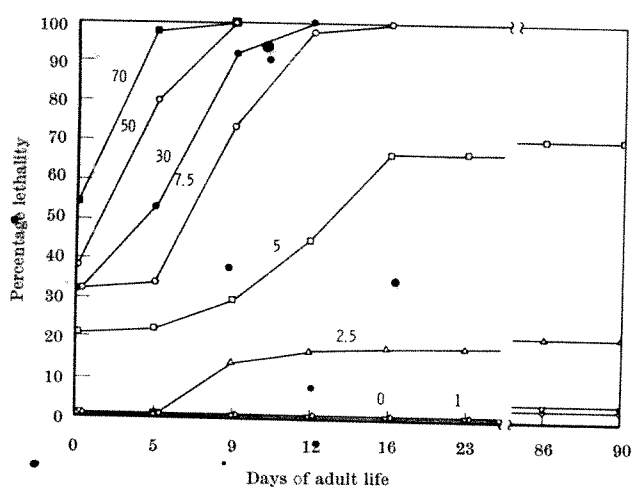


Fig. 1. Percentage lethality during adult life of *Tribolium confusum* irradiated in the pupal stage when 25 or 26 days old. Numbers on the curves refer to the dose in kr.

The exact causes of the mortality induced by radiation remain unknown. Nuclear effects induced by radiation are difficult to separate from cytoplasmic effects in this system; nevertheless, speculation from the data concerning these causes is interesting.

The 26-day-old pupae and day-old adults both required an X-ray dose of 4 kr. for induction of sterility[12], indicating no changes in meiotic activity between these developmental stages. Dick[13] and Erdman[14] found that *T. confusum* females were not sexually mature until day 5 of adult life; consequently, a differential radiosensitivity of germ cells was expected between sexually immature and mature adults. Actively dividing meiotic cells in sexually mature flour beetles (11 days old) were killed by 6 kr. (ref. 12). 100 per cent lethality was achieved with a minimal dose of 7·5 kr. (Fig. 1). This dose is similar to the 6 kr. required to kill dividing meiotic cells and suggests that lethality at 7·5 kr. may be due to adverse effects of radiation on cell division(s). Because 26-day-old pupae and 1- and 11-day-old adults died within a month of a dose of X-rays of 8 kr. (ref. 13), however, it was thought unlikely that cell divisions (mitosis) were factors in lethality. Until the necessary cytological investigations are carried out, the possible effects on lethality of the frequencies of cell divisions and their occurrence in specific tissue(s) cannot be discounted. Death in these irradiated pupae was probably due to chemical alteration of the cytoplasm by radiation and not to nuclear (chromosomal) effects, because pupae have adult structures and the most obvious changes into adults are movement and pigmentation. Antimetabolites could result from radiation-induced alterations in the chemistry of pigment formation. Certain biochemical processes continue from juvenile stages into the adult[15]. Interruption of such processes with subsequent induced cytoplasmic damage[16] could explain the adverse effects of irradiating the pupae on their metamorphosis and adult life span.

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Comparative Radioprotective Effectiveness of Compressed Gases

EBERT *et al.* showed that nitrogen and noble gases have a radioprotective effect in experiments on bean seedlings when under pressure. They consider this effect to result from the adsorption displacement of oxygen from radiosensitive cell structures[1–3]. This hypothesis has, however, been criticized by other workers[4–9]. Another mechanism is probably involved in the protective effect of compressed gases. Protection may be caused by inhibition of the diffusion oxygen which penetrates radiosensitive meristemal cells by way of the intracellular gas spaces[6–9].

This other hypothesis makes it possible to calculate the protective effectiveness of some gases if the oxygen diffusion coefficients for these gases are known. It is difficult to calculate the absolute pressure under which the protective effect is achieved, as many of the required parameters are unknown (path length of oxygen diffusion in extracellular gas spaces, loss of oxygen owing to consumption during diffusion, etc.). We therefore determined for the different gases the relative concentrations (pressures) which exert equal effects. These are directly proportional to the oxygen diffusion coefficients for these gases:

$$\frac{P_{N_2}}{D_{O_2\text{-}N_2}} = \frac{P_{Ar}}{D_{O_2\text{-}Ar}} = \frac{P_{He}}{D_{O_2\text{-}He}}$$

Here P_{N_2}, P_{Ar}, P_{He} are equally effective pressures of nitrogen, argon and helium. $D_{O_2\text{-}N_2}$, $D_{O_2\text{-}Ar}$, $D_{O_2\text{-}He}$ are the oxygen diffusion coefficients in these gases.

P_{N_2} was taken as 1. To check whether the values calculated are in accord with experimental results we determined the protective effect of the various gases on bean seedlings of *Vicia faba* 'Russkii chernii'. The apparatus was operated at 18 kVp. and 20 m.amp, producing 45 r./min. The seedlings were irradiated in rotating steel chambers. Dosimetry inside the chamber was carried out using an *RM-1M* roentgenometer. The average growth rate of the main root was taken as a measure of the degree of injury. Five measurements were taken of the growth rate of ten roots from the 4th to 9th day after irradiation. The gases used contained no more than 0·01 per cent of oxygen (the nitrogen samples contained less than 0·001 per cent oxygen). The gases were introduced into the chamber 10 min before irradiation and were left in the chamber for 5 min afterwards. Details of the technique are given in ref. 4. Fig. 1 shows dose curves for the determination of the radiosensitivity of seedlings in the presence of gases. From Fig. 1 we obtained values for the gas pressures which entirely or by 50 per cent suppress the oxygen-dependent radiosensitivity of the seedlings. These values were determined by interpolating or, in the case of helium, extrapolating the curves of Fig. 2.

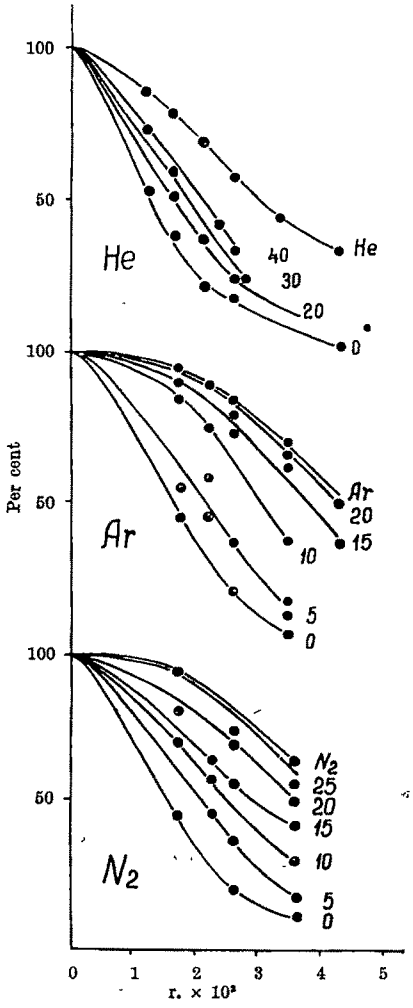


Fig. 1. Growth rate of bean seedlings under different gas pressures. Abscissa, dosage in r. Ordinate, growth rate as percentage of the growth rate of the control. Numbers on figure denote pressure (atm) of gases added to 1 atm air. Dose-effect curves for irradiation in the absence of air are denoted respectively by He, Ar and N_2.

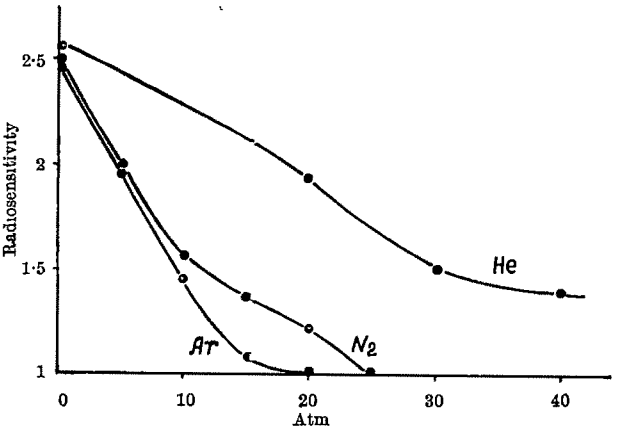


Fig. 2. Effect of helium, nitrogen and argon on the radiosensitivity of bean seedlings. Abscissa, gas pressure added to air (atm). Ordinate, radiosensitivity. Radiosensitivity in the absence of air is taken as unity.

Our experimental data are in good agreement with the theoretical predictions using the diffusion hypothesis. These results are not in accord with the data of Ebert *et al.* (Table 1).

Table 1. Calculated and Experimental Values for Equally Effective Pressures of Nitrogen, Argon and Helium

Gases		Nitrogen	Argon	Helium
Diffusion coefficients of oxygen $D_{O_2\text{-gas}}$ (cm²/sec)		0·241 (ref. 10)	0·224 (ref. 11)	0·661 (ref. 12)
Calculated relative equally effective pressures		1	0·93	2·74
Equally effective pressures according to Ebert (ref. 1) (atmospheres)	1. Complete repression of oxygen effect	21	5	110 extrapolation
	2. 50 per cent decrease of oxygen effect	12·5	2	55
	3. Relatively equally effective pressures (average of 1 and 2)	1	0·2	4·82
Equally effective pressures according to our data (atmospheres)	1. Complete repression of oxygen effect	25	20	67 extrapolation
	2. 50 per cent decrease of oxygen effect	8·1	6·9	23
	3. Relatively equally effective pressures (average of 1 and 2)	1	0·82	2·76

We conclude that the adsorption hypothesis for the protective action of compressed gases does not correspond with the results of experiment. The protection is caused by inhibition of the diffusion of oxygen in the extracellular gas spaces of the root.

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SOIL SCIENCE

Extraction of Organic Matter from Soils by means of Ultrasonic Dispersion in Aqueous Acetylacetone

THE extraction of organic matter from soils[1–4] in a form suitable for chemical examination requires a reagent in which the organic material is largely unaltered, uncontaminated with extraneous ions or compounds, and capable of ready purification. Martin and Reeve[5,6] found that acetylacetone was an effective extractant of organic matter from *B* horizons of podzol soils. Recent experiments have shown that the amounts of organic matter extracted in boiling anhydrous formic acid increase with addition of metal complexing agents such as hydrofluoric acid and acetylacetone[7]. The present investigation was begun to explore further the use of acetylacetone as a suitable extractant of organic matter, particularly the organic phosphorus component in soils.

The soils used consisted of eight surface samples as follows: (1) acid brown forest; (2) low humic gley; (3) and (4) iron podzol; (5) brown forest; (6) and (7) podzol; (8) chernozem. Samples 1–5 came from Scotland and samples 6–8 from Canada. All but No. 2 were well-drained. Samples 1, 2, 3 and 8 were clay loams, sample 4 was a coarse sandy loam, sample 5 a loam, and samples 6 and 7 were silt loams.

Total nitrogen was determined by Kjeldahl digestion, carbon by the method of Walkley and Black[8], and sulphur according to Butters and Chenery[9] with a seed suspension added to improve sensitivity. Organic phosphorus in the soils was determined according to Mehta *et al.*[10]. Organic phosphorus in the extracts was obtained as the difference between total phosphorus measured after digestion in perchloric acid[10] and inorganic phosphorus determined according to Martin and Doty[11]. All determinations were made in duplicate on each of two extracts.

In a preliminary investigation, boiling anhydrous formic acid containing acetylacetone[7] was tried out as an extractant, but was abandoned when it was found to extract only 11, 7, and 10 per cent of the total organic phosphorus in samples 1, 3 and 5 respectively. On the other hand, four successive 24 h extractions with aqueous 0·2 M acetylacetone (*p*H 7·3) following an acid pretreatment removed 63, 75 and 64 per cent of the total organic phosphorus in these three soils.

Table 1. EXTRACTION OF SOIL ORGANIC PHOSPHORUS (SOIL 1) USING ULTRASONIC DISPERSION AND 0·2 M ACETYLACETONE (*p*H 7·5)

Extract	Extraction time (h)	Organic phosphorus as a percentage of total 1 h ultrasonic	2 h ultrasonic
1	18	54	62
2	23	12	11
3	23	8	10
4	23	4	4
5*	18	8	6
Total		86	93

* Ultrasonic treatment repeated after fourth extraction.

Table 2. EFFECT OF HIGH SPEED CENTRIFUGATION ON CARBON, NITROGEN, SULPHUR AND ORGANIC PHOSPHORUS CONTENT OF ACETYLACETONE EXTRACTS AFTER ETHER EXTRACTION (SOIL 1)

Treatment	Organic phosphorus	Carbon	Nitrogen	Sulphur
	as percentage of the total			
Not centrifuged	101	103	98	100
Centrifuged	84	87	74	105

The 0·2 M acetylacetone at *p*H 7·3 was a more effective extractant of organic phosphorus than 1·0 M acetylacetone at *p*H 4·3, and adjustment of the *p*H of the 0·2 M solution to 8·0 or 8·5 with dilute sodium hydroxide extracted a further 5–11 per cent of the organic phosphorus from the three soils.

Because further increases in the *p*H of the extracting solution were not desirable, and longer shaking periods did not significantly increase the yield of organic phosphorus, the effect of dispersion of the soils with ultrasonic vibration[12,13] before extraction was investigated with one soil. Four successive extractions following initial ultrasonic treatment of 1 or 2 h removed 78 and 87 per cent, respectively, of the total organic phosphorus (Table 1) compared with 63 per cent removed by acetylacetone alone. Further ultrasonic treatments of 1 or 2 h followed by a fifth extraction increased the yield of organic phosphorus by 8 or 6 per cent, respectively. A large part of the extracted organic phosphorus was found in the initial extract.

Because the removal of excess acetylacetone and its complexes is an essential requirement of the method, the extracts were combined and extracted with ether. Analysis of the ether-insoluble material showed that the organic phosphorus content did not decline with ether extraction. Furthermore, it was found that the aqueous phase contained a large proportion of the soil carbon, nitrogen and sulphur as well as the organic phosphorus. Because the aqueous phase exhibited some cloudiness at this stage, portions were clarified by high speed centrifugation (about 16,000 r.p.m.). The carbon, nitrogen and organic phosphorus content of the extract declined, while sulphur remained about the same, following high speed centrifugation (Table 2). This indicated that some of the extracted carbon, nitrogen and organic phosphorus was closely associated with the finely suspended material, probably fine clay, removed by centrifugation.

The final procedure as developed for extraction of soil organic matter was as follows: Two-gram samples of soil (< 100 mesh) were mechanically shaken with 40 ml. of 0·1N hydrochloric acid for 30 min and centrifuged at about 3,000 r.p.m. Following a second acid treatment, the samples were transferred to 50 ml. beakers with 30 ml. of 0·2 M acetylacetone (*p*H 8·0) and given a 2-h ultrasonic treatment. Suspensions were transferred to centrifuge bottles with 10 ml. of acetylacetone solution, and the *p*H adjusted to *p*H 8·0 with 3N sodium hydroxide. Samples were then shaken for 16 h and centrifuged. The residues were then extracted four times with 40 ml. of acetylacetone using shaking periods of about 24 h. Before the fourth extraction the ultrasonic treatment was repeated. Acetylacetone and its complexes were removed from the combined extracts with diethyl ether in an upward displacement liquid-liquid extractor. The ether extracted solutions were clarified by high speed centrifugation (approximately 16,000 r.p.m.).

The organic matter of the eight soils described previously was extracted with this procedure, and analyses were made for carbon, nitrogen, sulphur and organic phosphorus.

The acetylacetone method removed a large proportion of the organic phosphorus from all soils (Table 3). Four of the values agreed closely, one was lower, whereas the remaining three were higher than those obtained for total phosphorus by the Mehta method. Although these

Table 3. TOTAL ORGANIC PHOSPHORUS, CARBON, NITROGEN AND SULPHUR CONTENT OF EIGHT SOILS AND AMOUNTS OF THESE CONSTITUENTS EXTRACTED WITH ACETYLACETONE AFTER ULTRASONIC DISPERSION

Sample	Organic phosphorus		Carbon		Nitrogen		Sulphur	
	Total	Acetylacetone	Total	Acetylacetone	Total	Acetylacetone	Total	Acetylacetone
	mg phosphorus/100 g soil		per cent		per cent		per cent	
(1)	81	78	3·8	3·44	0·39	0·25	0·065	0·052
(2)	31	31	2·3	2·06	0 20	0·11	0·035	0·031
(3)	72	86	5·0	3·40	0·36	0·21	0 049	0·044
(4)	59	67	3·1	3·11	0·26	0·16	0·055	0 049
(5)	49	42	2·3	2·02	0·22	0·11	0·037	0 035
(6)	35	35	1·9	1·94	0·18	0·10	0·020	0·026
(7)	41	43	2·7	2·41	0·24	0·11	0·029	0·029
(8)	48	58	6 6	5·54	0·59	0·40	0·059	0·050

high values (samples 3, 4 and 8) may suggest an overestimation of organic phosphorus obtained with acetylacetone, they were in agreement with total organic phosphorus values available for two of the soils (samples 3 and 8) by an ignition method. Thus, it would appear that the Mehta method may have underestimated the total organic phosphorus content of these particular samples.

In addition to the organic phosphorus, the method effectively extracted sulphur from all samples (85–100 per cent). The efficiency of this extraction was confirmed by the small amount of sulphur found in the soil residues. The method was least effective for the extraction of nitrogen, removing only 48–68 per cent of the total. Although the carbon values show an apparent extraction of 68–100 per cent of the total carbon in the different soils, incomplete removal of the extracting reagent may be expected to contribute to its overestimation. This was confirmed when it was found that the soil residues still retained 12–29 per cent of the total carbon.

The use of ultrasonic dispersion in conjunction with acetylacetone was found to increase the effectiveness of this reagent in extracting organic matter from a variety of soils. The main advantages of this method are its high efficiency in extracting organic phosphorus and sulphur, the mild nature of the extractant and the ease with which the extracted material can be prepared for chemical analysis.

This research was carried out while one of us (R.L.H.) was a visiting worker at the Macaulay Institute for Soil Research.

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MICROBIOLOGY

Role of Amino-acids in the Germination of Bacterial Spores by Subtilisin

BACTERIAL spores are characteristically resistant to desiccation, heat, irradiation, chemicals and to attack by enzymes. Resistance to enzymes is an attribute that must have important survival value for spores, because spores may come into contact with a variety of hydrolytic enzymes secreted by micro-organisms and other flora and fauna in their natural habitat, the soil. Previous reports that two lytic enzymes, lysozyme and an enzyme which can be extracted from *B. cereus* spores, will attack spores, were based on experiments in which the spores were first treated with reagents which rupture disulphide bonds[1,2]. This sensitization treatment was probably necessary to expose the substrates to the action of the enzymes[3,4].

A recent report[5] that the proteolytic enzyme subtilisin directly attacked spores of strains of *B. subtilis*, *B. cereus* and *B. licheniformis* was therefore of interest as the first report of a direct action of an enzyme on untreated bacterial spores. Spores incubated with subtilisin became dark when seen with the phase contrast microscope, and could be stained with methylene blue; they released dipicolinic acid and lost their resistance to heat, and the optical density of spore suspensions decreased. These reactions are characteristic of what is usually considered to be normal spore germination, that is, germination induced by germinants like L-alanine or inosine. It was presumed that subtilisin directly attacked some component of the spore, probably protein in the spore coat, and that this attack resulted in the germination-like changes[5].

During an investigation of the germination requirements of spores of a strain of *B. subtilis* (*globigii*), we incubated spores with subtilisin (Worthington Biochemical Corp., Freehold, New Jersey) and found them to germinate, confirming the observation of Sierra[5]. Spores of the *B. subtilis* (*globigii*) strain used normally require L-alanine in order to germinate, and their germination in L-alanine is inhibited by D-alanine[6,7]. When D-alanine was included with subtilisin, it was found to inhibit the germination induced by subtilisin (Table 1). It seemed possible that L-alanine was formed during incubation of subtilisin, for example by enzyme autodigestion or by degradation of some contaminating protein in the samples of subtilisin. L-Alanine so formed would then be expected to act as a germinant in the usual way. This seemed even more likely when it was found that when subtilisin was preincubated in buffer at 37° C for 1 h (to allow proteolysis to occur) and then added to the germination mixture it caused more rapid germination than when it was not preincubated (Table 1). Inosine, but not L-alanine, will germinate unheated spores of *B. cereus PX*; subtilisin had no effect on spores of this organism when tested under the conditions described in Table 1. Spores of *B. megaterium NCTC* 7581 will germinate in inosine plus L-alanine (or plus some other amino-acids) but not in inosine or L-alanine alone. Table 2 shows that, for germination of these spores, subtilisin could be substituted effectively for L-alanine, but not for inosine, and it was ineffective as a germinant alone.

Table 1. GERMINATION OF *B. subtilis* SPORES BY ALANINE AND BY SUBTILISIN

Mixtures*	Germination† (%) after incubation of spores in mixtures at 37° C for 30 min	60 min	90 min
No addition	2·0	2·5	1·5
L-alanine	18	34·5	41
D-alanine	1·5	2·0	2·0
Subtilisin	7·5	33	43·5
Subtilisin + D-alanine	2·5	2·5	2·0
Subtilisin preincubated‡	9·5	42	53
Subtilisin preincubated‡ + D-alanine	1·5	1·5	2 0

* All mixtures contained sodium phosphate buffer (0·1 M *p*H 8·0): isomers of alanine were used at 5 mM, and subtilisin at 40 mg/ml.
† Germination was estimated by counting the numbers of bright and dark spores at intervals using a phase contrast microscope.
‡ Spores or spores + D-alanine were added after preincubation of subtilisin solution at 37° C for 1 h.

Table 2. GERMINATION OF *B. megaterium* SPORES BY ALANINE, INOSINE AND SUBTILISIN

	Germination (%) after incubation of spores in mixtures at 37° C for 5 min	15 min
No addition	1	1
L-alanine	1	1
Inosine	1·5	2·0
L-alanine + inosine	65	98
Subtilisin	1	1
Subtilisin + L-alanine	2·5	9·5
Subtilisin + inosine	58	98·5

Experimental conditions as for Table 1. Inosine was used at mM.

Finally, subtilisin (40 mg/ml.) was incubated alone in buffer (0·1 M sodium phosphate at *p*H 8·0) at 37° C and samples taken at intervals over a period of 2·5 h. These were boiled for 10 min, dried at 100° C and extracted with butanol/phenol (2 : 3). Paper chromatography of the extracts revealed that a number of amino-acids were present, and these included one which was presumed from its R_F value to be alanine.

The experiments described here confirm the observation[5] that the *B. subtilis* proteolytic enzyme subtilisin will germinate spores of some *Bacillus* species. The experiments also indicate, however, that germination by subtilisin is not induced directly by the action of the enzyme

on spores, but by L-alanine and perhaps by other amino-acids which are probably released by the proteolytic action of the enzyme during its incubation.

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VIROLOGY

Marker Studies of Poliovirus

VARIATION in the chemical properties of the protein coat of different strains has been used as a marker test in investigations of the genetics of polioviruses. Bengtsson, Philipson, Persson and Laurent[1] recently showed that a group of these tests had a common basis. Small differences in the electrical charge of the protein of various strains of the same serotype resulted in different patterns of binding to charged adsorbents.

The *d* (ref. 2) and *m* (ref. 3) markers and the inhibition of plaque formation by dextran sulphate[4] measure depression of growth in the presence of polyanions, while the aluminium adsorption[5] and elution tests[6] and elution from DEAE cellulose[7] depend on the physical separation of infectious particles by differential adsorption.

For application on an epidemiological scale, a test of this property which is simple to perform and which conserves both materials and labour is desirable. The possibility that tube titration in the presence of a low concentration of dextran sulphate might meet these requirements was therefore investigated.

Primary rhesus monkey kidney cell cultures were used, with the control series maintained on medium 199 alone and the test series on medium 199 containing dextran sulphate 2000 (Pharmacia, molecular weight 2×10^6) in the concentrations shown in Tables 1 and 2. Virus dilutions were made at $\log_{10}$ intervals and two tissue culture tubes in each series were inoculated per dilution. The viruses tested are shown in Tables 1 and 2, and the results, calculated by the Kärber method, are based on readings for day 6.

Table 1 shows that the poliovirus type 1 vaccine strain (*LSc2ab*) can readily be differentiated from the type 1 Mahoney strain by titration in 0·05 per cent dextran sulphate. The poliovirus type 2 and type 3 prototype strains are not, however, clearly separated under any of the conditions tested by such a minimal technique. This is reminiscent of the findings of Wallis, Melnick and Wimberley[5], who showed large differences in the adsorption of poliovirus type 1 strains to aluminium salts but less satisfactory results for types 2 and 3.

Table 2 shows the results of tests on two groups of poliovirus type 1 strains: (*a*) strains isolated from patients with clinical poliomyelitis in the prevaccine years; (*b*) strains isolated from subjects given oral poliomyelitis vaccine.

The groups are clearly separated by the test, although the small range of values may limit its usefulness when applied to an individual strain. The titrations were carried out using minimum numbers of tubes. Greater accuracy would be obtained by increasing the number of tubes inoculated per dilution but this would mean corresponding increase in the labour and materials used.

Table 1. REDUCTION IN VIRUS TITRE OF PROTOTYPE POLIOVIRUS STRAINS IN THE PRESENCE OF VARIOUS CONCENTRATIONS OF DEXTRAN SULPHATE

Strain	Titre in 199	$\log_{10}$ reduction in the presence of dextran sulphate 0·005%		0·05%	0·5%
			Type 1		
Mahoney	$10^{6\cdot0}$	−1·0		0	−0·5
	$10^{6\cdot0}$	−0·5		−0·5	−0
	$10^{6\cdot0}$			+0·5	
	$10^{5\cdot5}$			0	
	$10^{6\cdot5}$			−0·5	
			Average	−0·1	
			Standard deviation	0·31	
Sabin 1	$10^{6\cdot5}$	−0·5		−3·0	−1·0
	$10^{6\cdot0}$	−1·5		−1·5	−2·5
	$10^{6\cdot0}$			−2·5	
	$10^{5\cdot5}$			−3·0	
	$10^{6\cdot0}$			−2·5	
			Average	−2·5	
			Standard deviation	0·55	
			Type 2		
YSK	$10^{5\cdot5}$	0		−2·0	−1·5
	$10^{5\cdot5}$	0		−0·5	−0·5
	$10^{5\cdot5}$			−1·5	
	$10^{5\cdot5}$	−1·0		−3·0	−1·0
Sabin 2	$10^{5\cdot5}$	−0·5		−2·0	−1·5
	$10^{5\cdot5}$			−2·0	
			Type 3		
Saukett	$10^{6\cdot5}$	+0·5		−1·0	−0·5
	$10^{6\cdot0}$	−0·5		0	0
	$10^{6\cdot5}$			0	
Sabin 3	$10^{5\cdot5}$	−1·0		0	0
	$10^{6\cdot0}$	−0·5		−1·0	−1·0
	$10^{6\cdot0}$			−1·5	

Table 2. COMPARISON OF NATURALLY DERIVED AND VACCINE STRAINS OF POLIOVIRUS TYPE 1

Virus reference lab. No. 3	Year	Clinical	Passage	Titre in 199	$\log_{10}$ reduction in 0·05% dextran sulphate
4657	1953	Paralytic polio	*MK*2	$10^{5\cdot5}$	+0·5
4862	1953	,,	,,	$10^{7\cdot0}$	+0·5
4863	1953	,,	,,	$10^{5\cdot5}$	0
5037	1953	,,	,,	$10^{5\cdot0}$	+1·0
5038	1953	,,	,,	$10^{7\cdot5}$	0
15	1957	,,	,,	$10^{7\cdot5}$	−1·0
17	1957	,,	,,	$10^{7\cdot5}$	0
20	1957	,,	,,	$10^{6\cdot5}$	+0·5
55	1957	,,	,,	$10^{5\cdot5}$	0
73	1957	,,	,,	$10^{7\cdot0}$	+0·5
				Average	+0·2
				Standard deviation	0·42
		Weeks post vaccination			
568	1962	1	*MK*1	$10^{5\cdot0}$	−2·0
569	1962	2	,,	$10^{5\cdot5}$	−1·5
570	1962	2	,,	$10^{5\cdot5}$	−2·0
183	1962	3	,,	$10^{6\cdot0}$	−2·0
474	1962	2	,,	$10^{5\cdot5}$	−2·5
496	1962	1	,,	$10^{7\cdot0}$	−2·0
498	1962	2	,,	$10^{7\cdot0}$	−2·0
551	1962	1	,,	$10^{6\cdot5}$	−1·0
751	1965	2	,,	$10^{7\cdot0}$	−2·0
812	1965	1	,,	$10^{5\cdot5}$	−1·0
				Average	−1·8
				Standard deviation	0·46

*MK*1 = first passage in rhesus monkey kidney.
*MK*2 = second ,, ,, ,, ,, ,,

The test should be applicable to the epidemiological study of type 1 polioviruses. This is the type mainly associated in the past with paralytic poliomyelitis. It has also been the cause of the limited outbreaks of poliomyelitis which have occurred in Britain since vaccination became widespread.

Y. E. COSSART

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Central Public Health Laboratory,
Colindale Avenue,
London.

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FORTHCOMING EVENTS

(Meetings marked with an asterisk are open to the public)

Tuesday, September 27

INSTITUTION OF MECHANICAL ENGINEERS, AUTOMOBILE DIVISION (at 1 Birdcage Walk, Westminster, London, S.W.1)—Symposium on "Ergonomics and Safety in Motor Car Design".

INSTITUTE OF PETROLEUM, EXPLORATION AND PRODUCTION GROUP (joint meeting with the Geological Society, at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Discussion Meeting on "The Origin of Oil".

SOCIETY OF INSTRUMENT TECHNOLOGY (at Manson House, 26 Portland Place, London, W.1), at 6 p.m.—Informal meeting on "Control Valve Characteristics and Sizing".

Wednesday, September 28

INSTITUTE OF MATHEMATICS AND ITS APPLICATIONS (in Lecture Theatre 2, Physics Department, Imperial College, London, S.W.7), at 5.30 p.m.—Dr. R. F. Churchhouse: "Using Computers to Discover New Results in the Theory of Numbers".

INSTITUTION OF ELECTRONIC AND RADIO ENGINEERS, TELEVISION GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Mr. R. I. Kinross: "Television Wire Broadcasting Systems".

Wednesday, September 28—Thursday, September 29

EUGENICS SOCIETY (in the Botany Lecture Theatre, University College, Gower Street, London, W.C.1), at 10.30 a.m. on Wednesday and 10 15 a.m. on Thursday—Symposium on "Social and Genetic Influences on Life and Death".

Thursday, September 29

RESEARCH AND DEVELOPMENT SOCIETY (at Imperial College, London, S.W.7)—Symposium on "Communicating the Results of Research and Development".

SOCIETY FOR ANALYTICAL CHEMISTRY, ATOMIC-ABSORPTION SPECTROSCOPY GROUP (at Thornton Research Centre, Chester), at 10 a.m.—Mr. D. J. Jenkins and Mr. W. Slavin: "Flames for Analysis".

TELEVISION SOCIETY (in the Conference Suite, I.T.A., 70 Brompton Road, London, S.W.3), at 7 p.m.—Mr. R. Knight: "13 Types of Picture Quality from 13 Television Programme Contractors".

Thursday, September 29—Friday, September 30

INSTITUTE OF BIOLOGY (at the Royal Geographical Society, Kensington Gore, London, S.W.7), at 10 a.m. daily—Symposium on "Biology and the Manufacturing Industries".

Friday, September 30

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—FOOD ENGINEERING PANEL (at the National College of Food Technology, Weybridge, Surrey), at 6.15 p.m.—Symposium on "Continuous Food Processing".

Saturday, October 1

BRITISH RADIOLOGICAL PROTECTION ASSOCIATION (at the Royal College of Surgeons, Lincoln's Inn Fields, London, W.C.2), at 9.45 a.m.—Symposium on "Radiation and Genetics" (organized by The Faculty of Radiologists).

Monday, October 3

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "Parameters Which Need to be Standardized to Specify a P.C.M. Interface" opened by Mr. R. O. Carter.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion Meeting on "The Mossbauer Spectrometer" opened by Mr. E. L. Bush and Mr. H. Grayson.

ROYAL SOCIETY OF MEDICINE (at 1 Wimpole Street, London, W.1), at 6 p.m.—Prof. E. Boyland: "A Chemist's View of Cancer Prevention" (The Michael Williams Lecture).

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION (at Shell Centre Theatre, Shell Centre, London, S.E.1), at 6.30 p.m.—Scientific Films.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SENIOR PHYSICIST (with at least five years' relevant postgraduate experience) to join a team of hospital physicists based at the Liverpool Clinic, and to undertake the duties of Radiological Protection Adviser to the hospitals of North Wales and also to take part in the development of radioisotope techniques at the Liverpool Centre—The Group Secretary, Central Wirral Hospital Management Committee, Clatterbridge Hospital, Bebington, Wirral, Cheshire (September 27).

ASSISTANT LIBRARIAN (graduate with appropriate academic and professional qualifications or experience)—The Registrar, University of Essex, Wivenhoe Park, Colchester, Essex (September 30).

RESEARCH ASSISTANT (preferably with a degree or Diploma in Technology which includes mathematics or statistics, and an interest in the application of computers to applied mathematical problems) IN THE POSTGRADUATE SCHOOL OF MATHEMATICS, to assist in an investigation of queues in which arrivals are partly regular and partly random (which is the situation in most real problems as opposed wholly random or wholly regular arrivals as in most theoretical solutions)—The Registrar, Bradford Institute of Technology, Bradford, 7 (October 2).

SCIENTISTS (preferably with research experience) for permanent posts concerned with fundamental work on brewing materials and fermentation—The Director, Brewing Industry Research Foundation, Nutfield, Redhill, Surrey (October 3).

TEMPORARY LECTURER or ASSISTANT LECTURER IN GEOPHYSICS IN THE DEPARTMENT OF EARTH SCIENCES—The Registrar, The University, Leeds, 2 (October 3).

CHAIRMAN OF THE DEPARTMENT OF CHEMISTRY, Trent University, Peterborough, Ontario, Canada—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 10).

PLANT ECOLOGIST (preferably an algologist); and an EMBRYOLOGIST or an INVERTEBRATE ZOOLOGIST IN THE DEPARTMENT OF BIOLOGY, Trent University, Peterborough, Ontario, Canada—The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (October 10).

READER IN ORAL SURGERY at University College Hospital Medical School—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 11).

LECTURER IN HAEMATOLOGY IN THE BLAND-SUTTON INSTITUTE OF PATHOLOGY—The Secretary, The Middlesex Hospital Medical School, London, W.1 (October 14).

ASSISTANT LIBRARIAN (CATALOGUER) (qualified librarian with a good honours degree)—The Registrar, Bradford Institute of Technology, Bradford, 7 (October 15).

CHAIR OF PURE MATHEMATICS IN THE SCHOOL OF MATHEMATICS AND PHYSICS—The Registrar, University of East Anglia, Earlham Hall, Norwich, Norfolk, NOR 88C (October 15).

DIRECTOR OF THE INSTITUTE—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire (October 15).

LECTURER IN MECHANICAL ENGINEERING and an ASSISTANT IN MECHANICAL ENGINEERING—The Secretary of the University Court, The University, Glasgow (October 15).

SECOND CHAIR IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, The University, Newcastle upon Tyne (October 15).

SCIENTIFIC OFFICER (biologist, with at least a good honours degree, and preferably a qualification or experience in microbiology) for research on pathogens of insects of agricultural importance—The Secretary, Rothamsted Experimental Station, Harpenden, Herts, quoting Ref. 1051/77 (October 17).

LECTURER or ASSISTANT LECTURER IN AGRICULTURAL EXTENSION at the University of the West Indies, Trinidad—The Inter-University Council, 33 Bedford Place, London, W.C.1 (October 21).

SENIOR LECTURER or LECTURER IN VETERINARY HISTOLOGY IN THE DEPARTMENT OF VETERINARY ANATOMY, University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 22).

CHAIR AND HEADSHIP OF THE DEPARTMENT OF PHYSIOLOGY—The Registrar, The University, Leeds, 2 (October 24).

CHAIR OF PHILOSOPHY at Bedford College—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 24).

SENIOR LECTURER or LECTURER IN SOCIAL ANTHROPOLOGY IN THE FACULTY OF ECONOMIC AND SOCIAL STUDIES, University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 33 Bedford Place, London, W.C.1 (October 25).

CHAIR OF HUMAN MORPHOLOGY; CHAIR OF BIOCHEMISTRY; CHAIR OF COMMUNITY HEALTH; and CHAIR OF MEDICINE IN THE NEW MEDICAL SCHOOL—The Registrar, The University, Nottingham (October 31).

FARADAY CHAIR OF ELECTROCHEMISTRY—The Deputy Secretary, The University, Southampton (October 31).

POSTDOCTORAL RESEARCH WORKER (with a primary qualification in physiology, physics or psychology (with understanding of statistical methods), and preferably some knowledge of computer programming) in the control and function of the small eye-movement of fixation and associated problems (stabilized retinal images, etc.)—The Assistant Bursar (Personnel), University of Reading, Reading, Berkshire (October 31).

SENIOR LECTURER/LECTURER (specialist in any field of philosophy) IN PHILOSOPHY at Flinders University of South Australia—The Registrar, Flinders University of South Australia, Bedford Park, South Australia, or The Association of Commonwealth Universities (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, October 31).

TECHNICAL OFFICER (with experience of modern vacuum technology and associated instrument making) IN THE DEPARTMENT OF PHYSICS, for duties which will include assisting research groups investigating ionization phenomena in gases and other projects, and the maintenance, development and operation of major instruments and associated vacuum equipment—The Registrar, University of New England, Armidale, New South Wales, Australia (October 31).

VETERINARY OFFICERS, GRADE II (members of the Royal College of Veterinary Surgeons with one year's appropriate post-graduate experience) with the Ministry of Agriculture, Fisheries and Food—The Civil Service Commission, 23 Savile Row, London, W.1, quoting 130/66/2 (November 4).

HEAD OF THE DEPARTMENT OF PSYCHOLOGY—Dr. P. D. McTaggart-Cowan, President, Simon Fraser University, Burnaby 2, British Columbia, Canada (November 15).

READER IN MEDICAL PARASITOLOGY at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (December 16).

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER (with a degree, Dip.Tech., H.N.C. or equivalent; if under 22, at least two G.C.E. "A" levels in scientific subjects) IN THE CHEMICAL MICROBIOLOGY DEPARTMENT, to work on proteins and enzymes of milk and cheese—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berkshire, quoting Ref. 66/29.

ASSISTANT (graduate or non-graduate) IN THE MEDICAL PROFESSORIAL UNIT for biochemical research involving automated amino acid analysis—The Governors, St. Bartholomew's Hospital, London, E.C.1, quoting Project No. 611, reference No. A30/736.

BIOCHEMIST (basic grade, probationary or post-probationary) for duties concerned primarily with blood gas analysis and biochemical aspects of investigation into metabolic disturbances associated with respiratory failure—The Personnel Officer, Royal Infirmary, Edinburgh, 3, Scotland.

MICROBIOLOGIST (Lecturer—medical or non-medical) to assist in the study of cross-infection and in an expanding programme of microbiology—The Secretary-Administrator, Institute of Laryngology and Otology, 330/332 Gray's Inn Road, London, W.C.1.

POSTDOCTORAL ASSISTANT to work under Prof. J. S. Anderson, F.R.S., on high-temperature inorganic chemistry—The Secretary, Inorganic Chemistry Laboratory, The University, South Parks Road, Oxford.

PROFESSOR AND HEAD OF THE DEPARTMENT OF OPHTHALMIC OPTICS—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff.

PROFESSOR OF PHARMACEUTICS—The Registrar, Welsh College of Advanced Technology, Cathays Park, Cardiff.

QUALIFIED PHYSICAL CHEMISTS to undertake research in inorganic thermochemistry and thermodynamics, and on vapour phase reactions partly at

high temperature—The Director of Research, Fulmer Research Institute, Stoke Poges, Bucks.

RESEARCH ASSOCIATE (candidate qualified in genetics or agriculture) IN ANIMAL BREEDING IN THE SCHOOL OF AGRICULTURE for work concerned with studies of the application of genetic theory to animal improvement—The Registrar, The University, Newcastle upon Tyne.

RESEARCH OFFICERS (graduates in biological sciences, preferably with an interest in mammalian systems, associated with a broader physico-chemical knowledge) for participation in research work in the field of safety evaluation of drugs and other chemicals—The Personnel Officer, Huntingdon Research Centre, Huntingdon, quoting Ref. GHW/3/G.

SCIENTIFIC ASSISTANT (graduate in science, preferably in biochemistry or microbiology) for microbiological assays in connexion with serum B_{12} estimations—The Consultant Haematologist, Glasgow Royal Infirmary, 84 Castle Street, Glasgow, C.4.

SENIOR TECHNICIAN (preferably with experience in high temperature techniques) to join a research group studying high-temperature chemical and electrical transport properties of refractory oxide materials—Dr. B. C. H. Steele, Metallurgy Department, Imperial College, London, S.W.7.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

General Register Office. The Registrar General's Quarterly Return for England and Wales, Quarter ended 31st March 1966. (No. 469, 1st Quarter 1966.) Pp. 47. (London: H.M. Stationery Office, 1966.) 2*s.* 6*d.* net. [257

Timber Research and Development Association. Annual Report for 1965. Pp. 40. (High Wycombe: Timber Research and Development Association, 1966.) [267

Mental Subnormality in London: a Survey of Community Care. Pp. 95. (London: Political and Economic Planning, 1966.) 7*s.* 6*d.* [267

Admiralty Marine Science Publication No. 8: Bathymetric and Magnetic Investigations, H.M.S. *Dalrymple*, 1961–1963. (Intergovernmental Oceanographic Commission—International Indian Ocean Expedition.) Pp. 13+27 profiles. (H.D. 566.) (London: Hydrographic Department, Ministry of Defence, 1966.) [277

Potato Marketing Board. Survey of Potato Research in the United Kingdom. Pp. ii+56. (London: Potato Marketing Board, 1966.) 2*s.* [277

British Scientific Instrument Research Association. Sira Annual Report 1965/66. Pp. 71. (Chislehurst, Kent: British Scientific Instrument Research Association, 1966.) [277

Industrial Chemistry—Inorganic: Ordinary and Advanced Levels. By D. M. Samuel. Pp. iv+46. (London: The Royal Institute of Chemistry, 1966.) 7*s.* 6*d.* [277

C-E-I-R Training Series 3: Management Technology. Pp. 20. (London: C-E-I-R, Ltd., 1966.) [277

Annual Report of the British Food Manufacturing Industries Research Association 1965. Pp. 67. (Leatherhead: British Food Manufacturing Industries Research Association, 1966.) [287

Royal Observatory Bulletins. No. 114: Radial-Velocity Observations of RR Lyrae Variables at Kottamia. By Sir Richard Woolley and Khairy Aly. Pp. E257–E276. No. 115: Photometric Observations of RR Lyrae Variables. By G. A. Harding and Margaret J. Penston. Pp. E277–E296. (London: H.M. Stationery Office, 1966.) 5*s.* 6*d.* net. [287

The Presentation of Technical Information Group. Proceedings of the Conference on the Training of Scientists and Technologists in Written Communication held at University College, London, 18th April, 1966. Pp. ii+13. (Babraham, Cambridge: A. K. Parker, Godolphin Cottage, Gog Magog Hills, 1966.) 6*s.* [287

The Cotton Silk and Man-Made Fibres Research Association. Shirley Institute Pamphlet No. 90: Moisture Measuring Instruments for Textiles. By G. J. Parish and M. R. Williams. Pp. 48. (Didsbury, Manchester: The Cotton Silk and Man-Made Fibres Research Association, 1966.) 15*s.* [297

Why Conservative? By Prof. David G. Shovelton. (An Inaugural Lecture delivered in the University of Birmingham on 8th March, 1966.) Pp. 15. (Birmingham: The University, 1966.) 2*s.* 6*d.* [297

Developments in the Continuous Casting of Copper-Tin Alloys Using the T.R.I. Process. By D. A. Robins and J. C. Prytherch. Pp. 7. (Publication 359.) (Greenford, Middx.: Tin Research Institute, 1966.) [297

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1110, Vol. 260 (28 July, 1966): A Discussion on Deformation of Solids by the Impact of Liquids, and Its Relation to Rain Damage in Aircraft and Missiles, to Blade Erosion in Steam Turbines and to Cavitation Erosion. Organized by F. P. Bowden, F.R.S. Pp. 73–315+plates 2 to 60. (London: The Royal Society, 1966.) 168*s.*; $25. [297

University of Bristol. Annual Report of the Long Ashton Agricultural and Horticultural Research Station (The National Fruit and Cider Institute), 1965. Pp. xiii+299+12 plates. (Long Ashton: The Research Station, University of Bristol, 1966.) 20*s.* [18

Department of Education and Science and the British Council. Scientific Research in British Universities and Colleges, 1965–66. Vol. 1: Physical Sciences. Pp. xix+413. 37*s.* 6*d.* net. Vol. 2: Life Sciences. Pp. xxi+426. 40*s.* net. (London: H.M. Stationery Office, 1966.) [18

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Other Countries

Records of the Australian Museum. Vol. 27, No. 2 (13 June, 1966): Studies of Polyzoa (Bryozoa) from the *Endeavour* Expedition, 1909–1914. 1: A New Lunulitiform Polyzoan (Ascohpora) from South-East Australia. 2: Colony Formation in *Selenaria nitida* Maplestone. By N. A. Powell. Pp. 17–32+plates 1 and 2. (Sydney: The Australian Museum, 1966.) 50 *c.* (5*s.*) [267

Rumania: a Country with a Modern Chemical Industry: Development of the Chemical Industry in Rumania. By Petru Ioan. Pp. 98. (Bucharest: Meridiane Publishing House, 1964.) [267

King Gustaf V Jubilee Clinic, Stockholm: The First Fifty Years: Radiumhemmet 1910–1937 and King Gustaf V Jubilee Clinic 1939–1960. By Elis Berven, Sven Hultberg, Hans-Ludvig Kottmeier, Rolf Sievert, Lars Santesson and Bengt Sylvén. Pp. 199. (*Acta Radiologica*, Supplementum 250.) (Stockholm: *Acta Radiologica*, 1965.) 30 Sw. kr. [267

FAO—WHO—OIE. Animal Health Yearbook 1965. Pp. vi+324. (Rome: Food and Agriculture Organization; London: H.M. Stationery Office, 1966.) 17*s.* 6*d.*; $3.50. [267

Annals of the New York Academy of Sciences. Vol. 132, Article 2: Kanamycin—Appraisal After Eight Years of Clinical Application. By S. G. Axline and 50 other authors. Pp. 771–1090. (New York: New York Academy of Sciences, 1966.) $8. [277

Réflexions pour 1985. Pp. 155. (Paris: La Documentation Française, 16 Rue Lord-Byron, 1966.) [277

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Federal Water Resources Research Program for Fiscal Year 1967: a Progress Report of the Committee on Water Resources Research. Pp. iii+20. (Washington, D.C.: Government Printing Office, 1966.) $0.15. [287

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Publications de l'Université Officielle du Congo. Vol. 10: La Structure, Phonetique du Français: Essai de Phonétique Quantique Appliquée. Par R. F. Mikus. Pp. 75. (Lubumbashi: Université Officielle du Congo, 1966.) [287

National Academy of Sciences—National Research Council. Publication 1408: Alternatives in Water Management. (A Report of the Committee on Water, Division of Earth Sciences.) Pp. viii+52. (Washington, D.C.: National Academy of Sciences—National Research Council, 1966.) $2. [287

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World Health Organization. Technical Report Series, No. 336: Sampling Methods in Morbidity Surveys and Public Health Investigations—Tenth Report of the WHO Expert Committee on Health Statistics. Pp. 29. (Geneva: World Health Organization; London: H.M. Stationery Office, 1966.) 2 Sw. francs; 3*s.* 6*d.*; $0·60. [18

Nuovi Criteri di Inquadramento e di Interpretazione Istogenetici su Basi Istopatologiche delle Neoplasie Primitive Renali. Da V. Gammafrota. (*Archivio Italiano di Patologia e Clinica dei Tumori*, Supplemento al Vol. 8, Fascicolo 3–4, Luglio-Dicembre 1965.) Pp. 198. (Milano: Istituto di Farmacologia della Università, 1966.) [18

International Atomic Energy Agency. The Agency's Budget for 1967. Pp. 44. Annual Report of the Board of Governors to the General Conference, 1 July 1965–30 June 1966. Pp. 85. The Agency's Programme for 1967–1968. Pp. 45. (Vienna: International Atomic Energy Agency, 1966.) [18

Northern Nigeria. Annual Report on the Forest Administration of Northern Nigeria, 1963–64. Pp. ii+47. (Kaduna: Government Printer, 1965. Available from Ministry of Information, Printing Division, Kaduna.) 3*s.* 6*d.* net. [28

Conseil National de la Politique Scientifique. Rapport Annuel 1965. Pp. 310. (Bruxelles: Conseil National de la Politique Scientifique, 1966.) [28

Deutsche Akademie der Wissenschaften zu Berlin: Geodätisches Institut Potsdam. Arbeiten aus dem Geodätischen Institut Potsdam. Nr. 10: Astronomische Zeit- und Breitenbestimmungen Empfangszeiten von Zeitsignalen 1961/1962. Pp. 75. Nr. 11. Relative Pendelmessungen Zwischen Potsdam und Rom. Von Claus Elstner und Rosemarie Schwarzberg. Pp. 18. Nr. 12: Sternkoordinatenkorrektionen aus dem Potsdamer Beobachtungsmaterial am Astrolab Danjon. Von Joachim Höpfner. Pp. 60. Veroffentlichungen des Geodätischen Instituts in Potsdam. Nr. 27: Die Bahnen der Kunstlichen Erdsatelliten in ihrer Abhängigkeit von den Schwereanomalien. Von Kurt Arnold. Pp. 53. Nr. 28: Der Neue 24-m-Interferenzkomparator des Geodätischen Instituts in Potsdam. Von R. Schuler und J. Rauhut. Pp. 59. Nr. 29: Die Bestimmung des Azimutes Potsdam-Bukarest aus Beobachtungen des Satelliten Echo I. Von Kurt Arnold und Dietrich Schoeps. Pp. 27. (Berlin: Deutsche Akademie der Wissenschaften zu Berlin, 1965 und 1966.) [28

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